UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

Maestría y Doctorado en Ciencias Bioquímicas

METAGENOMA EN MUESTRA CLÍNICA DE PACIENTES CON INFECCIONES DEL TRACTO RESPIRATORIO

TESIS

QUE PARA OPTAR POR EL GRADO DE:

Doctor en Ciencias

PRESENTA:

M. en Biociencias David Galeana Cadena

TUTOR PRINCIPAL

Dr. Xavier Soberón Mainero

Instituto de Biotecnología, UNAM

MIEMBROS DEL COMITÉ TUTOR

Dr. Carlos Federico Arias Ortiz

Instituto de biotecnología, UNAM

Dr. Joaquín Zúñiga Ramos

Instituto Nacional de Enfermedades Respiratorias

Ciudad de México. Octubre, 2024

CGEP/PMDCBQ/2101
B425BC-6654BDFD69794
Asunto: Jurado de examen

DAVID GALEANA CADENA
Estudiante de Doctorado en Ciencias Bioquímicas
Presente

Los miembros del Subcomité Académico en reunión ordinaria del **24 de mayo de 2024**, conocieron su solicitud de asignación de **JURADO DE EXAMEN** para optar por el grado de **Doctor en Ciencias**, con la réplica de la tesis **"Metagenoma en muestra clínica de pacientes con infecciones del tracto respiratorio"**, dirigida por el **Dr. FRANCISCO XAVIER SOBERÓN MAINERO**.

De su análisis se acordó nombrar el siguiente jurado integrado por los doctores:

YOLANDA LÓPEZ VIDAL	PMDCBQ	PRESIDENTE
FIDEL ALEJANDRO SANCHEZ FLORES	PMDCBQ	SECRETARIO
YALBI ITZEL BALDERAS MARTÍNEZ	PMDCBQ	VOCAL
AYARI FUENTES HERNANDEZ	PMDCBQ	VOCAL
PABLO VINUESA FLEISCHMANN	PMDCBQ	VOCAL

Sin otro particular por el momento, aprovecho la ocasión para enviarle un cordial saludo.

A t e n t a m e n t e
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Cd. Universitaria, Cd. Mx., a 27 de mayo de 2024

Coordinadora
Dra. Claudia Lydia Treviño Santa Cruz

PROTESTA UNIVERSITARIA DE
INTEGRIDAD Y HONESTIDAD ACADÉMICA
Y PROFESIONAL
(Graduación con trabajo escrito)

De conformidad con lo dispuesto en los artículos 87, fracción V, del Estatuto General, 68, primer párrafo, del Reglamento General de Estudios Universitarios y 26, fracción I, y 35 del Reglamento General de Exámenes, me comprometo en todo tiempo a honrar a la Institución y a cumplir con los principios establecidos en el Código de Ética de la Universidad Nacional Autónoma de México, especialmente con los de integridad y honestidad académica.

De acuerdo con lo anterior, manifiesto que el trabajo escrito titulado:

METAGENOMA EN MUESTRA CLÍNICA DE PACIENTES CON INFECCIONES DEL TRACTO RESPIRATORIO

que presenté para obtener el grado de Doctorado es original, de mi autoría y lo realicé con el rigor metodológico exigido por mi programa de posgrado, citando las fuentes de ideas, textos, imágenes, gráficos u otro tipo de obras empleadas para su desarrollo.

En consecuencia, acepto que la falta de cumplimiento de las disposiciones reglamentarias y normativas de la Universidad, en particular las ya referidas en el Código de Ética, llevará a la nulidad de los actos de carácter académico administrativo del proceso de graduación.

Atentamente

David Galeana Cadena
Número de cuenta 519022535

Miembros del Jurado

Dra. Yolanda López Vidal

Dr. Fidel Alejandro Sánchez Flores

Dra. Yalbi Itzel Balderas Martínez

Dra. Ayari Fuentes Hernández

Dr. Pablo Vinuesa Fleischmann

El presente trabajo fue realizado bajo la dirección del Dr. Xavier Soberón Mainero, miembro del Departamento de Ingeniería Celular y Biocatálisis del Instituto de Biotecnología de la Universidad Nacional Autónoma de México (IBT UNAM).

Esta investigación fue realizada gracias al financiamiento del Instituto de Biotecnología de la UNAM y el Instituto Nacional de Medicina Genómica.
También se contó con el apoyo de la beca de posgrado 2019-000002-01NACF-12598 por parte del Consejo Nacional de Humanidades Ciencias y Tecnología de México.

Agradecimientos.

Quiero expresar mi profundo agradecimiento a mis hijos por compartirme su alegría y brindarme valiosos consejos. A mis padres, por darme la oportunidad de nacer y por las enseñanzas que he recibido de ustedes a lo largo de mi vida. A mis hermanas, por su apoyo incondicional, y a *María*, por las diversas emociones que hemos disfrutado y superado juntos durante este proceso.

A mis amistades, por acompañarme en este andar de la vida.

A mis profesores y asesores, por ser una fuente constante de inspiración y enseñanza.

Al parecer, ante situaciones buenas un número de requisitos debe mantenerse simultáneamente, mientras que para llamar a una situación como mala una sola falla es suficiente.

Vladimir Arnold,

Teoría de las catástrofes.

Contenido

Índice de Figuras

Índice de Tablas

Abreviaturas

ACE2: Enzima Convertidora de Angiotensina 2

ADN: Ácido Desoxirribonucleico

ARN: Ácido Ribonucleico

BSL: Nivel de bioseguridad

NGS: Secuenciación de Nueva Generación

OMS: Organización Mundial de la Salud

PCR: Reacción en cadena de la polimerasa

RT-q PCR: PCR cuantitativa en tiempo real

ARNr: Ácido Ribonucleico ribosomal

SpO2: Saturación de Oxígeno Periférica

SDRA: Síndrome de dificultad respiratoria aguda

TAC: Tomografía Axial Computarizada

Resumen

El microbioma del tracto respiratorio, que incluye microorganismos y virus asociados a las estructuras anatómicas, proporciona información clave sobre la salud del huésped, particularmente en el contexto de enfermedades infecciosas como el COVID-19. Las alteraciones en el microbioma respiratorio durante infecciones virales se han relacionado con la severidad de la enfermedad, la respuesta inmunitaria y la susceptibilidad a infecciones secundarias. Este estudio analizó las alteraciones del microbioma nasofaríngeo en relación con la severidad del COVID-19 en pacientes mexicanos, utilizando el análisis del gen ARNr 16S. Además, se compararon los resultados con estudios previos en poblaciones de otros países.

Aunque no se encontraron diferencias estadísticamente significativas en los índices de diversidad evaluados entre los grupos de severidad, se identificaron géneros reportados como parte del microbioma oral que invaden la nasofaringe en pacientes con formas graves de la enfermedad. Los géneros *Corynebacterium, Streptococcus* y *Staphylococcus* fueron identificados como componentes nucleares del microbioma nasofaríngeo. Estos resultados sugieren que la enfermedad crítica por COVID-19 podría contribuir a la perturbación de los mecanismos de barrera y las condiciones fisicoquímicas de la nasofaringe, facilitando la invasión bacteriana. Si bien, los géneros nucleares coinciden con estudios de otras regiones geográficas, se observaron diferencias en otros géneros bacterianos, subrayando la importancia de estudios específicos para comprender su relevancia en la población mexicana.

Abstract

The respiratory tract microbiome, which includes microorganisms and viruses associated with anatomical structures, provides key insights into host health, particularly in the context of infectious diseases such as COVID-19. Alterations in the respiratory microbiome during viral infections have been linked to disease severity, immune response, and susceptibility to secondary infections. This study analyzed changes in the nasopharyngeal microbiome in relation to COVID-19 severity in Mexican patients, using 16S rRNA gene analysis. Additionally, the results were compared with previous studies conducted in populations from other countries.

Although no statistically significant differences were found in diversity indices between severity groups, genera previously reported as part of the oral microbiome were identified as invasive organisms of the nasopharynx in patients with severe forms of the disease. The genera *Corynebacterium, Streptococcus*, and *Staphylococcus* were identified as core components of the nasopharyngeal microbiome. These findings suggest that critical COVID-19 may contribute to the disruption of barrier mechanisms and the physicochemical conditions of the nasopharynx, facilitating bacterial invasion. While the core genera were consistent with studies from other geographic regions, differences in other bacterial genera were observed, highlighting the importance of region-specific studies to understand their relevance in the Mexican population.

1. Introducción

1.1 Infecciones del tracto respiratorio

La función respiratoria es esencial para la vida, aunque con frecuencia se pasan por alto los riesgos asociados a esta. Cada día inhalamos millones de microorganismos (Santacroce et al., 2020), sin que, en la mayoría de los casos, nuestro sistema respiratorio se vea afectado. Para lograrlo, el tracto respiratorio ha desarrollado estructuras anatómicas especializadas que crean un microambiente único (Man et al., 2017). Además, cuenta con mecanismos de defensa que incluyen barreras físicas y respuestas inmunológicas (Sözener et al., 2020). Sin embargo, en situaciones patológicas, como las enfermedades infecciosas respiratorias, se puede producir daño tisular, que varía en severidad, llegando incluso a ser letal en determinadas circunstancias (Choreño-Parra et al., 2022; Lanks et al., 2019). Ante estos daños, se desencadena una respuesta inflamatoria cuyo objetivo inicial es combatir la infección y, posteriormente, promover la reparación tisular.

El sistema respiratorio está formado por una amplia diversidad de células, como las epiteliales, endoteliales y mesenquimales, que constituyen una barrera física continua. Las células caliciformes, en particular, secretan complejos de proteínas que contienen mucina, citocinas, factores del complemento, péptidos antimicrobianos e IgA, desempeñando un papel importante en la prevención o propagación de infecciones (Mettelman et al., 2022).

Las citocinas y quimiocinas liberadas por las células que se encuentran en el tracto respiratorio pueden activar una respuesta inmunitaria que involucra a células especializadas, como las dendríticas, neutrófilos, células asesinas naturales (NK) y macrófagos (Netea et al., 2020). Esta respuesta puede intensificar la inflamación y conducir a una reacción inmunitaria más específica, en la que participan los linfocitos (Worbs et al., 2017). Como resultado, el proceso de daño e inflamación puede modificar las estructuras histológicas y las condiciones fisicoquímicas del microambiente, afectando las barreras protectoras lo cual puede contribuir al desarrollo de enfermedades infecciosas más severas (Galeana-Cadena et al., 2024).

Las infecciones respiratorias son un reto constante y dinámico para la salud pública, clasificadas como patologías emergentes, reemergentes, pandémicas o endémicas, según distintos criterios epidemiológicos (Parums, 2023). Estas infecciones abarcan una amplia variedad de enfermedades, que pueden categorizarse según la parte afectada del tracto respiratorio, ya sea superior o inferior, o por sitios anatómicos específicos, como rinitis, faringitis, bronquitis o neumonía (Niederman y Torres, 2022). Además, algunas infecciones pueden comenzar como un resfriado o gripe y luego evolucionar hacia enfermedades sistémicas.

Las etiologías reportadas de las infecciones del tracto respiratorio incluyen bacterias, hongos y virus. Hasta 2019, Streptococcus pneumoniae, *Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumanii, Haemophilus influenzae* tipo b, el Virus Sincicial Respiratorio y el virus de la Influenza eran destacados por sus altas tasas de incidencia y mortalidad a nivel mundial (GBD 2018-2019). No obstante, en 2019, surgieron los primeros casos de COVID-19 en Wuhan, China, dando lugar a

una enfermedad pandémica que ocupó los primeros lugares en morbilidad en muchas regiones del mundo hasta 2022.

1.1.1 COVID-19

La COVID-19 es una enfermedad infecciosa causada por el virus SARS-CoV-2. La principal vía de transmisión ocurre a través de gotas respiratorias expulsadas por individuos infectados al toser, estornudar o hablar. Sin embargo, también se ha documentado la transmisión por aerosoles y el contacto directo con superficies contaminadas (Harrison et al., 2020).

El SARS-CoV-2 pertenece a la familia Coronaviridae y está compuesto por una envoltura viral (E), una glicoproteína Spike (S) con dos subunidades (S1 y S2), proteínas de membrana (M), y una proteína de nucleocápside (N), con un genoma de ARN de aproximadamente 30,000 pares de bases. La glicoproteína S se une al receptor de la enzima convertidora de angiotensina 2 (ACE2) en las células humanas, lo que facilita la entrada del virus (Lamers & Haagmans, 2022).

Una vez que el virus entra en contacto con las células del tracto respiratorio superior, inicia su replicación y propagación en la cavidad nasal y oral (Moroni-Zengraf et al., 2023; Huang et al., 2021). El hospedero responde mediante interferones y la activación de células inmunitarias, lo que puede limitar la replicación viral y reducir la severidad de la enfermedad (Liu et al., 2022). En algunos casos, el virus puede migrar a la faringe, desencadenando una respuesta inflamatoria para limitar su avance (Lorenz Chua et al., 2020). La liberación de citocinas y quimiocinas en esta fase produce síntomas sistémicos como fiebre y malestar general (Elrobaa & New, 2021).

El epitelio respiratorio superior aumenta la secreción de moco para facilitar el aclaramiento viral, mientras que la inflamación convoca a células inmunitarias especializadas, iniciando así la respuesta inmunitaria adaptativa (Sette et al., 2023). Se considera que los individuos que no eliminan eficazmente el virus en estas etapas pueden experimentar formas más graves de la enfermedad al invadir el tracto respiratorio inferior (Merad et al., 2022) o desencadenan una respuesta descontrolada de citocinas y quimiocinas, también llamada tormenta de citocinas (Dharra et al., 2023).

A lo largo de la pandemia, se han identificado diversas variantes del SARS-CoV-2, algunas con mayor letalidad y otras con mayor capacidad de transmisión (Carabelli et al., 2023).

A pesar de los avances en el estudio de la COVID-19, persisten múltiples incógnitas, particularmente en torno a los factores que influyen en la severidad de la enfermedad. Factores cardiovasculares, metabólicos, inmunológicos y el microbioma respiratorio han sido señalados como elementos que podrían tener un impacto significativo en la evolución clínica de la infección.

1.2. Microbioma Humano

El término “microbioma” fue definido por Berg et al. en 2020 como el conjunto de microorganismos y sus genomas en un ambiente específico. En el contexto humano, el microbioma se refiere a la comunidad de bacterias, arqueas, hongos, protozoarios y virus que habitan en y sobre las distintas estructuras del cuerpo (Proctor et al., 2019; Lloyd-Price et al., 2016). Este ecosistema microbiano ha despertado gran interés,

especialmente en su relación con los procesos de salud y enfermedad a lo largo de la vida (Martino et al., 2022). Se ha sugerido que el microbioma desempeña un papel clave en el desarrollo del sistema inmunológico (Lubin et al., 2023), en la digestión de los alimentos (Fu et al., 2019), en la producción de metabolitos beneficiosos, como los ácidos grasos de cadena corta (He et al., 2020), y en la protección contra patógenos (Khan et al., 2021). Además, cada parte del cuerpo humano ofrece condiciones físicoquímicas particulares, creando microambientes específicos que permiten el desarrollo de distintas comunidades microbianas (Man et al., 2017).

1.2.1 Microbioma respiratorio

El tracto respiratorio exhibe características anatómicas y fisiológicas únicas en cada una de sus estructuras, a las cuales deben adaptarse los microorganismos y virus que lo colonizan. Estos agentes suelen provenir de otras personas con quienes se mantiene contacto cercano o se comparte un mismo espacio (Adair & Douglas, 2016). El sistema respiratorio sano alberga una microbiota residente, cuya composición puede verse influenciada por diversos factores como la genética, el tipo de parto, la edad, la dieta y la presencia de enfermedades (Galeana-Cadena et al., 2023).

En la cavidad nasal, se han identificado géneros como C*orynebacterium, Staphylococcus, Propionibacterium, Moraxella* y *Streptococcus*. En la nasofaringe, predominan *Moraxella, Staphylococcus, Corynebacterium, Dolosigranulum, Haemophilus* y *Streptococcus*. En la orofaringe, se han descrito *Streptococcus, Rothia, Veillonella, Prevotella, Leptotrichia, Neisseria, Bacteroides* y *Fusobacterium.* Mientras que en la cavidad oral se destacan *Streptococcus, Actinomyces, Corynebacterium, Fusobacterium, Leptotrichia, Neisseria, Porphyromonas, Prevotella, Alloprevotella, Veillonella, Rothia, Gemella, Granulicatella, Haemophilus*

(Figura 1). En el tracto respiratorio inferior, los géneros más comunes incluyen *Prevotella, Veillonella* y *Streptococcus* (Homan et al., 2017; Mark Welch et al., 2019; Natalini et al., 2022).

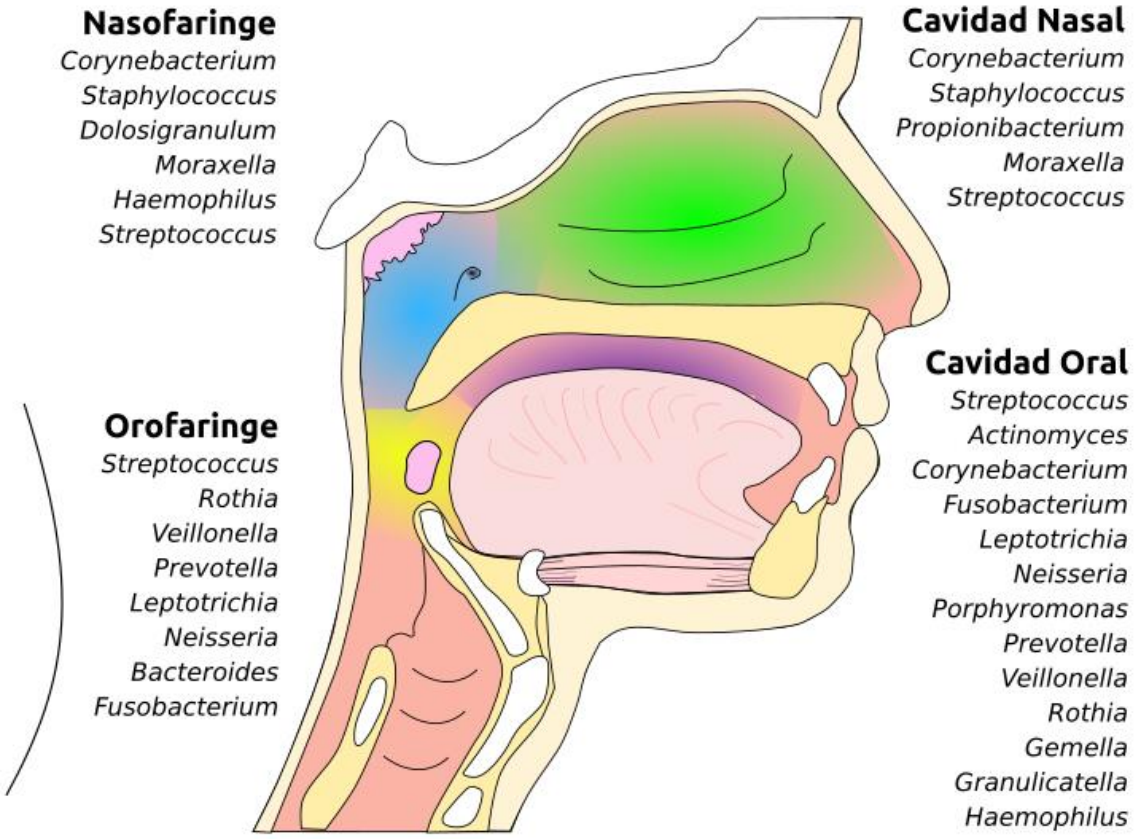


Figura 1 Géneros bacterianos reportados en las diferentes estructuras del tracto respiratorio superior.

La microbiota residente puede contribuir a la protección del hospedero de diversas formas. Proporciona protección ecológica directa al inhibir la colonización y el crecimiento de microorganismos patógenos mediante mecanismos de contención y/o eliminación (Chiu et al., 2017). Asimismo, promueve la producción y maduración de células inmunitarias, y participa en la regulación de respuestas inflamatorias (Lajqi et al., 2020). Cuando estos mecanismos se ven alterados, se abre la posibilidad de que ciertos microorganismos y virus desencadenen enfermedades infecciosas

respiratorias (Alvarado-Peña et al., 2023; Barbosa-Amezcua et al., 2023, Porto & Moraes, 2021).

Distinguir entre la colonización mutualista y la presencia de microorganismos potencialmente patógenos en el microbioma respiratorio es un desafío significativo. Además de la evaluación clínica, es crucial considerar diversos parámetros microbiológicos, como la morfología, fisiología y genómica, para lograr una identificación precisa de los microorganismos, evaluar su abundancia relativa y detectar la presencia de microorganismos dominantes (Funke et al., 1997; Church et al., 2020). Por ello, el análisis del microbioma respiratorio se realiza mediante enfoques complementarios, que incluyen tanto los cultivos microbiológicos tradicionales como técnicas avanzadas de biología molecular, como la secuenciación de nueva generación (NGS, por sus siglas en inglés) (Zhou et al., 2021).

2. Antecedentes

2.1 Aplicación de la NGS en el estudio del microbioma respiratorio

Las infecciones del tracto respiratorio han sido tradicionalmente estudiadas mediante cultivos microbiológicos, los cuales permiten obtener cultivos bacterianos puros, esenciales para investigar la virulencia y la susceptibilidad a los antibióticos (Lagier et al., 2015). Esta metodología ha sido clave para mejorar nuestra comprensión de las enfermedades respiratorias y desarrollar tratamientos eficaces. Sin embargo, para identificar todos los microorganismos presentes en una comunidad microbiana, incluidos aquellos que no son cultivables, es necesario emplear enfoques alternativos.

La NGS ha revolucionado el estudio del microbioma, destacándose dos metodologías principales: la secuenciación de amplicones y la secuenciación masiva aleatoria. La secuenciación de amplicones se utiliza para detectar grupos taxonómicos específicos, siendo el gen del ARNr 16S uno de los más empleados para identificar bacterias. Esta técnica permite estudiar tanto bacterias cultivables como no cultivables, evitando la interferencia del material genético del hospedero. Además, debido a su enfoque específico, requiere un menor número de secuencias, lo que la hace más rentable y facilita el análisis bioinformático. No obstante, su limitación radica en su especificidad, ya que no permite el estudio de otros grupos taxonómicos como hongos, parásitos o virus presentes en la muestra (Bharti & Grimm, 2021; Wensel et al., 2022; Regueira-Iglesias et al., 2023).

Para superar esta limitación, se puede emplear la secuenciación metagenómica masiva aleatoria. En este enfoque, el ADN total extraído de la muestra se fragmenta en secuencias cortas y se secuencia sin la necesidad de utilizar amplicones. La metagenómica permite el análisis de los genomas y genes presentes en las secuencias obtenidas, abarcando todos los organismos y virus en una muestra. Sin embargo, al no ser específica, la metagenómica puede requerir una gran cantidad de secuencias, particularmente en organismos y virus de baja abundancia, lo que incrementa significativamente los costos (Srinivas et al., 2022; Baharti & Grimm, 2021).

En 2019, Schlaberg et al. destacaron el potencial de la NGS en el ámbito clínico para revolucionar el diagnóstico de enfermedades infecciosas y mejorar la comprensión de su etiología. Dickson et al. (2020) demostraron que las variaciones en la microbiota pulmonar son predictivas del desenlace clínico en pacientes críticos. Este tipo de hallazgos ha impulsado un creciente interés en el análisis del microbioma respiratorio para el diagnóstico etiológico en neumonías (Romero-Espinoza et al., 2018; Qi et al., 2019).

2.2 Cambios en el microbioma respiratorio en infecciones virales

Los estudios sobre el microbioma respiratorio en pacientes con infecciones virales han revelado alteraciones taxonómicas que podrían tener un papel importante en la severidad de estas enfermedades. En infecciones virales como la influenza, se ha identificado la presencia de bacterias dominantes asociadas a patógenos

oportunistas, tales como *Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa* y *Streptococcus pneumoniae* (Kaul et al., 2020; Qin et al., 2020). Además, en adultos con infección por rinovirus, se observó una disminución de los géneros *Haemophilus* y *Neisseria,* junto con un aumento de *Propionibacterium* (Allen et al., 2014). En contraste, en infecciones por el virus sincicial respiratorio, Cuthbertson et al. (2021) no encontraron diferencias significativas en la composición bacteriana ni en la diversidad de las muestras orofaríngeas. Estos resultados subrayan la necesidad de continuar investigando las modificaciones del microbioma respiratorio en diferentes infecciones virales.

2.3 Microbioma respiratorio en pacientes con COVID-19

En el contexto de la COVID-19, Zhang et al. (2020) informaron una disminución de la diversidad alfa del microbioma estudiado mediante análisis metagenómico, en muestras de exudado nasofaríngeo y esputo de pacientes con COVID-19. Hernández-Terán et al. (2021) analizando el gen ARNr 16S describieron una disbiosis en pacientes con COVID-19 severo, caracterizada por la pérdida de complejidad estructural de la microbiota. Por su parte, Smith et al. (2021) observaron un incremento en los géneros *Staphylococcus, Peptostreptococcus* y *Prevotella* en pacientes con COVID crítico, sugiriendo que la disbiosis podría haber estado presente antes de la infección con SARS-CoV-2. Kumar et al. y Gauthier et al. (2022) no encontraron diferencias en la diversidad alfa entre pacientes con COVID y los controles, pero identificaron grupos taxonómicos asociados con la severidad de la enfermedad. En contraste, el estudio de Ventero et al. (2022), reportó una disminución en la diversidad del microbioma en pacientes que fallecieron por COVID-19.

3. Justificación

El microbioma respiratorio, es un ecosistema dinámico compuesto por microorganismos que coexisten en el tracto respiratorio. Desempeña un rol importante en la defensa directa contra patógenos y en la modulación del sistema inmunitario. Sin embargo, las infecciones virales pueden modificar este balance, desencadenando alteraciones de los grupos taxonómicos que potencialmente exacerban la severidad de la enfermedad. Aunque el papel del microbioma ha sido documentado en algunas enfermedades infecciosas virales, el impacto específico de los cambios en las comunidades microbianas del tracto respiratorio durante la infección por SARS-CoV-2, sigue siendo un tema importante de investigación en salud pública.

Durante la pandemia de COVID-19, el SARS-CoV-2 se convirtió en la principal causa de infecciones respiratorias entre 2020 y 2021. En México, de acuerdo a datos oficiales del Instituto Nacional de Estadística y Geografía (INEGI) la COVID-19 fue la principal causa de defunciones en 2021. Asimismo, de acuerdo con la Secretaría de Salud de México, hasta 2023 se han registrado 976,397 casos confirmados de COVID-19 y cerca de 240 mil defunciones atribuibles a esta enfermedad.

Ante este panorama, el presente proyecto propone estudiar cómo la infección por COVID-19 afecta la composición microbiana del tracto respiratorio y qué implicaciones tienen estos cambios en la severidad de la enfermedad, buscando la presencia de firmas microbianas predictivas de la severidad del COVID-19, lo que permitiría un manejo más personalizado de los pacientes y promover un mejor pronostico clínico.

4. Hipótesis

El análisis del microbioma de muestras respiratorias de pacientes mexicanos, identificará cambios taxonómicos en la nasofaringe relacionados con la severidad del COVID-19.

5. Objetivos

5.1 Objetivo General

Identificar cambios taxonómicos de la microbiota de la nasofaringe relacionados a la severidad COVID-19 en pacientes mexicanos, mediante el análisis bioinformático de muestras clínicas respiratorias.

5.2 Objetivos específicos

- Obtener muestras de exudado nasofaríngeo, de pacientes confirmados con COVID-19, familiares de los pacientes con COVID-19 y voluntarios sanos.
- Extraer ADN y ARN de las muestras y secuenciar el metagenoma por secuenciación aleatoria masiva.
- Extraer el ADN de las muestras y secuenciar el amplicón del gen ARNr 16S.
- Realizar análisis bioinformáticos para asignación taxonómica.
- Efectuar análisis estadísticos de asociación y correlación entre los grados de severidad y los grupos taxonómicos.

6. Consideraciones éticas y de bioseguridad

El proyecto fue aprobado por el Comité de Ética del Instituto Nacional de Enfermedades Respiratorias (INER) (No. 0352-2150) y los experimentos se llevaron a cabo en seguimiento de las Normas Oficiales Mexicanas vigentes y Lineamientos de bioseguridad nacionales e internacionales. Se elaboraron los consentimientos informados de acuerdo a la normatividad nacional e internacional vigente. Los datos recabados son resguardados con la finalidad de garantizar la protección a la privacidad y confidencialidad. Las técnicas moleculares fueron realizadas de manera ética y responsable. Los resultados están siendo comunicados de manera clara honesta y precisa.

7. Estrategia experimental

Población de estudio: Pacientes del INER con sospecha de COVID-19 con diferentes grados de severidad de la enfermedad.

Tipo de estudio: Transversal, exploratorio y descriptivo.

Tamaño de la muestra: 44 individuos

Tipo de muestreo: Por conveniencia

Tipo de muestra: Exudado nasofaríngeo

Controles: Voluntarios sanos, familiares del paciente con COVID-19, asintomáticos por 15 días y con resultado negativo de qrtPCR

Criterios de inclusión:

- Pacientes con diagnóstico confirmado de COVID por qrtPCR.

- Pacientes con qrtPCR negativa, pero con signos clínicos respiratorios compatibles con COVID-19.
- Aceptación del consentimiento informado.

Criterios de exclusión

- Epistaxis durante la toma de la muestra.
- Pólipos o tumores nasales bilaterales que impidan la toma de exudado nasofaringeo.

Criterios de eliminación

- Pacientes que retiren su consentimiento.
- Muy baja concentración de ácidos nucleicos en la muestra.
- Datos incorrectos de contacto, imposibilitando el seguimiento.

7.1. Diagrama del diseño experimental

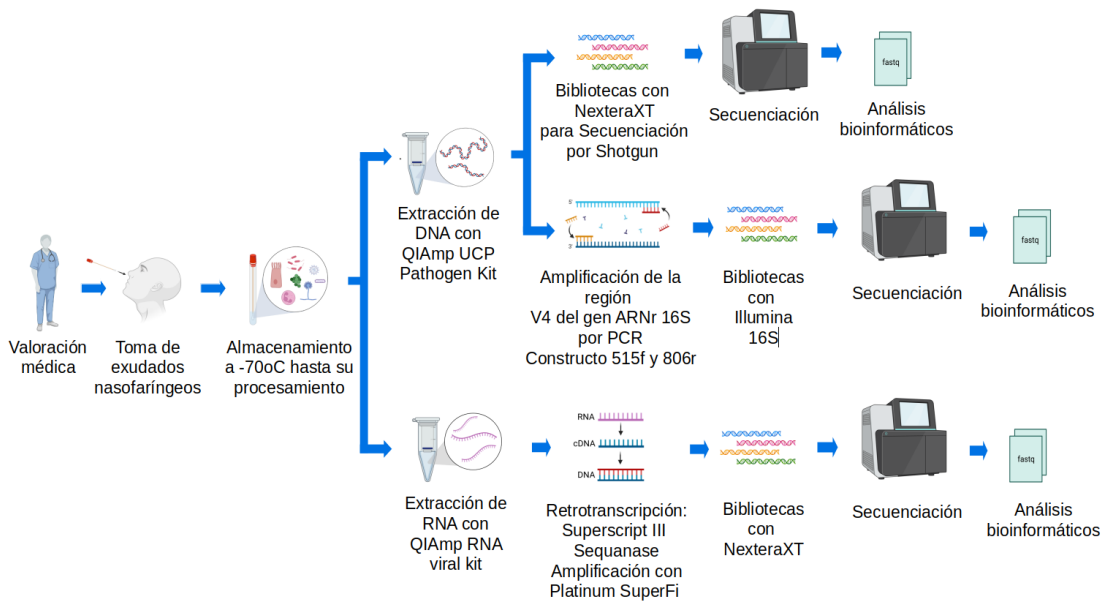


Figura 2. Diseño experimental general del proyecto para estudiar el microbioma de la nasofaringe de voluntarios sanos y pacientes con COVID-19

8. Materiales y métodos.

8.1. Población de estudio

La valoración clínica de los pacientes y voluntarios sanos fue realizada por personal médico calificado, quienes recabaron los datos clínicos relevantes mediante entrevistas, seguimiento telefónico y/o revisión de expedientes clínicos.

Posteriormente a su valoración clínica y la firma del consentimiento informado, personal capacitado obtuvo dos muestras de exudado nasofaríngeo pareadas en las instalaciones del INER. Las muestras fueron conservadas en medio UTM® Universal Transport Medium™ (Copan Diagnostics, Estados Unidos), y almacenadas a -70º C hasta su procesamiento. Para confirmar la presencia del virus de SARS-CoV-2, se realizó una RT-qPCR a una de las muestras pareadas de cada paciente.

La clasificación de los pacientes según la severidad de la COVID-19 se realizó de acuerdo con los criterios establecidos en la Guía para el manejo clínico del COVID-19 de la Organización Mundial de la Salud (OMS), publicada en 2020 (Tabla 1).

Tabla 1. Clasificación de la severidad de la COVID-19.

Clasificación	**Criterios**
Leve	Pacientes sintomáticos (fiebre, tos, fatiga, mialgias), confirmados con COVID por RT-qPCR, pero que no presentan neumonía o hipoxia.
Moderada	Signos clínicos de neumonía (fiebre, tos, disnea, taquipnea), SpO2 ≥90% con aire ambiente.
Severa	Signos clínicos de neumonía, más alguno de los siguientes: frecuencia respiratoria >30 inspiraciones /min, disnea grave: SpO2 <90% en aire ambiente.
Crítica	Síndrome de dificultad respiratoria aguda (SDRA), opacidades pulmonares bilaterales por radiografía o TAC, ventilación invasiva, choque séptico, embolia pulmonar.

En total, se recolectaron 205 muestras de exudado nasofaríngeo. Por razones presupuestarias, se seleccionaron 14 voluntarios sanos como controles, 9 pacientes con COVID-19 severo y 21 pacientes críticos.

8.2. Procesamiento de las muestras

La extracción de ácidos nucleicos se realizó en un gabinete de seguridad BSL II. Las muestras fueron divididas en dos partes: en una, se extrajo ADN utilizando el *kit QIAMP UCP Pathogen*; en la otra, se extrajo ARN viral mediante el Viral RNA Mini Kit, tras un filtrado con filtro de 0.22 µm. La retrotranscripción se efectuó con la *SuperScript III Reverse Transcriptase* siguiendo el protocolo reportado por Goya et al. (2018). La preparación de las librerías se realizó con el kit Nextera XT DNA. La secuenciación masiva aleatoria del metagenoma se llevó a cabo en la plataforma HiSeq de Illumina en la unidad de secuenciación del Instituto de Biotecnología (IBT) de la UNAM.

Para el análisis del gen ARNr 16S, el ADN fue amplificado utilizando los *primers* 515f y 806r reportados por Lopez-Filloy et al. (2022). Las muestras fueron codificadas y agrupadas siguiendo el protocolo Illumina 16S *Metagenomic Secuencing Library*. La secuenciación se realizó en la plataforma MiSeq del CIENI en el INER, con un tamaño de 250 pares de bases por lectura, de forma pareada.

8.3 Análisis bioinformáticos

Las secuencias metagenómicas obtenidas fueron sometidas a control de calidad, que incluyó la limpieza, filtrado y recorte de lecturas de baja calidad mediante el software Trimmomatic versión 0.39 (Bolger et al., 2014). La asignación taxonómica se realizó utilizando los programas Kraken2 (Wood et al., 2019) con la base de datos

maxikraken2_1903_140GB y DIAMOND versión 2.0.13 (Buchfink et al., 2017) con la base de datos *viruses nucleotide collection* nr/nt.

El análisis taxonómico de los amplicones de la región V4 del gen ARNr 16S se llevó a cabo en R versión 4.1.2 utilizando el paquete DADA-2 versión 1.14 (Callahan et al., 2016) y la base de datos SILVA v138, según lo detallado en Galeana-Cadena et al. (2024).

El *script* de los análisis en R del gen ARNr 16S se encuentra disponible en: https://github.com/David-microbiomics/Rscript/blob/main/Rscript_16SV4

8.4 Análisis estadísticos

Se realizó un análisis de correlación para explorar las relaciones entre las variables de interés. Se utilizó el coeficiente de correlación por rangos de Spearman para evaluar la fuerza y dirección de las asociaciones entre las abundancias relativas de los grupos taxonómicos y la severidad del COVID-19, categorizada en tres niveles ordinales: controles (0), pacientes con COVID-19 moderado (1) y casos críticos (2). Este análisis se realizó con el software R, estableciendo un nivel de significancia de $p < 0.05$.

Para los análisis de asociación, se empleó la prueba de Kruskal-Wallis para comparar la abundancia relativa de los grupos taxonómicos entre los diferentes niveles de severidad. Además, se realizó un análisis exploratorio para evaluar el impacto de covariables como comorbilidades, uso de antibióticos, administración de esteroides y ventilación mecánica mediante intubación orotraqueal. Estas comparaciones se efectuaron utilizando la prueba de Mann-Whitney, evaluando la relación entre las abundancias relativas de los grupos taxonómicos y las covariables mencionadas.

9. Resultados

9.1. Características demográficas y clínicas

El estudio incluyó a 44 adultos residentes de la Zona Metropolitana del Valle de México, con una edad media de 49 años (rango: 42-61), de los cuales 16 (36 %) eran mujeres. Las categorías de Índice de Masa Corporal (IMC) se distribuyeron de la siguiente manera: 5 personas (11 %) tenían peso normal, 19 (43 %) tenían sobrepeso, 16 (36 %) presentaban obesidad, y en 4 casos (9.1 %) no se reportó el IMC. Los pacientes se clasificaron de acuerdo con la severidad del COVID-19. Se observó que la obesidad era más frecuente en los pacientes con enfermedad severa y crítica (p = 0.002). Asimismo, se encontró una diferencia estadísticamente significativa en el historial de tabaquismo entre los grupos (p = 0.03) (Tabla 2).

Tabla 2. Características demográficas y comorbilidades de los participantes según la severidad del COVID-19.

	Controles, N=14	**Severos, N=9**	**Críticos, N=21**	**Valor de P***
Edad	52 (40, 60)	47 (47, 55)	48 (42, 65)	0.8
Género				>0.9
Femenino	6 (43%)	3 (33%)	7 (33%)	
Masculino	8 (57%)	6 (67%)	14 (67%)	
Altura	1.68 (1.54, 1.75)	1.67 (1.64, 1.68)	1.70 (1.60, 1.73)	0.9
Datos no disponibles	4	0	0	
Peso	72 (62, 81)	82 (74, 85)	80 (75, 91)	0.14
Datos no disponibles	4	0	0	
Clasificación por IMC				0.002
Peso normal	2 (14%)	1 (11%)	2 (9.5%)	
Obesidad	0 (0%)	5 (56%)	11 (52%)	
Sobrepeso	8 (57%)	3 (33%)	8 (38%)	
Datos no disponibles	4 (29%)	0 (0%)	0 (0%)	

Comorbilidades				
Obesidad	1 (7.1%)	0 (0%)	8 (38%)	0.031
DM2	1 (7.1%)	1 (11%)	6 (29%)	0.3
Hipertensión	1 (7.1%)	3 (33%)	9 (43%)	0.066
Cardiopatías	0 (0%)	0 (0%)	1 (4.8%)	>0.9
Insuficiencia renal	0 (0%)	0 (0%)	1 (4.8%)	>0.9
Inmunosupresión	0 (0%)	0 (0%)	0 (0%)	
Asma	1 (7.1%)	1 (11%)	0 (0%)	0.3
EPOC	0 (0%)	0 (0%)	0 (0%)	
VIH	0 (0%)	0 (0%)	0 (0%)	
ERG	2 (14%)	1 (11%)	0 (0%)	0.2
Rinitis alérgica	0 (0%)	2 (22%)	0 (0%)	0.038
Alcoholismo	0 (0%)	2 (22%)	5 (24%)	0.12
Tabaquismo	0 (0%)	1 (11%)	7 (33%)	0.031
Antibiótico previo	0	8 (89%)	15 (71%)	<0.001
Días hospitalizado	0 (0, 0)	11 (0, 16)	29 (13, 37)	<0.001
Días del inicio de síntomas hasta la toma de la muestra	0 (0, 0)	9 (7, 12)	10 (8, 14)	<0.001

* Prueba de suma de rangos de Kruskal-Wallis; prueba exacta de Fisher.
IMC: Índice de Masa Corporal, DM2: Diabetes Mellitus Tipo 2, EPOC: Enfermedad Pulmonar Obstructiva Crónica, GERD: Enfermedad por Reflujo Gastroesofágico, VIH: Virus de Inmunodeficiencia Humana.

Los síntomas más comunes en los pacientes con COVID-19 fueron fiebre, tos, disnea, artralgias y mialgias (Figura 3). Referente a los signos vitales, se observó una disminución significativa en la saturación de oxígeno (SpO2) en comparación con el grupo control $p<0.001$. En el análisis de biometría hemática, los pacientes con COVID-19 presentaron una reducción en los niveles de linfocitos ($p = 0.012$) y un aumento en los niveles de neutrófilos ($p = 0.010$) en relación con los controles. Además, la relación neutrófilo-linfocito mostró una diferencia estadísticamente significativa entre ambos grupos ($p = 0.001$).

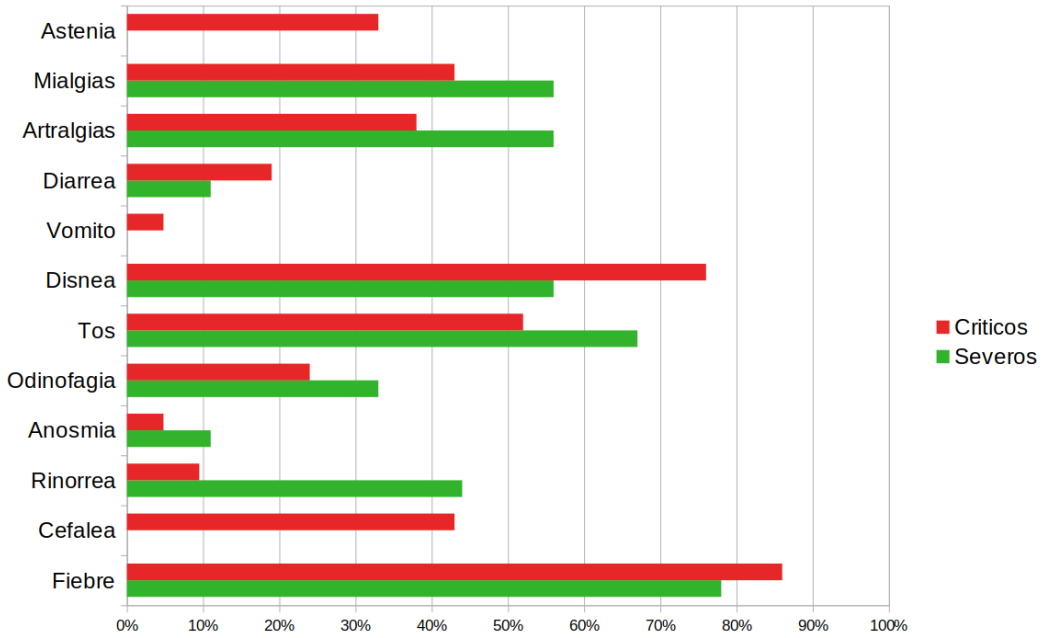


Figura 3. Prevalencia de síntomas en pacientes con COVID-19.

9.2 Análisis metagenómicos de las muestras

Durante el proceso de estandarización, se seleccionaron 8 muestras que fueron secuenciadas con una profundidad de 10 millones de lecturas, utilizando un tamaño de 2X75 pb. La asignación taxonómica inicial se realizó mediante Kraken2, observándose que un porcentaje considerable de las lecturas secuenciadas correspondía a *Homo sapiens* (Figura 4).

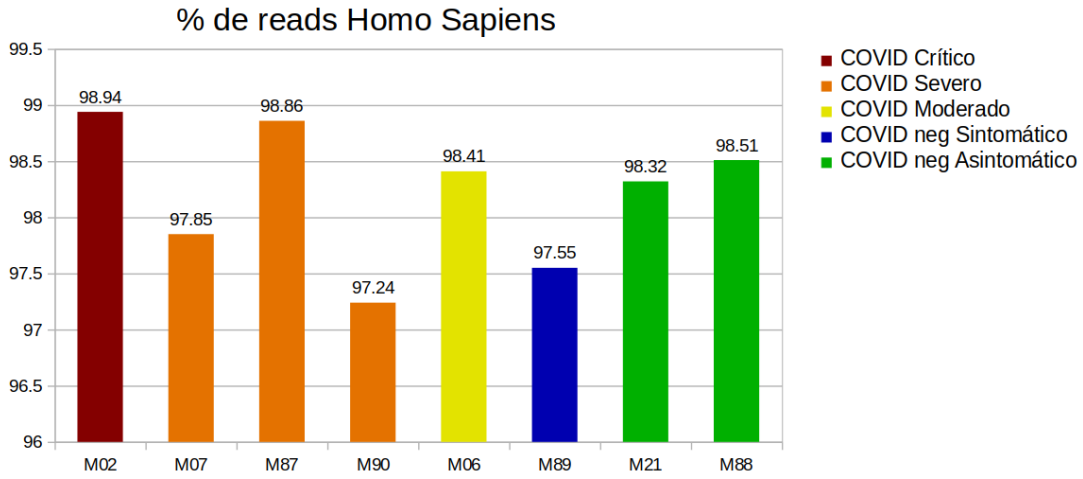


Figura 4. Porcentaje de secuencias de *Homo sapiens* en las muestras.

Dado el elevado porcentaje de lecturas humanas, se empleó el *software* Bowtie2 para filtrar aquellas que alineaban con el genoma humano GRCh38. Una vez eliminadas las secuencias asignadas al ADN humano, las lecturas restantes fueron clasificadas nuevamente utilizando Kraken2, cuyos resultados se resumen en la Tabla 3.

Tabla 3. Resultados de asignación taxonómica mediante Kraken 2 posterior al retiro de secuencias humanas.

Especie	M02	M07	M87	M90	M06	M89	M21	M88
Cutibacterium acnes	23	179	81	452	96	106	86	67
Staphylococcus epidermidis		31	21	153	41	59	40	335
Acinetobacter johnsonii		37			10	12	20	
Klebsiella variicola	25		23	24		22	20	15
Staphylococcus aureus			541					14
Dolosigranulum pigrum								1602
Corynebacterium segmentosum				1269				846
Streptococcus pyogenes			20	18		14	17	
Pseudomonas fluorescens	37		34	34		33	29	22
Malassezia restricta								105
Corynebacterium kefirresidentii						27		
Klebsiella pneumoniae						15		
Enterococcus faecalis						37		
Staphylococcus virus SEP1				7				

COVID Crítico
COVID Severo
COVID Moderado
COVID neg Sintomático
COVID neg Asintomático

Adicionalmente, a partir de la retrotranscripción del RNA extraído de las muestras, se secuenciaron una selección de muestras con el objetivo de detectar la presencia de virus. Los resultados indicaron que solo se detectó la presencia de SARS-CoV-2, con lecturas mínimas asociadas a virus del papiloma humano (Tabla 4).

Tabla 4. Virus detectados en las muestras seleccionadas

		Número de lecturas asignadas por muestra				
Familia	**Especie**	**MC02 DNA**	**MC07 DNA**	**MC07 RNA**	**MC198 RNA**	**MH78 RNA**
Inoviridae	Inoviridae sp. Cti YN10	0	0	0	2	0
Papilomaviridae	Human papilomavirus	2	0	16	0	0
Parvovirinae	Parvovirus NIH-CQV	0	0	4	0	0

Flaviviridae	Pestivirus A	0	0	0	0	8
Coronaviridae	SARS-CoV-2	37	58	16133	26	8
Astroviridae	Human astrovirus	0	0	0	0	2
Retroviridae	Human endogenous retrovirus	0	0	0	0	2

Debido al alto porcentaje de secuencias humanas en las muestras y considerando que el objetivo principal del estudio era analizar el microbioma respiratorio, se evaluaron alternativas para optimizar el proceso de secuenciación. Entre las opciones consideradas estaban el aumento de la profundidad de secuenciación y la implementación de métodos de depleción del ADN del hospedero. No obstante, estas alternativas implicaban un aumento significativo en los costos del proyecto. Tras valorar estos factores, se decidió llevar a cabo el análisis del microbioma utilizando el gen ARNr 16S para la totalidad del estudio.

9.3 Análisis de Diversidad del Microbioma nasofaríngeo en pacientes con COVID-19 por análisis del gen ARNr 16S.

En el análisis de los índices de alfa diversidad se observó que el índice de Shannon mostró un aumento en la diversidad bacteriana en pacientes críticos con COVID-19 ($p = 0.03$) (Figura 5c). Sin embargo, el resto de los índices alfa no revelaron diferencias significativas entre los pacientes severos, críticos y los controles (Figura 5a y 5b). Para evaluar la diversidad beta, se utilizó el escalado multidimensional no métrico (NMDS) con el índice de disimilitud de Bray-Curtis, encontrándose diferencias significativas entre los pacientes críticos y los controles ($R^2 = 0.09$, $p = 0.01$) (Fig. 5d).

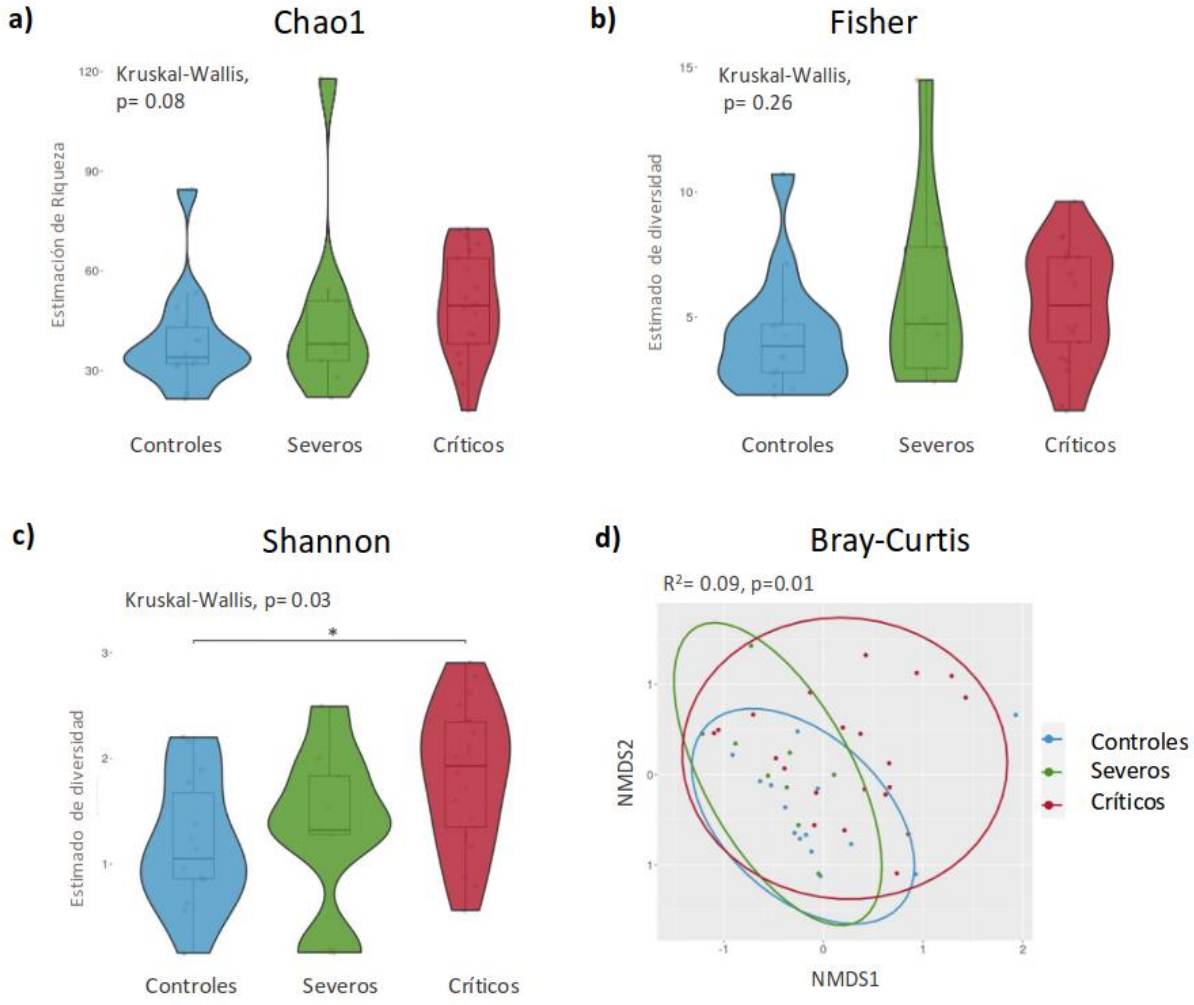


Figura 5. Índices de riqueza y diversidad alfa. a) Índice de riqueza Chao1, b) Índice de Fisher sin diferencias significativas. c) Índice de Shannon que muestra una diferencia significativa entre el grupo control y los pacientes críticos con COVID-19 (p = 0.03). d) Diversidad beta del microbioma nasofaríngeo entre los diferentes grupos de gravedad del COVID-19. La significancia se determinó mediante la prueba de Kruskal-Wallis con comparaciones múltiples de Dunn, con un intervalo de confianza del 95 %. El gráfico NMDS se basa en la disimilitud de Bray-Curtis, y las estadísticas PERMANOVA indican una diferencia significativa entre los tres grupos. Cada color representa un grupo específico analizado. Modificado de Galeana-Cadena et al. (2024)

9.4. Impacto de la Severidad del COVID-19 en la Composición de Grupos Taxonómicos

A nivel de género, *Corynebacterium* (0.24 [0.06, 0.50]) y *Staphylococcus* (0.14 [0.03, 0.39]) fueron los más abundantes (Figura 6a), sin diferencias significativas entre los grupos (Figura 6b y c).

Se observó una disminución significativa en la abundancia relativa de los géneros *Lawsonella* y *Cutibacterium* en pacientes con COVID-19 severo y crítico ($p < 0.001$) (Figura 6d y e). En contraste, los géneros S*treptococcus, Actinomyces, Peptostreptococcus, Atopobium, Granulicatella* y *Mogibacterium* presentaron un aumento en su abundancia relativa en estos pacientes ($p < 0.01$) (Figura 7a-f). Además, los géneros *Veillonella, Prevotella_7, Rothia, Gemella, Alloprevotella* y *Solobacterium* incrementaron su presencia exclusivamente en pacientes críticos comparados con los controles ($p < 0.01$) (Figura 7g-l).

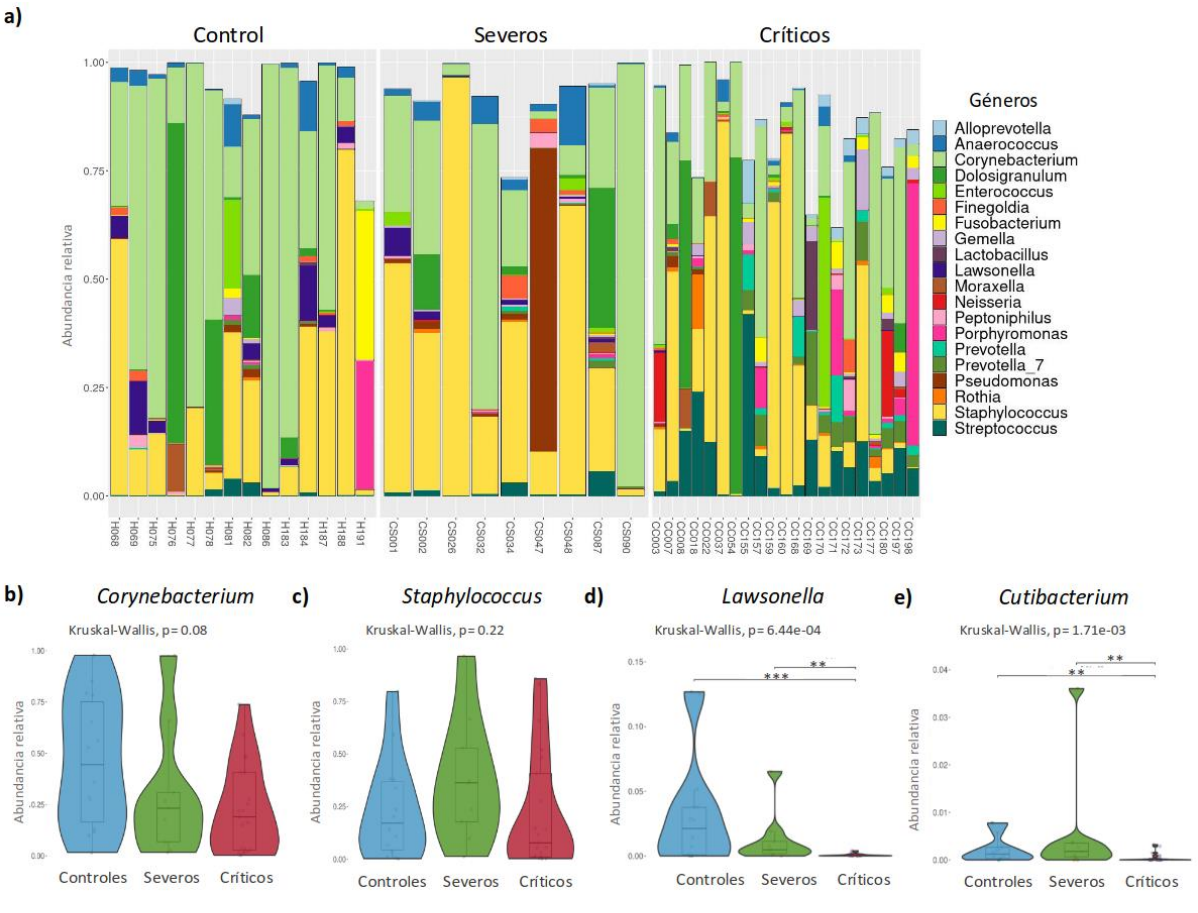


Figura 6. Comparación de los principales grupos taxonómicos por severidad. a) Gráfico de barras que muestra los 20 géneros bacterianos más abundantes clasificados por abundancia relativa en pacientes del grupo control, severos y críticos. b) y c) Gráfico de violín que muestra la abundancia relativa de los géneros *Staphylococcus* y *Corynebacterium*. c) y d) Disminución en la abundancia relativa de los géneros *Lawsonella* y *Cutibacterium* en pacientes críticos con COVID-19. La significancia se determinó utilizando la prueba de Kruskal-Wallis con comparaciones múltiples de Dunn, con un intervalo de confianza del 95 %, donde * $p \leq 0.05$, ** $p \leq 0.01$, y *** $p \leq 0.001$. Modificado de Galeana-Cadena et al. (2024)

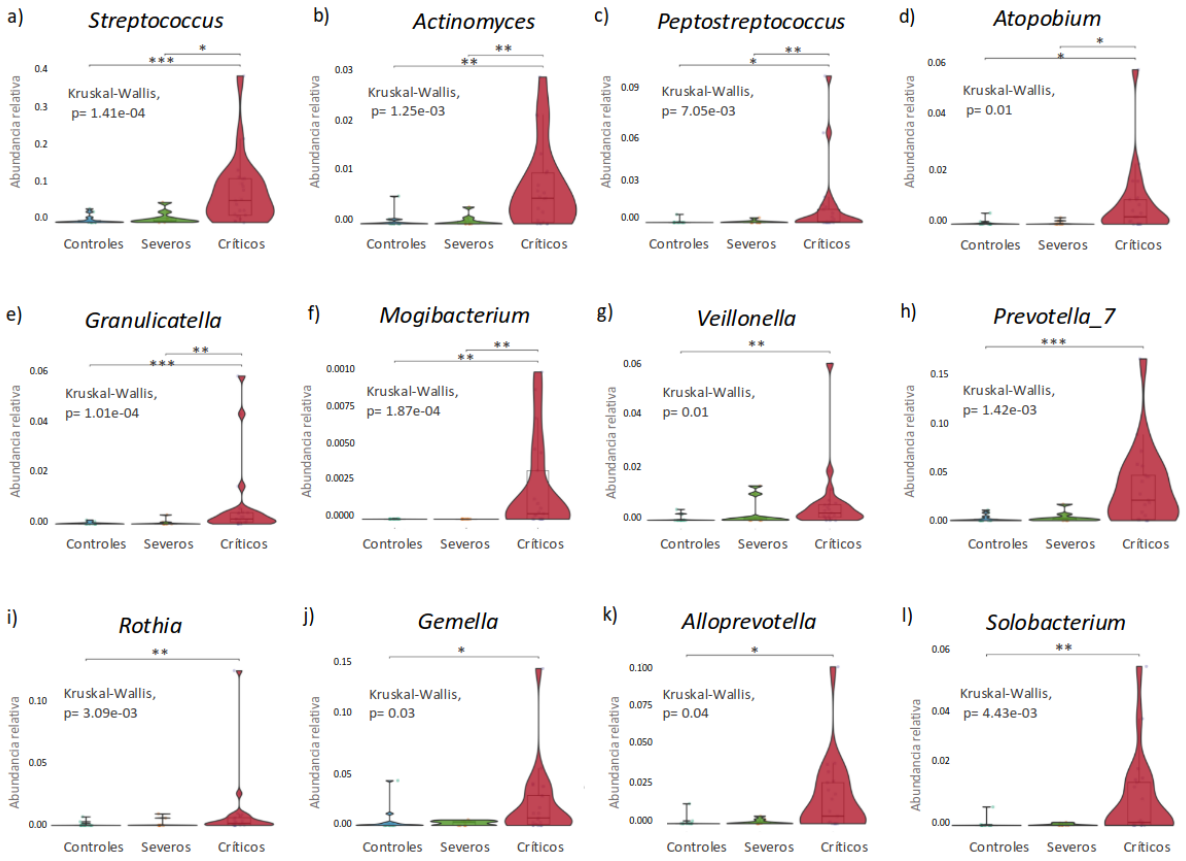


Figura 7. Géneros con abundancia relativa incrementada en pacientes críticos con COVID-19. La significancia se determinó utilizando la prueba de Kruskal-Wallis con comparaciones múltiples de Dunn, con un intervalo de confianza del 95 %, donde * p ≤ 0.05, ** p ≤ 0.01, y *** p ≤ 0.001. Modificado de Galeana-Cadena et al. (2024)

El análisis de correlación de Spearman reveló correlaciones negativas para *Corynebacterium* (rho = −0.334, p = 0.026), *Cutibacterium* (rho = −0.509, p = 0.0004) y *Lawsonella* (rho = −0.562, p = 7.192e-05) con la severidad del COVID-19. En contraste, *Streptococcus* mostró una correlación positiva fuerte con la severidad de la enfermedad (rho = 0.637, p = 3.315e-06) (Figura 8), mientras que otros géneros como *Prevotella, Actinomyces, Solobacterium, Atopobium, Mogibacterium, Alloprevotella, Veillonella, Rothia, Granulicatella* y *Peptostreptococcus* presentaron correlaciones positivas moderadas con la severidad del COVID-19.

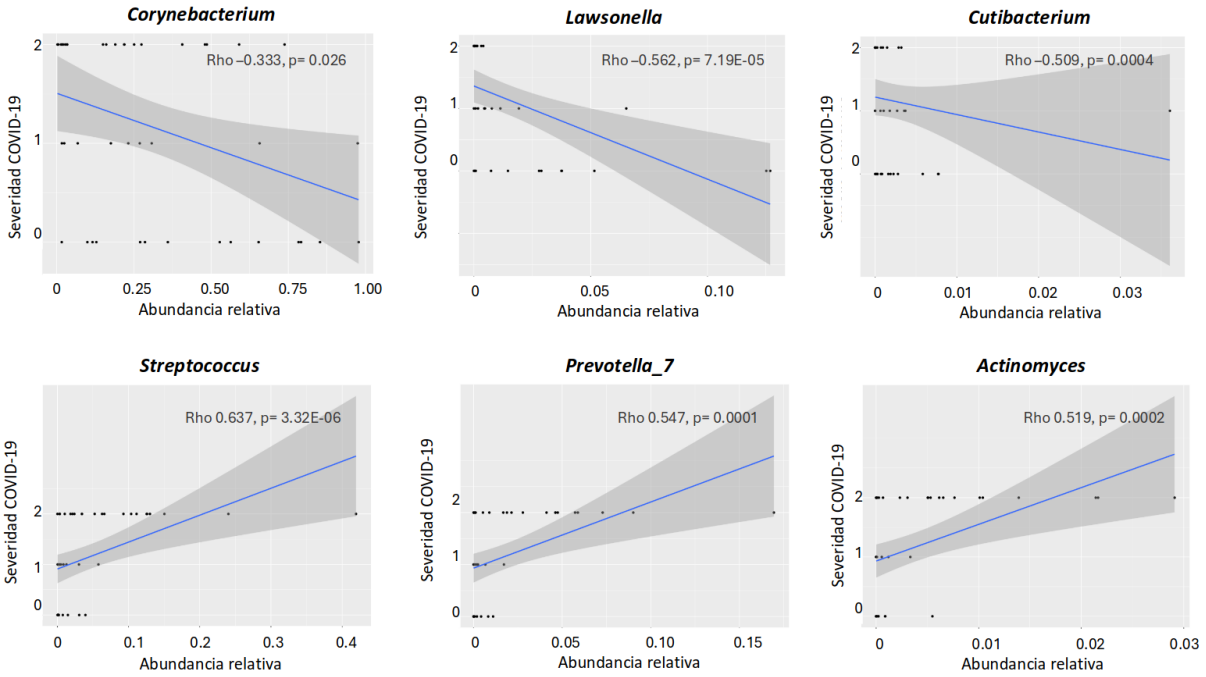


Figura 8. Correlaciones de Spearman entre la abundancia relativa de géneros bacterianos y la gravedad del COVID-19. La severidad del COVID-19 fue categorizada en tres niveles ordinales: controles (valor 0), COVID-19 moderado (valor 1) y COVID-19 crítico (valor 2).

El análisis exploratorio para evaluar el impacto de covariables como comorbilidades, uso de antibióticos, administración de esteroides y ventilación mecánica mediante intubación orotraqueal no mostró una influencia significativa en los resultados obtenidos en función de la severidad de la enfermedad. Para un análisis más detallado consultar Galeana Cadena et al. (2024).

9.5 Géneros bacterianos nucleares en la nasofaringe

En el análisis del microbioma nasofaríngeo de los pacientes con COVID-19, se identificaron *Corynebacterium, Streptococcus* y *Staphylococcus* como los géneros bacterianos nucleares predominantes. Los controles sanos y los casos graves de COVID-19 compartieron los géneros *Dolosigranulum, Lawsonella* y *Anaerococcus.* Además de estos, se identificaron los géneros bacterianos *Peptoniphilus,*

Pseudomonas y *Acinetobacter* en los casos severos de COVID-19. Por otro lado, los pacientes en estado crítico mostraron un la presencia distintiva de los géneros *Gemella, Prevotella, Veillonella, Atopobium, Granulicatella, Actinomyces, Oribacterium, Fusobacterium* y *Porphyromonas*.(Figura 9).

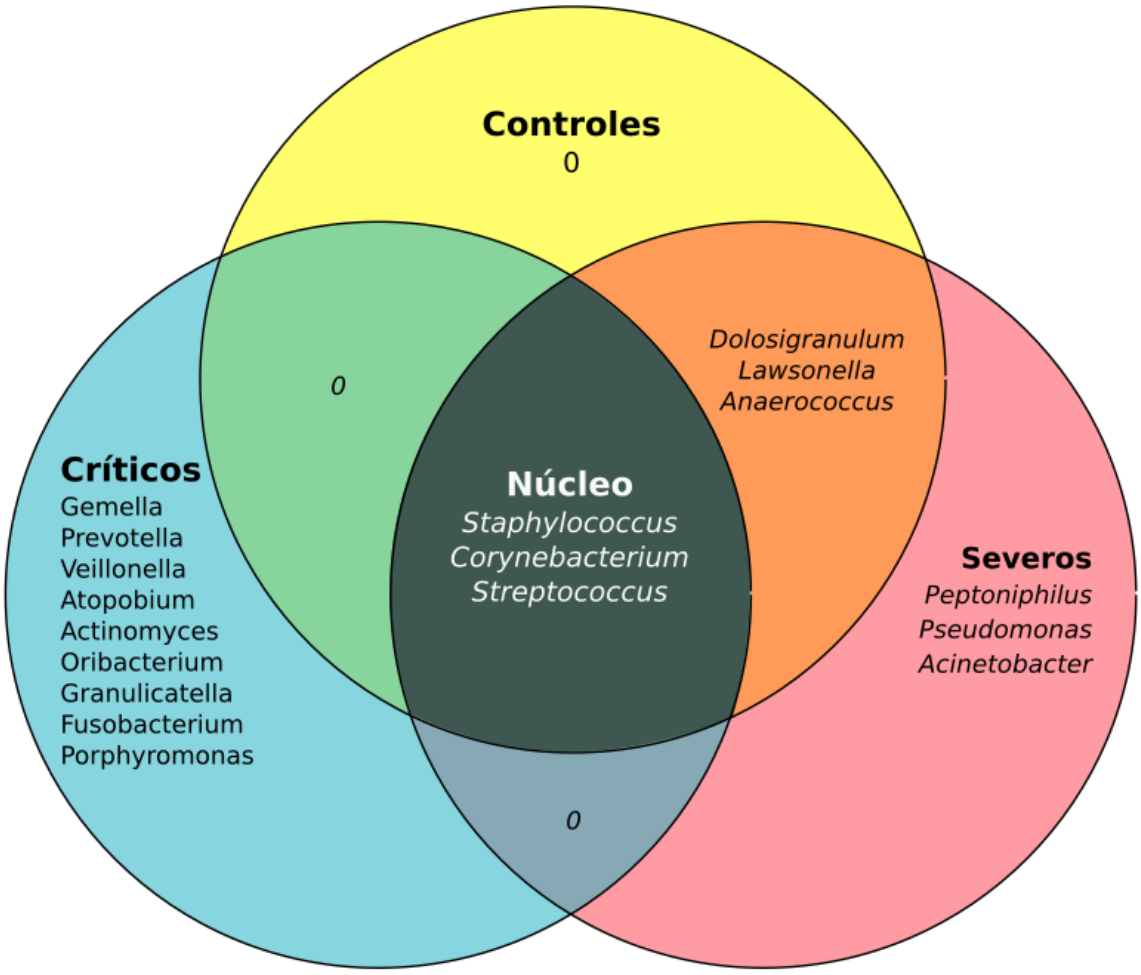


Figura 9. Microbioma nuclear de la nasofaringe. Diagrama de Venn con los microorganismos compartidos entre los grupos de estudio. Los microorganismos están presentes en al menos el 60 % de las muestras del grupo, con una abundancia relativa del 0.001 %.

9.6. Comparación de resultados con otras regiones geográficas

El análisis de los géneros nucleares del microbioma nasofaríngeo en pacientes con COVID-19 reveló que *Corynebacterium, Streptococcus* y *Staphylococcus* son consistentemente los más predominantes en diversas regiones del mundo, incluidas las poblaciones mexicanas. No obstante, se observan variaciones en la composición de la microbiota entre diferentes regiones geográficas (Tabla 5).

Tabla 5. Comparación con otros estudios previamente publicados

Características	**Smith et al. [18]**	**Hurst et al. [40]**	**Este estudio**
COVID-19 asintomáticos	0	5	0
COVID-19 moderados	15	22	0
COVID-19 severos	11	0	9
COVID-19 criticos	23	0	21
Voluntarios Sanos	12	4	14
Total de sujetos	61	31	44
Tipo de muestra	Exudado Nasofaringeo	Exudado Nasofaringeo	Exudado Nasofaringeo
Sitio Geográfico	Francia	USA	México
Año	2021	2022	2024
Tipo de análisis	Gen ARNr 16S, V3-V4	Gen ARNr 16S, V4	Gen ARNr 16S, V4
Géneros con aumento de la abundancia relativa en función de la gravedad del COVID-19	*Staphylococcus, Veillonella*	*no significant difference*	*Streptococcus, Actinomyces, Prevotella_ 7, Peptostreptococccus, Veillonella, Rothia, Solobacterium, Atopobium, Granullicatella, Gemella, Mogibacterium, Alloprevotella*
Géneros con disminución de la abundancia relativa en función de la gravedad del COVID-19	*Corynebacterium, Dolosigranulum, Lawsonella*	*Fusobacterium*	*Lawsonella, Cutibacterium*
Géneros nucleares en el microbioma de la nasofaringe en voluntarios sanos	*Corynebacterium, Staphylooccus, Escherichia-Shigella, Methylobacterium-Methylorubrum, Acinetobacter, Streptococcus, Pseudomonas, Lawsonella*	*Corynebacterium, Staphyloccus, Dolosigranuum, Lawsonella, Anaerococcus, Streptococcus*	*Staphylococcus, Corynebacterium, Dolosigranulum, Streptococcus, Lawsonella, Anaerococcus,*
Acceso a datos en SRA-NCBI	PRJNA714242	PRJNA703574	PRJNA981220

10. Discusión

La infección por COVID-19 puede alterar la composición del microbioma del tracto respiratorio, especialmente en pacientes con cuadros severos o críticos, donde los cambios en la microbiota se asocian a procesos inflamatorios sistémicos y locales (Merenstein et al., 2022). Este estudio tuvo como objetivo principal investigar los cambios taxonómicos en la microbiota de la nasofaringe asociados con la severidad del COVID-19 en pacientes mexicanos, un grupo poblacional aún subrepresentado en la investigación mundial sobre el microbioma respiratorio. Los hallazgos obtenidos contribuyen a una mejor comprensión de cómo la infección viral puede influir en las comunidades microbianas y cómo éstas podrían afectar la progresión de la enfermedad.

En los resultados de metagenómica, observamos que más del 90% de las lecturas correspondían a ADN humano, un desafío común en estudios del microbioma respiratorio (Marotz et al., 2018). Esta alta proporción de material genético humano puede limitar la detección de microorganismos menos abundantes, disminuyendo la capacidad para obtener una visión completa de las comunidades microbianas. Aunque técnicas como la depleción de células eucariotas podrían mejorar la recuperación de secuencias microbianas, este enfoque incrementaría significativamente los costos del proyecto y presentaría nuevos desafíos, como el riesgo de eliminar inadvertidamente poblaciones bacterianas importantes durante el proceso de depleción.

En el artículo principal de este proyecto (Galeana-Cadena et al., 2024), se observó un aumento en la colonización de la nasofaringe por microorganismos del microbioma oral, lo cual podría ser consecuencia de una disrupción significativa de las barreras físico-químicas, debida al proceso inflamatorio exacerbado característico de los casos con mayor severidad de COVID-19. Estudios previos han demostrado que las alteraciones del microbioma, inducidas por la inflamación y los cambios inmunológicos, pueden facilitar la sobrecolonización por bacterias oportunistas o patógenos secundarios, exacerbando el deterioro clínico (Sulaiman et al., 2021).

Un hallazgo destacado de este estudio es la identificación de los géneros *Corynebacterium, Streptococcus* y *Staphylococcus* como componentes nucleares del microbioma nasofaríngeo, observados de manera consistente tanto en nuestra cohorte mexicana como en otras poblaciones estudiadas alrededor del mundo. Estos géneros son fundamentales en la ecología del tracto respiratorio superior, participando en la exclusión competitiva de patógenos y en el mantenimiento de la homeostasis microbiana. Sin embargo, es notable que, aunque compartimos estos géneros con otras regiones, también observamos variaciones importantes en la abundancia relativa y la presencia de otros géneros microbianos en los pacientes mexicanos (Galeana-Cadena et al., 2024). Estas diferencias podrían reflejar influencias ambientales, genéticas, dietéticas y socioeconómicas, lo que subraya la importancia de investigar poblaciones específicas para captar las particularidades del microbioma local.

Es importante reconocer las limitaciones de este estudio, particularmente el uso de la secuenciación del gen ARNr 16S, que, si bien es robusta y ampliamente utilizada,

presenta limitaciones en cuanto a la resolución taxonómica, especialmente a nivel de especie. Además, este enfoque no permite capturar componentes virales o fúngicos, que podrían tener un papel relevante en la interacción con el sistema inmunológico del huésped.

El tamaño limitado de la muestra es una restricción a considerar. Aunque nuestros resultados proporcionan información valiosa, una muestra más grande permitiría obtener una mayor robustez estadística y la posibilidad de realizar análisis más detallados, incluyendo subgrupos basados en factores como la edad, el sexo, comorbilidades preexistentes. Estos subgrupos podrían revelar patrones específicos de alteraciones en el microbioma que no fueron evidentes en nuestro análisis inicial. Asimismo, factores externos como la dieta, el uso de antibióticos y antivirales, y el manejo clínico durante la hospitalización también pueden influir en los resultados obtenidos. Por ejemplo, se ha demostrado que el tratamiento con antibióticos en pacientes con COVID-19 puede tener un impacto directo en la composición del microbioma respiratorio y gastrointestinal (Langford et al., 2021), lo que podría haber contribuido a las alteraciones observadas en algunos pacientes críticos de nuestra cohorte. En futuros estudios, sería valioso incluir un mayor número de pacientes en cada grupo de antibióticos administrados, con el fin de permitir una evaluación más robusta en los análisis estadísticos.

Una línea de investigación futura que consideramos clave es la realización de estudios longitudinales. Actualmente, la mayoría de los estudios, incluido el nuestro, se basan en muestras tomadas en un momento específico de la enfermedad. No obstante, sería de gran valor seguir la evolución del microbioma nasofaríngeo a lo

largo del tiempo, desde la fase aguda hasta la recuperación, con el fin de identificar si los cambios observados son transitorios o si persisten a largo plazo. Este enfoque permitiría evaluar si las alteraciones en la composición del microbioma predisponen a los pacientes a infecciones recurrentes o aumentan su susceptibilidad a otras enfermedades respiratorias.

Asimismo, será recomendable ampliar los futuros estudios para incluir un enfoque multicéntrico, que involucre una mayor diversidad de pacientes y regiones dentro de México y otros países. Un diseño multicéntrico permitiría aumentar el tamaño de la muestra y realizar comparaciones geográficas, lo que podría aclarar si las diferencias observadas en nuestra cohorte mexicana se replican en otras poblaciones. Además, promovería una mayor estandarización en las definiciones y metodologías utilizadas, lo que facilitaría la comparación de resultados entre estudios y permitiría desarrollar estrategias terapéuticas basadas en el microbioma que sean aplicables a diversas regiones y condiciones socioeconómicas.

Un punto importante a explorar a futuro es la intervención terapéutica basada en la modulación del microbioma. Aunque nuestros resultados no permiten establecer una relación causal directa entre las alteraciones taxonómicas de la nasofaringe y la progresión del COVID-19, estudios previos sugieren que la manipulación del microbioma, a través de probióticos, prebióticos o trasplante de microbiota, podría ofrecer una vía para mejorar el pronóstico clínico en pacientes con infecciones respiratorias graves (Baud et al., 2020). Sería interesante evaluar en el futuro si intervenciones tempranas que promuevan la estabilidad y diversidad del microbioma

respiratorio podrían mitigar la respuesta inflamatoria del COVID-19 y mejorar la recuperación de los pacientes.

En resumen, nuestros hallazgos proporcionan una visión inicial del impacto del COVID-19 en el microbioma nasofaríngeo de pacientes mexicanos y refuerzan la necesidad de continuar investigando este tema desde una perspectiva multidisciplinaria y con diferentes metodologías. La identificación de géneros microbianos clave, la comprensión de su variabilidad geográfica, y la exploración de nuevas herramientas terapéuticas basadas en el microbioma representan caminos prometedores para mejorar el tratamiento y pronóstico de los pacientes con COVID-19 y otras enfermedades respiratorias.

11. Conclusiones

Nuestro estudio sugiere que la enfermedad crítica por COVID-19 podría contribuir a la perturbación de los mecanismos de barrera y condiciones fisicoquímicas, facilitando así la colonización e invasión de bacterias provenientes de la orofaringe a estructuras anatómicas adyacentes.

12. Referencias

Adair, K. L., & Douglas, A. E. (2017). ScienceDirect Making a microbiome : the many determinants of host-associated microbial community composition. Current Opinion in Microbiology, 35, 23–29. https://doi.org/10.1016/j.mib.2016.11.002

Allen, E. K., Koeppel, A. F., Hendley, J. O., Turner, S. D., Winther, B., & Sale, M. M. (2014). Characterization of the nasopharyngeal microbiota in health and during rhinovirus challenge. Microbiome, 2(1), 1–11. https://doi.org/10.1186/2049-2618-2-22

Alvarado-Peña, N., Galeana-Cadena, D., Gómez-García, I. A., Mainero, X. S., & Silva-Herzog, E. (2023). The microbiome and the gut-lung axis in tuberculosis: interplay in the course of disease and treatment. Frontiers in Microbiology, 14(October), 1–12. https://doi.org/10.3389/fmicb.2023.1237998

Barbosa-Amezcua, M., Galeana-Cadena, D., Alvarado-Peña, N., & Silva-Herzog, E. (2022). The Microbiome as Part of the Contemporary View of Tuberculosis Disease. Pathogens, 11(5), 1–15. https://doi.org/10.3390/pathogens11050584

Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M. C. C., Charles, T., Chen, X., Cocolin, L., Eversole, K., Corral, G. H., Kazou, M., Kinkel, L., Lange, L., Lima, N., Loy, A., Macklin, J. A., Maguin, E., Mauchline, T., McClure, R., … Schloter, M. (2020). Microbiome definition re-visited: old concepts and new challenges. Microbiome. 2020;8(1):1–22. https://doi.org/10.1186/s40168-020-00875-0

Bharti, R., & Grimm, D. G. (2021). Current challenges and best-practice protocols for microbiome analysis. Briefings in Bioinformatics, 22(1), 178–193. https://doi.org/10.1093/bib/bbz155

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics, 30(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Buchfink, B., Xie, C., & Huson, D. H. (2014). Fast and sensitive protein alignment using DIAMOND. Nature Methods, 12(1), 59–60. https://doi.org/10.1038/nmeth.3176

Carabelli, A. M., Peacock, T. P., Thorne, L. G., Harvey, W. T., Hughes, J., de Silva, T. I., Peacock, S. J., Barclay, W. S., de Silva, T. I., Towers, G. J., & Robertson, D. L. (2023). SARS-CoV-2 variant biology: immune escape, transmission and fitness. Nature Reviews Microbiology, 21(3), 162–177. https://doi.org/10.1038/s41579-022-00841-7

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016). "DADA2: High-resolution sample inference from Illumina amplicon data." Nature Methods, 13, 581-583. doi:10.1038/nmeth.3869.

Celebi Sözener, Z., Cevhertas, L., Nadeau, K., Akdis, M., & Akdis, C. A. (2020). Environmental factors in epithelial barrier dysfunction. Journal of Allergy and Clinical Immunology, 145(6), 1517–1528. https://doi.org/10.1016/j.jaci.2020.04.024

Chiu, L., Bazin, T., Truchetet, M. E., Schaeverbeke, T., Delhaes, L., & Pradeu, T. (2017). Protective microbiota: From localized to long-reaching co-immunity. Frontiers in Immunology, 8(DEC), 1–19. https://doi.org/10.3389/fimmu.2017.01678

Choreño-Parra, J. A., Jiménez-Álvarez, L. A., Cruz-Lagunas, A., Rodríguez-Reyna, T. S., Ramírez-Martínez, G., Sandoval-Vega, M., Hernández-García, D. L., Choreño-Parra, E. M., Balderas-Martínez, Y. I., Martinez-Sánchez, M. E., Márquez-García, E., Sciutto, E., Moreno-Rodríguez, J., Barreto-Rodríguez, J. O., Vázquez-Rojas, H., Centeno-Sáenz, G. I., Alvarado-Peña, N., Salinas-Lara, C., Sánchez-Garibay, C., … Zúñiga, J. (2021). Clinical and Immunological Factors That Distinguish COVID-19 From Pandemic Influenza A(H1N1). Frontiers in Immunology, 12(April), 1–19. https://doi.org/10.3389/fimmu.2021.593595

Chua, R. L., Lukassen, S., Trump, S., Hennig, B. P., Wendisch, D., Pott, F., Debnath, O., Thürmann, L., Kurth, F., Völker, M. T., Kazmierski, J., Timmermann, B., Twardziok, S., Schneider, S., Machleidt, F., Müller-Redetzky, H., Maier, M., Krannich, A., Schmidt, S., … Eils, R. (2020). COVID-19 severity correlates with airway epithelium–immune cell interactions identified by single-cell analysis. Nature Biotechnology, 38(8), 970–979. https://doi.org/10.1038/s41587-020-0602-4

Church, D. L., Cerutti, L., Gürtler, A., Griener, T., Zelazny, A., & Emler, S. (2020). Performance and Application of 16S rRNA Gene Cycle Sequencing for Routine Identification of Bacteria in the. American Society for Microbiology, 33(4), 1–74.

Cuthbertson, L., Walker, A. W., Oliver, A. E., Rogers, G. B., Rivett, D. W., Hampton, T. H., Ashare, A., Elborn, J. S., De Soyza, A., Carroll, M. P., Hoffman, L. R., Lanyon, C., Moskowitz, S. M., O'Toole, G. A., Parkhill, J., Planet, P. J., Teneback, C. C., Tunney, M. M., Zuckerman, J. B., … Van Der Gast, C. J. (2020). Lung function and microbiota diversity in cystic fibrosis. Microbiome, 8(1), 1–13. https://doi.org/10.1186/s40168-020-00810-3

Dharra, R., Kumar Sharma, A., & Datta, S. (2023). Emerging aspects of cytokine storm in COVID-19: The role of proinflammatory cytokines and therapeutic

prospects. Cytokine, 169(June), 156287. https://doi.org/10.1016/j.cyto.2023.156287

Elrobaa, I. H., & New, K. J. (2021). COVID-19: Pulmonary and Extra Pulmonary Manifestations. Frontiers in Public Health, 9(September), 1–26. https://doi.org/10.3389/fpubh.2021.711616

Fu, X., Liu, Z., Zhu, C., Mou, H., & Kong, Q. (2019). Nondigestible carbohydrates, butyrate, and butyrate-producing bacteria. Critical Reviews in Food Science and Nutrition, 59(S1), S130–S152. https://doi.org/10.1080/10408398.2018.1542587

Galeana-Cadena, D., Gómez-García, I. A., Lopez-Salinas, K. G., Irineo-Moreno, V., Jiménez-Juárez, F., Tapia-García, A. R., Boyzo-Cortes, C. A., Matías-Martínez, M. B., Jiménez-Alvarez, L., Zúñiga, J., & Camarena, A. (2023). Winds of change a tale of: asthma and microbiome. Frontiers in Microbiology, 14(December). https://doi.org/10.3389/fmicb.2023.1295215

Galeana-Cadena, D., Ramirez-Martínez, G., Alberto Choreño-Parra, J., Silva-Herzog, E., Margarita Hernández-Cárdenas, C., Soberón, X., & Zúñiga, J. (2024). Microbiome in the Nasopharynx: Insights into the Impact of COVID-19 Severity. Heliyon, 10(May), e31562. https://doi.org/10.1016/j.heliyon.2024.e31562

Gauthier, N. P. G., Locher, K., MacDonald, C., Chorlton, S. D., Charles, M., & Manges, A. R. (2022). Alterations in the nasopharyngeal microbiome associated with SARS-CoV-2 infection status and disease severity. PLoS ONE, 17(10 October), 1–13. https://doi.org/10.1371/journal.pone.0275815

GBD. (2018). Estimates of the global , regional , and national morbidity , mortality , and aetiologies of lower respiratory infections in 195 countries , 1990 – 2016 : a systematic analysis for the Global Burden of Disease Study 2016. Lancet Infect Dis, 3099(18), 1–20. https://doi.org/10.1016/S1473-3099(18)30310-4

Goya, S., Valinotto, L. E., Tittarelli, E., Rojo, G. L., Nabaes Jodar, M. S., Greninger, A. L., Zaiat, J. J., Marti, M. A., Mistchenko, A. S., & Viegas, M. (2018). An optimized methodology for whole genome sequencing of RNA respiratory viruses from nasopharyngeal aspirates. PLoS ONE, 13(6), 1–15. https://doi.org/10.1371/journal.pone.0199714

Harrison, A. G., Lin, T., & Wang, P. (2020). Mechanisms of SARS-CoV-2 Transmission and Pathogenesis. Trends in Immunology, 41(12), 1100–1115. https://doi.org/10.1016/j.it.2020.10.004

He, L. H., Ren, L. F., Li, J. F., Wu, Y. N., Li, X., & Zhang, L. (2020). Intestinal Flora as a Potential Strategy to Fight SARS-CoV-2 Infection. Frontiers in Microbiology, 11(June). https://doi.org/10.3389/fmicb.2020.01388

Hernández-Terán, A., Mejía-Nepomuceno, F., Herrera, M. T., Barreto, O., García, E., Castillejos, M., Boukadida, C., Matias-Florentino, M., Rincón-Rubio, A., Avila-Rios, S., Mújica-Sánchez, M., Serna-Muñoz, R., Becerril-Vargas, E., Guadarrama-Pérez, C., Ahumada-Topete, V. H., Rodríguez-Llamazares, S., Martínez-Orozco, J. A., Salas-Hernández, J., Pérez-Padilla, R., & Vázquez-Pérez, J. A. (2021). Dysbiosis and structural disruption of the respiratory microbiota in COVID-19 patients with severe and fatal outcomes. Scientific Reports, 11(1). https://doi.org/10.1038/s41598-021-00851-0

Huang, Y., Tang, J. hua, Cai, Z., Qi, Y., Jiang, S., Ma, T. ting, Yue, Y., Huang, F., Yang, H., & Ma, Y. yun. (2022). Alterations in the nasopharyngeal microbiota associated with active and latent tuberculosis. Tuberculosis, 136(July), 102231. https://doi.org/10.1016/j.tube.2022.102231

Kaul, D., Rathnasinghe, R., Ferres, M., Tan, G. S., Barrera, A., Pickett, B. E., Methe, B. A., Das, S., Budnik, I., Halpin, R. A., Wentworth, D., Schmolke, M., Mena, I., Albrecht, R. A., Singh, I., Nelson, K. E., García-Sastre, A., Dupont, C. L., & Medina, R. A. (2020). Microbiome disturbance and resilience dynamics of the upper respiratory tract during influenza A virus infection. Nature Communications, 11(1). https://doi.org/10.1038/s41467-020-16429-9

Khan, I., Bai, Y., Zha, L., Ullah, N., Ullah, H., Shah, S. R. H., Sun, H., & Zhang, C. (2021). Mechanism of the Gut Microbiota Colonization Resistance and Enteric Pathogen Infection. Frontiers in Cellular and Infection Microbiology, 11(December), 1–19. https://doi.org/10.3389/fcimb.2021.716299

Lagier, J. C., Edouard, S., Pagnier, I., Mediannikov, O., Drancourt, M., & Raoult, D. (2015). Current and past strategies for bacterial culture in clinical microbiology. Clinical Microbiology Reviews, 28(1), 208–236. https://doi.org/10.1128/CMR.00110-14

Lajqi, T., Pöschl, J., Frommhold, D., & Hudalla, H. (2020). The Role of Microbiota in Neutrophil Regulation and Adaptation in Newborns. Frontiers in Immunology, 11(September), 1–10. https://doi.org/10.3389/fimmu.2020.568685

Lamers, M. M., & Haagmans, B. L. (2022). SARS-CoV-2 pathogenesis. Nature Reviews Microbiology, 20(5), 270–284. https://doi.org/10.1038/s41579-022-00713-0

Lanks, C. W., Musani, A. I., & Hsia, D. W. (2019). Community-acquired Pneumonia and Hospital-acquired Pneumonia. Medical Clinics of North America, 103(3), 487–501. https://doi.org/10.1016/j.mcna.2018.12.008

Liu, Q., Chi, S., Dmytruk, K., Dmytruk, O., & Tan, S. (2022). Coronaviral Infection and Interferon Response: The Virus-Host Arms Race and COVID-19. Viruses, 14(7). https://doi.org/10.3390/v14071349

Lloréns-Rico, V., Gregory, A. C., Van Weyenbergh, J., Jansen, S., Van Buyten, T., Qian, J., Braz, M., Menezes, S. M., Van Mol, P., Vanderbeke, L., Dooms, C., Gunst, J., Hermans, G., Meersseman, P., Van Herck, Y., Wilmer, A., Casaer, M., Rex, S., Lorent, N., … Raes, J. (2021). Clinical practices underlie COVID-19 patient respiratory microbiome composition and its interactions with the host. Nature Communications, 12(1), 1–12. https://doi.org/10.1038/s41467-021-26500-8

Lloyd-Price, Abu-Ali, G., & Huttenhower, C. (2016). The healthy human microbiome. Genome Medicine, 8(1), 1–11. https://doi.org/10.1186/s13073-016-0307-y

López-Filloy, M., Cortez, F. J., Gheit, T., Cruz y Cruz, O., Cruz-Talonia, F., Chávez-Torres, M., Arteaga-Gómez, C., Mancilla-Herrera, I., Montesinos, J. J., Cortés-Morales, V. A., Aguilar, C., Tommasino, M., Pinto-Cardoso, S., & Rocha-Zavaleta, L. (2022). Altered Vaginal Microbiota Composition Correlates With Human Papillomavirus and Mucosal Immune Responses in Women With Symptomatic Cervical Ectopy. Frontiers in Cellular and Infection Microbiology, 12(May), 1–15. https://doi.org/10.3389/fcimb.2022.884272

Lubin, J. B., Green, J., Maddux, S., Denu, L., Duranova, T., Lanza, M., Wynosky-Dolfi, M., Flores, J. N., Grimes, L. P., Brodsky, I. E., Planet, P. J., & Silverman, M. A. (2023). Arresting microbiome development limits immune system maturation and resistance to infection in mice. Cell Host and Microbe, 31(4), 554-570.e7. https://doi.org/10.1016/j.chom.2023.03.006

Man, W. H., De Steenhuijsen Piters, W. A. A., & Bogaert, D. (2017). The microbiota of the respiratory tract: Gatekeeper to respiratory health. Nature Reviews Microbiology, 15(5), 259–270. https://doi.org/10.1038/nrmicro.2017.14

Mark Welch, J. L., Dewhirst, F. E., & Borisy, G. G. (2019). Biogeography of the oral microbiome: The site-specialist hypothesis. Annual Review of Microbiology, 73, 335–358. https://doi.org/10.1146/annurev-micro-090817-062503

Marotz, C. A., Sanders, J. G., Zuniga, C., Zaramela, L. S., Knight, R., & Zengler, K. (2018). Improving saliva shotgun metagenomics by chemical host DNA depletion. Microbiome, 6(1), 1–9. https://doi.org/10.1186/s40168-018-0426-3

Martino, C., Dilmore, A. H., Burcham, Z. M., Metcalf, J. L., Jeste, D., & Knight, R. (2022). Microbiota succession throughout life from the cradle to the grave. Nature Reviews Microbiology, 20(12), 707–720. https://doi.org/10.1038/s41579-022-00768-z

Merad, M., Blish, C. A., Sallusto, F., & Iwasaki, A. (2022). The immunology and immunopathology of COVID-19. Science, 375(6585), 1122–1127. https://doi.org/10.1126/science.abm8108

Merenstein, C., Bushman, F. D., & Collman, R. G. (2022). Alterations in the respiratory tract microbiome in COVID-19: current observations and potential significance. Microbiome, 10(1), 1–14. https://doi.org/10.1186/s40168-022-01342-8

Mettelman, R. C., Allen, E. K., & Thomas, P. G. (2022). Mucosal immune responses to infection and vaccination in the respiratory tract. Immunity, 55(5), 749–780. https://doi.org/10.1016/j.immuni.2022.04.013

Moroni-Zentgraf, P., Keller, C., Eschenfelder, C. C., Walter Müller, H., Sigmund, R., Galeana-Cadena, D., Márquez-García, J. E., Moncada-Morales, A., & Zúñiga, J. A. (2023). Salivette® Cortisol versus oropharyngeal swabbing for the detection of SARS-CoV-2 infection. Expert Review of Molecular Diagnostics, 23(11), 1011–1014. https://doi.org/10.1080/14737159.2023.2260308

Natalini, J. G., Singh, S., & Segal, L. N. (2023). The dynamic lung microbiome in health and disease. Nature Reviews Microbiology, 21(4), 222–235. https://doi.org/10.1038/s41579-022-00821-x

Netea, M. G., Domínguez-Andrés, J., Barreiro, L. B., Chavakis, T., Divangahi, M., Fuchs, E., Joosten, L. A. B., van der Meer, J. W. M., Mhlanga, M. M., Mulder, W. J. M., Riksen, N. P., Schlitzer, A., Schultze, J. L., Stabell Benn, C., Sun, J. C., Xavier, R. J., & Latz, E. (2020). Defining trained immunity and its role in health and disease. Nature Reviews Immunology, 20(6), 375–388. https://doi.org/10.1038/s41577-020-0285-6

Niederman, M. S., & Torres, A. (2022). Respiratory infections. European Respiratory Review, 31(166), 1–2. https://doi.org/10.1183/16000617.0150-2022

Parums, D. V. (2023). Editorial: Outbreaks of Post-Pandemic Childhood Pneumonia and the Re-Emergence of Endemic Respiratory Infections. Medical Science Monitor, 29, e943312. https://doi.org/10.12659/MSM.943312

Porto, B. N., & Moraes, T. J. (2021). The triad: respiratory microbiome–virus–immune response in the pathophysiology of pulmonary viral infections. Expert Review of Respiratory Medicine, 15(5), 635–648. https://doi.org/10.1080/17476348.2021.1893168

Proctor, L. M., Creasy, H. H., Fettweis, J. M., Lloyd-Price, J., Mahurkar, A., Zhou, W., Buck, G. A., Snyder, M. P., Strauss, J. F., Weinstock, G. M., White, O., & Huttenhower, C. (2019). The Integrative Human Microbiome Project. Nature, 569(7758), 641–648. https://doi.org/10.1038/s41586-019-1238-8

Qi, C., Hountras, P., Pickens, C. O., Walter, J. M., Kruser, J. M., Singer, B. D., Seed, P., Green, S. J., & Wunderink, R. G. (2019). Detection of respiratory pathogens in clinical samples using metagenomic shotgun sequencing. Journal of Medical Microbiology, 1–7. https://doi.org/10.1099/jmm.0.000968

Qin, T., Wang, Y., Deng, J., Xu, B., Zhu, X., Wang, J., Zhou, H., Zhao, N., Jin, F., Ren, H., Wang, H., Li, Q., Xu, X., Guo, Y., Li, R., Xiong, Y., Wang, X., Guo, J., Zheng, H., … Xu, J. (2022). Super Dominant Pathobiontic Bacteria in the Nasopharyngeal Microbiota Cause Secondary Bacterial Infection in COVID-19 Patients. Microbiology Spectrum, 10(3). https://doi.org/10.1128/spectrum.01956-21

Regueira-Iglesias, A., Balsa-Castro, C., Blanco-Pintos, T., & Tomás, I. (2023). Critical review of 16S rRNA gene sequencing workflow in microbiome studies: From primer selection to advanced data analysis. Molecular Oral Microbiology, 38(5), 347–399. https://doi.org/10.1111/omi.12434

Romero-Espinoza, J. A., Moreno-Valencia, Y., Coronel-Tellez, R. H., Castillejos-Lopez, M., Hernandez, A., Dominguez, A., Miliar-Garcia, A., Barbachano-Guerrero, A., Perez-Padilla, R., Alejandre-Garcia, A., & Vazquez-Perez, J. A. (2018). Virome and bacteriome characterization of children with pneumonia and asthma in Mexico City during winter seasons 2014 and 2015. PLoS ONE, 13(2), 1–14. https://doi.org/10.1371/journal.pone.0192878

Santacroce, L., Charitos, I. A., Ballini, A., Inchingolo, F., Luperto, P., De Nitto, E., & Topi, S. (2020). The human respiratory system and its microbiome at a glimpse. Biology, 9(10), 1–16. https://doi.org/10.3390/biology9100318

berg, R., Chiu, C. Y., Miller, S., Procop, G. W., & Weinstock, G. (2019). Validation of metagenomic next-generation sequencing tests for universal pathogen detection. Archives of Pathology and Laboratory Medicine, 141(6), 776–786. https://doi.org/10.5858/arpa.2016-0539-RA

Sette, A., Sidney, J., & Crotty, S. (2023). T cell responses to SARS-COV-2. Annual Review of Immunology, 202, 183–217. https://doi.org/https://doi.org/10.1146/annurev-immunol-101721- 061120

Smith, N., Goncalves, P., Charbit, B., Grzelak, L., Beretta, M., Planchais, C., Bruel, T., Rouilly, V., Bondet, V., Hadjadj, J., Yatim, N., Pere, H., Merkling, S. H., Ghozlane, A., Kernéis, S., Rieux-Laucat, F., Terrier, B., Schwartz, O., Mouquet, H., … Di Santo, J. P. (2021). Distinct systemic and mucosal immune responses during acute SARS-CoV-2 infection. Nature Immunology, 22(11), 1428–1439. https://doi.org/10.1038/s41590-021-01028-7

Srinivas, M., O'Sullivan, O., Cotter, P. D., Sinderen, D. van, & Kenny, J. G. (2022). The Application of Metagenomics to Study Microbial Communities and Develop Desirable Traits in Fermented Foods. Foods, 11(20), 1–28. https://doi.org/10.3390/foods11203297

Wensel, C. R., Pluznick, J. L., Salzberg, S. L., & Sears, C. L. (2022). Next-generation sequencing: insights to advance clinical investigations of the

microbiome. Journal of Clinical Investigation, 132(7). https://doi.org/10.1172/JCI154944

Wood, D. E., Lu, J., & Langmead, B. (2019). Improved metagenomic analysis with Kraken 2. Genome Biology, 20(1), 1–13. https://doi.org/10.1186/s13059-019-1891-0

Worbs, T., Hammerschmidt, S. I., & Förster, R. (2017). Dendritic cell migration in health and disease. Nature Reviews Immunology, 17(1), 30–48. https://doi.org/10.1038/nri.2016.116

Zhang, H., Ai, J.-W., Yang, W., Zhou, X., He, F., Xie, S., Zeng, W., Li, Y., Yu, Y., Gou, X., Li, Y., Wang, X., Su, H., Zhu, Z., Xu, T., & Zhang, W. (2020). Metatranscriptomic Characterization of Coronavirus Disease 2019 Identified a Host Transcriptional Classifier Associated With Immune Signaling. Clinical Infectious Diseases. https://doi.org/10.1093/cid/ciaa663

Zhou, H., Larkin, P. M. K., Zhao, D., Ma, Q., Yao, Y., Wu, X., Wang, J., Zhou, X. H., Li, Y., Wang, G., Feng, M., Wu, L., Chen, J., Zhou, C., Hua, X., Zhou, J., Yang, S., & Yu, Y. (2021). Clinical Impact of Metagenomic Next-Generation Sequencing of Bronchoalveolar Lavage in the Diagnosis and Management of Pneumonia: A Multicenter Prospective Observational Study. Journal of Molecular Diagnostics, 23(10), 1259–1268. https://doi.org/10.1016/j.jmoldx.2021.06.007

13. Anexos

13.1 Artículos de investigación derivados de esta tesis

Durante la etapa del doctorado se publicaron seis artículos, una publicación con los resultados principales del proyecto, dos son colaterales del trabajo del doctorado y tres revisiones son directamente pertinentes al trabajo. A continuación, se adjuntan los trabajos mencionados.

Heliyon 10 (2024) e31562

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Microbiome in the nasopharynx: Insights into the impact of COVID-19 severity

David Galeana-Cadena [a,d], Gustavo Ramirez-Martínez [a], José Alberto Choreño-Parra [a], Eugenia Silva-Herzog [b], Carmen Margarita Hernández-Cárdenas [c], Xavier Soberón [d,**,1], Joaquín Zúñiga [a,e,*,1]

[a] Laboratorio de Inmunobiología y Genética, Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas (INER), Mexico City, Mexico
[b] Unidad de Vinculación Científica Facultad de Medicina UNAM-INMEGEN, Instituto Nacional de Medicina Genómica (INMEGEN), Mexico City, Mexico
[c] Unidad de Cuidados Intensivos y Dirección General, Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas (INER), Ciudad de México, Mexico
[d] Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Mexico
[e] Tecnologico de Monterrey, Escuela de Medicina y Ciencias de la Salud, Mexico City, Mexico

ARTICLE INFO

Keywords:
Nasopharyngeal microbiome
Nasopharynx
COVID-19
Staphylococcus
Corynebacterium
Streptococcus
Lawsonella
Cutibacterium Core microbiome
Intubation

ABSTRACT

Background: The respiratory tract harbors a variety of microbiota, whose composition and abundance depend on specific site factors, interaction with external factors, and disease. The aim of this study was to investigate the relationship between COVID-19 severity and the nasopharyngeal microbiome.

Methods: We conducted a prospective cohort study in Mexico City, collecting nasopharyngeal swabs from 30 COVID-19 patients and 14 healthy volunteers. Microbiome profiling was performed using 16S rRNA gene analysis. Taxonomic assignment, classification, diversity analysis, core microbiome analysis, and statistical analysis were conducted using R packages.

Results: The microbiome data analysis revealed taxonomic shifts within the nasopharyngeal microbiome in severe COVID-19. Particularly, we observed a significant reduction in the relative abundance of *Lawsonella* and *Cutibacterium* genera in critically ill COVID-19 patients ($p < 0.001$). In contrast, these patients exhibited a marked enrichment of *Streptococcus, Actinomyces, Peptostreptococcus, Atopobium, Granulicatella, Mogibacterium, Veillonella, Prevotella_7, Rothia, Gemella, Alloprevotella*, and *Solobacterium* genera ($p < 0.01$). Analysis of the core microbiome across all samples consistently identified the presence of *Staphylococcus*, *Corynebacterium*, and *Streptococcus*.

Conclusions: Our study suggests that the disruption of physicochemical conditions and barriers resulting from inflammatory processes and the intubation procedure in critically ill COVID-19 patients may facilitate the colonization and invasion of the nasopharynx by oral microorganisms.

* Corresponding author. Laboratorio de Inmunobiología y Genética, Instituto Nacional de Enfermedades Respiratorias (INER), Ciudad de México, Mexico.
** Corresponding author.
E-mail addresses: xavier.soberon@ibt.unam.mx (X. Soberón), joaquin.zuniga@iner.gob.mx (J. Zúñiga).
[1] Postal address: Calzada de Tlalpan 4502, Col. Sección XVI, Delegación de Tlalpan, CP 14080, Ciudad de México, México.

https://doi.org/10.1016/j.heliyon.2024.e31562
Received 12 July 2023; Received in revised form 15 May 2024; Accepted 17 May 2024
Available online 18 May 2024

1. Introduction

Respiratory tract is characterized by unique anatomical structures, performing various functions and harboring distinct microenvironments. The nasal cavity, nasopharynx, and oropharynx are part of the upper respiratory tract (URT). Despite their anatomical proximity, these structures exhibit differences in their microbiota. Genera such as *Staphylococcus*, *Propionibacterium*, *Cutibacterium*, *Corynebacterium*, *Moraxella*, and *Streptococcus* have been identified as predominant in the nasal cavity [1,2]. Nasopharynx, with its unique microenvironment characterized by specific pH, temperature, relative humidity, and the presence of pharyngeal tonsils, is permissive to the colonization by different microorganisms, including *Dolosigranulum*, *Neisseria*, and *Haemophilus* [3]. Due to the proximity of its connection with the mouth, the oropharynx harbors a distinct microbial community that includes *Rothia, Veillonella, Prevotella, Atopobium, Gemella,* and *Streptococcus*, amongst others [4]. This structure has the highest bacterial load in the respiratory tract, with many microorganisms originating from oral microbiota [5].

It is well known that respiratory microbiome is a complex ecosystem, comprising diverse microorganisms that participate in cooperative and competitive interactions among themselves, and with the host, thereby impacting respiratory health and disease. The coexistence of two microbial species in the same niche from airways ecosystems, with identical needs usually is not feasible [6]. Each anatomical site within the respiratory tract signifies a distinct niche influenced by interactions with the host immune system and diverse external factors. Respiratory viral infections, provoke changes in the physicochemical and immunological conditions of the epithelial barrier that impact in microbial competition for specific ecological niches [7], leading to modifications in microbial colonization. In this context, COVID-19, a highly infectious respiratory disease caused by the SARS-CoV-2 virus, causes inflammation in the respiratory tract, and in severe cases, respiratory tract damage may progress to respiratory failure and acute respiratory distress syndrome (ARDS) [8–10] triggering pathogenic immune response associated to epithelial respiratory tract and lung tissue damage [11,12]. These inflammatory and physicochemical changes in the mucosal membranes may provoke the translocation of microorganisms from different compartments, such as the gut to the blood [13] or the mouth to the respiratory tract [14]. This phenomenon could be a cause of secondary infections or co-infections by microorganisms such as *Staphylococcus, Streptococcus, Pseudomonas, Klebsiella*, and *Acinetobacter*, thereby contributing to the severity of COVID-19 [15,16]. These complications often result in extended hospital stays and can even lead to fatal outcomes [17]. Previous studies have focused on comparing the diversity of the nasopharyngeal microbiome in COVID-19 [18,19]. Prasad et al. [20] and Mostafa et al. [21] reported a decrease in alpha diversity, while Braun et al. [18] and Feehan et al. [22] did not find significant differences in alpha diversity indices. Taxonomic changes have been reported in the relative abundance of *Corynebacterium, Dolosigranulum, Staphylococcus, Fusobacterium, Anaerococcus, Enterobacter, Peptostreptococcus, Prevotella, Pseudomonas, Mycoplasma, Alloprevotella, Solobacterium, Veillonella, Streptococcus*, and *Actinomyces* genera in COVID-19 patients compared to healthy individuals [15,22–25]. In general, the results of these studies are still non consistent.

In this study, the relationship between the severity of COVID-19 and the nasopharyngeal microbiome was evaluated. Thus, we analyzed the bacterial microbiome of patients with different degree of COVID-19 disease severity and compared them to healthy volunteers. Our analysis, along with the identification of the core microbiome and the changes in nasopharyngeal genera, might contribute to our understanding of microbial interactions in patients with different clinical presentations of COVID-19.

2. Materials and methods

2.1. Study population

We conducted a prospective cohort study in 44 adult subjects, including 30 SARS-CoV-2 positive individuals by real-time PCR and 14 healthy volunteers negative for SARS-CoV-2 infection. Patients were recruited from the emergency room unit at the Instituto Nacional de Enfermedades Respiratorias "Ismael Cosío Villegas" (INER) in Mexico City, which is a referral center for respiratory diseases in Mexico. Nasopharyngeal swabs were collected from fall 2020 to fall 2021 and only patients with positive real-time PCR analysis for SARS-CoV-2 were recruited. Samples from healthy donors were collected in parallel. Healthy volunteers had no respiratory symptoms and were negative for the RT-PCR test, at the time of sample collection and for 4 weeks thereafter. COVID-19 severity was categorized according to WHO's Living Guideline Clinical Management of COVID-19 (January 13th, 2023), 9 patients had severe COVID-19 disease and 21 were classified as critically ill requiring mechanical ventilation. The list of the samples and clinical and demographic information of patients are summarized in Supplementary Table 1.

2.2. Sample processing

Nasopharyngeal swab samples were taken according to the standard guidelines. The sample was placed in UTM®: Universal Transport Medium™ (Copan Diagnostics, United States) and stored at −70°C until its DNA extraction was performed. Samples were centrifuged for 20 min at 14,000 RPM, the supernatant was removed and discarded. The pellet was resuspended in 500 μl of Sputolysin® (Calbiochem, Germany) and was mixed on a vortex mixer for 30 s, the suspended mixture stood at room temperature for 15 min. Subsequently, it was centrifuged for 5 min at 1500 rpm, discarding the supernatant. DNA was extracted with the QIAmp UCP pathogen miniKit (Qiagen), using the mechanical pre-lysis with spin protocol for swabs described in the QIAmp UCP Pathogen Mini Handbook. The incubation time with Proteinase K was performed during 20 min. Subsequently, the DNA V4 region of 16S rRNA was amplified by PCR using the primers 515f and 806r [26]. Samples were pooled and barcoded following Illumina 16S metagenomics protocol (Illumina, USA). The barcodes were pooled in equimolar concentration and sequenced on the Illumina Miseq platform using the 2 × 250 pair-end method.

2.3. Bioinformatics analysis and statistics

Sequencing data was processed with R version 4.2.1. Quality trimming, sample inference, paired reads merging, chimera removal, and taxonomy assignment were performed using DADA2 R package version 3.17 [27]. Silva v138 database was utilized for taxonomic assignment. ASV abundances were normalized using the Wrench method [28]. The analysis of alpha diversity was performed using the Microbiome R package version 1.18 [29]. For beta diversity analysis Non-metric multidimensional scaling (NMDS) was used, using the Bray-Curtis dissimilarity index, and permanova analysis ADONIS2 was used to determine significance. Statistical tests and charts were performed using Ggstatsplot R package version 0.9.4 [30] and Gtsummary R package version 1.6.2 [31] to evaluate the differences between groups and demographic characteristics, comorbidities, clinical data, laboratory tests, severity of illness, and clinical outcome. The core microbiome was defined as taxonomic groups that were present in at least 60 % of the samples with 0.001 % relative abundance, using the Eulerr, Microbiome, and Microbiomeutilities R packages. Correlation analysis was performed to explore the relationships between variables of interest. Spearman's rank correlation coefficient was utilized to assess the strength and direction of associations between the relative abundances of taxonomic groups and severity of COVID-19. The severity of COVID-19 was categorized into three ordinal levels: controls (assigned a value of 0), moderate COVID-19 cases (assigned a value of 1), and critical COVID-19 cases (assigned a value of 2). Correlation analysis was conducted using statistical software R with significance set at $p < 0.05$.

Additionally, we conducted exploratory data analysis to assess the impact of covariates, including comorbidities, antibiotic usage, and steroid administration prior to sampling, further the effect of orotracheal intubation for mechanical ventilation. Our analysis involved a comparative examination of each covariate using the relative abundances of taxonomic groups. To conduct this analysis, we employed Mann-Whitney statistical tests, utilizing the Ggstatsplot R package (version 0.9.4) and the Gtsummary R package (version 1.6.2).

2.4. Bioinformatic analyses based in databases reported in other studies

Furthermore, to strengthen our analysis, a systematic search was conducted in PubMed of the National Center for Biotechnology Information (NCBI) to identify studies focusing on the nasopharyngeal microbiota and COVID-19 severity. A total of six relevant studies were identified, all of which included healthy volunteers and employed high-throughput amplicon sequencing based on the 16S rRNA gene to analyze the microbiome. After reviewing these six studies, we found that only two of them had accessible data on samples from the nasopharynx in both COVID-19 patients and healthy volunteers. Specifically, Smith et al. [25] conducted their study

Table 1
Demographic characteristics and comorbidities.

	Control, N = 14	Severe, N = 9	Critical, N = 21	P-value*
Age	52 (40, 60)	47 (47, 55)	48 (42, 65)	0.8
Gender				>0.9
female	6 (43 %)	3 (33 %)	7 (33 %)	
male	8 (57 %)	6 (67 %)	14 (67 %)	
Height	1.68 (1.54, 1.75)	1.67 (1.64, 1.68)	1.70 (1.60, 1.73)	0.9
Unknown	4	0	0	
Weight	72 (62, 81)	82 (74, 85)	80 (75, 91)	0.14
Unknown	4	0	0	
BMI categories				0.002
Normal weight	2 (14 %)	1 (11 %)	2 (9.5 %)	
Obesity	0 (0 %)	5 (56 %)	11 (52 %)	
Overweight	8 (57 %)	3 (33 %)	8 (38 %)	
No data	4 (29 %)	0 (0 %)	0 (0 %)	
Comorbidities				
Obesity	1 (7.1 %)	0 (0 %)	8 (38 %)	0.031
DM2	1 (7.1 %)	1 (11 %)	6 (29 %)	0.3
Hypertension	1 (7.1 %)	3 (33 %)	9 (43 %)	0.066
Heart disease	0 (0 %)	0 (0 %)	1 (4.8 %)	>0.9
Renal insufficiency	0 (0 %)	0 (0 %)	1 (4.8 %)	>0.9
Immunosuppression	0 (0 %)	0 (0 %)	0 (0 %)	
Asthma	1 (7.1 %)	1 (11 %)	0 (0 %)	0.3
COPD	0 (0 %)	0 (0 %)	0 (0 %)	
HIV	0 (0 %)	0 (0 %)	0 (0 %)	
GERD	2 (14 %)	1 (11 %)	0 (0 %)	0.2
Allergic rhinitis	0 (0 %)	2 (22 %)	0 (0 %)	0.038
Alcoholism	0 (0 %)	2 (22 %)	5 (24 %)	0.12
Smoking	0 (0 %)	1 (11 %)	7 (33 %)	0.031
Initial Antibiotic	0	8 (89 %)	15 (71 %)	<0.001
Days hospitalized	0 (0, 0)	11 (0, 16)	29 (13, 37)	
Days onset sampling	0 (0, 0)	9 (7, 12)	10 (8, 14)	

Median (IQR); n (%); *Kruskal-Wallis rank sum test; Fisher's exact test.
BMI Body Mass Index, DM2 Diabetes Mellitus Type 2, GERD Gastroesophageal reflux disease.

in France, while Hurst et al. [32] conducted an interesting study on patients from the United States. Both studies provided complete metadata and available sequences, enabling us to examine nasopharyngeal samples across varying severities of COVID-19 and compare them to those from healthy volunteers. Thus, in order to determine differences in microbial abundance in patients and controls and compare their reports with our data, we processed the data obtained from these studies. The sequencing data and meta data were downloaded from the SRA database for projects PRJNA714242 and PRJNA703574. Subsequent preprocessing steps involved quality control measures, including the removal of adapter sequences, elimination of low-quality reads, and filtering out sequences containing ambiguous bases using the DADA2 package in R. For Smith et al. [25], sequences, we used the filterAndTrim parameters truncLen = c(262,230), maxN = 0, maxEE = c(2,2), truncQ = 2, and rm. phix = TRUE. For Hurst et al. [32], we used the filter parameters truncLen = c(150,160), maxN = 0, maxEE = c(2,2), truncQ = 2, and rm. phix = TRUE. Following preprocessing, the sequences underwent error correction, dereplication, merging of paired reads, identification of unique sequence variants, and taxonomy annotation using our bioinformatic pipeline.

For more specific details see Supplementary Materials and Methods.

3. Results

A total of 44 subjects were included in the study, all of them were adults residing in Mexico, with a median age of 49 (42–61) years, and 16 (36 %) were female. Body Mass Index (BMI) categories were as follows: 5 (11 %) had normal weight, 19 (43 %) were overweight, 16 (36 %) presented obesity, and 4 (9.1 %) BMI was not reported. The studied groups were classified according to COVID-19 severity. Severe and critical COVID-19 patients were more frequently obese ($p = 0.002$). We found a statistically significant difference between the groups in terms of medical history related to tobacco use ($p = 0.03$) (Table 1). Fever, cough, dyspnea, arthralgias, myalgias, and decreased SpO2 were the most prevalent symptoms in COVID-19 patients. We found decreased levels of lymphocytes ($p = 0.012$) and high levels of neutrophils ($p = 0.010$) in COVID-19 patients when compared to controls. The neutrophil-lymphocyte ratio (NLR) was statistically significantly different between groups ($p = 0.001$).

3.1. Respiratory microbiome composition in COVID-19 patients with different clinical presentations

The abundance of amplicon sequence variants (ASV) at the phylum level, revealed an increase in *Bacteroidota* ($p = 0.010$) and

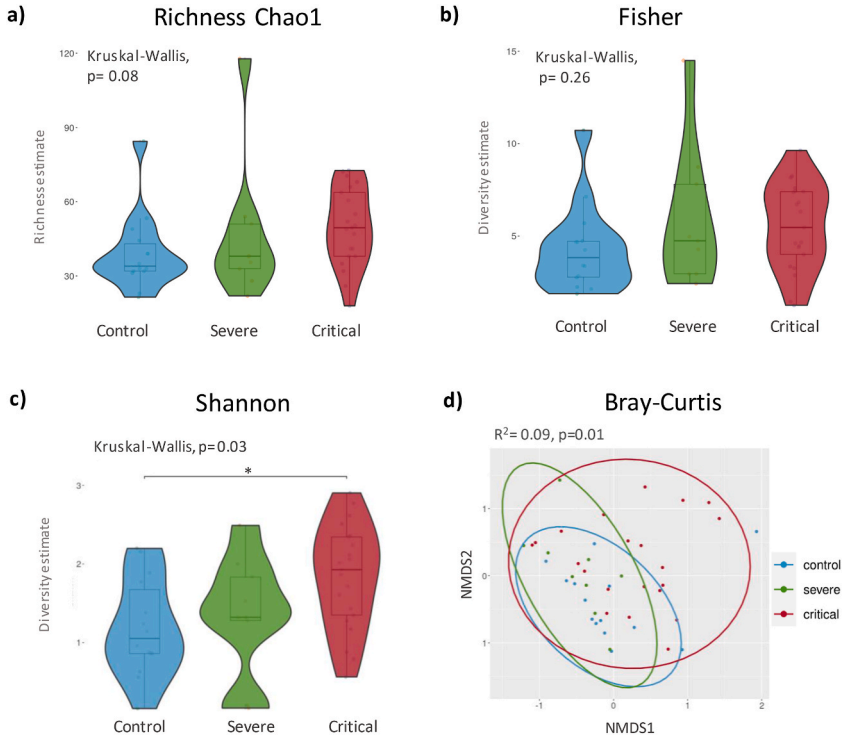


Fig. 1. Richness and alpha diversity indices. a) Richness Chao1 index, b) Fisher index showing no significant differences. c) Shannon index revealing a significant difference between the control and critical COVID-19 ($p = 0.03$). d) Beta diversity of the nasopharyngeal microbiome among different severity groups of COVID-19. Significance was determined using Kruskal-Wallis test with Dunn's multiple comparison with a 95 % confidence interval. The NMDS plot is based on Bray-Curtis dissimilarity, and PERMANOVA statistics indicate a significant difference among the three groups. Each color represents a specific analyzed group. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fusobacteria (p = 0.026) in samples from COVID-19 patients. *Actinobacteria* and *Firmicutes* phyla were the most abundant in COVID-19 and healthy controls but showed no significant differences amongst these groups. Shannon index showed an increase in the bacterial diversity in critically ill COVID-19 patients (p = 0.03) (Fig. 1c) however the remaining alpha indexes did not show significant differences between critically ill and severe COVID-19 patients and controls (Fig. 1a and b). Non-metric dimensional scaling (NMDS) was used to compare beta diversity and revealed significant differences between critically ill COVID-19 patients and controls ($R^2 = 0.09$ p = 0.01) (Fig. 1d). At genus level, *Corynebacterium* (0.24 [0.06, 0.50]) and *Staphylococcus* (0.14 [0.03, 0.39]) were the most abundant (Fig. 2a), with no significant differences between groups (Fig. 2b and c). Importantly, the presence of the genera *Lawsonella* and *Cutibacterium* was low in severe and critically ill COVID-19 patients (p < 0.001) (Fig. 2d and e). Contrariwise, *Streptococcus, Actinomyces, Peptostreptococcus, Atopobium, Granulicatella*, and *Mogibacterium* (Fig. 3 a-f) showed an increase in severe and critical COVID-19 patients (p < 0.01). Other genera that exhibited increased abundance only in critical COVID-19 compared to controls were *Veillonella, Prevotella_7, Rothia, Gemella, Alloprevotella*, and *Solobacterium* (p < 0.01) (Fig. 3 g-l).

To assess the strength and direction of the association between different genera and the severity of COVID-19 illness, a Spearman rank correlation analysis was done. We observed moderate to strong negative correlations of *Corynebacterium* (rho = −0.334, p-value = 0.026), *Cutibacterium* (rho = −0.509, p-value = 0.0004), and *Lawsonella* (rho = −0.562, p-value = 7.192e-05) with COVID-19 severity. In Contrast, *Streptococcus* exhibited a strong positive correlation with COVID severity (rho = 0.637, p-value = 3.315e-06), while several other genera including *Prevotella, Actinomyces, Solobacterium, Atopobium, Mogibacterium, Alloprevotella, Veillonella, Rothia, Granulicatella*, and *Peptostreptococcus* demonstrated moderate positive correlations with COVID-19 severity (Supplementary Fig. 1).

3.2. Analysis of confounding covariates

The analysis of antibiotic use before sampling revealed a statistically significant effect (p < 0.05) among the genera *Prevotella 7*, *Alloprevotella*, *Rothia*, and *Granulicatella* between the control group and COVID-19 patients. Regarding comorbidities, only *Solobacterium* showed statistical significance (p = 0.03). However, the previous administration of steroids demonstrated a significant effect across almost all genera of interest in this study (p < 0.05) (Supplementary Table 2). Notably, *Staphylococcus* and *Cutibacterium* did not exhibit significant differences based on steroid administration.

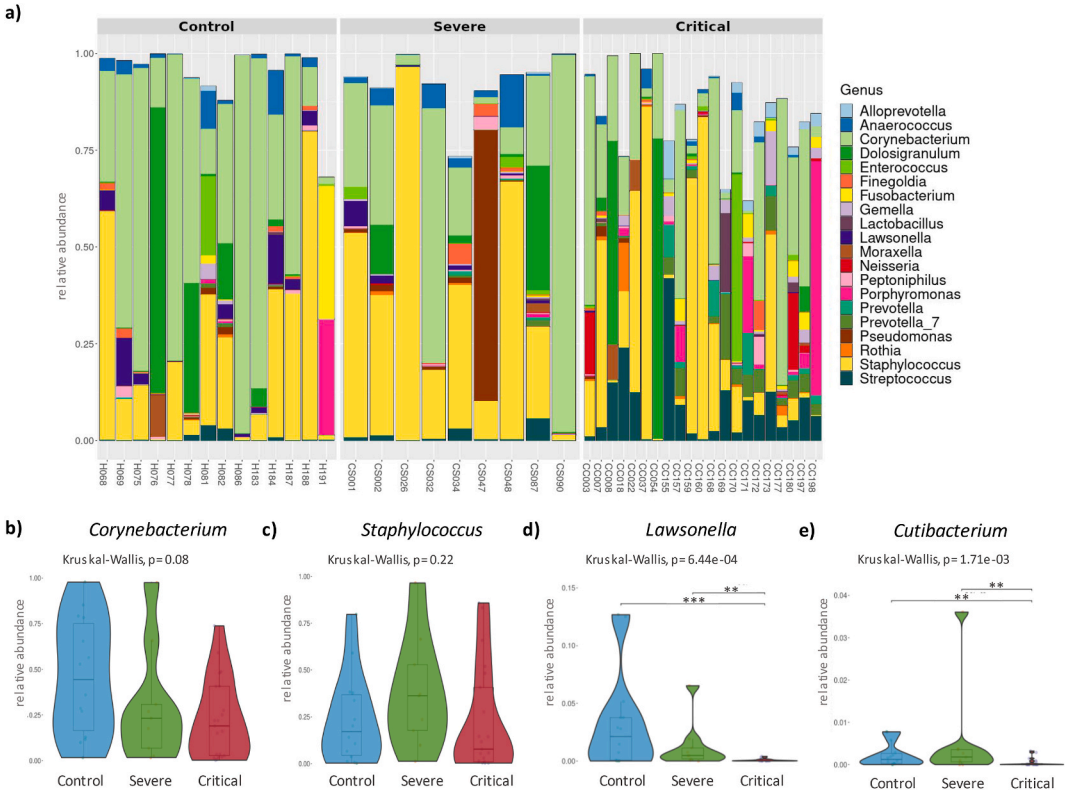


Fig. 2. a) Taxonomy bar plot showing the top 20 microbiome profiles at the genus level, ranked by relative abundance, in control, severe, and critical patients. b) and c) Violin plot displaying the relative abundance of *Staphylococcus* and *Corynebacterium* genera. No significant differences were observed between severity groups. c) and d) Decrease in the relative abundance of *Lawsonella* and *Cutibacterium* genera in critical COVID-19 patients. Significance was determined using the Kruskal-Wallis test with Dunn's multiple comparison, 95 % confidence interval, where * p ≤ 0.05, ** p ≤ 0.01, and *** p ≤ 0.001.

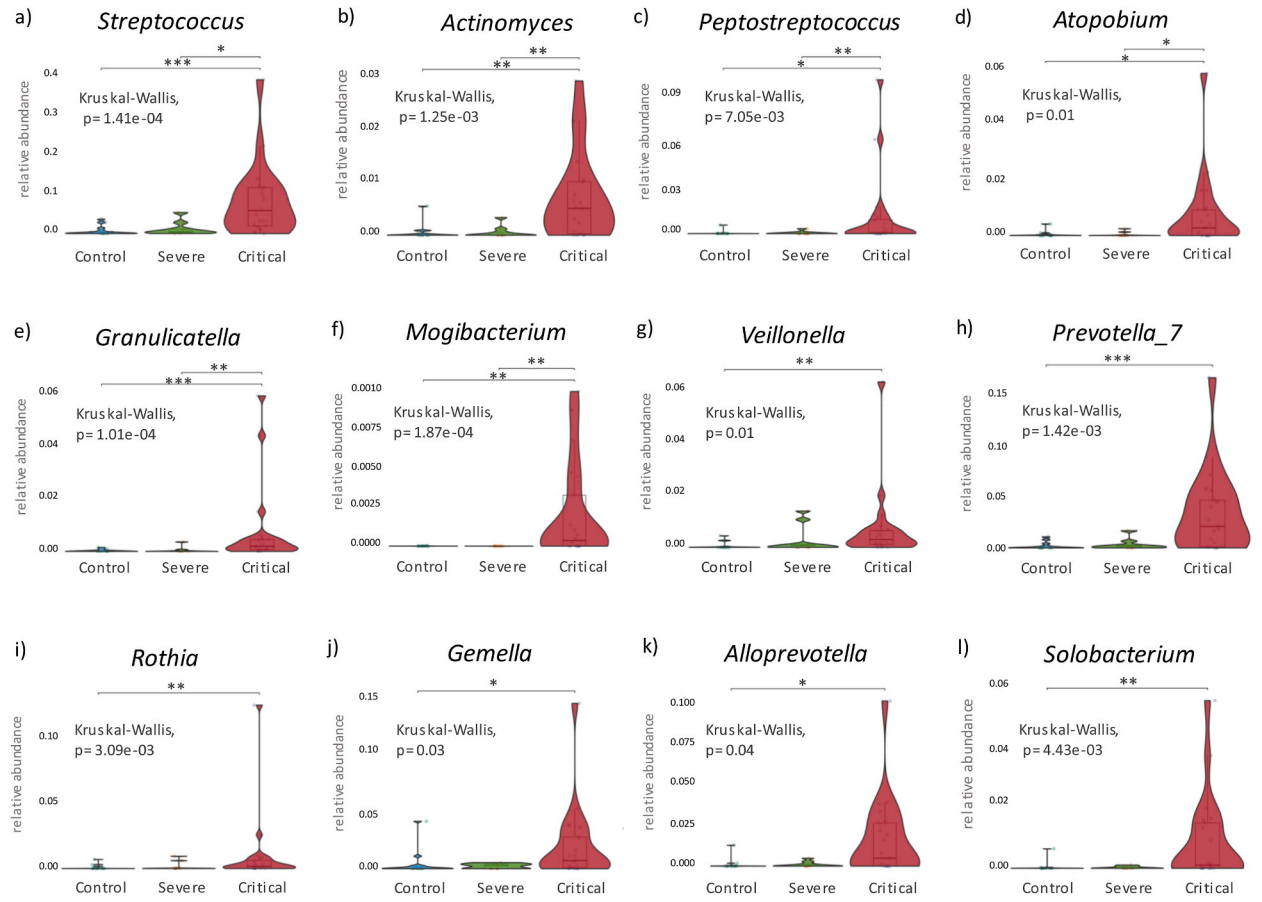


Fig. 3. Genera with increased relative abundance in critical COVID-19 patients. Significance was determined using the Kruskal-Wallis test with Dunn's multiple comparison 95 % confidence interval, where * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$.

3.3. Microbiome composition after intubation

Since previous studies have indicated that the use of invasive respiratory devices can disrupt the microenvironment and alter microbial populations [33], we decided to investigate whether such changes occurred in our studied groups of patients. For this analysis, we divided the group of critical patients into two subgroups: Pre-intubation and Post-Intubation, based on the timing of sample collection about the time of intubation for mechanical ventilatory support. The first subgroup comprised patients sampled before intubation [n = 7], with an average time of 1.85 days (range 0–5 days) between sample collection and orotracheal intubation. The second subgroup consisted of patients sampled after intubation (n = 14), with an average time of 2 days (range 0–7 days) between orotracheal intubation and sample collection. It is crucial to note that each subgroup consists of different patients. For additional details about the critical patient's intubation time line refer to Supplementary Fig. 2. Based on this analysis we found a statistically significant increase in the alpha diversity indices: Chao1 ($p = 0.001$), Fisher ($p = 0.03$), and Shannon ($p = 0.05$) (Supplementary Figs. 3a, 3b, and 3c) among critically ill COVID-19 patients sampled before and after receiving mechanical ventilation support. Furthermore, beta diversity analysis using the Bray-Curtis index demonstrated significant differences between the Pre-intubation and Post-intubation samples ($R2 = 0.08$, $p = 0.03$) (Supplementary Fig. 3d). Analysis of the genus composition after intubation revealed that the distribution of *Corynebacterium, Staphylococcus, Lawsonella, Cutibacterium*, *Streptococcus, Veillonella, Rothia,* and *Granulicatella* was not different between the two groups. Interestingly, *Prevotella_7, Atopobium* ($p < 0.001$), Solobacterium ($p = 0.003$), and *Actinomyces* ($p = 0.008$), exhibited an increased abundance after placement of the orotracheal tube (Supplementary Fig. 4).

3.4. Nasopharyngeal core microbiome

One of the challenges in microbiome research is to identify microorganisms that are consistently and stably present in most samples, within a specific niche. In our analysis, the common core of the microbes presents in the analyzed samples, consisted of the genera *Staphylococcus, Corynebacterium,* and *Streptococcus*. Healthy controls and COVID-19 severe cases shared the genera *Dolosigranulum, Lawsonella,* and *Anaerococcus*. Regardless of this, additional bacterial genera *Peptoniphilus, Pseudomonas,* and *Acinetobacter* were identified in COVID-19 severe cases. While critically ill patients showed an enrichment of the genera *Gemella, Prevotella,*

Veillonella, Atopobium, Granulicatella, Actinomyces, Oribacterium, Fusobacterium, and *Porphyromonas* (Fig. 4).

3.5. Insights from other studies of nasopharyngeal microbiome in COVID-19 patients

After the COVID-19 epidemic, people started looking into how the body's microbiome affects the disease. As a result, we identified different studies with accessible raw data, obtained from nasopharynx samples from COVID-19 patients and healthy volunteers [25, 32]. From these studies, we selected and downloaded raw data to perform comparative bioinformatic analyses with our data. In the analysis that we performed from the data published by Smith et al. [25], we observed an increase in the abundance of the genera *Staphylococcus* and *Veillonella*, accompanied by a decrease in *Corynebacterium, Dolosigranulum*, and *Lawsonella* among critically ill COVID-19 patients. In contrast, the study conducted by Hurst et al. [32], did not reveal statistically significant decreases in any genera between severity groups and healthy volunteers; only an increase in *Fusobacterium* was noted in moderate COVID-19 patients. Importantly, *Corynebacterium, Staphylococcus*, and *Streptococcus* were consistently present in all samples from healthy volunteers across both studies (Table 2).

4. Discussion

Here, we conducted a comparative analysis of the diversity and composition of the nasopharyngeal microbiome to explore its potential relationship with COVID-19 severity. Also, using a taxonomic profiling approach, we identified core microbiome genera associated with the nasopharynx in both healthy volunteers and COVID-19 patients.

Our main findings were 1) alpha diversity indexes were not different between severe and critically ill COVID-19 patients and controls and 2) COVID-19 patients present significant changes in the relative abundance of genera *Lawsonella* and *Cutibacterium* that were diminished, whereas genera *Streptococcus* and *Actinomyces* amongst other genera from oral microbiota were increased in severe but particularly in critically ill patients.

In line with our results, previous studies performed in other populations also described homogeneous alpha indexes of diversity in COVID-19 severe patients [18,22,34]. In this regard, Feehan et al. [22], described that alpha diversity did not differ in COVID-19 independently of the clinical status, but interestingly the alpha index was modified by other factors such as age and tobacco use. Contrasting those findings Shilts et al. and Ventero et al. [35,36], reported a decrease in alpha diversity in critically ill COVID-19 patients or those with fatal outcomes.

Another relevant finding of our study was that genera Staphylococcus, Streptococcus, and Corynebacterium were present as a core in all individuals including controls and COVID-19 patients. Furthermore, we identified in COVID-19 severe cases genera *Peptoniphilus, Pseudomonas,* and *Acinetobacter* and critically ill patients an enrichment of the genera *Gemella, Prevotella, Veillonella, Atopobium,*

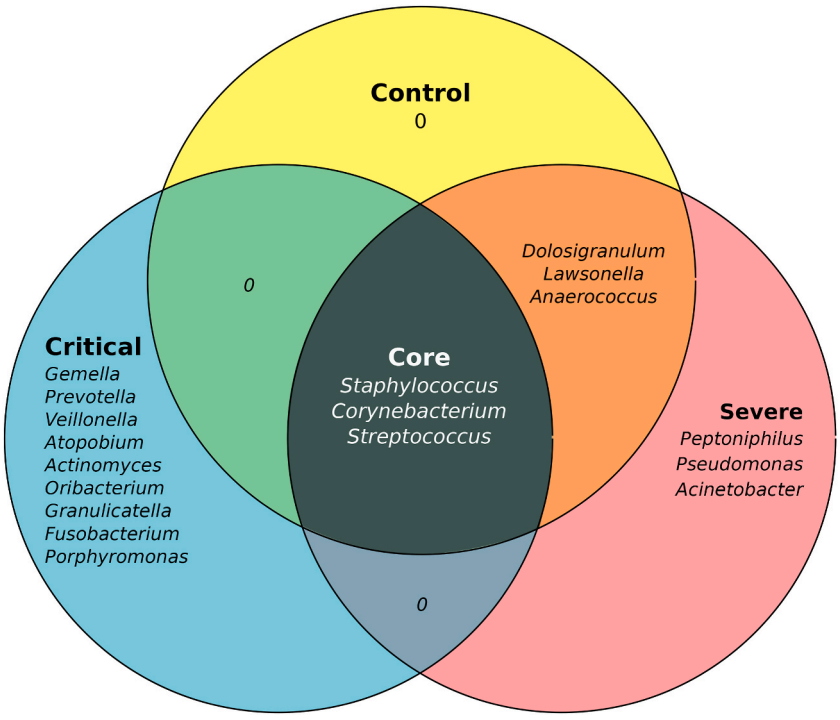


Fig. 4. Nasopharyngeal Core Microbiome. Microorganisms shared across communities, are present in at least 60 % of the samples with 0.001 % of relative abundance.

Table 2
| Comparison with other previously published studies.

Data Set	Smith et al. [25]	Hurst et al. [32]	This study
COVID-19 asymptomatic	0	5	0
COVID-19 moderate patients	15	22	0
COVID-19 severe patients	11	0	9
COVID-19 critical patients	23	0	21
Healthy volunteers	12	4	14
Total Subjects	61	31	44
Source	Nasopharyngeal	Nasopharyngeal	Nasopharyngeal
Geography	France	USA	Mexico
Year	2021	2022	2023
Analysis	16S rRNA gene, V3–V4	16S rRNA gene, V4	16S rRNA gene, V4
Genus with Increased Relative Abundance by COVID Severity	*Staphylococcus, Veillonella*	*no significant difference*	*Streptococcus, Actinomyces, Prevotella_ 7, Peptostreptococcus, Veillonella, Rothia, Solobacterium, Atopobium, Granulicatella, Gemella, Mogibacterium, Alloprevotella*
Genus with decreased relative abundance by COVID Severity	*Corynebacterium, Dolosigranulum, Lawsonella*	*Fusobacterium*	*Lawsonella, Cutibacterium*
Nasopharynx Core Microbiome in Healthy Individuals	*Corynebacterium, Staphylococcus, Escherichia-Shigella, Methylobacterium- Methylorubrum, Acinetobacter, Streptococcus, Pseudomonas, Lawsonella*	*Corynebacterium, Staphylococcus, Dolosigranulum, Lawsonella, Anaerococcus, Streptococcus*	*Staphylococcus, Corynebacterium, Dolosigranulum, Streptococcus, Lawsonella, Anaerococcus,*
Accession number	PRJNA714242	PRJNA703574	PRJNA981220

Granulicatella, Actinomyces, Oribacterium, Fusobacterium, and *Porphyromonas.* The meta-analysis of Reubens et al. [37] regarding studies on the diversity of the upper respiratory tract (URT) microbiome is consistent with our results, in that they find a trend in COVID-19 infections showing a reduction in some bacterial genera and an increase in others. However, the inclusion of both nasopharyngeal and oropharyngeal samples in their meta-analysis is a limitation that could explain discrepancies between their study and ours such as the observed higher abundance of *Cutibacterium* in their population.

We meticulously considered the potential influence of covariates beyond COVID-19 severity. Despite the presence of these covariates, we were able to identify discernible alterations in taxonomic composition among critically ill COVID-19 patients, suggesting that *Lawsonella* and *Cutibacterium* decrease additionally to *Streptococcus* increase was independent of confusing factors such as comorbidities, intubation, and prior antibiotic intake. We also observed statistically significant differences between groups by severity of COVID-19 related to the previous administration of steroids. However, it is important to note that steroids were primarily administered to critically ill patients.

Additionally, we found a moderate negative correlation between the severity of COVID-19 and the genera *Lawsonella* and *Cutibacterium*. Interestingly, although the genus *Corynebacterium* did not exhibit significant differences among groups, it showed a negative correlation with COVID-19 severity. In contrast, we observed a significant positive correlation between the severity of COVID-19 and the presence of oral cavity-associated genera in nasopharyngeal samples.

In this context, we suggest that this invasion of oral microorganisms may result from the disruption of physical-chemical barriers caused by inflammation during COVID-19, leading to ecological niche competition. Particularly, critically ill COVID-19 patients experience increased mouth breathing before mechanical ventilation support, facilitating the entry of microorganisms such as *Streptococcus, Rothia, Veillonella*, and *Granulicatella* from the oral cavity into the respiratory tract. Moreover, intubation disrupts barriers, allowing the invasion of microorganisms such as *Prevotella_7*, *Actinomyces, Solobacterium*, and *Atopobium* into the nasopharynx.

Some of these bacterial genera are considered part of the healthy human microbiome and are found in various anatomical sites [38], primarily in the oral microbiome [39]. These bacteria have also been linked to oral diseases and pathologies in other organs or systems when there is ectopic colonization [39–41]. In the case of the *Streptococcus* genus in the oral cavity, its presence has been documented on the tongue, mucous membranes, saliva, and dentogingival plaque. Interestingly, different species colonize distinct parts of the mouth [39]. Importantly, *Streptococcus pneumoniae* has been associated with high case-fatality rates in COVID-19 patients [16].

Rothia is primarily found on the dorsum of the tongue and is associated with nitrate reduction in the oral cavity [39]. However, its translocation to other parts of the body can lead to endocarditis or pneumonia [42]. *Veillonella* is present in almost the entire oral cavity of healthy individuals [39]. Additionally, an increase in its abundance has been observed in patients with caries [43] and has been reported as a cause of lung abscesses with empyema [44]. *Granulicatella* is part of the periodontal microbiota in healthy patients, and its decrease has been described in patients with periodontitis [45]. Nevertheless, its translocation to the bloodstream can lead to endocarditis [46].

Prevotella has been described in various anatomical sites [38], as well as throughout the mouth and saliva of healthy individuals,

being the second most abundant genus after *Streptococcus* [47]. It is associated with both periodontal disease and endodontic infections [47] and has been reported as a cause of aspiration pneumonia [48] and Lemierre syndrome characterized by vein thrombosis, oropharyngeal infection, and metastatic septic emboli [49]. Furthermore, in the murine model, *Prevotella intermedia* has a synergistic effect on pneumococcal pneumonia, increasing inflammatory cytokine levels, bacterial loads in the lungs, and mortality [50]. *Actinomyces* is present in subgingival plaque and on the tongue [39] and is associated with dental caries [51]. It is important to mention that *Actinomyces israelii* is the causative agent of actinomycosis, a chronic suppurative granulomatous infection and, in some cases, can be complicated by presenting microabscesses, pneumonia, or septicemia [52].

The potential clinical implications of our findings suggest that the invasion of microorganisms from the oral cavity initiates before intubation, highlighting the clinical relevance of oral hygiene. Oral hygiene is already recognized as an important approach to reducing the risk of ventilator-associated pneumonia [53]. Sampson et al. [54], suggest that there is a link between poor oral health and COVID-19 complications. We highlighted the need for further research to explore strategies like oral hygiene in severe COVID-19 patients to mitigate the risk of critical illness.

Our analysis of data from other studies involving COVID-19 patients categorized by severity revealed varying taxonomic changes, suggesting potential influences of geographic variables, genetics, and diet. Understanding the stability of the microbiome is crucial for elucidating its role in host health and disease susceptibility. Across multiple studies from different countries, we consistently observed the presence *of Staphylococcus, Corynebacterium*, and *Streptococcus* genera in samples from healthy volunteers. While some species within these genera are known to be beneficial, others may have pathogenic effects, raising questions about their functional significance in respiratory health.

Lawsonella and *Cutibacterium* are commonly found in the nasal cavity [55], facial skin [56], and nasopharynx [57]. *Cutibacterium* has been reported to promote skin health and produce substances with antimicrobial properties [58], suggesting potential roles as commensals within the respiratory tract. Nevertheless, it has also been reported as a human pathogen [59,60]. Furthermore, it is possible that these bacteria were carried out during sampling via nasopharyngeal swabs. Hence, the presence of some taxonomic groups in the nasopharynx or potential consideration as contaminants from the nasal cavity or skin warrants further investigation.

In other viral respiratory infections, such as influenza, specific dominant taxonomic groups are recognized, often linked with pathobionts or opportunistic pathogens such as *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Streptococcus pneumoniae* [61,62]. In adults with Rhinovirus infection, Allen et al. observed a decline in *Haemophilus* and *Neisseria*, alongside an increase in *Propionibacterium* [63]. Additionally, in adults with Respiratory Syncytial Virus (RSV) infection, Cuthbertson et al. found no statistically significant differences in taxonomic groups or diversity in oropharyngeal samples [64]. These findings highlight the importance of continuing research to shed light on how each viral infection alters physicochemical conditions, immune responses, the microbiome, and anatomical barriers.

Our study has some limitations including, the small sample size, temporal considerations due to variations in the circulating COVID-19 variants, and limited taxonomic resolution. However, it is important to highlight that recruitment of cases of severe and critically ill COVID-19 was done carefully and using strict inclusion criteria in order to avoid bias in the selection of patients and controls.

In conclusion, our study suggests that inflammatory processes and intubation procedures in critically ill COVID-19 patients may disrupt physicochemical conditions, immune responses, microbiome, and barriers. This disruption could potentially facilitate the colonization and invasion of oral microorganisms in the nasopharynx and may be correlated with the severity of COVID-19.

Funding source

This work was supported by internal resources of Instituto de Biotecnología and Instituto Nacional de Medicina Genómica. DGC was supported by a scholarship 2019-000002-01NACF-12598 from Consejo Nacional de Humanidades Ciencias y Tecnología, México.

Ethical approval statement

The study was approved by the Ethics Committee of the INER from Mexico (approval number No.0352-2150). Informed consent was obtained from all subjects.

Data availability statement

The raw V4 region of 16S rRNA sequencing data of this study has been deposited in the NCBI Bioproject database under accession number **PRJNA981220.** The R script used for this analysis has been uploaded on https://github.com/David-microbiomics/Rscript/blob/main/Rscript_16SV4.

CRediT authorship contribution statement

David Galeana-Cadena: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gustavo Ramirez-Martínez:** Writing – original draft, Supervision, Methodology, Formal analysis. **José Alberto Choreño-Parra:** Supervision, Formal analysis, Conceptualization. **Eugenia Silva-Herzog:** Writing – original draft, Supervision, Conceptualization. **Carmen Margarita Hernández-Cárdenas:** Writing – original draft, Supervision, Conceptualization. **Xavier Soberón:** Writing – review & editing, Writing – original draft, Validation, Supervision,

Conceptualization. **Joaquín Zúñiga:** Writing – review & editing, Writing – original draft, Validation, Supervision, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to express their gratitude for the invaluable assistance provided by the healthcare workers at the INER, as well as the sequencing unit at the CIENI led by Sandra Maria Pinto Cardoso. We would also like to acknowledge Carlos Federico Arias Ortiz, Pavel Isa, and Humberto Flores Soto for their support and training. Our thanks go to Jerome Verleyen for his technical assistance and for granting us access to the HPC infrastructure at the Unidad Universitaria de Secuenciación Masiva y Bioinformática, Instituto de Biotecnología (UNAM), which is part of the Laboratorio Nacional de Apoyo Tecnológico a las Ciencias Genómicas (CONACyT). Lastly, we would like to thank the Instituto de Biotecnología-UNAM for granting us access to their computer cluster.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e31562.

References

[1] M. Flynn, Z. Lyall, G. Shepherd, O.N.Y. Lee, I. Marianna Da Fonseca, Y. Dong, S. Chalmers, J. Hare, J. Thomson, F. Millar, Interactions of the bacteriome, virome, and immune system in the nose, FEMS Microbes 3 (2022) 1–6, https://doi.org/10.1093/femsmc/xtac020.
[2] B.L. Hardy, D.S. Merrell, Friend or foe: Interbacterial competition in the nasal cavity, J. Bacteriol. 203 (2020), https://doi.org/10.1128/JB.00480-20.
[3] D.W. Cleary, S.C. Clarke, The nasopharyngeal microbiome, Emerg. Top. Life Sci. 1 (2017) 297–312, https://doi.org/10.1042/ETLS20170041.
[4] L. Ren, Y. Wang, J. Zhong, X. Li, Y. Xiao, J. Li, J. Yang, G. Fan, L. Guo, Z. Shen, L. Kang, L. Shi, Q. Li, J. Li, L. Di, H. Li, C. Wang, Y. Wang, X. Wang, X. Zou, J. Rao, L. Zhang, J. Wang, Y. Huang, B. Cao, J. Wang, M. Li, Dynamics of the upper respiratory tract microbiota and its association with mortality in COVID-19, Am. J. Respir. Crit. Care Med. 204 (2021) 1379–1390, https://doi.org/10.1164/rccm.202103-0814OC.
[5] C. Bacali, R. Vulturar, S. Buduru, A. Cozma, A. Fodor, A. Chis, O. Lucaciu, L. Damian, M.L. Moldovan, Oral microbiome : Getting to know and befriend neighbors , a biological approach, Biomedicines (2022) 1–22, https://doi.org/10.3390/biomedicines10030671.
[6] J.I. Prosser, B.J.M. Bohannan, T.P. Curtis, R.J. Ellis, M.K. Firestone, R.P. Freckleton, J.L. Green, L.E. Green, K. Killham, J.J. Lennon, A.M. Osborn, M. Solan, C. J. van der Gast, J.P.W. Young, The role of ecological theory, Nat. Rev. Microbiol. 5 (2007) 384–392.
[7] R.A. Quinn, W. Comstock, T. Zhang, J.T. Morton, R. Da Silva, A. Tran, A. Aksenov, L.F. Nothias, D. Wangpraseurt, A.V. Melnik, G. Ackermann, D. Conrad, I. Klapper, R. Knight, P.C. Dorrestein, Niche partitioning of a pathogenic microbiome driven by chemical gradients, Sci. Adv. 4 (2018), https://doi.org/10.1126/sciadv.aau1908.
[8] R. Bale, A. Iida, M. Yamakawa, C.G. Li, M. Tsubokura, Quantifying the COVID19 infection risk due to droplet/aerosol inhalation, Sci. Rep. 12 (2022) 1–15, https://doi.org/10.1038/s41598-022-14862-y.
[9] C. Menni, A.M. Valdes, L. Polidori, M. Antonelli, S. Penamakuri, A. Nogal, P. Louca, A. May, J.C. Figueiredo, C. Hu, E. Molteni, L. Canas, M.F. Österdahl, M. Modat, C.H. Sudre, B. Fox, A. Hammers, J. Wolf, J. Capdevila, A.T. Chan, S.P. David, C.J. Steves, S. Ourselin, T.D. Spector, Symptom prevalence, duration, and risk of hospital admission in individuals infected with SARS-CoV-2 during periods of omicron and delta variant dominance: a prospective observational study from the ZOE COVID Study, Lancet (London, England) 399 (2022) 1618–1624, https://doi.org/10.1016/S0140-6736(22)00327-0.
[10] L. Ball, P.L. Silva, D.R. Giacobbe, M. Bassetti, G.R. Zubieta-Calleja, P.R.M. Rocco, P. Pelosi, Understanding the pathophysiology of typical acute respiratory distress syndrome and severe COVID-19, Expert Rev. Respir. Med. 16 (2022) 437–446, https://doi.org/10.1080/17476348.2022.2057300.
[11] N.K. Sharma, S.C. Sarode, Low pH and temperature of airway surface liquid are key determinants that potentiate SARS-CoV-2 infectivity, Curr. Mol. Med. 22 (2021) 471–477, https://doi.org/10.2174/1566524021666210816095557.
[12] C.M. Capelle, S. Ciré, O. Domingues, I. Ernens, F. Hedin, A. Fischer, C.J. Snoeck, W. Ammerlaan, M. Konstantinou, K. Grzyb, A. Skupin, C.L. Carty, C. Hilger, G. Gilson, A. Celebic, P. Wilmes, A. Del Sol, I.M. Kaplan, F. Betsou, T. Abdelrahman, A. Cosma, M. Vaillant, G. Fagherazzi, M. Ollert, F.Q. Hefeng, Combinatorial analysis reveals highly coordinated early-stage immune reactions that predict later antiviral immunity in mild COVID-19 patients, Cell Reports Med 3 (2022), https://doi.org/10.1016/j.xcrm.2022.100600.
[13] A. Oliva, M.C. Miele, F. Di Timoteo, M. De Angelis, V. Mauro, R. Aronica, D. Al Ismail, G. Ceccarelli, C. Pinacchio, G. d'Ettorre, M.T. Mascellino, C. M. Mastroianni, Persistent systemic microbial translocation and intestinal damage during coronavirus disease-19, Front. Immunol. 12 (2021) 1–9, https://doi.org/10.3389/fimmu.2021.708149.
[14] S. Ma, F. Zhang, F. Zhou, H. Li, W. Ge, R. Gan, H. Nie, B. Li, Y. Wang, M. Wu, D. Li, D. Wang, Z. Wang, Y. You, Z. Huang, Metagenomic analysis reveals oropharyngeal microbiota alterations in patients with COVID-19, Signal Transduct. Target. Ther. 6 (2021), https://doi.org/10.1038/s41392-021-00614-3.
[15] S. Rattanaburi, V. Sawaswong, S. Chitcharoen, P. Sivapornnukul, P. Nimsamer, N. Suntronwong, J. Puenpa, Y. Poovorawan, S. Payungporn, Bacterial microbiota in upper respiratory tract of COVID-19 and influenza patients, Exp. Biol. Med. 247 (2022) 409–415, https://doi.org/10.1177/15353702211057473.
[16] Z. Amin-Chowdhury, F. Aiano, A. Mensah, C.L. Sheppard, D. Litt, N.K. Fry, N. Andrews, M.E. Ramsay, S.N. Ladhani, Impact of the coronavirus disease 2019 (COVID-19) pandemic on invasive pneumococcal disease and risk of pneumococcal coinfection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): prospective national cohort study, england, Clin. Infect. Dis. 72 (2021) E65–E75, https://doi.org/10.1093/cid/ciaa1728.
[17] C. Feldman, R. Anderson, The role of co-infections and secondary infections in patients with COVID-19, Pneumonia 13 (2021), https://doi.org/10.1186/s41479-021-00083-w.
[18] T. Braun, S. Halevi, R. Hadar, G. Efroni, E. Glick Saar, N. Keller, A. Amir, S. Amit, Y. Haberman, SARS-CoV-2 does not have a strong effect on the nasopharyngeal microbial composition, Sci. Rep. 11 (2021) 8922, https://doi.org/10.1038/s41598-021-88536-6.
[19] P.A. Engen, A. Naqib, C. Jennings, S.J. Green, A. Landay, A. Keshavarzian, R.M. Voigt, Nasopharyngeal microbiota in SARS-CoV-2 positive and negative patients, Biol. Proced. Online 23 (2021) 1–6, https://doi.org/10.1186/s12575-021-00148-6.
[20] P. Prasad, S. Mahapatra, R. Mishra, K.C. Murmu, S. Aggarwal, M. Sethi, P. Mohapatra, A. Ghosh, R. Yadav, H. Dodia, S.A. Ansari, S. De, D. Singh, A. Suryawanshi, R. Dash, S. Senapati, T.K. Beuria, S. Chattopadhyay, G.H. Syed, R. Swain, S.K. Raghav, A. Parida, Long-read 16S-seq reveals nasopharynx

microbial dysbiosis and enrichment of Mycobacterium and Mycoplasma in COVID-19 patients: a potential source of co-infection, Mol. Omi. 18 (2022) 490–505, https://doi.org/10.1039/d2mo00044j.
[21] H.H. Mostafa, J.A. Fissel, B. Fanelli, Y. Bergman, V. Gniazdowski, M. Dadlani, K.C. Carroll, R.R. Colwell, P.J. Simner, Metagenomic next-generation sequencing of nasopharyngeal specimens collected from confirmed and suspect covid-19 patients, mBio 11 (2020) 1–13, https://doi.org/10.1128/mBio.01969-20.
[22] A.K. Feehan, R. Rose, D.J. Nolan, A.M. Spitz, K. Graubics, R.R. Colwell, J. Garcia-Diaz, S.L. Lamers, Nasopharyngeal microbiome community composition and structure is associated with severity of COVID-19 disease and breathing treatment, Appl. Microbiol. 1 (2021) 177–188, https://doi.org/10.3390/applmicrobiol1020014.
[23] A. Hernández-Terán, F. Mejía-Nepomuceno, M.T. Herrera, O. Barreto, E. García, M. Castillejos, C. Boukadida, M. Matias-Florentino, A. Rincón-Rubio, S. Avila-Rios, M. Mújica-Sánchez, R. Serna-Muñoz, E. Becerril-Vargas, C. Guadarrama-Pérez, V.H. Ahumada-Topete, S. Rodríguez-Llamazares, J.A. Martínez-Orozco, J. Salas-Hernández, R. Pérez-Padilla, J.A. Vázquez-Pérez, Dysbiosis and structural disruption of the respiratory microbiota in COVID-19 patients with severe and fatal outcomes, Sci. Rep. 11 (2021), https://doi.org/10.1038/s41598-021-00851-0.
[24] C. Merenstein, G. Liang, S.A. Whiteside, A.G. Cobián-Güemes, M.S. Merlino, L.J. Taylor, A. Glascock, K. Bittinger, C. Tanes, J. Graham-Wooten, L.A. Khatib, A. S. Fitzgerald, S. Reddy, A.E. Baxter, J.R. Giles, D.A. Oldridge, N.J. Meyer, E.J. Wherry, J.E. McGinniss, F.D. Bushman, R.G. Collman, Signatures of COVID-19 severity and immune response in the respiratory tract microbiome, mBio 13 (2022), https://doi.org/10.1128/mbio.02293-22.
[25] N. Smith, P. Goncalves, B. Charbit, L. Grzelak, M. Beretta, C. Planchais, T. Bruel, V. Rouilly, V. Bondet, J. Hadjadj, N. Yatim, H. Pere, S.H. Merkling, A. Ghozlane, S. Kernéis, F. Rieux-Laucat, B. Terrier, O. Schwartz, H. Mouquet, D. Duffy, J.P. Di Santo, Distinct systemic and mucosal immune responses during acute SARS-CoV-2 infection, Nat. Immunol. 22 (2021) 1428–1439, https://doi.org/10.1038/s41590-021-01028-7.
[26] M. López-Filloy, F.J. Cortez, T. Gheit, O. Cruz y Cruz, F. Cruz-Talonia, M. Chávez-Torres, C. Arteaga-Gómez, I. Mancilla-Herrera, J.J. Montesinos, V.A. Cortés-Morales, C. Aguilar, M. Tommasino, S. Pinto-Cardoso, L. Rocha-Zavaleta, Altered vaginal microbiota composition correlates with human papillomavirus and mucosal immune responses in women with symptomatic cervical ectopy, Front. Cell. Infect. Microbiol. 12 (2022) 1–15, https://doi.org/10.3389/fcimb.2022.884272.
[27] B.J. Callahan, P.J. McMurdie, M.J. Rosen, A.W. Han, A.J.A. Johnson, S.P. Holmes, DADA2: high-resolution sample inference from Illumina amplicon data, Nat. Methods 13 (2016) 581–583, https://doi.org/10.1038/nmeth.3869.
[28] M.S. Kumar, E.V. Slud, K. Okrah, S.C. Hicks, S. Hannenhalli, H. Corrada Bravo, Analysis and correction of compositional bias in sparse sequencing count data, BMC Genom. 19 (2018) 1–23, https://doi.org/10.1186/s12864-018-5160-5.
[29] S. Lahti, L, Shetty, Microbiome R package, Bioconductor (2017), https://doi.org/10.18129/B9.bioc.microbiome.
[30] I. Patil, Visualizations with statistical details: the “ggstatsplot” approach, J. Open Source Softw. 6 (2021) 3167, https://doi.org/10.21105/joss.03167.
[31] D.D. Sjoberg, K. Whiting, M. Curry, J.A. Lavery, J. Larmarange, Reproducible summary tables with the gtsummary package, R J 13 (2021) 570–580, https://doi.org/10.32614/rj-2021-053.
[32] J.H. Hurst, A.W. McCumber, J.N. Aquino, J. Rodriguez, S.M. Heston, D.J. Lugo, A.T. Rotta, N.A. Turner, T.S. Pfeiffer, T.C. Gurley, M.A. Moody, T.N. Denny, J. F. Rawls, J.S. Clark, C.W. Woods, M.S. Kelly, Age-related changes in the nasopharyngeal microbiome are associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and symptoms among children, adolescents, and young adults, Clin. Infect. Dis. 75 (2022) E928–E937, https://doi.org/10.1093/cid/ciac184.
[33] B.J. Kelly, I. Imai, K. Bittinger, A. Laughlin, B.D. Fuchs, F.D. Bushman, R.G. Collman, Composition and dynamics of the respiratory tract microbiome in intubated patients, Microbiome 4 (2016) 1–13, https://doi.org/10.1186/s40168-016-0151-8.
[34] C. Merenstein, F.D. Bushman, R.G. Collman, Alterations in the respiratory tract microbiome in COVID-19: current observations and potential significance, Microbiome 10 (2022) 1–14, https://doi.org/10.1186/s40168-022-01342-8.
[35] M.H. Shilts, C. Rosas-Salazar, B.A. Strickland, K.S. Kimura, M. Asad, E. Sehanobish, M.H. Freeman, B.C. Wessinger, V. Gupta, H.M. Brown, H.H. Boone, V. Patel, M. Barbi, D. Bottalico, M. O'Neill, N. Akbar, S.V. Rajagopala, S. Mallal, E. Phillips, J.H. Turner, E. Jerschow, S.R. Das, Severe COVID-19 is associated with an altered upper respiratory tract microbiome, Front. Cell. Infect. Microbiol. 11 (2022) 1–11, https://doi.org/10.3389/fcimb.2021.781968.
[36] M.P. Ventero, O. Moreno-Perez, C. Molina-Pardines, A. Paytuví-Gallart, V. Boix, I. Escribano, I. Galan, P. González-delaAleja, M. López-Pérez, R. Sánchez-Martínez, E. Merino, J.C. Rodríguez, Nasopharyngeal Microbiota as an early severity biomarker in COVID-19 hospitalised patients, J. Infect. 84 (2022) 329–336, https://doi.org/10.1016/j.jinf.2021.12.030.
[37] R.C. Reuben, R. Beugnon, S.D. Jurburg, COVID-19 alters human microbiomes: a meta-analysis, Front. Cell. Infect. Microbiol. 13 (2023) 1–10, https://doi.org/10.3389/fcimb.2023.1211348.
[38] J. Lloyd-Price, A. Mahurkar, G. Rahnavard, J. Crabtree, J. Orvis, A.B. Hall, A. Brady, H.H. Creasy, C. McCracken, M.G. Giglio, D. McDonald, E.A. Franzosa, R. Knight, O. White, C. Huttenhower, Strains, functions and dynamics in the expanded human microbiome project, Nature 550 (2017) 61–66, https://doi.org/10.1038/nature23889.
[39] J.L. Baker, J.L. Mark Welch, K.M. Kauffman, J.S. McLean, X. He, The oral microbiome: diversity, biogeography and human health, Nat. Rev. Microbiol. (2023), https://doi.org/10.1038/s41579-023-00963-6.
[40] X. Peng, L. Cheng, Y. You, C. Tang, B. Ren, Y. Li, X. Xu, X. Zhou, Oral microbiota in human systematic diseases, Int. J. Oral Sci. 14 (2022) 1–11, https://doi.org/10.1038/s41368-022-00163-7.
[41] J.R. Willis, T. Gabaldón, The human oral microbiome in health and disease: from sequences to ecosystems, Microorganisms 8 (2020) 1–28, https://doi.org/10.3390/microorganisms8020308.
[42] M. Fatahi-Bafghi, Characterization of the Rothia spp. and their role in human clinical infections, Infect. Genet. Evol. 93 (2021) 104877, https://doi.org/10.1016/j.meegid.2021.104877.
[43] N.D. Teixeira, A.K. Almeida de Lima, T. Do, C.M. Stefani, Meta-analysis using NGS data: the Veillonella species in dental caries, Front. Oral Heal. 2 (2021), https://doi.org/10.3389/froh.2021.770917.
[44] K. Martinez, G.K. Mangat, N. Sherwani, M. Glover Do, M. Silver Md, Veillonella intrapulmonary abscess with empyema, Cureus 15 (2023) 1–8, https://doi.org/10.7759/cureus.45210.
[45] B. Liu, L.L. Faller, N. Klitgord, V. Mazumdar, M. Ghodsi, D.D. Sommer, T.R. Gibbons, T.J. Treangen, Y.C. Chang, S. Li, O.C. Stine, H. Hasturk, S. Kasif, D. Segrè, M. Pop, S. Amar, Deep sequencing of the oral microbiome reveals signatures of periodontal disease, PLoS One 7 (2012), https://doi.org/10.1371/journal.pone.0037919.
[46] C. Albano, S. Bagarello, S. Giordano, M.F. Sanfilippo, C. Comparato, G. Scardino, V. Garbo, G. Boncori, A. Condemi, A. Cascio, C. Colomba, Granulicatella spp., a causative agent of infective endocarditis in children, Pathogens 11 (2022) 1–9, https://doi.org/10.3390/pathogens11121431.
[47] A. Tett, E. Pasolli, G. Masetti, D. Ercolini, N. Segata, Prevotella diversity, niches and interactions with the human host, Nat. Rev. Microbiol. 19 (2021) 585–599, https://doi.org/10.1038/s41579-021-00559-y.
[48] X. Wang, C. Xu, Z. Yang, T. Zhang, F. Du, X. Zhou, Y. Wang, Aspiration pneumonia caused by Prevotella causing prothorax after pulmonary puncture: a case report, Clin. Lab. 69 (2023), https://doi.org/10.7754/Clin.Lab.2023.230605.
[49] A. Sharma, Z. Mahajan, S. Mehta, A. Puri, Lemierre syndrome: a diagnostic dilemma of critical care in the post-COVID era, BMJ Case Rep. 17 (2024) e257143, https://doi.org/10.1136/bcr-2023-257143.
[50] K. Nagaoka, K. Yanagihara, Y. Morinaga, S. Nakamura, T. Harada, H. Hasegawa, K. Izumikawa, Y. Ishimatsu, H. Kakeya, M. Nishimura, S. Kohnoa, Prevotella intermedia induces severe bacteremic pneumococcal pneumonia in mice with upregulated platelet-activating factor receptor expression, Infect. Immun. 82 (2014) 587–593, https://doi.org/10.1128/IAI.00943-13.
[51] A.C.R. Tanner, C.A. Kressirer, S. Rothmiller, I. Johansson, N.I. Chalmers, The caries microbiome: implications for reversing dysbiosis, Adv. Dent. Res. 29 (2018) 78–85, https://doi.org/10.1177/0022034517736496.
[52] E. Könönen, W.G. Wade, Actinomyces and related organisms in human infections, Clin. Microbiol. Rev. 28 (2015) 419–442, https://doi.org/10.1128/CMR.00100-14.

[53] T. Zhao, X. Wu, Q. Zhang, C. Li, W. Hv, F. Hua, T. Zhao, X. Wu, Q. Zhang, C. Li, W. Hv, F. Hua, T. Zhao, X. Wu, Q. Zhang, C. Li, H. Worthington, F. Hua, Oral hygiene care for critically ill patients to prevent ventilator-associated pneumonia, Cochrane Database Syst. Rev. 12 (12) (2020) 1–139, https://doi.org/10.1002/14651858.CD008367.pub4.www.cochranelibrary.com. John Wiley and Sons Ltd. 2020.
[54] V. Sampson, N. Kamona, A. Sampson, Could there be a link between oral hygiene and the severity of SARS-CoV-2 infections? Br. Dent. J. 228 (2020) 971–975, https://doi.org/10.1038/s41415-020-1747-8.
[55] J.G. Natalini, S. Singh, L.N. Segal, The dynamic lung microbiome in health and disease, Nat. Rev. Microbiol. 21 (2023) 222–235, https://doi.org/10.1038/s41579-022-00821-x.
[56] J.H. Kim, S.M. Son, H. Park, B.K. Kim, I.S. Choi, H. Kim, C.S. Huh, Taxonomic profiling of skin microbiome and correlation with clinical skin parameters in healthy Koreans, Sci. Rep. 11 (2021) 1–15, https://doi.org/10.1038/s41598-021-95734-9.
[57] M. Yasir, H.A. Al-Sharif, T. Al-Subhi, A.A. Sindi, D.H. Bokhary, M.M. El-Daly, B. Alosaimi, M.E. Hamed, A.M. Karim, A.M. Hassan, M.M. AlShawdari, M. Alawi, S.A. El-Kafrawy, E.I. Azhar, Analysis of the nasopharyngeal microbiome and respiratory pathogens in COVID-19 patients from Saudi Arabia, J. Infect. Public Health 16 (2023) 680–688, https://doi.org/10.1016/j.jiph.2023.03.001.
[58] M. Rozas, A.H. de Ruijter, M.J. Fabrega, A. Zorgani, M. Guell, B. Paetzold, F. Brillet, From dysbiosis to healthy skin: major contributions of cutibacterium acnes to skin homeostasis, Microorganisms 9 (2021) 1–18, https://doi.org/10.3390/microorganisms9030628.
[59] R. Ramesh, M. Assi, Z. Esquer Garrigos, M.R. Sohail, Lawsonella clevelandensis: an emerging cause of vascular graft infection, BMJ Case Rep. 14 (2021), https://doi.org/10.1136/bcr-2020-237350.
[60] J. Prinz, B. Schmid, R. Zbinden, P.O. Zingg, I. Uçkay, Y. Achermann, P.P. Bosshard, Fast and sensitive multiplex real-time quantitative PCR to detect cutibacterium periprosthetic joint infections, J. Mol. Diagnostics. 24 (2022) 666–673, https://doi.org/10.1016/j.jmoldx.2022.03.003.
[61] D. Kaul, R. Rathnasinghe, M. Ferres, G.S. Tan, A. Barrera, B.E. Pickett, B.A. Methe, S. Das, I. Budnik, R.A. Halpin, D. Wentworth, M. Schmolke, I. Mena, R. A. Albrecht, I. Singh, K.E. Nelson, A. García-Sastre, C.L. Dupont, R.A. Medina, Microbiome disturbance and resilience dynamics of the upper respiratory tract during influenza A virus infection, Nat. Commun. 11 (2020), https://doi.org/10.1038/s41467-020-16429-9.
[62] T. Qin, T. Geng, H. Zhou, Y. Han, H. Ren, Z. Qiu, X. Nie, T. Du, J. Liang, P. Du, W. Jiang, T. Li, J. Xu, Super-dominant pathobiontic bacteria in the nasopharyngeal microbiota as causative agents of secondary bacterial infection in influenza patients, Emerg. Microbes Infect. 9 (2020) 605–615, https://doi.org/10.1080/22221751.2020.1737578.
[63] E.K. Allen, A.F. Koeppel, J.O. Hendley, S.D. Turner, B. Winther, M.M. Sale, Characterization of the nasopharyngeal microbiota in health and during rhinovirus challenge, Microbiome 2 (2014) 1–11, https://doi.org/10.1186/2049-2618-2-22.
[64] L. Cuthbertson, P. James, M.S. Habibi, R.S. Thwaites, A. Paras, C. Chiu, P.J.M. Openshaw, W.O.C. Cookson, M.F. Moffatt, Resilience of the respiratory microbiome in controlled adult RSV challenge study, Eur. Respir. J. 59 (2022), https://doi.org/10.1183/13993003.01932-2021.

Expert Review of Molecular Diagnostics

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iero20

Salivette® Cortisol versus oropharyngeal swabbing for the detection of SARS-CoV-2 infection

Petra Moroni-Zentgraf, Christoph Keller, Christoph C. Eschenfelder, Hanns Walter Müller, Ralf Sigmund, David Galeana-Cadena, José Eduardo Márquez-García, Angélica Moncada-Morales & Joaquin A. Zúñiga

To cite this article: Petra Moroni-Zentgraf, Christoph Keller, Christoph C. Eschenfelder, Hanns Walter Müller, Ralf Sigmund, David Galeana-Cadena, José Eduardo Márquez-García, Angélica Moncada-Morales & Joaquin A. Zúñiga (2023) Salivette® Cortisol versus oropharyngeal swabbing for the detection of SARS-CoV-2 infection, Expert Review of Molecular Diagnostics, 23:11, 1011-1014, DOI: 10.1080/14737159.2023.2260308

To link to this article: https://doi.org/10.1080/14737159.2023.2260308

Published online: 24 Oct 2023.

EXPERT REVIEW OF MOLECULAR DIAGNOSTICS
2023, VOL. 23, NO. 11, 1011–1014
https://doi.org/10.1080/14737159.2023.2260308

Taylor & Francis
Taylor & Francis Group

ORIGINAL RESEARCH

Salivette® Cortisol versus oropharyngeal swabbing for the detection of SARS-CoV-2 infection

Petra Moroni-Zentgraf[a], Christoph Keller[b], Christoph C. Eschenfelder[a], Hanns Walter Müller[c], Ralf Sigmund[d], David Galeana-Cadena[e], José Eduardo Márquez-García[e], Angélica Moncada-Morales[e] and Joaquin A. Zúñiga[e,f]

[a]Human Pharma Medicine, Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany; [b]HP Country Pharmacovigilance, Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany; [c]Non-Clinical Statistics, Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany; [d]Biostatistics and Data Sciences, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany; [e]Laboratory of Immunobiology and Genetics, Instituto Nacional de Enfermedades Respiratorias Ismaesl Cosío Villegas, Mexico City, Mexico; [f]National Institute of Respiratory Diseases-Mexico (INER), Tecnologico de Monterrey, Escuale ade Medicina y Ciencias de las Salud, Monterrey, Mexico

ABSTRACT

Background: Detecting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by naso/oropharyngeal swabbing may expose health-care workers to the virus and is technically challenging. The Salivette® is an alternative saliva-collection device with an oral cotton swab containing citric acid to stimulate saliva production, which may have an unpleasant taste. We present a pilot study comparing the Salivette® Cortisol (SC), which uses a synthetic swab without citric acid, against oropharyngeal swabbing for the detection of SARS-CoV-2 by reverse transcription quantitative polymerase chain reaction (RT-qPCR).

Research design and methods: Symptomatic SARS-CoV-2-positive patients were sampled at various timepoints. The number of patients positive/negative for SARS-CoV-2 in oropharyngeal swab and SC samples and the percentage of patients testing true positive/true negative for SARS-CoV-2 from SC samples were determined. Positivity was defined by RT-qPCR amplification of 2/3 target SARS-CoV-2 N, ORF1, and S gene sequences.

Results: SC demonstrated 100% specificity, 52.2% sensitivity, and positive correlation with oropharyngeal swabbing for the detection of the SARS-CoV-2 S gene. In later-stage disease, lower viral load was observed in SC samples compared with oropharyngeal swabs.

Conclusions: The SC may be an alternative for SARS-CoV-2 detection where naso/oropharyngeal swabbing is not feasible/available. This technique also confirms observations that the detection of SARS-CoV-2 in the upper airway may vary due to viral load over the disease course.

Trial registration: NCT04599959

ARTICLE HISTORY
Received 21 July 2023
Accepted 10 September 2023

KEYWORDS
SARS-CoV-2; COVID-19; oropharyngeal swabbing; saliva sampling; viral load; Salivette Cortisol

1. Introduction

Coronavirus disease 2019 (COVID-19) has been pandemic since March 2020 [1], and the World Health Organization has identified breaking the chain of transmission of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as paramount in controlling the spread of the disease. To achieve this, large-scale, accurate, and rapid testing is crucial, and the sampling method is a critical aspect of this. The standard method of detection of SARS-CoV-2 is reverse transcription quantitative polymerase chain reaction (RT-qPCR) of naso/oropharyngeal swab samples [2]. This is an established procedure but may cause discomfort to the individual and/or lead to coughing or sneezing, exposing health-care workers to airborne virus droplets [3,4]. Naso/oropharyngeal swabbing (the standard procedure for self-testing when the study reported here was carried out) is also technically challenging when self-sampling [5]. Studies have shown that sampling of saliva, chiefly by spitting in a tube or gargling, may provide an easier and potentially more sensitive approach to testing [6,7]. However, this also generates aerosols and can be challenging to perform, again particularly in children [8]. The Salivette® is an inexpensive saliva-collection device containing a cotton swab that is inserted by the user into the mouth [9]. It has been used successfully to collect saliva for SARS-CoV-2 detection by PCR, with results comparable to nasopharyngeal swabbing [4,10,11]. However, at the time the study presented here was carried out, the cotton swab contained citric acid to stimulate saliva production, which has an intense taste that may be perceived as unpleasant. The Salivette® Cortisol [12] contains a synthetic swab instead of cotton, without the addition of citric acid. It is licensed for the collection of saliva samples, particularly small volumes, for cortisol measurement in children and, in addition to an improved taste compared with the cotton Salivette®, benefits from almost 100% saliva recovery for analysis [12,13].

Here, we present a pilot study comparing collection of saliva with the Salivette® Cortisol (hereafter SC) versus oropharyngeal swabbing for detecting SARS-CoV-2 infection by RT-qPCR (NCT04599959). The trial was conducted in accordance with the

CONTACT Petra Moroni-Zentgraf petra.moroni-zentgraf@boehringer-ingelheim.com Human Pharma Medicine, Boehringer Ingelheim Pharma GmbH & Co. KG, Binger Strasse 173, Ingelheim 55216, Germany

trial protocol, the principles of the Declaration of Helsinki, and the Harmonized Tripartite Guideline for Good Clinical Practice from the International Conference on Harmonisation. The study was approved by the Federal Committee for Protection from Sanitary Risks (Comisión Federal para la Protección contra Riesgos Sanitarios; COFEPRIS). Written informed consent was obtained from all patients before study entry.

2. Methods

The study was carried out in participants with a diagnosis of COVID-19 ($N = 151$; median [IQR] age: 41.5 [23.0] years; 47.0% male; 72.9% respiratory symptoms, 7.3% gastrointestinal [GI] symptoms, 7.3% GI with respiratory symptoms, and 13.3% asymptomatic) who were recruited from a tertiary hospital in Mexico City, Mexico, and aimed to carry out oropharyngeal and matching SC sampling up to 5 days after the verification of SARS-CoV-2 infection by PCR. All recruited participants were from Mexican ancestry and were recruited from a COVID-19 referral center in Mexico City. All participants fulfilled the clinical criteria for COVID-19 diagnosis, and all virologic confirmations by standard methods were performed. No participant follow-up was carried out. Baseline and clinical characteristics are described in Table 1. Primary outcomes were (1) the number of participants testing positive or negative for SARS-CoV-2 virus from oropharyngeal swab and SC samples (processed in Mexico) and (2) the percentage of participants testing true positive (both swab and SC samples testing positive [sensitivity]) or true negative (both swab and SC samples testing negative [specificity]) for SARS-CoV-2 virus from saliva samples (processed in Ingelheim, Germany). Positivity/negativity was determined by RT-qPCR of three SARS-CoV-2 target gene sequences: Nucleocapsid (N), Open Reading Frame 1 (ORF1), and Spike (S). Positivity was defined as a target sequence copy threshold (ct) <45 in 2/3 target sequences. Nucleic acid extraction and target sequence amplification were carried out using methodologies optimized and validated for the purpose of detecting SARS-CoV-2 virus RNA in clinical samples. Oropharyngeal sample collection was carried out by a hospital health-care professional (HCP). The SC was self-administered under supervision by an HCP, who then collected the sample from the participant.

3. Results

A total of 151 swabs were analyzed, of which 85 (56.3%) were positive and 66 (43.7%) were negative. Forty-eight SC samples were not analyzed due to negative PCR result in the swab PCR (Mexico) or lack of saliva, resulting in 103 usable samples, of which 13 (12.6%) were positive, 89 (86.4%) were negative, and 1 (1%) was ambiguous. For analysis of sensitivity and specificity, 141 participants provided matched swab and SC samples, which were then stored and shipped at −80°C to Germany for PCR analysis as above. Sixty-nine of 141 (48.9%) swabs were positive, of which 36 had matching positive SC samples, yielding a sensitivity of 52.2%. Seventy-two swab samples (51.1%) were negative, and all had matching negative SC samples, yielding a specificity of 100%. There was a positive cycle threshold correlation across the three target sequences between matched positive swabs and Salivettes, and viral load was lower in the SC samples compared with swab samples by approximately 10 amplification cycles (Figure 1).

Table 1. Baseline and clinical characteristics of participants enrolled into the study.

	n	%
Gender		
Female	80	53.00
Male	71	47.00
Age[a]		
Median (IQR), years	41.5 (23.0)	
Mean (SD), years	43.4 (14.7)	
18–30 years	33	21.85
31–40 years	38	25.17
41–50 years	34	22.52
51–60 years	24	15.89
61–70 years	13	8.61
>70 years	8	5.30
Symptoms		
Respiratory	110	72.85
Gastrologic	11	7.28
Respiratory and gastrological	11	7.28
Asymptomatic	20	13.25
Onset of symptoms prior to sampling, days[b]		
0	1	0.66
1	17	11.26
2	18	11.92
3	21	13.91
4	13	8.61
5	8	5.30
6	2	1.32
7	3	1.99
8	8	5.30
9	8	5.30
≥10	32	21.19

[a]One patient had no year of birth recorded. [b]Onset of symptoms data were not available for 20 participants.

Our study had aimed to sample participants no later than 5 days after the confirmation of SARS-CoV-2 infection during the early course of the disease when viral load in the saliva is high [7,11]. However, at the time of study start, the incidence rate in Germany was low due to warmer climate conditions and countermeasures taken by government such as social distancing, hygiene rules, and wearing of masks. Therefore, the study was placed in a high-incidence region, in this case Mexico. Here, participants were in a tertiary hospital setting and at a more advanced stage of disease, with 35.1% at more than 5 days after symptom onset at study entry, of which 60% were at ≥10 days. A proportion of swabs and Salivettes (43.7% and 86.4%, respectively) tested negative for SARS-CoV-2. This mirrors previous findings showing a decrease in viral load in nasopharyngeal swabs and saliva over time from onset of symptoms [7,11] as the virus migrates from the upper to the lower airway over the course of the disease and reflects the later-stage disease of the participants in our study. Previous comparable studies have demonstrated sensitivity of SC sampling ranging from approximately 33% up to 100% [4,10]. Melo-Costa and colleagues [4] found a sensitivity of 33.3% in a sub-population of patients who were enrolled during follow-up after a positive SARS-CoV-2 result. These patients were sampled a median of 8 days from the onset of symptoms compared with 0 days for the overall population and were more symptomatic. Our study found an SC sensitivity of 52.2%, which would also suggest a more symptomatic

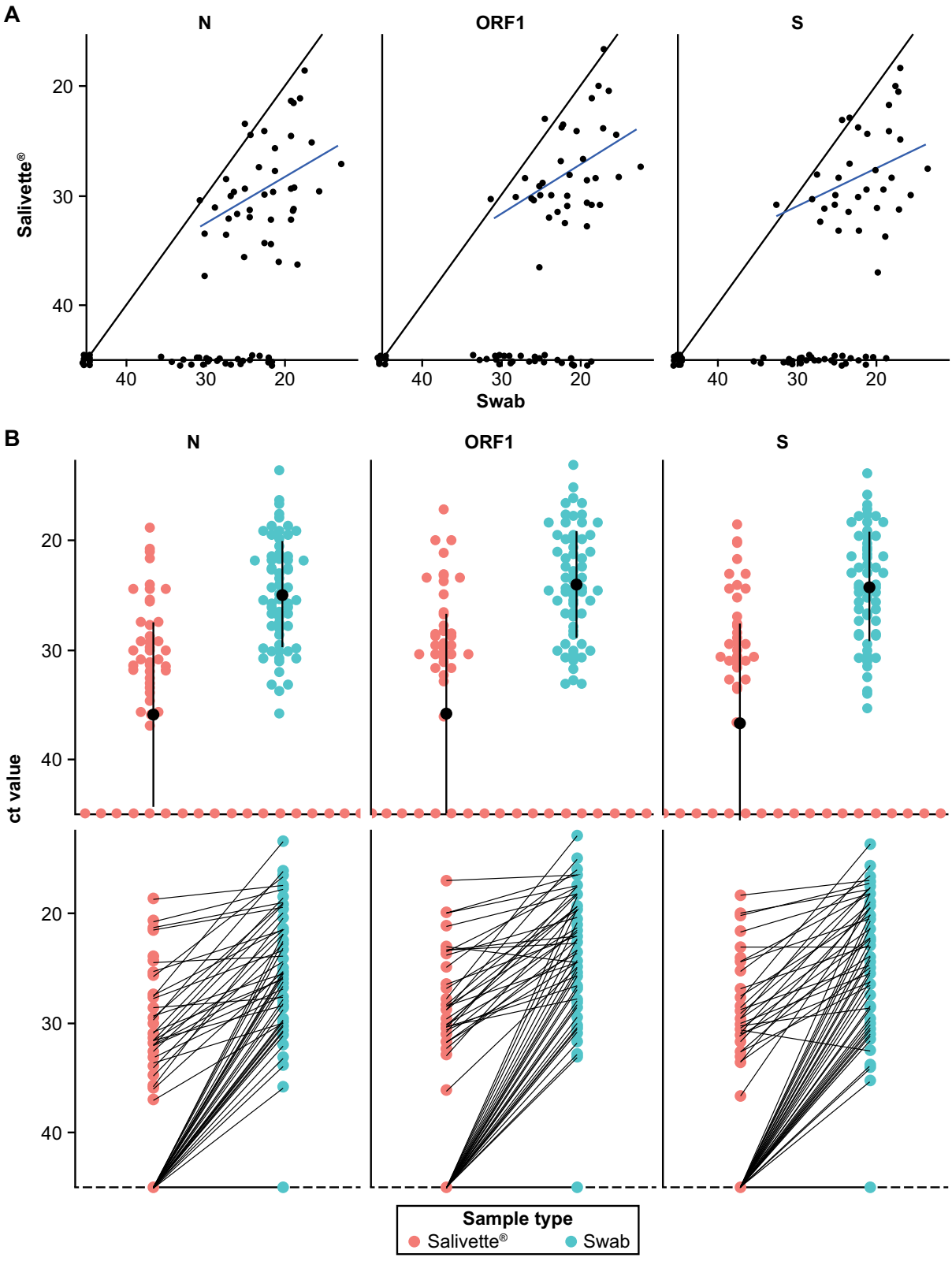


Figure 1. RT-qPCR across N, ORF1, and S target sequences, showing cycle threshold (ct) correlation (blue line) (A), and mean and individual paired cycle threshold values (B) for oropharyngeal swab and SC samples. In (B), black circles denote the mean value and error bars denote 1 standard deviation.

population sampled later in the disease course. RT-qPCR results from our study (Figure 1) mirror the previous study by Basso and colleagues [11] that showed a high degree of correlation in *S* gene ct between cotton SC saliva samples and nasopharyngeal swabs. RT-qPCR results showing a lower viral load in SC saliva samples than in swabs in participants in later-stage disease are also broadly in agreement with a previous study showing a decrease in samples testing positive for SARS-CoV-2 in saliva samples compared with nasopharyngeal swabs in patients ≥11 days after COVID-19 diagnosis [7].

4. Conclusion

In conclusion, our study using the Salivette® Cortiso adds evidence that saliva is a relevant matrix for SARS-CoV-2 detection by RT-qPCR. A specificity of 100% indicates that there were no false positives and that PCR of saliva from SARS-CoV -2-negative individuals sampled by SC consistently indicated the absence of the virus. The SC technique may therefore be an alternative for convenient sample collection where nasopharyngeal swabbing is not available, particularly to confirm negativity of a SARS-CoV-2 result and that a subject is no longer infectious. Furthermore, our study reinforces observations that the detection of SARS-CoV-2 in the upper airway may vary due to viral load behavior over the course of the disease and that the sensitivity of the SC technique may also vary due to disease course and severity. Further trials are needed in earlier timepoints of infection than in this setting to confirm the potential usefulness of Salivette® Cortisol to break the chain of SARS-CoV-2 transmission.

Funding

Funding was provided by Boehringer Ingelheim Pharma GmbH & Co. KG, Germany.

Declaration of interests

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

Author contributions

J Zúñiga was the Principal Investigator for this study. P Moroni-Zentgraf, C Eschenfelder, C Keller and J Zúñiga conceptualized the work entailed in this manuscript. P Moroni-Zentgraf, C Eschenfelder, C Keller, R Sigmund and H Walter Mueller designed the methodology for the study. J Zúñiga provisioned the resources and study materials for this study. J Zúñiga was responsible for data curation. Formal analyses of the data were undertaken by R Sigmund, H Walter Mueller and J Zúñiga. H Walter Mueller performed visualization and presentation of the data. Data validation, including reproducibility of research outputs, was performed by the Boehringer Ingelheim programming team. All authors were involved in the writing, critical reviewing, revision and editing of the manuscript. P Moroni-Zentgraf, C Eschenfelder, C Keller, R Sigmund, H Walter Mueller and J Zúñiga supervised the project and were responsible for all research activity-related planning and execution. Project administration, including management and co-ordination of research activities, was handled by P Moroni-Zentgraf, C Keller and C Eschenfelder.

Acknowledgments

The authors would like to thank the study participants, investigators and coordinators, Bioscientia and the Occupational Healthcare Service Team at Boehringer Ingelheim. Medical writing assistance, in the form of the preparation and revision of the manuscript, was supported financially by Boehringer Ingelheim and provided by Lee Kempster, PhD at MediTech Media (London, UK), under the authors' conceptual direction and based on feedback from the authors.

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Astuti II. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): an overview of viral structure and host response. Diabetes Metab Syndr. 2020;14(4):407–412. doi: 10.1016/j.dsx.2020.04.020
2. Hussein HA, Hassan RYA, Chino M, et al. Point-of-care diagnostics of COVID-19: from current work to future perspectives. Sensors (Basel). 2020;20(15):4289. doi: 10.3390/s20154289
3. Scheuch G. Breathing is enough: for the spread of influenza virus and SARS-CoV-2 by breathing only. J Aerosol Med Pulm Drug Deliv. 2020;33(4):230–234. doi: 10.1089/jamp.2020.1616
4. Melo Costa M, Benoit N, Dormoi J, et al. Salivette®, a relevant saliva sampling device for SARS-CoV-2 detection. J Oral Microbiol. 2021;13(1):1920226. doi: 10.1080/20002297.2021.1920226

•• **This study found a sensitivity of 33.3% for Salivette® sampling in a population of patients sampled during a follow-up after SARS-CoV-2 detection and a median of 8 days from the onset of symptoms.**

5. Liao WT, Hsu MY, Shen CF, et al. Home sample self-collection for COVID-19 patients. Adv Biosyst. 2020;4(11):e2000150. doi: 10.1002/adbi.202000150
6. da Silva RC M, Nogueira Marinho LC, de Araujo Silva DN, et al. Saliva as a possible tool for the SARS-CoV-2 detection: a review. Travel Med Infect Dis. 2020;38:101920. doi: 10.1016/j.tmaid.2020.101920

• **Study showing that saliva is an appropriate matrix for SARS-CoV-2 detection.**

7. Wyllie AL, Fournier J, Casanovas-Massana A, et al. Saliva or nasopharyngeal swab specimens for detection of SARS-CoV-2. N Engl J Med. 2020;383(13):1283–1286. doi: 10.1056/NEJMc2016359

•• **Study showing that saliva is an appropriate matrix for SARS-CoV-2 detection, and a decrease in viral load in nasopharyngeal swabs and saliva over time from the onset of symptoms.**

8. Yee R, Truong TT, Pannaraj PS, et al. Saliva is a promising alternative specimen for the detection of SARS-CoV-2 in children and adults. J Clin Microbiol. 2021;59(2):e02686–20. doi: 10.1128/JCM.02686-20

• **Study showing that saliva is an appropriate matrix for SARS-CoV-2 detection.**

9. Sarstedt AG & Co. Salivette® hygenic saliva collection for diagnostics and monitoring. Available from: https://www.sarstedt.com/fileadmin/user_upload/99_Broschueren/Englisch/156_Salivette_GB_0813.pdf
10. Braz-Silva PH, Mamana AC, Romano CM, et al. Performance of at-home self-collected saliva and nasal-oropharyngeal swabs in the surveillance of COVID-19. J Oral Microbiol. 2020;13(1):1858002. doi: 10.1080/20002297.2020.1858002

• **Study showing that saliva is an appropriate matrix for SARS-CoV-2 detection.**

11. Basso D, Aita A, Padoan A, et al. Salivary SARS-CoV-2 antigen rapid detection: a prospective cohort study. Clin Chim Acta. 2021;517:54–59. doi: 10.1016/j.cca.2021.02.014

•• **Study showing a high degree of correlation in S gene copy threshold between cotton Salivette® saliva samples and nasopharyngeal swabs.**

12. Sarstedt AG & Co. Cortisol-Salivette® saliva collection device evaluation report. Available from: https://www.sarstedt.com/fileadmin/user_upload/99_Literatur/Englisch/448_a_Salivette_Eval_GB.pdf
13. Groschl M, Rauh M. Influence of commercial collection devices for saliva on the reliability of salivary steroids analysis. Steroids. 2006;71(13–14):1097–1100. doi: 10.1016/j.steroids.2006.09.007

frontiers in Immunology

ORIGINAL RESEARCH
published: 21 April 2021
doi: 10.3389/fimmu.2021.593595

Clinical and Immunological Factors That Distinguish COVID-19 From Pandemic Influenza A(H1N1)

*José Alberto Choreño-Parra[1,2], Luis Armando Jiménez-Álvarez[2], Alfredo Cruz-Lagunas[2], Tatiana Sofía Rodríguez-Reyna[3], Gustavo Ramírez-Martínez[2], Montserrat Sandoval-Vega[4], Diana Lizzeth Hernández-García[5], Eduardo M. Choreño-Parra[6], Yalbi I. Balderas-Martínez[2], Mariana Esther Martinez-Sánchez[2], Eduardo Márquez-García[2], Edda Sciutto[7], José Moreno-Rodríguez[8], José Omar Barreto-Rodríguez[9], Hazel Vázquez-Rojas[9], Gustavo Iván Centeno-Sáenz[9], Néstor Alvarado-Peña[10], Citlaltepetl Salinas-Lara[11], Carlos Sánchez-Garibay[11], David Galeana-Cadena[2], Gabriela Hernández[3], Criselda Mendoza-Milla[12], Andrea Domínguez[2,13], Julio Granados[14], Lula Mena-Hernández[15], Luis Ángel Pérez-Buenfil[16], Guillermo Domínguez-Cheritt[13,17], Carlos Cabello-Gutiérrez[18], Cesar Luna-Rivero[19], Jorge Salas-Hernández[20], Patricio Santillán-Doherty[21], Justino Regalado[21], Angélica Hernández-Martínez[22], Lorena Orozco[22], Federico Ávila-Moreno[23], Ethel A. García-Latorre[1], Carmen M. Hernández-Cárdenas[5,13], Shabaana A. Khader[24], Albert Zlotnik[25] and Joaquín Zúñiga[2,13]**

[1] Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico, [2] Laboratory of Immunobiology and Genetics, Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, Mexico City, Mexico, [3] Department of Immunology and Rheumatology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico, [4] Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, Mexico City, Mexico, [5] Intensive Care Unit, Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, Mexico City, Mexico, [6] Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, Mexico City, Mexico, [7] Department of Immunology, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico, [8] Direccion de Enseñanza e Investigación, Hospital Juárez de Mexico, Mexico City, Mexico, [9] Subdirección de Medicina, Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, Mexico City, Mexico, [10] Coordinación de Infectología y Microbiología, Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, Mexico City, Mexico, [11] Departamento de Neuropatología, Instituto Nacional de Neurología y Neurocirugía "Manuel Velasco Suarez", Mexico City, Mexico, [12] Departamento de Fibrosis Pulmonar, Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, Mexico City, Mexico, [13] Tecnologico de Monterrey, Escuela de Medicina y Ciencias de la Salud, Mexico City, Mexico, [14] Department of Transplantation, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico, [15] Department of Dermatology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico, [16] Department of Education, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico, [17] Critical Care Unit, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico, [18] Department of Virology, Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, Mexico City, Mexico, [19] Department of Pathology, Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, Mexico City, Mexico, [20] General Direction, Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, Mexico City, Mexico, [21] Department of Medical Direction, Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, Mexico City, Mexico, [22] Laboratorio Inmunogenómica y Enfermedades Metabólicas, Instituto Nacional de Medicina Genómica, Mexico City, Mexico, [23] Biomedicine Research Unit (UBIMED), Lung Diseases and Cancer Epigenomics Laboratory, Facultad de Estudios Superiores (FES) Iztacala, Universidad Nacional Autónoma de México (UNAM), Tlalnepantla de Baz, Mexico, [24] Department of Molecular Microbiology, Washington University School of Medicine in St Louis, St Louis, MO, United States, [25] Department of Physiology & Biophysics School of Medicine, Institute for Immunology, University of California, Irvine, CA, United States

OPEN ACCESS

Edited by:
Bin Su,
Capital Medical University, China

Reviewed by:
Zhenlong Liu,
McGill University, Canada
Marina Cella,
Washington University School of Medicine in St. Louis, United States
Amit Upadhyay,
Emory University, United States

***Correspondence:**
Joaquín Zúñiga
joazu@yahoo.com

Specialty section:
This article was submitted to Viral Immunology, a section of the journal Frontiers in Immunology

Received: *11 August 2020*
Accepted: *25 March 2021*
Published: *21 April 2021*

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), is a global health threat with the potential to cause severe disease manifestations in the lungs. Although COVID-19 has been extensively characterized clinically, the factors distinguishing SARS-CoV-2 from other

respiratory viruses are unknown. Here, we compared the clinical, histopathological, and immunological characteristics of patients with COVID-19 and pandemic influenza A (H1N1). We observed a higher frequency of respiratory symptoms, increased tissue injury markers, and a histological pattern of alveolar pneumonia in pandemic influenza A (H1N1) patients. Conversely, dry cough, gastrointestinal symptoms and interstitial lung pathology were observed in COVID-19 cases. Pandemic influenza A(H1N1) was characterized by higher levels of IL-1RA, TNF-α, CCL3, G-CSF, APRIL, sTNF-R1, sTNF-R2, sCD30, and sCD163. Meanwhile, COVID-19 displayed an immune profile distinguished by increased Th1 (IL-12, IFN-γ) and Th2 (IL-4, IL-5, IL-10, IL-13) cytokine levels, along with IL-1β, IL-6, CCL11, VEGF, TWEAK, TSLP, MMP-1, and MMP-3. Our data suggest that SARS-CoV-2 induces a dysbalanced polyfunctional inflammatory response that is different from the immune response against pandemic influenza A (H1N1). Furthermore, we demonstrated the diagnostic potential of some clinical and immune factors to differentiate both diseases. These findings might be relevant for the ongoing and future influenza seasons in the Northern Hemisphere, which are historically unique due to their convergence with the COVID-19 pandemic.

Keywords: SARS-CoV-2, COVID-19, Influenza A(H1N1) pdm09, pandemic influenza, acute respiratory distress syndrome

INTRODUCTION

The novel SARS-CoV-2 has submerged the world into a public health crisis of unprecedented features. With more than 113.4 million infected people and 2.5 million deaths, SARS-CoV-2 continues spreading worldwide (1). Although other emerging pathogens have caused similar outbreaks in the past, the pandemic influenza A(H1N1) pdm09 virus is the immediate antecedent reference for the global spread of a new zoonotic respiratory pathogen. This virus emerged in Mexico in 2009, causing approximately 151,700-575,400 deaths worldwide during the first year after its appearance (2–4). Ever since, the influenza A (H1N1) pdm09 virus has continued circulating globally, acquiring a seasonal transmission pattern (5). Notably, the emergence of SARS-CoV-2 in December of 2019 (6–8), occurred when several countries were at the peak of the flu season. This hampered differentiating COVID-19 and influenza during the early days of the current pandemic. With improved understanding of the clinical characteristics and pathobiology of COVID-19 (9–12), the overall identification of positive cases drastically improved.

Despite this, only a few comparisons of the characteristics of COVID-19 and influenza have been conducted (13–16). This is crucial as both entities are converging at several regions of the Northern hemisphere. In this context, the accurate identification of the causative pathogen has important therapeutic implications, including the selection of adequate antiviral drugs. A better understanding of the host factors implicated in protective vs. pathogenic immunity against SARS-CoV-2 is also crucial to guide immunotherapeutic interventions for patients in critical conditions. Unfortunately, what we currently comprehend about the immunopathology of severe COVID-19 is a paradox: the adaptive response is overactive but unable to control the virus. In fact, patients with COVID-19 display a pro-inflammatory (IL-1β, IL-6, IL-7, IL-8, IL-9, FGF, G-CSF, GM-CSF, IFN-γ, CXCL10, CCL2, CCL3, CCL4, PDGF, TNFα, and VEGF) and regulatory cytokine profile (IL-10 and TGFβ; cytokine storm) (17, 18). Interestingly, unlike other cytokine storm syndromes, the polyfunctional immune activation of COVID-19 is accompanied by lymphopenia, reduced T cell numbers, and strong infiltration of immune cells into the lung (19–21). Thus, the lung damage associated with COVID-19 may be caused both by the virus and hyperinflammation.

Comparing the immune profiles of COVID-19 with other respiratory pathogens may dissipate prevailing controversies about the immunopathology of SARS-CoV-2 infection. For this reason, here we evaluated clinical and immunological factors distinguishing critically ill COVID-19 and pandemic influenza A(H1N1) patients. We also compared histopathological changes and expression of immune markers in the lungs of patients with both diseases. Our results reveal crucial differences in the clinical characteristics of the two infections. Furthermore, our analyses clearly show that the human immune response elicited after SARS-CoV-2 is completely different from the immune responses against the influenza A (H1N1) pdm09 virus. Our study may support the use of some of these distinctive traits to differentiate COVID-19 from pandemic influenza A(H1N1) reliably.

MATERIALS AND METHODS

Participants

We conducted a prospective cohort study in patients with an acute respiratory illness that attended the emergency department of the Instituto Nacional de Ciencias Médicas y Nutrición

Salvador Zubirán (INCMNSZ), and the Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas (INER) in Mexico City. Individuals with laboratory-confirmed COVID-19 requiring hospital admission were eligible. Detection of SARS-CoV-2 was performed by real-time polymerase chain reaction (RT-PCR) in swab samples, bronchial aspirates (BA), or bronchoalveolar lavage (BAL) specimens, as previously described (22). Briefly, viral RNA was extracted from clinical samples with the MagNA Pure 96 system (Roche, Penzberg, Germany). The RT-PCR reactions were performed in a total volume of 25μL, containing 5μL of RNA, 12.5μL of 2 × reaction buffer provided with the Superscript III one-step RT-PCR system with Platinum Taq Polymerase (Invitrogen, Darmstadt, Germany; containing 0.4 mM of each deoxyribose triphosphates (dNTP) and 3.2 mM magnesium sulfate), 1μL of reverse transcriptase/Taq mixture from the kit, 0.4μL of a 50 mM magnesium sulfate solution (Invitrogen), and 1μg of nonacetylated bovine serum albumin (Roche). Primer and probe sequences, as well as optimized concentrations, are shown in **Supplemental Table 1**. All oligonucleotides were synthesized and provided by Tib-Molbiol (Berlin, Germany). Thermal cycling was performed at 55°C for 10 min for reverse transcription, followed by 95°C for 3 min and then 45 cycles of 95°C for 15 s, 58°C for 30 s.

Individuals with COVID-19 were further categorized into two groups: a) moderate COVID-19 group (n=10), that included patients with respiratory symptoms that did not require mechanical ventilation (MV); and b) severe COVID-19 group (n=24), consisting of patients requiring invasive MV and admission to the intensive care unit (ICU). Our comparative cohort included patients with influenza-like illness (ILI) that attended to the INER in Mexico City during the immediately preceding 2019/2020 flu season. Individuals with confirmed influenza A(H1N1) pdm09 virus infection that progressed to acute respiratory distress syndrome (ARDS), requiring MV and admission to the ICU were included. ILI was defined as an acute respiratory illness with a measured temperature of ≥ 38°C and cough, with onset within the past ten days. These subjects were first screened for influenza A virus infection using the Fuji dri-chem immuno AG cartridge FluAB kit (Fujifilm Corp, Tokyo, Japan) rapid influenza diagnostic test (RIDT) in fresh respiratory swab specimens. In positive cases, further molecular characterization of the causative influenza A virus subtype was assessed by RT-PCR. All influenza cases enrolled in the study were infected with the pandemic influenza A(H1N1) pdm09 virus. None of the participants had human immunodeficiency virus (HIV) infection.

Data Retrieval

Microsoft Excel (MS Excel 365) was used for data collection. Clinical and demographic data were retrieved from all participants´ medical records. These data included age, gender, anthropometrics, comorbidities, symptoms, triage vital signs, the severity of illness scores at admission [Sequential Organ Failure Assessment (SOFA), and Acute Physiology And Chronic Health Evaluation II (APACHE II)], and initial laboratory tests. Initial laboratory tests were defined as the first test results available (typically within 24 hours of admission) and included white blood cell counts, liver and kidney function, gasometric parameters at admission, and other tissue-injury biomarkers.

Cytokine Determinations

Peripheral blood samples were obtained from all participants at hospital admission. Serum levels of different cytokines, chemokines, growth factors, and other immune mediators were determined by Luminex assays using the Luminex platform Bio-Plex Multiplex 200 (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Serum samples from 13 healthy donors were used as controls. The immune mediators that were quantified are listed as follows: IFN-α, interferon-alpha, IFN-β; interferon-beta; IFN-γ, interferon-gamma; TNF-α, tumor necrosis factor-alpha; IL-1β, interleukin 1beta; IL-1RA, interleukin 1 receptor antagonist; IL-2, interleukin 2; IL-4, interleukin 4; IL-5, interleukin 5; IL-6, interleukin 6; IL-7, interleukin 7; IL-8, interleukin 8; IL-9, interleukin 9; IL-10, interleukin 10; IL-12 (p40), interleukin 12 p40 subunit; IL-12p70, interleukin 12 p70 subunit; IL-13, interleukin 13; IL-15, interleukin 15; IL-17A, interleukin 17A; IL-26, interleukin 26; IL-32, interleukin 32; CXCL10, C-X-C motif chemokine ligand 10, CCL2, C-C motif chemokine ligand 2; CCL3, C-C motif chemokine ligand 3; CCL4, C-C motif chemokine ligand 4; CCL5, C-C motif chemokine ligand 5; CCL11, C-C motif chemokine ligand 11; G-CSF, granulocyte colony-stimulating factor; bFGF, basic fibroblast growth factor; PDGF-BB, platelet-derived growth factor bb; VEGF, vascular endothelial growth factor; APRIL/TNFSF13, A proliferation-inducing ligand/tumor necrosis factor ligand superfamily member 13; BAFF/TNFSF13B, B-cell activating factor/tumor necrosis factor ligand superfamily member 13B; sCD30/TNFRSF8, soluble CD30/ tumor necrosis factor ligand superfamily member 8; sCD163, soluble CD163; chitinase 3/like1; gp130/sIL-6Rβ, glycoprotein of 130 kDa/soluble IL-6 receptor beta; sIL-6Rα, soluble IL-6 receptor alpha; MMP-1, matrix metalloprotease 1; MMP-2, matrix metalloprotease 2; MMP-3, matrix metalloprotease 3; osteocalcin; ostepontin; pentraxin-3; sTNF-R1, soluble tumor necrosis factor receptor 1; sTNF-R2, soluble tumor necrosis factor receptor 2; TSLP, thymic stromal lymphopoietin; TWEAK/TNFSF12, tumor necrosis factor-like weak inducer of apoptosis/tumor necrosis factor ligand superfamily member 12.

Histopathological Analysis

Formalin-fixed and paraffin-embedded lung autopsy specimens from patients who died of pandemic influenza A(H1N1) or COVID-19 (N=2 patients per group) were obtained from the Pathology Department of the INER. Sections of 3-5μm were processed for hematoxylin-eosin (H&E) staining for histopathological analysis. For immunohistochemistry (IHQ), lung sections were mounted on silane-covered slides, deparaffinized in xylenes, and hydrated with a series of graded alcohol-to-water dilutions. The endogenous peroxidase was blocked with 3% hydrogen peroxide for 30 minutes. Sections were incubated overnight at room temperature with optimal dilutions (1:100) of the following antibodies: anti-IFN-γ (Anti-Interferon gamma antibody, ab9657, Abcam, UK), anti-IL-1β (IL-1β Antibody (H-153): sc-7884, Santa Cruz Biotechnology Inc., Santa Cruz, CA), anti-IL-4 (IL-4 Antibody (OX81):

sc-53084, Santa Cruz Biotechnology Inc., Santa Cruz, CA), and anti-IL-17A (Anti-IL-17 antibody (ab91649), Abcam, UK). Secondary biotinylated antibodies labeled with peroxidase were added, and those attached were revealed with diaminobenzidine (DAB) for 5 minutes (MACH 1 Universal HRP-Polymer Detection Kit, Biocare Medical, LLC). Slides were counter-stained with hematoxylin.

Statistical Analysis

Descriptive statistics were used to characterize the study population clinically. Frequencies and proportions were calculated for categorical data. Means, medians, standard deviations (SD), interquartile ranges (IQR), and 95% confidence intervals were used for continuous variables. Differences between groups were assessed by the Fisher exact, Chi-square test, Mann-Whitney U test, or Kruskal-Wallis test with *post hoc* Dunn´s test, as appropriate. Multiple linear regression analyses using Spearman rank correlation coefficients were used to determine correlations between continuous variables. ROC curves were constructed to estimate the diagnostic utility of different variables to differentiate between participant groups in terms of their area under the curve (AUC). The prognostic value of the different clinical and immunological parameters expressed in terms of odds ratio (OR) values for adverse outcomes (intubation, death) was estimated using binomial logistic regression analyses.

Principal component analyses (PCAs) were conducted to analyze how the study participants clustered together according to the interplay between their clinical and immunological characteristics. Furthermore, a Linear Discriminant Analysis (LDA) without and with "leave-one-out" type cross-validation was performed to assess whether the linear combination of different variables allowed differentiating individuals according to their diagnosis. The variables included were AST, ALT, LDH, ALP, procalcitonin, SOFA, IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-7, IL-12, IL-13, IL- 17A, TNF-α, CCL3, CCL11, G-CSF, and VEGF. A Wilks 'Lambda test was performed to evaluate the discriminatory power of each variable in the LDA. Variables were transformed to log10 to meet the LDA assumptions and were scaled to prevent the scale of each variable from influencing the analysis results. Individuals with missing data were omitted from PCA and LDA analyses. All analyses were conducted using GraphPad Prism 8 (La Jolla, CA), R Statistical Software (Foundation for Statistical Computing, Vienna, Austria) packages Factoextra and MASS, and Python packages pandas v0.23.4 and seaborn v0.10.1. Specific analysis tests are also mentioned in figure legends. P values ≤0.05 were considered as significant: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$.

Study Approval

The Institutional Review Boards of the INCMNSZ (approval number: 3349) and the INER (approval number: B28-16 and B09-20) in Mexico City approved the study. All participants or their legal guardians provided written informed consent in accordance with the Declaration of Helsinki for Human Research. Clinical samples were managed according to the Mexican Constitution law NOM-012-SSA3-2012, which establishes the criteria for the execution of clinical investigations in humans.

RESULTS

Participant Characteristics

The main demographic characteristics of enrolled patients were similar (**Table 1**), although the proportion of males tended to be higher in both groups of COVID-19 subjects, as reported before (9, 11, 12, 17, 23, 24). Obesity was more frequent in pandemic influenza A(H1N1) patients, whereas other comorbidities (diabetes, systemic arterial hypertension (SAH), chronic obstructive pulmonary disease (COPD), and obstructive sleep apnea syndrome (OSA)) were equally distributed across groups. Fever was the most frequent symptom among all participants, followed by cough, fatigue, myalgia, arthralgia, and headache. Dyspnea occurred in 10% of patients with moderate COVID-19 and in ~80% of individuals with severe COVID-19 and pandemic influenza A(H1N1). Rhinorrhea, sore throat, thoracic pain, and sputum production were more common during pandemic influenza A(H1N1), whereas dry cough, diarrhea, and vomit were more frequent among COVID-19 patients. This finding suggests that some symptoms could differentiate these infectious entities. We performed a logistic regression analysis with the symptoms reported by pandemic influenza A(H1N1) and COVID-19 patients at hospital admission. Fever and rhinorrhea were associated with pandemic influenza A(H1N1), whereas dry cough predicted COVID-19 (**Supplemental Figure 1** and **Supplemental Table 2**). Sore throat and thoracic pain were marginally associated with pandemic influenza A(H1N1) but did not reach statistical significance. Similarly, gastrointestinal symptoms exhibited higher, but not significant odds ratio (OR) values for COVID-19 (**Supplemental Figure 1** and **Supplemental Table 2**). Overall, patients in the moderate COVID-19 group attended earlier after symptoms onset than individuals with severe pandemic influenza A(H1N1) and COVID-19 (**Table 1**).

Laboratory Parameters of Pandemic Influenza A(H1N1) and COVID-19

White blood cells (WBC), neutrophil counts, neutrophil to lymphocyte ratio (NLR), glucose, total bilirubin, and aspartate aminotransferase (AST) levels were similar in both pandemic influenza A(H1N1) and severe COVID-19 groups, but lower in the moderate COVID-19 group (**Table 2**). Low lymphocyte counts were observed among all participants, indicating that lymphopenia is not a unique feature of severe COVID-19. Renal function parameters did not differ between groups. However, levels of some tissue injury markers, such as alkaline phosphatase (ALP), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine phosphokinase (CPK), and procalcitonin, were higher in pandemic influenza A(H1N1) as compared to COVID-19 patients. We also observed that the SOFA and APACHE II scores were higher in pandemic influenza A(H1N1) patients. Importantly, both groups presented similar rates of complications, and received equal supportive medical interventions (**Table 3**). Despite this, the mortality of our cohort of critically ill pandemic influenza A(H1N1) patients was significantly lower (21%) than the mortality of severely ill COVID-19 patients (62%). No fatality cases were observed in the group of moderated COVID-19.

TABLE 1 | Clinical characteristics of patients with COVID-19 and pandemic influenza.

Characteristic	Influenza A(H1N1) pdm09 A N = 23	*p*-value A vs. B	Moderate COVID-19 B N = 10	p-value B vs. C	Severe COVID-19 C N = 24	p-value A vs. C
Age (years), median (range)	49 (29 - 77)	0.2385	34.5 (28 – 71)	0.1706	52 (30 – 73)	>0.9999
Gender						
Males	14 (60.86)	0.7098	7 (70)	0.5659	19 (79.16)	0.1703
Females	9 (39.13)		3 (30)		5 (20.83)	
BMI	33.6 (29.6 - 42.4)	0.0004	25.3 (22.5 – 29.3)	0.1164	29.6 (25.3 – 33.4)	0.0592
Relevant co-morbidities						
Smoking	8 (34.78)	0.0715	0 (0)	0.0720	8 (33.33)	0.9165
Biomass exposure	5 (21.73)	0.2911	0 (0)	0.2958	4 (16.66)	0.7238
Diabetes	5 (21.73)	>0.9999	2 (20)	>0.9999	6 (25)	0.7918
SAH	6 (26.08)	>0.9999	2 (20)	>0.9999	4 (16.66)	0.4936
OSA	2 (8.69)	>0.9999	0 (0)	1	0 (0)	0.2340
COPD	2 (8.69)	>0.9999	1 (10)	0.2941	0 (0)	0.2340
Cancer	0 (0)	0.0852	2 (20)	0.0802	0 (0)	1
Clinical findings at onset						
Fever	21 (91.3)	0.3605	8 (80)	0.7541	18 (75)	0.1371
Myalgia	17 (73.91)	0.7077	8 (80)	0.5809	17 (70.83)	0.8135
Arthralgia	17 (73.91)	0.4438	6 (60)	0.7109	16 (66.66)	0.5871
Headache	11 (47.82)	0.9086	5 (50)	0.8245	11 (45.83)	0.8911
Dyspnea	18 (78.26)	0.0004	1 (10)	0.0002	19 (79.16)	0.9395
Nasal congestion	3 (13.04)	0.5363	0 (0)	>0.9999	2 (8.33)	0.6662
Rhinorrhea	11 (47.82)	0.0129	0 (0)	0.1478	6 (25)	0.1035
Sore throat	8 (34.78)	0.0321	0 (0)	0.1478	6 (25)	0.4635
Thoracic pain	4 (17.39)	0.2890	0 (0)	1	0 (0)	0.0496
Cough	19 (82.6)	0.4155	7 (70)	0.2226	21 (87.5)	0.6378
Sputum	11 (47.82)	0.0129	0 (0)	0.2958	4 (16.66)	0.0220
Dry cough	8 (34.78)	0.0619	7 (70)	0.9612	17 (70.83)	0.0133
Fatigue	19 (82.6)	>0.9999	8 (80)	0.9563	19 (79.16)	0.7643
Diarrhea	2 (8.69)	0.1493	3 (30)	0.3943	4 (16.66)	0.6662
Nausea	2 (8.69)	0.1493	3 (30)	0.1380	2 (8.33)	>0.9999
Vomit	0 (0)	0.0220	3 (30)	0.3284	3 (12.5)	0.0797
Illness onset - hospital admission (days)	7 (4 - 8.5)	0.0583	3 (0 – 5.7)	0.0158	6 (5 – 13.2)	>0.9999
Vital signs at admission						
Body temperature (°C)	37 (36.8 – 37)	0.5195	36.5 (36.3 – 37.2)	0.02	37 (37 – 37.7)	0.2878
Respiratory rate (bpm)	26 (22 – 30)	0.0018	20 (16.7 – 21.7)	0.0518	24 (22 – 26)	0.48
Hearth rate (bpm)	93 (80 – 103)	>0.9999	90 (75.7 – 99.7)	0.7698	84 (72 – 90)	0.1295
MAP (mmHg)	82 (73.5 – 94.8)	>0.9999	87 (80.7 – 88.7)	0.1820	75 (70.2 – 84.5)	0.3202

Data are displayed as n (%) or median (IQR). N is the total number of patients with available data. BMI, body mass index; bpm, breaths/beats per minute; COPD, chronic obstructive pulmonary disease; IQR, interquartile range; ICU, intensive care unit; MAP, mean arterial pressure; OSA, obstructive sleep apnea syndrome; SAH, systemic arterial hypertension; SD, standard deviation. Differences in continuous variables were estimated using the Kruskal Wallis with post hoc Dunn's test. Differences in categorical variables were calculated using the Fisher's exact or the Chi-square test as appropriate.

Immune Profiles of Pandemic Influenza A (H1N1) and COVID-19 Patients

The severity of pandemic influenza A(H1N1) and COVID-19 has been systematically attributed to an exacerbated production of pro-inflammatory cytokines (cytokine storm syndrome (CSS)) (25, 26). More recently, some researchers have also proposed that immune depression, rather than an exuberant immune activation, is responsible for the clinical pathology of severe COVID-19 (27). Comparing the immune responses elicited by SARS-CoV-2 and influenza A(H1N1) pdm09 virus may be more helpful in identifying unique immune mechanisms associated with morbidity and mortality in COVID-19. Thus, we determined the circulating levels of several immune mediators in pandemic influenza A(H1N1) and COVID-19 patients. Also, we correlated cytokine levels with clinical findings and disease outcomes. Our results showed that critically ill COVID-19 patients had increased serum levels of IL-1β, IL-1RA, IL-6, IL-9, and CXCL10, and lower levels of IL-2 and IL17A as compared to healthy volunteer donors (**Figure 1** and **Supplemental Figure 2**). These findings are coincident with the immune profiles that were reported in Chinese patients with COVID-19 (9, 17, 28). Levels of pro-inflammatory (IFN-γ, IL-1β, IL-6, IL-9, IL-12p70, CCL11) and anti-inflammatory (IL-4, IL-5, IL-10, IL-13) cytokines, as well as VEGF, were higher in severely ill COVID-19 patients as compared to pandemic influenza A(H1N1) subjects. In contrast, levels of IL-1RA, IL-2, TNF-α, CCL3, and G-CSF were more increased among pandemic influenza A(H1N1) patients (**Figure 1** and **Supplemental Figure 2**).

These serum cytokine profiles indicate that, besides a higher production of pro-inflammatory and Th1 cytokines, SARS-CoV-2, but not influenza A(H1N1) pdm09 infection, parallelly induces Th2 responses. This may suggest that a lack of sufficient regulation and balancing of the type of immune response triggered after SARS-CoV-2 infection might

TABLE 2 | Laboratory parameters of participants at admission.

Parameter	Influenza A(H1N1) pdm09A N = 23	*p*-value A vs. B	Moderate COVID-19B N = 10	*p*-value B vs. C	Severe COVID-19C N = 24	*p*-value A vs. C
Glucose (mg/dL)	132.1 (111 – 207.4)	0.1241	96.5 (85.2 – 112)	0.3025	124.3 (99 – 163.7)	>0.9999
Blood count						
White blood cells (10^9/L)	7.3 (5.8 – 11.9)	0.0069	4.0 (3.5 – 5.7)	0.0007	9.5 (6.4 – 13.1)	>0.9999
Neutrophils (10^9/L)	5.7 (4.6 – 9.9)	0.0057	2.9 (1.8 – 3.9)	0.0184	7.4 (4.1 – 10.1)	>0.9999
Lymphocytes (10^9/L)	0.7 (0.4 – 0.9)	0.5050	0.9 (0.6 – 1.2)	>0.9999	0.9 (0.6 – 1.2)	0.3522
NLR	8.4 (5.1 – 17.1)	0.0120	3.1 (1.6 – 5.7)	0.0702	8.7 (3.7 – 13.4)	>0.9999
Hgb (g/dL)	14.2 (13 – 17.3)	0.6994	15.4 (14.2 - 16.4)	0.0755	13.7 (13.2 – 15.1)	0.5663
Platelets (10^9/L)	173 (141 – 205)	>0.9999	192 (137 – 224)	0.7794	208 (165 – 258)	0.1547
Renal function						
Cr (mg/dL)	1.1 (0.9 – 2.2)	0.5773	1.0 (0.8 – 1.1)	>0.9999	0.9 (0.8 – 1.5)	0.4344
BUN (mg/dL)	22.2 (16.2 – 34.3)	0.1106	14.5 (10.5 – 18.8)	0.6716	18 (11.9 – 26.8)	0.7558
Na (mmol/L)	135.2 (132.5 – 139.3)	>0.9999	137 (136 – 139)	0.4082	139.8 (136.2 – 141.7)	0.0161
K (mmol/L)	4.2 (3.8 – 4.6)	0.7951	4.0 (3.7 – 4.2)	0.6386	4.2 (4 – 4.5)	>0.9999
Liver function						
Total bilirubin (mg/dL)	0.6 (0.4 – 0.8)	0.0344	0.3 (0.3 – 0.4)	0.0183	0.5 (0.4 – 0.8)	>0.9999
AST (U/L)	60.9 (39.6 – 84.1)	0.0026	22.6 (15 – 38.5)	0.0170	43.5 (29 – 90.7)	>0.9999
ALT (U/L)	29.2 (23.3 – 47.5)	0.4934	21.7 (17.2 – 32.1)	0.0435	40.2 (28.8 – 56.8)	0.5891
ALP (U/L)	122.7 (86.1 – 169.7)	0.0016	72.5 (58.2 – 80.5)	0.6821	78 (63.4 – 88.2)	0.0104
Other biomarkers						
LDH (U/L)	643.8 (452.2 – 804.7)	<0.0001	186 (165.8 – 251.5)	0.0070	414.5 (318 .4– 494.8)	0.0269
CPK (U/L)	274.4 (158.2 – 771.2)	0.0277	73 (49.7 – 161.3)	0.1225	160.3 (74.6 – 1419)	>0.9999
Procalcitonin (ng/mL)	0.6 (0.2 – 3.6)	<0.0001	0.05 (0.05 – 0.08)	0.1652	0.1 (0.09 – 0.17)	0.0008
Gasometric parameters						
pH	7.37 (7.32 – 7.45)	0.2325	7.43 (7.41 – 7.46)	0.3277	7.41 (7.33 – 7.45)	>0.9999
PaO2 mmHg	51 (38 – 67)	0.0768	65 (55 – 91)	0.1260	51 (42 – 65)	>0.9999
PCO2 mmHg	35 (30 – 48)	>0.9999	34 (29 – 37)	>0.9999	34 (27 – 47)	>0.9999
Lactate (mmol/L)	1.2 (0.8 – 1.5)	ND	ND	ND	0.9 (0.8 – 1.1)	0.1309
HCO3 (mEq/L)	22.2 (17.9 – 27.4)	>0.9999	22.6 (21.5 – 25.8)	0.4845	21.1 (19 – 22.8)	0.9628
PaO_2/FiO_2	96 (62.8 – 160)	<0.0001	314 (262 – 433)	0.001	127 (96 – 155)	0.5999
Mild (PaO_2/FiO_2 201 - 300)	1 (4.34)	0.0002	7 (70)	0.0019	3 (12.5)	0.6085
Moderate (PaO_2/FiO_2 101-200)	11 (47.82)	0.3410	3 (30)	0.0836	15 (62.5)	0.3118
Severe (PaO_2/FiO_2 <100)	11 (47.82)	0.0129	0 (0)	0.0815	6 (25)	0.1035
Severity of illness scores						
SOFA	8 (7 – 13)	<0.0001	1 (0 – 2)	0.0031	6 (3 – 8)	0.0398
APACHE II	11 (5 – 18)	0.0405	4 (0 – 7.5)	0.3546	7 (4 – 10)	0.6420

Data are displayed as n (%) or median (IQR). N is the total number of patients with available data. ALP, alkaline phosphatase; APACHE-II, Acute Physiology and Chronic Health Evaluation II; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood ureic nitrogen; CPK, creatine phosphokinase; Cr, creatinine; FiO2, fraction of inspired oxygen; HCO3, bicarbonate; Hgb, hemoglobin; IQR, interquartile range; LDH, lactate dehydrogenase; ND, not determined; NLR, neutrophil/lymphocyte ration; PaO2, partial pressure of oxygen in arterial blood; PCO2, partial pressure of carbon dioxide in the blood; SD, standard deviation; SOFA, Sequential Organ Failure Assessment. Differences in continuous variables were estimated using the Kruskal Wallis with post hoc Dunn's test. Differences in categorical variables were calculated using the Fisher's exact or the Chi-square test as appropriate.

contribute to the immune dysfunction reported during COVID-19. Also, the proinflammatory and profibrotic immune profile observed in COVID-19 patients may contribute to the extensive tissue damage and poor outcomes reported during SARS-CoV-2 infection (27, 29). Other cytokines similarly increased in patients with severe pandemic influenza A(H1N1) and COVID-19 included IL-7, IL-15, IL8, and CXCL10 (**Supplemental Figure 2**).

Histopathological Characteristics of the Lungs of Pandemic Influenza A(H1N1) and COVID-19 Patients

Parallel histopathological comparative analyses of the lungs of COVID-19 and pandemic influenza A(H1N1) patients have not been conducted. Here, we obtained lung autopsy specimens from individuals that succumbed to either of these diseases and analyze their pathological features. Our analysis revealed that pandemic influenza A(H1N1) induces alveolar edema and intra-alveolar inflammatory infiltrates in the lungs, sparing the integrity of alveolar walls and the micro-architecture of the organ (**Figure 2A**, left panel). These findings are compatible with a typical pattern of alveolar pneumonia. The inflammatory infiltrates observed in the lungs of pandemic influenza A(H1N1) patients were composed of macrophages, polymorphonuclear cells, and scarce lymphocytes scattered between areas of intra-alveolar edema, hemorrhage, and fibrin mucoid exudates. Furthermore, although conserved, the alveolar walls showed capillaries with vasodilation and congestion (**Figure 2A**, right panel). Meanwhile, SARS-CoV-2 induced intense and extensive inflammatory lung infiltrates, as well as thickness of alveolar walls, hemorrhages, and partial loss of the histological architecture of the lung. These changes are compatible with interstitial pneumonia (**Figure 2B**, left panel). The inflammatory infiltrates observed in the lungs of COVID-19 patients were mainly composed of macrophages. Notably, the lungs infected with SARS-CoV-2 showed scarce lymphocytes and detachment

TABLE 3 | Complications and treatment of study participants.

Parameter	Influenza A(H1N1) pdm09 A N = 23	*p*-value A vs. B	Moderate COVID-19 B N = 10	*p*-value B vs. C	Severe COVID-19 C N = 24	*p*-value A vs. C
Complications						
Acute myocardial infarction	3 (13.04)	0.5363	0 (0)	1	0 (0)	0.1092
Deep vein thrombosis	1 (4.34)	>0.9999	0 (0)	1	0 (0)	0.4894
Acute kidney injury	10 (43.47)	0.0148	0 (0)	0.0815	6 (25)	0.1814
Secondary infection	14 (60.86)	0.0014	0 (0)	0.0169	10 (41.66)	0.1880
Medical treatment						
Oseltamivir	23 (100)	0.0012	6 (60)	0.9283	14 (58.33)	0.0005
Antibiotic therapy	23 (100)	1	10 (100)	1	24 (100)	1
No. of antibiotics per patient, median (range)	3.5 (2 – 10)	0.0077	2 (2 – 3)	0.0252	4 (3 – 5)	>0.9999
Chloroquine/hydroxychloroquine	0 (0)	<0.0001	10 (100)	<0.0001	23 (95.83)	<0.0001
Azithromycin	0 (0)	<0.0001	10 (100)	0.0049	13 (54.16)	<0.0001
Corticosteroids	4 (17.39)	0.2890	0 (0)	0.2908	5 (20.83)	0.7643
Respiratory support						
Nasal cannula	0 (0)	<0.0001	10 (100)	<0.0001	0 (0)	1
MV	23 (100)	<0.0001	0 (0)	<0.0001	24 (100)	1
Prone position	14 (60.86)	0.0014	0 (0)	0.0135	11 (45.83)	0.3017
ECMO	2 (8.69)	0.3360	0 (0)	1	0 (0)	0.2340
Renal replacement therapy	6 (26.08)	0.1445	0 (0)	0.2908	5 (20.83)	0.4252
Mortality	5 (21.73)	0.2911	0 (0)	0.0135	15 (62.5)	0.0077

Data are displayed as n (%) or median (IQR). N is the total number of patients with available data. ECMO, extracorporeal membrane oxygenation; IQR, interquartile range; MV, mechanical ventilation; SD, standard deviation. Differences in continuous variables were estimated using the Kruskal Wallis with post hoc Dunn´s test. Differences in categorical variables were calculated using the Fisher's exact or the Chi-square test as appropriate.

of pneumocytes, which showed hyperplasia, cellular changes, and prominent nucleoli (**Figure 2B**, right panel).

Interestingly, our IHQ analysis showed that IFN-γ, IL-1β, and IL-17A were expressed in the lungs of patients with both diseases, mainly inside macrophages and pneumocytes (**Figure 3**). However, the intensity of expression of IFN-γ and IL-17A was higher in patients infected with SARS-CoV-2. Strikingly, IL-4, a Th2 cytokine, was absent in the lungs of pandemic influenza A(H1N1) patients but expressed in COVID-19 subjects (**Figure 3**). These findings are in line with the combined Th1/Th2 immune profile detected only in the serum of our cohort of patients infected with SARS-CoV-2 but not in pandemic influenza A(H1N1) subjects.

Clinical and Immunological Markers Distinguishing Pandemic Influenza A (H1N1) and COVID-19

To determine which clinical and immunological characteristics contributed more to the differences between pandemic influenza A(H1N1) and COVID-19, we performed PCA. The analysis showed that pandemic influenza A(H1N1) patients cluster apart from the combined cohort of COVID-19 subjects in the PC2 (**Figure 4A**). Of note, clinical characteristics contributed to 31.2% of the total variance explained by the two first PCs (12.51% to PC1 and 50.03% to PC2). Meanwhile, serum cytokine levels contributed to 68.7% of the total variance explained by the two first PCs (87.48% to PC1 and 49.96% to PC2). These data indicate that immunological characteristics may be more useful than clinical variables to discriminate between both diseases. Thus, we performed additional PCAs using only clinical or immunological characteristics. We observed that patients with severe pandemic influenza A (H1N1) were not separated from severely ill COVID-19 patients by their clinical features, but they clustered apart from moderate COVID-19 subjects (**Supplemental Figure 3a**). Age, neutrophils, ALP, CPK, bilirubin, LDH, PaO2/FiO2, and SOFA were the clinical variables that contribute more to the first two PCs of this analysis. Conversely, pandemic influenza A(H1N1) patients clustered apart from the entire COVID-19 cohort in a PCA using only serum cytokine levels (**Supplemental Figure 3b**). IFN-γ, IL-1RA, IL-5, IL-9, IL-10, and G-CSF levels contribute to the first two PCs of this PCA.

Using logistic regression analyses, we further evaluated which clinical and immune factors differentiate our two cohorts of severely ill pandemic influenza A(H1N1) and COVID-19 patients. IFN-γ was not included in this analysis, as it showed perfect discrimination of severe COVID-19 from pandemic influenza A(H1N1). We identified that LDH, ALP, procalcitonin, SOFA score, IL-1RA, IL-2, IL-7, TNF-α, CCL3, and G-CSF levels were significantly associated with severe pandemic influenza A(H1N1). In contrast, IL-1β, IL-4, IL-5, IL-12p70, IL-13, IL-17A, CCL11, and VEGF levels predicted severe COVID-19 (**Figures 4B, C**). Some of these factors, along with PaO2/FiO2 index, the incidence of acute kidney injury (AKIN), co-infections, APACHE-II score, IFN-γ, IL-15, and CCL5, also contributed to differentiate the entire COVID-19 cohort from pandemic influenza A(H1N1) subjects (**Supplemental Figure 4**).

An LDA showed that some of these selected parameters, along with AST and ALT, used together, accurately differentiate between severe pandemic influenza A(H1N1), moderate COVID-19, and severe COVID-19 groups (**Figures 5A, B**). Since it would be impractical to assess all these factors combined to differentiate both diseases, we analyze the results of the LDA using the Wilk´s Lambda test. This analysis showed that ALT, ALP, SOFA, IL-2, and TNF-α were crucial for the

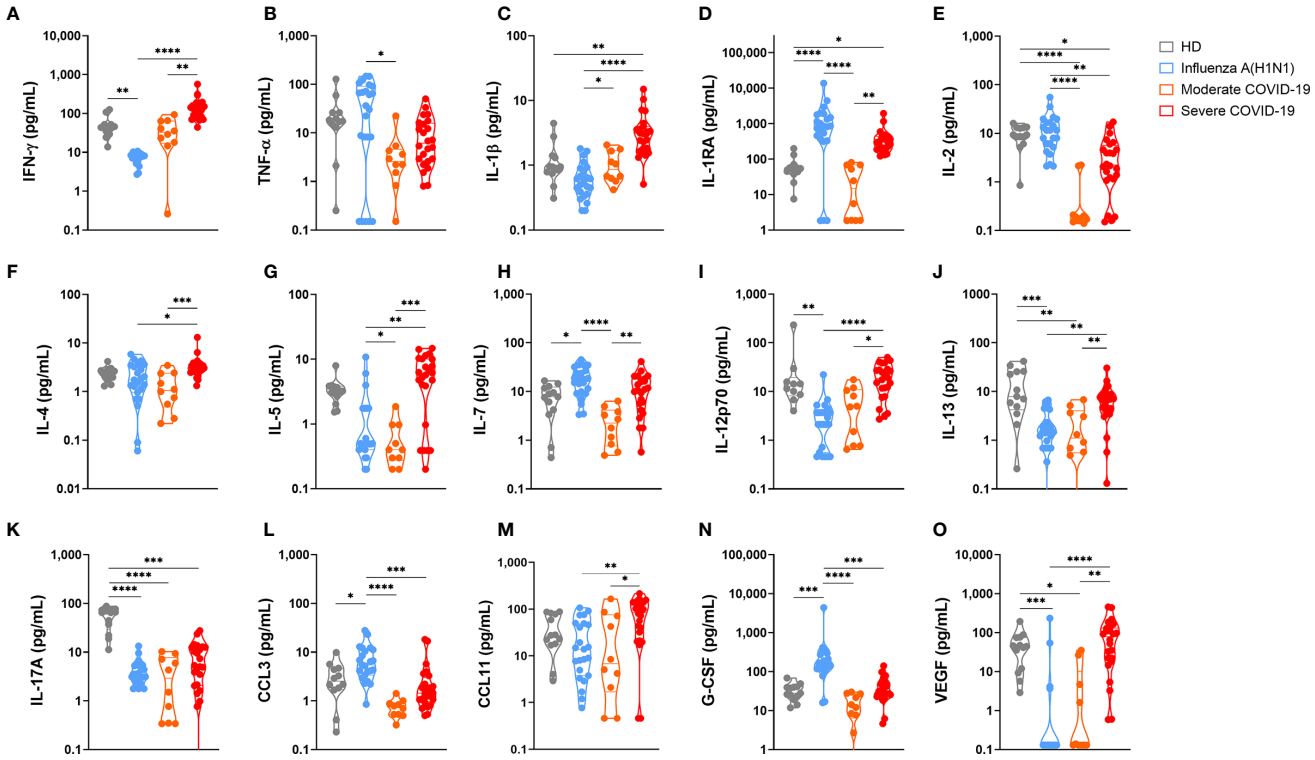


FIGURE 1 | Serum cytokine levels in pandemic influenza A(H1N1) and COVID-19 patients. Serum levels of cytokines, chemokines, and growth factors in healthy volunteer donors (HD, n=13), patients with COVID-19 (n=10 moderate, 24 severe), and influenza (n=23), were assessed by Luminex assay. Violin plots display medians and interquartile ranges (IQR). Differences between groups we estimated using the Kruskal-Wallis test with *post hoc* Dunn´s test. Significant differences are denoted by bars and asterisks: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001. **(A)** IFN-γ, interferon-gamma; **(B)** TNF-α, tumor necrosis factor-alpha; **(C)** IL-1β, interleukin 1beta; **(D)** IL-1RA, interleukin 1 receptor antagonist; **(E)** IL-2, interleukin 2; **(F)** IL-4, interleukin 4; **(G)** IL-5, interleukin 5; **(H)** IL-7, interleukin 7; **(I)** IL-12p70, interleukin 12 p70 subunit; **(J)** IL-13, interleukin 13; **(K)** IL-17A, interleukin 17A; **(L)** CCL3, C-C motif chemokine ligand 3; **(M)** CCL11, C-C motif chemokine ligand 11; **(N)** G-CSF, granulocyte colony-stimulating factor; **(O)** VEGF, vascular endothelial growth factor.

discriminative power of our LDA model (**Figure 5C**). Furthermore, receiver operating characteristics (ROC) curve analyses showed that IFN-γ, IL-1β, IL-12p70, G-CSF, and VEGF had the highest diagnostic performance to distinguish severe COVID-19 and pandemic influenza A(H1N1) (**Figure 6**).

Clinical and Immunological Prognostic Factors in Pandemic Influenza A(H1N1) and COVID-19

We also evaluated the prognostic value of clinical and immunological factors in pandemic influenza A(H1N1) and COVID-19. Among COVID-19 patients, the duration of symptoms before admission, WBC, neutrophil counts, LDH, and SOFA score predicted severe disease defined as the need for intubation (**Figure 7A**). IL-4, IL-7, IL-8, IL-12p70, IL-15, and VEGF were also associated with increased risk of intubation in COVID-19 subjects. IL-6 showed increased but not significant OR values for severity in the combined COVID-19 cohort, contrasting with previous studies that indicate that IL-6 is significantly associated with severe COVID-19 (18, 30). Using a similar approach, we observed that WBC, and SOFA score conferred a higher risk of death after SARS-CoV-2 infection in the entire cohort of COVID-19 patients (**Figure 7B**). Likewise, the need for renal replacement therapy (OR 32, 3 – 849.9 95% CI, $p = 0.0029$), and the use of steroids (OR 25.5, 2.1 – 698.4 95% CI, $p = 0.0091$), were associated with mortality risk after pandemic influenza A(H1N1) (**Supplemental Figure 5**), as reported before (31, 32). However, none of the evaluated cytokines were associated with mortality in COVID-19 and pandemic influenza A(H1N1) patients (**Figure 7** and **Supplemental Figure 5**). At the time of patient recruitment, there was no consensus regarding the use of steroids for COVID-19, and the RECOVERY trial had not been published (33). Hence, only some of our COVID-19 patients were treated with steroids.

Additional Immune Markers Distinguishing Pandemic Influenza A(H1N1) From COVID-19

Finally, we analyzed another set of immune mediators in the blood of 25 moderate and 24 severe COVID-19 patients, as well as in 22 pandemic influenza A(H1N1) subjects, from which we were able to obtain plasma samples (**Figure 8** and **Supplemental Figure 6**). Plasma levels of these factors showed only a few correlations with clinical characteristics and serum cytokine

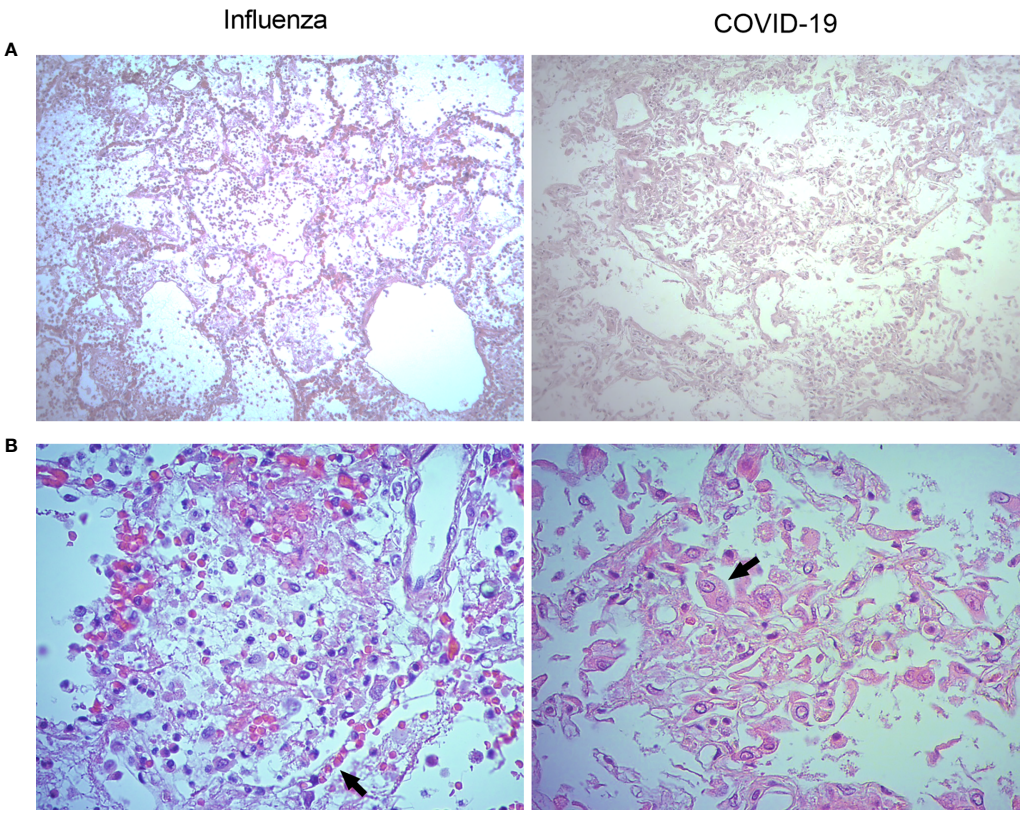


FIGURE 2 | Histological characteristics of the lungs of patients with pandemic influenza A(H1N1) and COVID-19. Lung tissue autopsy specimens were obtained from patients that died of pandemic influenza A(H1N1) and COVID-19. **(A)** The histological changes induced in the lungs during pandemic influenza A(H1N1) were mainly characterized by intra-alveolar inflammatory infiltrates that did not compromise the integrity of alveolar walls (left panel). Meanwhile, the morphological changes of COVID-19 consisted of extensive inflammation, thickening of the alveolar walls, and partial loss of the histological architecture (right panel). H&E staining, x100. **(B)** The inflammatory infiltrates observed in the lungs of pandemic influenza A(H1N1) patients consisted of macrophages, polymorphonuclear cells, and scarce lymphocytes scattered between areas of edema, hemorrhage, and fibrin deposits. Also, congestive, and vasodilated capillaries (arrow) were observed in the alveolar walls of influenza patients (left panel). Conversely, the inflammatory infiltrates found in the lung of COVID-19 patients were dominated by macrophages. Furthermore, the detachment of alveolar epithelial cells, which showed atypical characteristics such as large nucleoli (arrow), was also notable in COVID-19 patients (right panel). H&E staining, x400.

levels (**Supplemental Figure 7**). The overall profile of these correlations was different in pandemic influenza A(H1N1) and COVID-19 patients, suggesting distinct immune mechanisms underlying clinical manifestations of both diseases.

Although levels of plasma type I interferons were below the levels of reliable detection, IFN-α, and IFN-β were increased among all participant groups as compared to healthy controls (**Figure 8**). Furthermore, a slight increase in the levels of IFN-β was noticed in pandemic influenza A(H1N1) patients as compared to COVID-19 patients. Remarkably, although elevated, the levels of APRIL/TNFSF13, sCD30, sCD163, sTNF-R1, and sTNF-R2 were lower in COVID-19 than in pandemic influenza A(H1N1) patients. APRIL/TNFSF13 is crucial for plasma cell survival (34). Thus, plasma cell responses could be downregulated in COVID-19 as compared to pandemic influenza A(H1N1). Soluble CD30 has been proposed as a marker of T cell activation during solid organ transplant rejection (35), whereas sCD163 is a readout of macrophage activation (36). Hence, our data may indicate a depletion of activated lymphocytes and macrophages from the circulation during SARS-CoV-2 infection, despite the high levels of inflammatory mediators found in COVID-19 patients. Soluble TNF-R1 and sTNF-R2 act as decoy receptors for TNF-α (37); as such, patients with COVID-19 might be less capable of balancing pathogenic TNF-α activities than individuals with pandemic influenza A(H1N1).

TWEAK, TSLP, MMP-1, and MMP-3 were elevated in COVID-19 cases. TWEAK is a stimulator of IL-6, IL-8, CXCL10, and MMP-1 (38, 39). As such, high levels of TWEAK might expand the inflammatory response observed in COVID-19 patients. TSLP is a promoter of allergic inflammation and Th2 responses (40). Indeed, high TSLP levels coincide with a Th2 cytokine profile in our COVID-19 cohort. Our results also indicate a possible role for MMP-1 and MMP-3 in lung injury associated with COVID-19, two matrix metalloproteases implicated in tissue damage underlying other lung diseases (41–43).

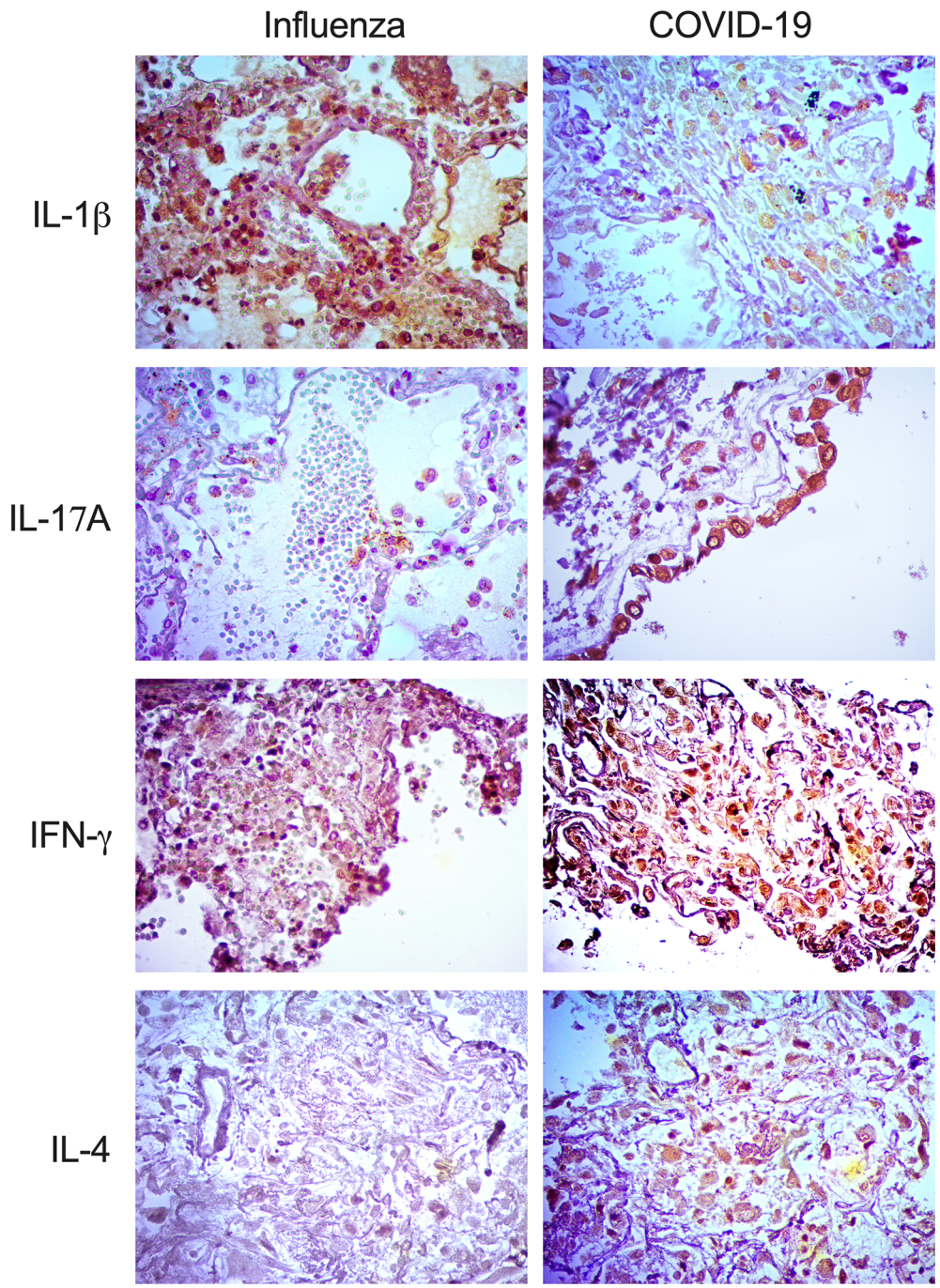


FIGURE 3 | Expression of immune markers in the lungs of pandemic influenza A(H1N1) and COVID-19 patients. Expression of different immune markers in lung autopsy specimens from pandemic influenza A(H1N1) and COVID-19 patients was assessed using specific antibodies by immunohistochemistry (IHQ), x400.

DISCUSSION

The ongoing winter in the Northern hemisphere has been one of the most challenging public health crises in recent history due to the convergence of influenza and COVID-19. This situation could be further aggravated at settings of high pandemic influenza A(H1N1) circulation. Thus, a better understanding of the clinical and immunopathological characteristics that differentiate both diseases is still required to guide specific therapeutic approaches. This includes the selection of adequate antiviral drugs and appropriate immunological therapeutics for each case. Unfortunately,

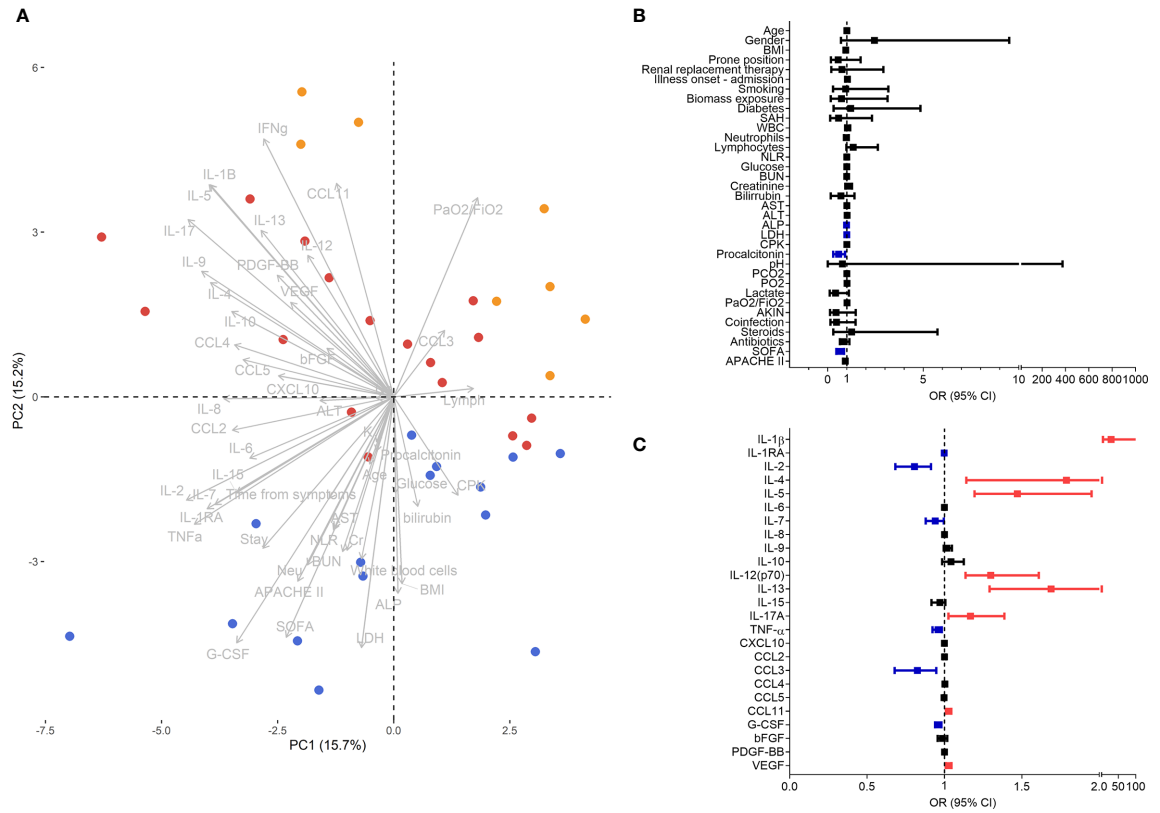


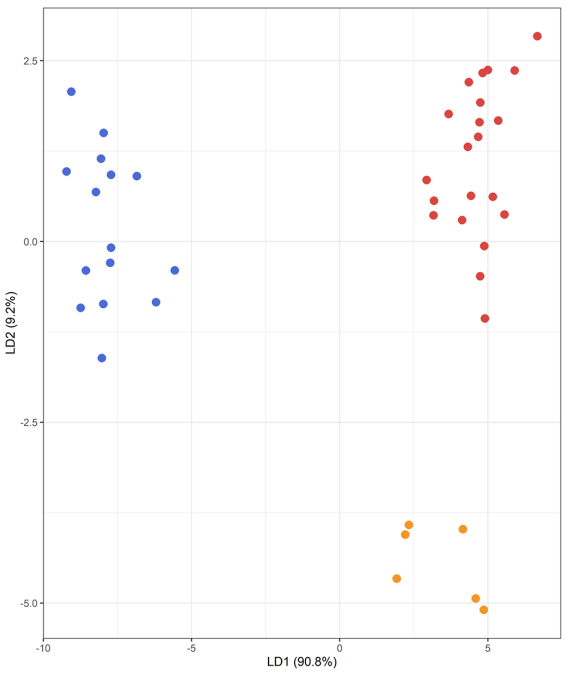
FIGURE 4 | Clinical and immunological factors that distinguish pandemic influenza A(H1N1) and severe COVID-19. **(A)** Principal component analysis (PCA) of the clinical and immunological characteristics of study participants. Each dot represents a single individual, and each color represents a group of participants: blue for pandemic influenza A(H1N1), orange for moderate COVID-19, and red for severe COVID-19. **(B, C)** Bivariate logistic regression analysis of the clinical and immunological characteristics associated with the causative pathogen in the two cohorts of patients with severe influenza and COVID-19. The forest plots show the odds ratio (OR) and 95% CI interval values that were non-significant (black) and significant for severe COVID-19 (red). OR values of factors inversely associated with severe COVID-19 that instead predict pandemic influenza A(H1N1) are shown in blue color. Absolute OR values are also presented in **Supplementary Table 3**.

whereas our knowledge of the immunopathogenesis of pandemic influenza A(H1N1) has improved over the last decade, the current lack of understanding of the COVID-19 pathobiology remains incomplete. This is a barrier to the identification of targets for drug and vaccine development. The inevitable co-circulation of influenza viruses and SARS-CoV-2 and the potential scenarios of viral co-infection may further represent an aggravation of the COVID-19 morbidity and mortality. However, we do not know if an infection with SARS-CoV-2 in patients already infected with influenza viruses would result in worse or better clinical outcomes. The outcomes of the opposite scenario are also speculative. Despite this, it is essential to have reliable indicators to differentiate these conditions, especially in settings of limited resources to perform RT-PCR tests.

Some recent literature reviews have tried to highlight differences between patients infected with SARS-CoV-2 and seasonal influenza viruses (14, 15). However, these retrospective comparisons carry the risk of biased conclusions due to differences in the genetic background, sociocultural characteristics, and access to medical attention of populations from different regions. Thus, parallel comparisons of influenza and COVID-19 cases in geographical settings with similar health care resources would provide a better perspective of the main differences between these entities. In this context, Mexico is an ideal place to conduct comparative studies between pandemic influenza A(H1N1) and COVID-19, as this country was the site of origin of the influenza A(H1N1) pdm09 virus (2–4). Since its emergence in 2009, hospitals around Mexico have acquired ample experience in the management of severe cases of this viral infection, which has resulted in progressive decreases in mortality rates over the last ten years (44). On February 28th, 2020, Mexico confirmed its first two cases of SARS-CoV-2. Ever since, the epidemiological curve of COVID-19 shows a continuous increase in the number of positive cases, with more than 2.2 million cases and 207,000 deaths reported on March 2nd of 2021 (45).

Here, we compared the clinical, histopathological, and immune characteristics of pandemic influenza A(H1N1) and COVID-19 patients. One of the most striking findings of our study was that most of the clinical and laboratory parameters routinely evaluated in emergency departments were similar between both infections in severe disease. Nonetheless, some

A



B

Group	% of individuals correctly classified	
	Result	Cross-validation
Influenza A(H1N1)	100	93.33
Moderate COVID-19	100	83.33
Severe COVID-19	100	95.23
Total	100	92.85

C

Variable	Wilk's Lambda	F (2,20)	*p*
AST	0.863	1.586	0.229
ALT	0.682	4.671	**0.022**
LDH	0.855	1.695	0.209
ALP	0.694	4.414	0.026
Procalcitonin	0.932	0.726	0.496
SOFA	0.580	7.242	0.004
IL-1β	0.762	3.125	0.066
IL-1RA	0.749	3.359	0.055
IL-2	0.491	10.381	0.001
IL-4	0.861	1.616	0.224
IL-5	0.975	0.254	0.778
IL-7	0.915	0.924	0.413
IL-12	0.741	3.498	0.050
IL-13	0.807	2.391	0.117
IL-17A	0.877	1.408	0.268
TNF-α	0.676	4.801	0.020
CCL3	0.921	0.855	0.440
CCL11	0.776	2.885	0.079
G-CSF	0.959	0.423	0.661
VEGF	0.927	0.791	0.467

FIGURE 5 | Selected clinical and immunological characteristics that better distinguish pandemic influenza A(H1N1) from COVID-19. **(A)** Linear discriminant analysis (LDA) plot of the first two discriminant functions showing the separation of the different groups of study participants according to a set of selected clinical and immunological characteristics used in combination. Each dot represents a single individual, and each color represents a group of participants: blue for pandemic influenza A(H1N1), orange for moderate COVID-19, and red for severe COVID-19. **(B)** Accuracy of the LDA results before and after a "leave-one-out" cross-validation. **(C)** The discriminant potential of each individual variable included in the LDA was estimated using the Wilk´s Lambda test. The table displays values of Wilk´s lambda, F, and *p* for each variable.

features separated well moderate COVID-19 patients from severe COVID-19 and pandemic influenza A(H1N1) subjects. Interestingly, our data reveal that respiratory symptoms are more common during pandemic influenza A(H1N1), whereas dry cough and gastrointestinal symptoms are distinctive characteristics associated with COVID-19. These clinical differences may traduce distinct infective capacities of both viruses to affect several organs besides the lungs. In this sense, influenza viruses are thought to be primary respiratory pathogens that rarely cause extrapulmonary dissemination (46). Meanwhile, it is accepted that SARS-CoV-2 has a broad infective capacity to invade several tissues and organs (47). The expression of the angiotensin I converting enzyme 2 (ACE2), the transmembrane serine protease 2 (TMPRSS2), furin, cathepsin L, and other viral entry factors in human organs determine the tissue tropism of SARS-CoV-2. These factors are expressed in the lungs; nonetheless, their expression is even higher at several parts of the upper and lower gastrointestinal tract (48). This might explain the clinical differences observed in our study.

We also found that levels of ALP, ALT, LDH, CPK, procalcitonin, as well as SOFA and APACHE II scores were higher in pandemic influenza A(H1N1) as compared to both groups of COVID-19 patients. Meanwhile, the PaO2/FiO2 upon arrival was similar in severe COVID-19 and severe pandemic influenza A(H1N1) patients. These findings coincide with the results of a previous study evaluating the differences in clinical presentations between Chinese ARDS patients infected with either SARS-CoV-2 or influenza A(H1N1) (13). The researchers also found that ground-glass opacities were more common in radiological studies of COVID-19 patients, whereas consolidation opacities were more frequent in influenza subjects. Ground-glass opacities are typically associated with an interstitial inflammatory process of the lung, whereas consolidations traduce intra-alveolar exudates (49). Here, we found that the histopathological pattern induced after lung infection with SARS-CoV-2 is mainly characterized by an interstitial inflammatory infiltrate. Meanwhile, pandemic influenza A(H1N1) induces changes compatible with alveolar pneumonia. Together, both studies highlight that the two diseases display crucial differences in the histological characteristics of the infected lungs that may also translate into distinctive clinical manifestations.

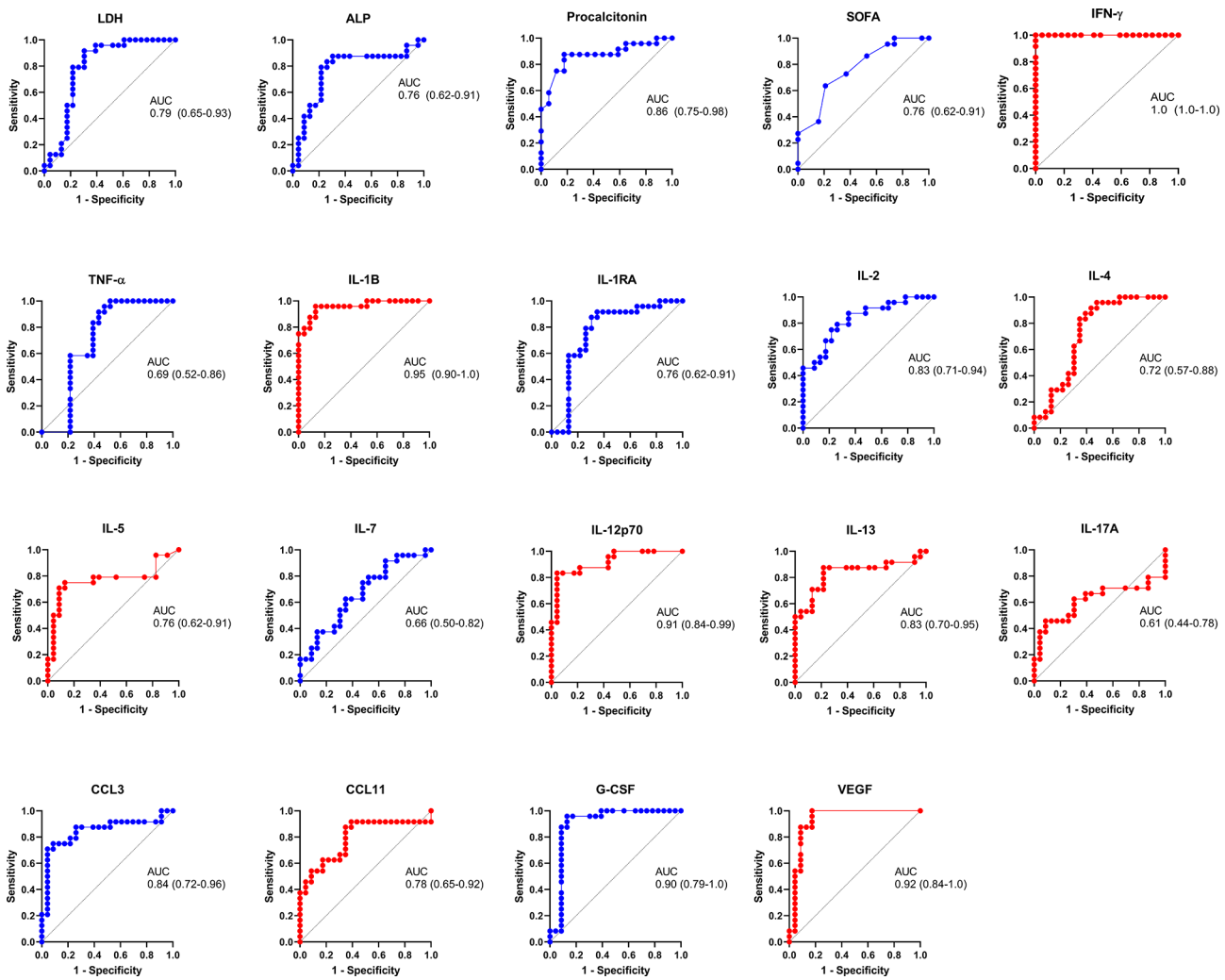


FIGURE 6 | Diagnostic value of the clinical and immunological factors that distinguish between severe COVID-19 and pandemic influenza A(H1N1). Receiver operating characteristic (ROC) curves were constructed for the clinical and immunological characteristics that showed significant OR values in the bivariate logistic regression analysis. ROC curves of variables associated with pandemic influenza A(H1N1) are shown in blue color, whereas variables associated with severe COVID-19 are displayed in red color. The graphs show area under the curve (AUC) and 95% CI interval values.

The immune response against SARS-CoV-2 is not well comprehended so far. The prevailing paradigm to explain the morbidity and mortality of COVID-19 patients is that SARS-CoV-2 elicits an exuberant immune reaction characterized by a dysregulated cytokine production. This phenomenon, known as "cytokine storm," is thought to be responsible for mediating tissue injury in patients with COVID-19 that progress to severe illness (19, 28, 50, 51). The immune receptors that recognize the viral infection and initiate the immune responses against SARS-CoV-2 are unknown. As this virus is genetically related to SARS-CoV-1, it is presumed that both viruses share mechanisms of infection. In this sense, SARS-CoV-1 is recognized by the toll-like receptors (TLR) TLR3 and TLR4, which induce an immune reaction *via* MyD88 and TRIF pathways (52, 53). Furthermore, SARS-CoV-1 triggers the production of IL-1β through the activation of the inflammasome (54). It is also possible that SARS-CoV-2 activates the inflammasome, as high levels of IL-1β have been observed in COVID-19 patients (55). Other immune mediators exaggeratedly produced in response to SARS-CoV-2 include IL2, IL-6, IL7, IL10, G-SCF, CXCL10, CCL2, CCL3, and TNF-α (9, 17, 28). Similar immune signatures were detected in our cohort of COVID-19 patients. Strikingly, our study, and two recent investigations carrying out single cell RNA sequencing of immune cells and cytokine determinations in BAL (16, 56), converge in a major pathogenic role of IL-1 β, IL-6, and CCL2 in patients who develop severe COVID-19 compared to people with less severe disease.

Meanwhile, the pathogenicity and virulence of the influenza A(H1N1) pdm09 virus are due to acquired properties contributing to alter the regulation of inflammatory responses

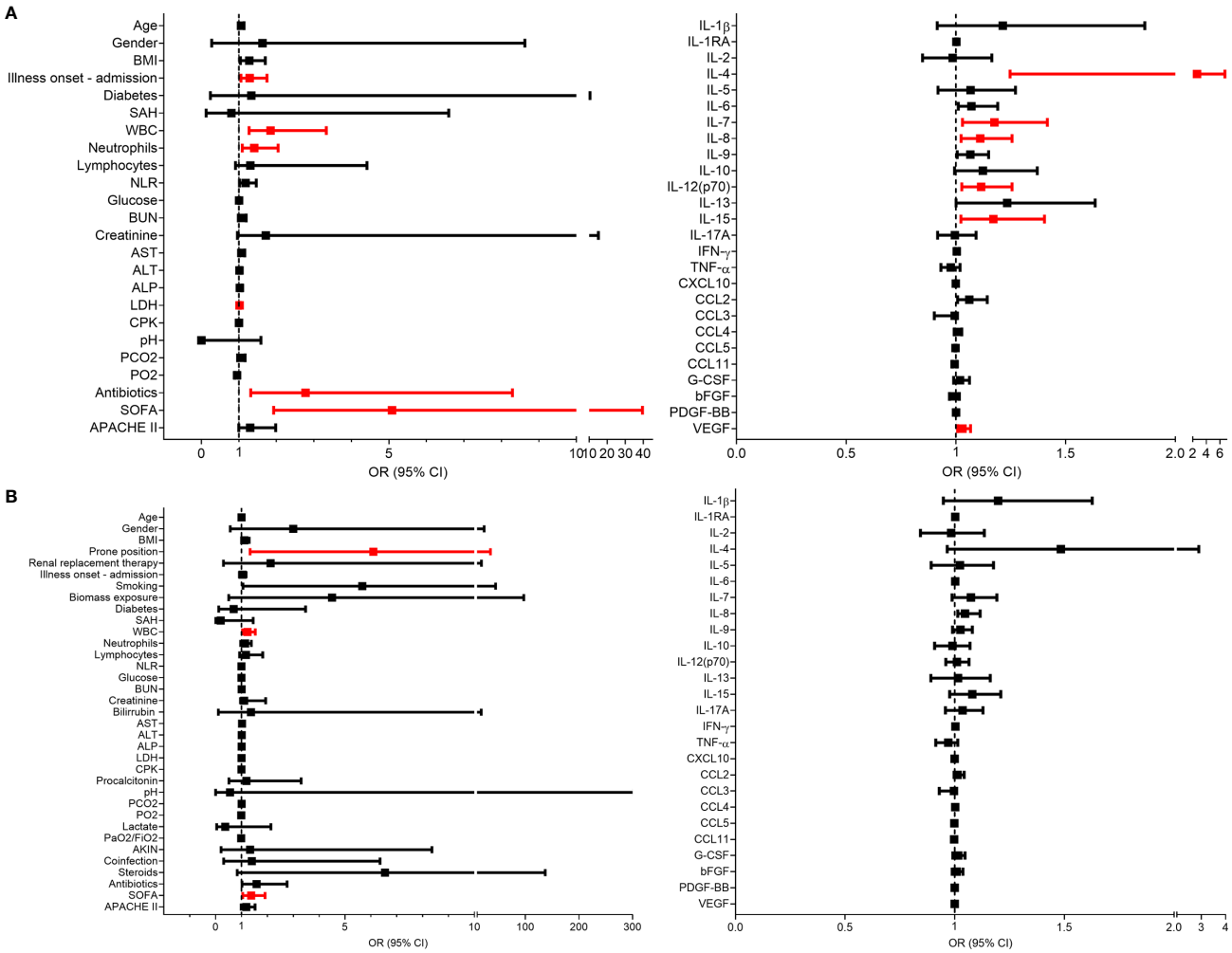


FIGURE 7 | Clinical and immunological factors associated with disease outcomes in patients with COVID-19. **(A)** Bivariate logistic regression analysis of the clinical and immunological characteristics associated with intubation in patients COVID-19. **(B)** Clinical and immunological factors associated with mortality in patients with COVID-19. The forest plots show the odds ratio (OR) and 95% CI interval values. OR values that did not include the null value in the 95% CI were considered significant for intubation/mortality and are shown in red color. Absolute OR values are also presented in **Supplemental Table 5**.

and evade antiviral immunity. Previously, we have described that pandemic, but not seasonal influenza A strains, downregulate the expression of the suppressors of cytokine signaling 1 (SOCS-1) and increase the production of IL-6, IL-8, TNF-α, IL-10, CCL3, CCL4, and CCL5 in experimental infection assays of human lung A549 epithelial cells and human macrophages (57). Levels of IL-6, IL-8, TNF-α, and CCL3 were also increased in our cohort of pandemic influenza A(H1N1) patients, validating our previous observations. The influenza A(H1N1) pdm09 also suppresses the expression of the retinoid-inducible gene I (RIG-I) and induces lower levels of type I interferons in human macrophages and human lung epithelial cells, as compared to seasonal influenza A strains (57). In this sense, it is possible that blocking type I interferon responses might be a strategy of SARS-CoV-2 to evade antiviral immune mechanisms, as we found very low induction of plasma IFN-α and IFN-β in both pandemic influenza A(H1N1) and COVID-19 patients. A similar type I interferon deficiency was observed in the blood of French critically ill COVID-19 patients (58). Conversely, another study from Korea reveals that type I interferon expression is increased in BAL immune cells from severe COVID-19 patients (16), indicating that antiviral interferon responses against SARS-CoV-2 might be highly compartmentalized into the lungs and barely detectable in the blood.

Notably, despite the dysregulated production of other immune mediators, an ample range of immune cell subtypes are depleted from the circulation of patients with severe SARS-CoV-2 infection. These cells include monocytes, dendritic cells, CD4+ T cells, CD8+ T cells, B cells, and NK cells (59). Furthermore, the few adaptive lymphocytes that remain in the blood express markers of functional exhaustion (29). These data suggest that severe COVID-19 is a state of immunosuppression

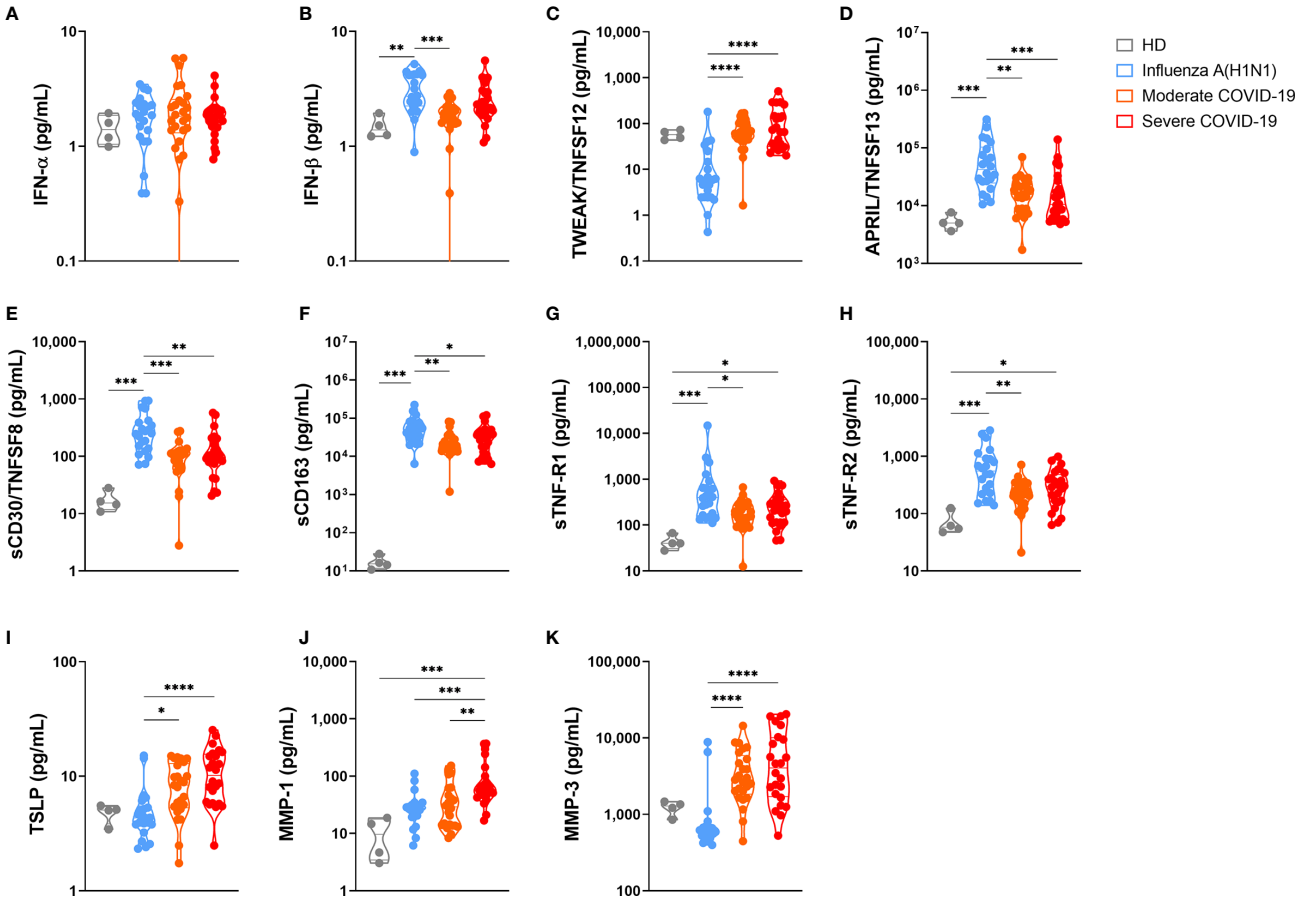


FIGURE 8 | Immune mediators in the plasma of patients with pandemic influenza A(H1N1) and COVID-19. Levels of different soluble immune mediators in plasma samples from patients with COVID-19 (n=25 moderate, 24 severe) and pandemic influenza A(H1N1) (n=23), as well as in samples from healthy volunteer donors (HD, n=4) were assessed by Luminex assay. Violin plots display medians and interquartile ranges (IQR). Differences between groups we estimated using the Kruskal-Wallis test with *post hoc* Dunn´s test. Significant differences are denoted by bars and asterisks: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001. **(A)** IFN-α, interferon-alpha; **(B)** IFN-β, interferon-beta; **(C)** TWEAK/TNFSF12, tumor necrosis factor-like weak inducer of apoptosis/tumor necrosis factor ligand superfamily member 12; **(D)** APRIL/TNFSF13, A proliferation-inducing ligand/tumor necrosis factor ligand superfamily member 13; **(E)** sCD30/TNFRSF8, soluble CD30/tumor necrosis factor ligand superfamily member 8; **(F)** sCD163, soluble CD163; **(G)** sTNF-R1, soluble tumor necrosis factor receptor 1; **(H)** sTNF-R2, soluble tumor necrosis factor receptor 2; **(I)** TSLP, thymic stromal lymphopoietin; **(J)** MMP-1, metalloprotease 1; **(K)** MMP-3, metalloprotease 3.

similar to the known sepsis-induced immunosuppression (60). Notably, a recent study by Remy and collaborators has shown that the immunosuppression observed in COVID-19 is even more profound than in critically ill patients with sepsis of other causes (27). These researchers demonstrated that the production of IFN-γ by peripheral blood T cells of COVID-19 patients was impaired as compared with T cells from healthy individuals and septic patients after anti-CD3/anti-CD28 antibody stimulation. Furthermore, a reduced production of TNF-α by stimulated monocytes from COVID-19 patients was noticed. These findings led the researchers to propose that the primary immune mechanism underlying the morbidity and mortality of COVID-19 is immunosuppression rather than hyperinflammation.

In this context, our study confirms that the immune response against SARS-CoV-2 is entirely different from the response against pandemic influenza A(H1N1). Indeed, our analyses bring forward a set of immunological markers with the potential to differentiate COVID-19 from pandemic influenza A(H1N1) successfully. Measuring some of these markers might improve the diagnostic approach and subsequent therapeutic decision for ARDS patients. Also, our study may provide additional evidence useful to clarify current controversies about the immunopathology of COVID-19. Based on our results and previous investigations, we propose that hyperinflammation and immunosuppression are not mutually exclusive in COVID-19. First, our data showed some indirect readouts of immunosuppression in individuals infected with SARS-CoV-2. For instance, we found that TNF-α levels were lower in the serum of COVID-19 patients as compared to pandemic influenza A(H1N1) patients. This coincides with the limited capacity of monocytes from COVID-19 patients to produce TNF-α upon stimulation described by Remy et al. (27). We also observed lower plasma levels of the macrophage activation marker sCD163, although macrophages infiltrating

the lungs of COVID-19 patients expressed several cytokines. Furthermore, we found low levels of IL-2 and APRIL/TNFSF13 (two immune mediators crucial for T-cell and plasma cell survival), as well as sCD30 (a marker of lymphocyte activation) in the circulation of COVID-19 but not pandemic influenza A(H1N1) patients. Similarly, we observed a lack of lymphocytes in the inflammatory infiltrates found in lung autopsy specimens from patients that died of COVID-19. These findings may reflect a depletion of activated lymphocytes and monocytes from the circulation during SARS-CoV-2 infection and poor recruitment of lymphocytes to the lungs.

At the same time, we have described that an exacerbated polyfunctional immune response prevails in the circulation of COVID-19 patients. Such a response is characterized by higher levels of Th1 as well as Th2 cytokines as compared to pandemic influenza A(H1N1) patients. Conversely, although pandemic influenza A(H1N1) subjects also display elevated levels of some inflammatory mediators, these individuals may have enough regulatory mechanisms that counteract the detrimental effects of hyperinflammation. The higher levels of IL-1RA observed here in pandemic influenza A(H1N1) patients as compared to COVID-19 subjects well exemplify this. Furthermore, we found higher serum levels of the C-X-C motif chemokine ligand 17 (CXCL17), a mucosal chemokine with anti-inflammatory properties, in pandemic influenza A(H1N1) but not COVID-19 patients (61). In addition, the serum cytokine pattern of COVID-19 resembles the inflammatory profile of rheumatoid arthritis patients with interstitial lung disease (62), and the polyfunctional inflammatory response of the cytokine release syndrome (CRS) that occurs after chimeric antigen receptor (CAR) T-cell therapy (63). Immunosuppression and hyperinflammation are also a hallmark of both of these conditions.

Of note, the higher levels of Th2 cytokines, particularly IL-4 and IL-5, might inhibit Th1 protective antiviral responses in COVID-19 patients. Thus, our data indicate that a lack of immune balance of the type of effector response is another crucial determinant of the collapse of the host protective immunity against SARS-CoV-2. This Th2 biased response may generate interstitial infiltrates of Th2 cells, neutrophils, eosinophils, and type 2 innate lymphoid cells, mediating lung inflammation, and tissue damage. In fact, critically ill COVID-19 patients usually show interstitial lung infiltrates, some of which resemble several forms of progressive interstitial lung disease like cryptogenic organizing pneumonia and non-specific interstitial pneumonia (9, 64–66). Here, we also observed interstitial inflammation and expression of IL-4 in the lungs of COVID-19 patients but not pandemic influenza A(H1N1) subjects. These deleterious effects of Th2 responses could also explain the abnormalities in lung function, and progression to pulmonary fibrosis observed in more than 45% of COVID-19 patients discharged from hospitals (67), particularly in older patients. Hence, it would be of great interest to characterize the cytokine profile of COVID-19 patients that subsequently develop any form of interstitial lung disease, as they would benefit from specific and anti-fibrotic therapeutics.

We propose that ideal immune therapeutics for COVID-19 should be directed not only to blocking or enhancing specific immune signaling pathways to counteract hyperinflammation or reverting immunosuppression. Instead, immune therapies must re-establish a convenient immune balance that promotes protective immunity against SARS-CoV-2. Under the light of this hypothesis, several immune mediators and immune cell subsets could be targeted. For instance, type 2 innate lymphoid cells (ILC2s) have been identified as the leading producers of Th2 cytokines in the lungs, contributing to potent allergen-induced airway inflammation even in lymphopenic hosts (68). Thus, ILC2s may constitute novel targets to inhibit Th2 responses in COVID-19 patients. The potential pathogenic effects of Th2-biased responses in COVID-19 may also be counteracted with monoclonal antibodies. For instance, dupilumab, a monoclonal antibody against IL-4, has been safely used in patients with atopic dermatitis and COVID-19, without increased risk of severe complications of the infection. Remarkably, some patients receiving dupilumab that later acquired the infection with SARS-CoV-2 did not show respiratory symptoms (69–71). Finally, TSLP could be another target to inhibit Th2 responses in COVID-19 patients, as this molecule promotes allergic inflammation (40), and indeed, high levels of TSLP were observed in our cohort of COVID-19 but not pandemic influenza A(H1N1) subjects.

LIMITATIONS

A limitation of our study is that we did not recruit patients infected with seasonal influenza virus subtypes. Thus, our observations are only useful to distinguish between influenza A (H1N1) pdm09 and SARS-CoV-2 infection. The clinical and immunological characteristics of SARS-CoV-2 and seasonal influenza have been compared in a recent study by Mudd et al. (72). In such a study, researchers found that COVID-19, as compared to seasonal influenza, is characterized by lower mean cytokine levels in serum. Conversely, we found that cytokine levels were higher in COVID-19 patients than in individuals with pandemic influenza A(H1N1). These discrepancies are probably related to variations in the virulence and capacity to induce inflammatory immune responses of seasonal and pandemic influenza viruses. Lee et al. (16), also compared single cell RNA sequencing of BAL immune cells from COVID-19 and influenza A patients. Although these researchers did not specify the subtype of influenza A virus infection, their results coincide with our data with regards to the higher induction of IL-1β in COVID-19 than influenza. However, differential roles of TNF and type I interferon signaling during the two diseases are important discrepancies between their and our study. The source and time of sample collection can potentially be a reason for these differences. Finally, another limitation of our study is that we did not measure cytokine levels in serial serum/plasma samples from our two cohorts of pandemic influenza A (H1N1) and COVID-19 patients. Thus, future investigations should compare differences in the kinetics of immune responses against both diseases. Despite this, our study provides important insights into the differences between the

two most important respiratory pathogens that have caused pandemics of international concern in recent years.

CONCLUSIONS

In conclusion, our results demonstrate significant differences in the immune responses elicited after SARS-CoV-2 and influenza A(H1N1) pdm09 virus. Our data support the use of specific clinical characteristics, laboratory parameters, and immunological markers to differentiate SARS-CoV-2 infection from pandemic influenza A(H1N1). These data may also contribute to the discovery of novel therapeutic targets to counteract harmful immune mechanisms underlying the immunopathology of COVID-19 and pandemic influenza A(H1N1).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Boards of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ, approval number: 3349) and the Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas (INER, approval number: B28-16 and B09-20). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Design of the research study: JC-P, TR-R, SK, AZ, and JZ. Recruited patients: JC-P, TR-R, MS-V, DH-G, EM-G, ES, JM-R, JB-R, HV-R, G-CS, NA-P, DG-C, GH, JG, LM-H, LP-B, GD-C, and CH-C. Retrieved clinical data: JC-P, TR-R, MS-V, DH-G, N-AP, GH, LM-H, CH-C, AH-M, and LO. Collected and processed blood samples: JC-P, LJ-A, AC-L, TR-R, GR-M, EM-G, NA-P, GH, CM-M, AD, and LM-H. Obtained and processed lung autopsy specimens: CS-L, CS-G, and CL. Conducted cytokine determinations: L-JA, AC-L, GR-M, and EM-G. Conducted histological and immunohistochemistry analyses: JC-P, CS-L, and CS-G. Performed statistical analyses of the data: JC-P, EC-P, YB-M, and MM-S. Provided reagents: LJ-A, TR-R, CS-L, JG, LM-H, LP-B, GD-C, CC-G, JS-H, PS-D, JR, FA-M, EG-L, CH-C, SK, AZ, and JZ. Discussed the manuscript: JC-P, TR-R, FA-M, SK, AZ, and JZ. Drafted the manuscript: JC-P, SK, AZ, and JZ. All authors contributed to the article and approved the submitted version.

FUNDING

JC-P was supported by the National Council of Science and Technology of Mexico (CONACyT) to achieve his PhD degree (CONACyT-CVU 737347). The current study received funding from institutional research grants of INER, from the UNAM-INER interinstitutional collaboration agreement (UNAM: 43355-3065-17-XI-15, to FA-M), from the Dirección General de Personal Académico de la Universidad Nacional Autónoma de México (UNAM, grant #IV201020 to ES), and from CONACyT under the research contracts: CONACYT-Support for scientific research, technological development and innovation in health during COVID-19 contingency (CONACyT-COVID-19), with the project numbers 313517 and 00311999 to TR-R; SECTEI/050/2020, Secretaría de Ciencia, Tecnología e Innovación de la Ciudad de México (SECTEI CDMX); FORDECYT/10SE/2020/05/14-06 and FORDECYT/10SE/2020/05/14-07 from the Fondo Institucional de Fomento Regional para el Desarrollo Científico y Tecnológico y de Innovación (FORDECYT) to JZ.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.593595/full#supplementary-material

REFERENCES

1. World Health Organization. *CCOVID-19 Weekly Epidemiological Update - 2*. (2021), World Health Organization. Available at: https://www.who.int/publications/m/item/weekly-epidemiological-update—2-march-2021.
2. Shrestha SS, Swerdlow DL, Borse RH, Prabhu VS, Finelli L, Atkins CY, et al. Estimating the burden of 2009 pandemic influenza A (H1N1) in the United States (April 2009-April 2010). *Clin Infect Dis* (2011) 52 Suppl 1(suppl_1): S75–82. doi: 10.1093/cid/ciq012
3. Centers for Disease C. Prevention. Swine influenza A (H1N1) infection in two children–Southern California, March-April 2009. *MMWR Morbidity Mortality Weekly Rep* (2009) 58(15):400–2.
4. Novel Swine-Origin Influenza AVIT, Dawood FS, Jain S, Finelli L, Shaw MW, Lindstrom S, et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. *N Engl J Med* (2009) 360(25):2605–15. doi: 10.1056/NEJMoa0903810
5. Castillejos M, Cabello-Gutierrez C, Alberto Choreno-Parra J, Hernandez V, Romo J, Hernandez-Sanchez F, et al. High performance of rapid influenza diagnostic test and variable effectiveness of influenza vaccines in Mexico. *Int J Infect Dis* (2019) 89:87–95. doi: 10.1016/j.ijid.2019.08.029
6. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* (2020) 382 (8):727–33. doi: 10.1056/NEJMoa2001017
7. Wu F, Zhao S, Yu B, Chen Y-M, Wang W, Song Z-G, et al. A new coronavirus associated with human respiratory disease in China. *Nature* (2020) 579 (7798):265–9. doi: 10.1038/s41586-020-2008-3
8. Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable

bat origin. *Nature* (2020) 579(7798):270–3. doi: 10.1038/s41586-020-2012-7
9. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* (2020) 395 (10223):497–506. doi: 10.1016/S0140-6736(20)30183-5
10. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* (2020) 395(10223):507–13. doi: 10.1016/S0140-6736(20)30211-7
11. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med* (2020) 382(18):1708–20. doi: 10.1056/NEJMoa2002032
12. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *Jama* (2020). doi: 10.1001/jama.2020.1585
13. Tang X, Du R-H, Wang R, Cao T-Z, Guan L-L, Yang C-Q, et al. Comparison of Hospitalized Patients With ARDS Caused by COVID-19 and H1N1. *Chest* (2020). S0012-3692(20)30558-4. doi: 10.1016/j.chest.2020.03.032
14. Jiang C, Yao X, Zhao Y, Wu J, Huang P, Pan C, et al. Comparative review of respiratory diseases caused by coronaviruses and influenza A viruses during epidemic season. *Microbes Infection* (2020) 22(6-7):236–44. doi: 10.1016/j.micinf.2020.05.005
15. Petersen E, Koopmans M, Go U, Hamer DH, Petrosillo N, Castelli F, et al. Comparing SARS-CoV-2 with SARS-CoV and influenza pandemics. *Lancet Infect Dis* (2020) 20(9):e238–e44. doi: 10.1016/S1473-3099(20)30484-9
16. Lee JS, Park S, Jeong HW, Ahn JY, Choi SJ, Lee H, et al. Immunophenotyping of COVID-19 and influenza highlights the role of type I interferons in development of severe COVID-19. *Sci Immunol* (2020) 5(49):eabd1554. doi: 10.1126/sciimmunol.abd1554
17. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest.* (2020) 130(5):2620–9. doi: 10.1172/JCI137244
18. Zhu Z, Cai T, Fan L, Lou K, Hua X, Huang Z, et al. Clinical value of immune-inflammatory parameters to assess the severity of coronavirus disease 2019. *Int J Infect Dis* (2020) 95:332–9. doi: 10.1016/j.ijid.2020.04.041
19. McGonagle D, Sharif K, O'Regan A, Bridgewood C. The Role of Cytokines including Interleukin-6 in COVID-19 induced Pneumonia and Macrophage Activation Syndrome-Like Disease. *Autoimmun Rev* (2020) 19(6):102537. doi: 10.1016/j.autrev.2020.102537
20. Ahmadpoor P, Rostaing L. Why the immune system fails to mount an adaptive immune response to a COVID-19 infection. *Transpl Int* (2020) 33 (7):824–5. doi: 10.1111/tri.13611
21. Moore JB, June CH. Cytokine release syndrome in severe COVID-19. *Science* (2020) 368(6490):473–4. doi: 10.1126/science.abb8925
22. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill* (2020) 25(3):2000045. doi: 10.2807/1560-7917.ES.2020.25.3.2000045
23. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, et al. Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. *JAMA* (2020) 323(20):2052–9. doi: 10.1001/jama.2020.6775
24. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, et al. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. *JAMA Intern Med* (2020) 180(7):934–43. doi: 10.1001/jamainternmed.2020.0994
25. Coperchini F, Chiovato L, Croce L, Magri F, Rotondi M. The cytokine storm in COVID-19: An overview of the involvement of the chemokine/chemokine-receptor system. *Cytokine Growth Factor Rev* (2020) 53:25–32. doi: 10.1016/j.cytogfr.2020.05.003
26. Liu Q, Zhou YH, Yang ZQ. The cytokine storm of severe influenza and development of immunomodulatory therapy. *Cell Mol Immunol* (2016) 13 (1):3–10. doi: 10.1038/cmi.2015.74
27. Remy KE, Mazer M, Striker DA, Ellebedy AH, Walton AH, Unsinger J, et al. Severe immunosuppression and not a cytokine storm characterizes COVID-19 infections. *JCI Insight* (2020) 5(17):e140329. doi: 10.1172/jci.insight.140329
28. Yang Y, Shen C, Li J, Yuan J, Wei J, Huang F, et al. Plasma IP-10 and MCP-3 levels are highly associated with disease severity and predict the progression of COVID-19. *J Allergy Clin Immunol* (2020) 146(1):119–27.e4. doi: 10.1016/j.jaci.2020.04.027
29. Diao B, Wang C, Tan Y, Chen X, Liu Y, Ning L, et al. Reduction and Functional Exhaustion of T Cells in Patients With Coronavirus Disease 2019 (COVID-19). *Front Immunol* (2020) 11(827):827. doi: 10.3389/fimmu.2020.00827
30. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Med* (2020) 46(5):846–8. doi: 10.1007/s00134-020-05991-x
31. Ni YN, Chen G, Sun J, Liang BM, Liang ZA. The effect of corticosteroids on mortality of patients with influenza pneumonia: a systematic review and meta-analysis. *Crit Care* (2019) 23(1):99. doi: 10.1186/s13054-019-2395-8
32. Bautista E, Arcos M, Jimenez-Alvarez L, Garcia-Sancho MC, Vazquez ME, Pena E, et al. Angiogenic and inflammatory markers in acute respiratory distress syndrome and renal injury associated to A/H1N1 virus infection. *Exp Mol Pathol* (2013) 94(3):486–92. doi: 10.1016/j.yexmp.2013.03.007
33. Group RC, Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, et al. Dexamethasone in Hospitalized Patients with Covid-19. *N Engl J Med* (2021) 384(8):693–704. doi: 10.1056/NEJMoa2021436
34. Benson MJ, Dillon SR, Castigli E, Geha RS, Xu S, Lam KP, et al. Cutting edge: the dependence of plasma cells and independence of memory B cells on BAFF and APRIL. *J Immunol* (2008) 180(6):3655–9. doi: 10.4049/jimmunol.180.6.3655
35. Schlaf G, Altermann WW, Rothhoff A, Seliger B. Soluble CD30 serum level–an adequate marker for allograft rejection of solid organs? *Histol Histopathol* (2007) 22(11):1269–79. doi: 10.1093/ndt/gfm397
36. Moller HJ, de Fost M, Aerts H, Hollak C, Moestrup SK. Plasma level of the macrophage-derived soluble CD163 is increased and positively correlates with severity in Gaucher's disease. *Eur J Haematol* (2004) 72(2):135–9. doi: 10.1046/j.0902-4441.2003.00193.x
37. Pennica D, Lam VT, Weber RF, Kohr WJ, Basa LJ, Spellman MW, et al. Biochemical characterization of the extracellular domain of the 75-kilodalton tumor necrosis factor receptor. *Biochemistry* (1993) 32(12):3131–8. doi: 10.1021/bi00063a027
38. Chicheportiche Y, Chicheportiche R, Sizing I, Thompson J, Benjamin CB, Ambrose C, et al. Proinflammatory activity of TWEAK on human dermal fibroblasts and synoviocytes: blocking and enhancing effects of anti-TWEAK monoclonal antibodies. *Arthritis Res* (2002) 4(2):126–33. doi: 10.1186/ar388
39. Saas P, Boucraut J, Walker PR, Quiquerez AL, Billot M, Desplat-Jego S, et al. TWEAK stimulation of astrocytes and the proinflammatory consequences. *Glia* (2000) 32(1):102–7. doi: 10.1002/1098-1136(200010)32:1<102::AID-GLIA100>3.0.CO;2-U
40. Ito T, Liu YJ, Arima K. Cellular and molecular mechanisms of TSLP function in human allergic disorders–TSLP programs the "Th2 code" in dendritic cells. *Allergol Int* (2012) 61(1):35–43. doi: 10.2332/allergolint.11-RAI-0376
41. Dahlen B, Shute J, Howarth P. Immunohistochemical localisation of the matrix metalloproteinases MMP-3 and MMP-9 within the airways in asthma. *Thorax* (1999) 54(7):590–6. doi: 10.1136/thx.54.7.590
42. Greenlee KJ, Werb Z, Kheradmand F. Matrix metalloproteinases in lung: multiple, multifarious, and multifaceted. *Physiol Rev* (2007) 87(1):69–98. doi: 10.1152/physrev.00022.2006
43. D'Armiento J, Dalal SS, Okada Y, Berg RA, Chada K. Collagenase expression in the lungs of transgenic mice causes pulmonary emphysema. *Cell* (1992) 71 (6):955–61. doi: 10.1016/0092-8674(92)90391-O
44. Salto-Quintana JN, Rivera-Alfaro G, Sanchez-Ramos EL, Gomez-Gomez A, Noyola DE. Post-pandemic influenza-associated mortality in Mexico. *Pathog Glob Health* (2019) 113(2):67–74. doi: 10.1080/20477724.2019.1589211
45. *Dirección General de Epidemiología. Covid-19 México 2021*. Available at: https://coronavirus.gob.mx/datos/.
46. Mansfield KG. Viral tropism and the pathogenesis of influenza in the Mammalian host. *Am J Pathol* (2007) 171(4):1089–92. doi: 10.2353/ajpath.2007.070695
47. Behzad S, Aghaghazvini L, Radmard AR, Gholamrezanezhad A. Extrapulmonary manifestations of COVID-19: Radiologic and clinical overview. *Clin Imaging* (2020) 66:35–41. doi: 10.1016/j.clinimag.2020.05.013
48. Muus C, Luecken MD, Eraslan G, Waghray A, Heimberg G, Sikkema L, et al. Integrated analyses of single-cell atlases reveal age, gender, and smoking status

associations with cell type-specific expression of mediators of SARS-CoV-2 viral entry and highlights inflammatory programs in putative target cells. (2020) 2020.04.19.049254.
49. Van Mieghem IM, De Wever WF, Verschakelen JA. Lung infection in radiology: a summary of frequently depicted signs. *JBR-BTR* (2005) 88 (2):66–71.
50. Conti P, Ronconi G, Caraffa A, Gallenga CE, Ross R, Frydas I, et al. Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by Coronavirus-19 (COVI-19 or SARS-CoV-2): anti-inflammatory strategies. *J Biol Regul Homeost Agents.* (2020) 34(2):327–31. doi: 10.23812/CONTI-E
51. Wan S, Yi Q, Fan S, Lv J, Zhang X, Guo L, et al. Characteristics of lymphocyte subsets and cytokines in peripheral blood of 123 hospitalized patients with 2019 novel coronavirus pneumonia (NCP). (2020). 2020.02.10.20021832.
52. Sheahan T, Morrison TE, Funkhouser W, Uematsu S, Akira S, Baric RS, et al. MyD88 is required for protection from lethal infection with a mouse-adapted SARS-CoV. *PloS Pathog* (2008) 4(12):e1000240. doi: 10.1371/journal.ppat.1000240
53. Totura AL, Whitmore A, Agnihothram S, Schafer A, Katze MG, Heise MT, et al. Toll-Like Receptor 3 Signaling via TRIF Contributes to a Protective Innate Immune Response to Severe Acute Respiratory Syndrome Coronavirus Infection. *mBio* (2015) 6(3):e00638–15. doi: 10.1128/mBio.00638-15
54. Shi CS, Nabar NR, Huang NN, Kehrl JH. SARS-Coronavirus Open Reading Frame-8b triggers intracellular stress pathways and activates NLRP3 inflammasomes. *Cell Death Discovery* (2019) 5:101. doi: 10.1038/s41420-019-0181-7
55. Ong EZ, Chan YFZ, Leong WY, Lee NMY, Kalimuddin S, Haja Mohideen SM, et al. A Dynamic Immune Response Shapes COVID-19 Progression. *Cell Host Microbe* (2020) 27(6):879–82 e2. doi: 10.1016/j.chom.2020.03.021
56. Liao M LY, Yuan J, Wen Y, Xu G, Zhao J, Cheng L, et al. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. *Nat Med* (2020) 26(6):842–4. doi: 10.1038/s41591-020-0901-9
57. Ramírez-Martínez G, Cruz-Lagunas A, Jiménez-Alvarez L, Espinosa E, Ortíz-Quintero B, Santos-Mendoza T, et al. Seasonal and pandemic influenza H1N1 viruses induce differential expression of SOCS-1 and RIG-I genes and cytokine/chemokine production in macrophages. *Cytokine* (2013) 62 (1):151–9. doi: 10.1016/j.cyto.2013.01.018
58. Hadjadj J, Yatim N, Barnabei L, Corneau A, Boussier J, Smith N, et al. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. *Science* (2020) 369(6504):718–24. doi: 10.1126/science.abc6027
59. Wang W, Su B, Pang L, Qiao L, Feng Y, Ouyang Y, et al. High-dimensional immune profiling by mass cytometry revealed immunosuppression and dysfunction of immunity in COVID-19 patients. *Cell Mol Immunol* (2020) 17(6):650–2. doi: 10.1038/s41423-020-0447-2
60. Ono S, Tsujimoto H, Hiraki S, Aosasa S. Mechanisms of sepsis-induced immunosuppression and immunological modification therapies for sepsis. *Ann Gastroenterol Surg* (2018) 2(5):351–8. doi: 10.1002/ags3.12194
61. Choreño-Parra JA, Jiménez-Álvarez LA, Ramírez-Martínez G, Sandoval-Vega M, Salinas-Lara C, Sánchez-Garibay C, et al. CXCL17 Is a Specific Diagnostic Biomarker for Severe Pandemic Influenza A(H1N1) That Predicts Poor Clinical Outcome. *Front Immunol* (2021) 12:633297. doi: 10.3389/fimmu.2021.633297
62. Shen H, Xia L, Lu J. Interleukin-4 in rheumatoid arthritis patients with interstitial lung disease: a pilot study. *Indian J Med Res* (2013) 138(6):919–21.
63. Teachey DT, Lacey SF, Shaw PA, Melenhorst JJ, Maude SL, Frey N, et al. Identification of Predictive Biomarkers for Cytokine Release Syndrome after Chimeric Antigen Receptor T-cell Therapy for Acute Lymphoblastic Leukemia. *Cancer Discovery* (2016) 6(6):664–79. doi: 10.1158/2159-8290.CD-16-0040
64. Polak SB, Van Gool IC, Cohen D, von der Thusen JH, van Paassen J. A systematic review of pathological findings in COVID-19: a pathophysiological timeline and possible mechanisms of disease progression. *Mod Pathol* (2020) 33(11):2128–38. doi: 10.1038/s41379-020-0603-3
65. Carsana L, Sonzogni A, Nasr A, Rossi RS, Pellegrinelli A, Zerbi P, et al. Pulmonary post-mortem findings in a series of COVID-19 cases from northern Italy: a two-centre descriptive study. *Lancet Infect Diseases* (2020) 20(10):1135–40. doi: 10.1016/S1473-3099(20)30434-5
66. Cui N, Zou X, Xu L. Preliminary CT findings of coronavirus disease 2019 (COVID-19). *Clin Imaging* (2020) 65:124–32. doi: 10.1016/j.clinimag.2020.04.042
67. Mo X, Jian W, Su Z, Chen M, Peng H, Peng P, et al. Abnormal pulmonary function in COVID-19 patients at time of hospital discharge. *Eur Respir J* (2020) 55(6):2001217. doi: 10.1183/13993003.01217-2020
68. Halim TY, Krauss RH, Sun AC, Takei F. Lung natural helper cells are a critical source of Th2 cell-type cytokines in protease allergen-induced airway inflammation. *Immunity* (2012) 36(3):451–63. doi: 10.1016/j.immuni.2011.12.020
69. Ferrucci S, Romagnuolo M, Angileri L, Berti E, Tavecchio S. Safety of dupilumab in severe atopic dermatitis and infection of Covid-19: two case reports. *J Eur Acad Dermatol Venereol* (2020) 34(7):e303–e4. doi: 10.1111/jdv.16527
70. Caroppo F, Biolo G, Belloni Fortina A. SARS-CoV-2 asymptomatic infection in a patient under treatment with dupilumab. *J Eur Acad Dermatol Venereol* (2020) 34(8):e368. doi: 10.1111/jdv.16619
71. Carugno A, Raponi F, Locatelli AG, Vezzoli P, Gambini DM, Di Mercurio M, et al. No evidence of increased risk for Coronavirus Disease 2019 (COVID-19) in patients treated with Dupilumab for atopic dermatitis in a high-epidemic area - Bergamo, Lombardy, Italy. *J Eur Acad Dermatol Venereol* (2020) 34(9): e433–e4. doi: 10.1111/jdv.16552
72. Mudd PA, Crawford JC, Turner JS, Souquette A, Reynolds D, Bender D, et al. Distinct inflammatory profiles distinguish COVID-19 from influenza with limited contributions from cytokine storm. *Sci Adv* (2020) 6(50):eabe3024. doi: 10.1126/sciadv.abe3024

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer MC declared a shared affiliation, with no collaboration with the author SK, to the handling editor, at the time of review.

Citation: *Choreño-Parra JA, Jiménez-Álvarez LA, Cruz-Lagunas A, Rodríguez-Reyna TS, Ramírez-Martínez G, Sandoval-Vega M, Hernández-García DL, Choreño-Parra EM, Balderas-Martínez YI, Martinez-Sánchez ME, Márquez-García E, Sciutto E, Moreno-Rodríguez J, Barreto-Rodríguez JO, Vázquez-Rojas H, Centeno-Sáenz GI, Alvarado-Peña N, Salinas-Lara C, Sánchez-Garibay C, Galeana-Cadena D, Hernández G, Mendoza-Milla C, Domínguez A, Granados J, Mena-Hernández L, Pérez-Buenfil LÁ, Domínguez-Cheritt G, Cabello-Gutiérrez C, Luna-Rivero C, Salas-Hernández J, Santillán-Doherty P, Regalado J, Hernández-Martínez A, Orozco L, Ávila-Moreno F, García-Latorre EA, Hernández-Cárdenas CM, Khader SA, Zlotnik A and Zúñiga J (2021) Clinical and Immunological Factors That Distinguish COVID-19 From Pandemic Influenza A(H1N1).*
Front. Immunol. 12:593595. doi: 10.3389/fimmu.2021.593595

frontiers | Frontiers in Microbiology

TYPE Review
PUBLISHED 31 October 2023
DOI 10.3389/fmicb.2023.1237998

OPEN ACCESS

EDITED BY
Abolfazl Fateh,
Pasteur Institute of Iran (PII), Iran

REVIEWED BY
Sunil Banskar,
University of Arizona, United States
Harindra Sathkumara,
James Cook University, Australia

*CORRESPONDENCE
Eugenia Silva-Herzog
✉ esilvaherzog@inmegen.gob.mx

RECEIVED 10 June 2023
ACCEPTED 09 October 2023
PUBLISHED 31 October 2023

CITATION
Alvarado-Peña N, Galeana-Cadena D, Gómez-García IA, Mainero XS and Silva-Herzog E (2023) The microbiome and the gut-lung axis in tuberculosis: interplay in the course of disease and treatment. *Front. Microbiol.* 14:1237998. doi: 10.3389/fmicb.2023.1237998

The microbiome and the gut-lung axis in tuberculosis: interplay in the course of disease and treatment

Néstor Alvarado-Peña [1], David Galeana-Cadena [2], Itzel Alejandra Gómez-García [2,3], Xavier Soberón Mainero [4] and Eugenia Silva-Herzog [5]*

[1]Clínica de Tuberculosis, Instituto Nacional de Enfermedades Respiratorias "Ismael Cosío Villegas", México City, Mexico, [2]Laboratorio de Inmunobiología y Genética, Instituto Nacional de Enfermedades Respiratorias, México City, Mexico, [3]Tecnológico de Monterrey, Escuela de Medicina y Ciencias de la Salud, México City, Mexico, [4]Departamento de Ingeniería Celular y Biocatálisis, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Mexico, [5]Laboratorio de Vinculación Científica, Facultad de Medicina-Universidad Nacional Autonoma de México-Instituto Nacional de Medicina Genomica, México City, Mexico

Tuberculosis is a chronic infectious disease caused by *Mycobacterium tuberculosis* (MTB) that remains a significant global health challenge. The extensive use of antibiotics in tuberculosis treatment, disrupts the delicate balance of the microbiota in various organs, including the gastrointestinal and respiratory systems. This gut-lung axis involves dynamic interactions among immune cells, microbiota, and signaling molecules from both organs. The alterations of the microbiome resulting from anti-TB treatment can significantly influence the course of tuberculosis, impacting aspects such as complete healing, reinfection, and relapse. This review aims to provide a comprehensive understanding of the gut-lung axis in the context of tuberculosis, with a specific focus on the impact of anti-TB treatment on the microbiome.

KEYWORDS

tuberculosis, *Mycobacterium tuberculosis* (MTB), gut-lung axis, microbiome, microbiota, anti-tuberculosis treatment

Introduction

The human body contains a broad diversity of microorganisms, collectively known as the microbiota, which form a dynamic and functional system that evolves alongside its host. Although the gut harbors the largest population of microorganisms, they are also present throughout the body, including the entire digestive tract, skin, mucous membranes, urogenital and respiratory tract. This wide distribution underscores the significance of the microbiota in shaping and impacting various aspects of human health and physiology (Turnbaugh et al., 2007).

The millions of microbial cells in the human body play important roles in physicochemical and physiological functions, including intestinal development, barrier integrity and function, metabolism, immunity, inflammation, and neurological signaling regulation (Marsland et al., 2015; Enaud et al., 2020). The gut microbiome is highly dynamic and can be modified or disturbed by many factors, such as genetics, age, circadian

rhythm, dietary habits, use of antibiotics, and other environmental factors (Nicholson et al., 2012; Dickson et al., 2016b). Furthermore, these factors play a role in the susceptibility, pathogenesis, and development of both non-transmissible and infectious diseases (Dang and Marsland, 2019; Naidoo et al., 2019; Wypych et al., 2019). In particular, malnutrition, diabetes, obesity, alcoholism, smoking, and HIV are some of the conditions that result in gut microbiome dysbiosis and altered immune function, that are associated with increased susceptibility to disease (Zevin et al., 2016; Weiss and Hennet, 2017; Iddrisu et al., 2021; Bach et al., 2023).

The increased intestinal permeability derived from this altered immune response and chronic inflammation allows metabolites and microorganisms to leak into the bloodstream, where they can affect other anatomical parts of the body, including the respiratory system (Usuda et al., 2021). Likewise, clinical studies on chronic lung diseases suggest that pulmonary disorders may be implicated in intestinal diseases (Rutten et al., 2014; Gui et al., 2021). Interestingly, the respiratory and gastrointestinal epithelia have structural similarities (Budden et al., 2017) and, in fact, several pulmonary and intestinal diseases exhibit many overlapping components, including common risk factors like mucus reduction, increased permeability, and low expression of tight-junction proteins, that can exacerbate the progression of infections (Duarte et al., 2018).

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis* (MTB) that persists as one of the top 13 causes of death worldwide (World Health Organization [WHO], 2022a). TB mainly affects pulmonary parenchyma presenting sustained weight loss, night sweats, fever, chronic cough, wasting, and hemoptysis. Diagnosis relies on identifying the microorganism through an automated PCR test (Xpert MTB/RIF and Xpert Ultra) (World Health Organization [WHO], 2021b). However, the heterogeneity of the TB clinical spectrum delays diagnosis and, therefore, anti-TB treatment (Cadena et al., 2017). Furthermore, anti-TB treatment represents one of the longest-duration antibiotic regimens used globally. This treatment includes combinations of at least four specific and broad-range antibiotics in schedules that range from four to more than 20 months, depending on the strain of MTB infection (World Health Organization [WHO], 2022b,c). Regardless of the regime, anti-TB treatment is associated with alterations of the gut microbiota in patients and animal models; the effect of these alterations in the lung microbiome and the underlying immune system response is the focus of many studies (Langdon et al., 2016; Namasivayam et al., 2017; Naidoo et al., 2019; Wang et al., 2020). This review aims to present a picture of recent studies on anti-TB treatment alterations of the microbiota in the course of the disease and its effect on the gut-lung axis.

Gut-lung axis

The microbiome is a dynamic community of microorganisms that is in constant interaction with the host and its environment. Under physiological conditions, the microbiome is resilient to changes, benefiting both host and microbial communities, and it is considered to be in eubiosis (Giulio, 2021). On the other hand, the reduction of the adaptive capacity of a microbiome to changes that cause unfavorable alterations for the host is referred to as dysbiosis (Barbosa-Amezcua et al., 2022). All the different microbiomes in the human body: gut, lung, mouth, skin, genitals, liver and other barrier sites, are unique communities with specific interactions with the immune system and other organs in the body (Belkaid and Naik, 2013).

In particular, the host-associated gut microbiota is involved in several critical physiological functions such as absorption of nutrients, fermentation of food, vitamin production, and importantly, stimulating and training the immune system (Shreiner et al., 2015; Hillman et al., 2017; Al Nabhani et al., 2019). The gut microbiota includes bacteria, archaea, fungi, protozoa and viruses. Its composition is dominated by six bacterial phyla: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia, and two fungi phyla: Ascomycota and Basidiomycota (Nash et al., 2017). Although the composition changes with geographic location, diet, and age, it reaches a stable composition in absence of antibiotic treatment (Ferrer et al., 2017).

The interaction among all the organ systems is essential for the proper functioning of the body. Traditionally, this communication has been studied in the context of the autonomic nervous system, immune responses, and the endocrine system. However, recent research highlights a novel dimension of bidirectional communication between the gut microbiome and other organs such as the brain, skin, and lungs. These interactions constitute what is now recognized as the gut-brain axis, gut-skin axis, and gut-lung axis of microbiome communication, with each axis playing a significant role in maintaining overall health (Enaud et al., 2020; De Pessemier et al., 2021; Giulio, 2021). Despite the physical separation of the gut and lungs, microorganisms and immune cells communicate with each other resulting in immune tolerance to innocuous stimuli, host defense against potentially harmful external agents and pathogens as well as prevention of commensals from over-exploitation of host resources (Lazar et al., 2018; Yoo et al., 2020; Zheng et al., 2020).

Although the precise mechanisms of communication between the gut and lungs are not yet fully understood, emerging evidence points to the involvement of various pathways, including neuroendocrine and immune systems, as well as the translocation of microorganisms (**Table 1** and **Figure 1**). These pathways often involve the release of metabolites, including microbiome-derived, that can shape immune responses, and modulate intestinal homeostasis and hematopoietic precursors in the bone marrow (Dang and Marsland, 2019). The vagus nerve, which connects the brain to multiple organs, including the lungs and gastrointestinal tract, is an essential conduit for this communication (Yuan and Silberstein, 2016). Onyszkiewicz et al. (2019) reported that butyric acid, a short-chain fatty acid (SCFA) produced by gut microbiota, lowers arterial blood pressure via colon-vagus nerve signaling. Furthermore, recent evidence has shown that the gut microbiota influences the hypothalamic-pituitary-adrenal (HPA) axis and the body's response to stress (Frankiensztajn et al., 2020). In particular the intake of *Lactococcus lactis* was shown to lower the basal activity of the HPA axis, improve sleep, mental health and immune response through the activation of MQs and NK cells (Jin et al., 2020; Matsuura et al., 2022).

The interaction between the gut microbiome and the respiratory system through the immune system is complex and dynamic; the microbiome exposes immune cells to a diverse range

TABLE 1 Mechanisms of gut-lung axis communication.

Mechanism			Model	Key findings	Study
Neuroendocrine					
	Vagus nerve		mice	Vagal nerve stimulation prevents acute lung injury after trauma-hemorrhagic shock via the intestinal barrier protective effects provided by stimulation of the enteric nervous system.	Reys et al., 2013
	HPA axis		mice	*E. coli* and their LPS production can increase the occurrence of anxiety by inducing NF- kB activation.	Jang et al., 2018
Immune response					
	Immune education		mice	Early-life exposure to microbiota is important for the development of a normal and equilibrated immune system.	Al Nabhani et al., 2019
			mice	Innate lymphoid cells (ILCs) undergo maturation through the lung-gut axis to obtain proper function. A defect of ILCs development in the lung significantly impacts the count and function of ILCs in the gut.	Zhao et al., 2022
			mice	Comensal microbiota regulates generation of virus specific CD4 and CD8 T cells after influenza infection. Comensal microbiota leads to expression of IL-1beta, pro-IL18; activation of inflammasome.	Ichinohe et al., 2011
	Immune modification		mice	Commensal bacteria-derived ATP activates CD70 high CD11c low cells in the lamina propria to induce IL-6 and IL-23 production as well as TGF-b activation, thereby leading to local differentiation of T H 17 cell	Atarashi et al., 2008
			67 patients with asthma	Expression of TH 17-related genes was associated with Proteobacteria	Huang et al., 2015
Signaling molecules					
	SCFAs				
		Acetate	mice	Acetate-GPR43 interactions profoundly affect inflammatory responses. Stimulation of GPR43 by acetate was necessary for the normal resolution of colitis, arthritis and asthma	Maslowski et al., 2009
		Propionate	mice	Propionate on Ozone exposure induce airway hyperresponsiveness	Cho et al., 2018
		Butyrate	rat	Butyric acid lowers blood pressure via colon vagus	Onyszkiewicz et al., 2019
	Tryptophan and derivatives				
		Indole	mice	The microbiome metabolite indole reduced pulmonary and extrapulmonary bacterial burden, restored immune responses, and improved cellular trafficking required for host defense.	Samuelson et al., 2021
Translocation of microorganisms					
			mice and 68 patients with acute respiratory distress syndrome	Gut–lung translocation and alteration of the lung microbiome may represent a mechanism of pathogenesis in sepsis and ARD	Dickson et al., 2016b
			patient of intensive care unit (ICU)	Lung colonization in the ICU was driven by the translocation of *Pseudomonas aeruginosa* from the gut.	Wheatley et al., 2022

Summary of the main mechanisms associated with the communication in the gut-lung axis (Atarashi et al., 2008; Maslowski et al., 2009; Ichinohe et al., 2011; Cho and Blaser, 2012; Reys et al., 2013; Huang et al., 2015; Dickson et al., 2016a; Jang et al., 2018; Al Nabhani et al., 2019; Onyszkiewicz et al., 2019; Samuelson et al., 2021; Wheatley et al., 2022; Zhao et al., 2022).

of antigens and microbial molecules, shaping its development and function, whereas the immune system maintains a permissive environment for the microbiota (Belkaid and Naik, 2013; Zheng et al., 2020). Both branches of the immune system participate in this communication. The innate immune system confers compartmentalization, preventing microbial translocation

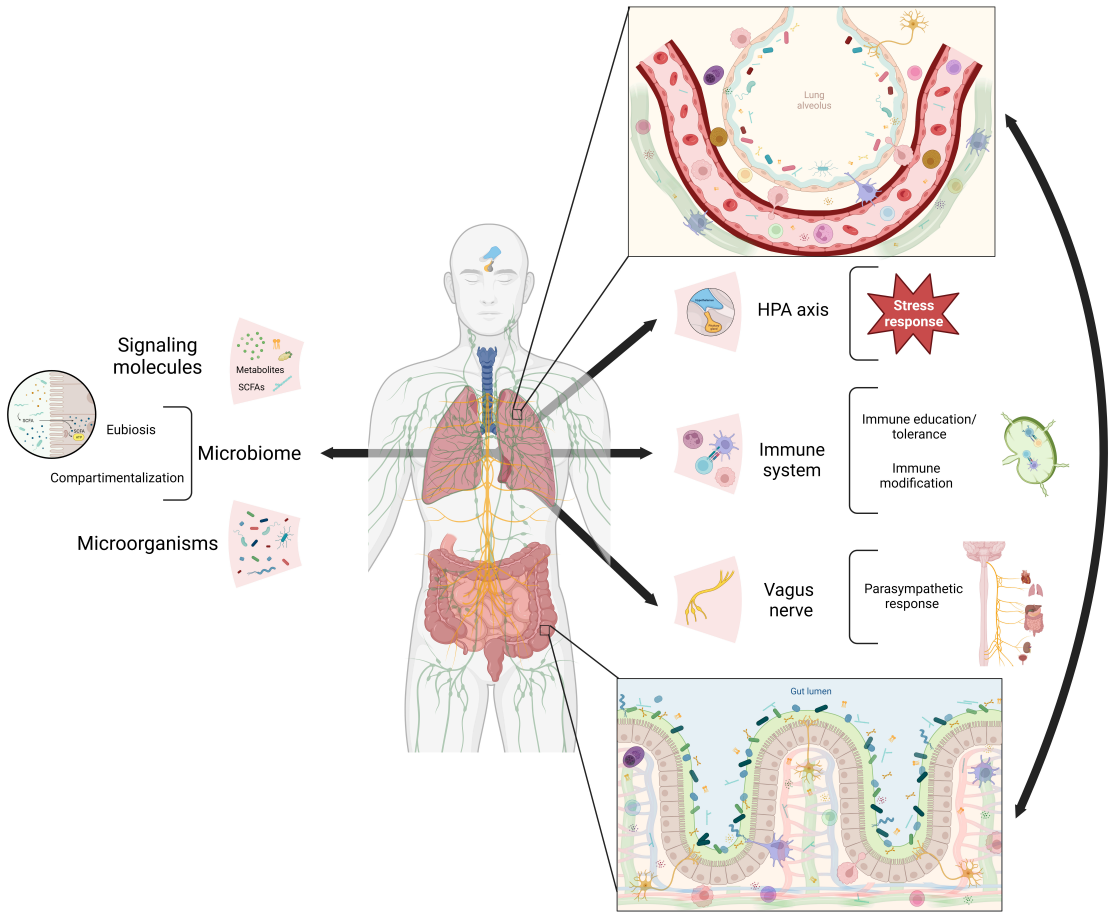


FIGURE 1
Mechanisms of gut-lung axis communication. Gut and lung communication pathways reported so far in literature (arrows). The neuroendocrine system via the hypothalamic-pituitary-adrenal (HPA) axis and the vagus nerve plus the immune system, cellular and soluble factors. All of them interact with microbiome signals from microorganisms and their metabolites. In both epitheliums, pathways' components link up (zoom in) resulting in adequate stress and parasympathetic responses, immune education and modification, eubiosis and microorganisms compartmentalization (brackets). SCAFs (short-chain fatty acids). Created with BioRender.com.

through a dense mucus layer, antimicrobial peptides (AMP), and tight junction proteins that preserve the epithelial barrier (Thaiss et al., 2016). Furthermore, the response of innate immune cells such as macrophages, dendritic cells, neutrophils, innate lymphoid cells, and epithelial cells respond to both commensal microbes signals and microbe-associated molecular patterns (MAMPs) (Chunxi et al., 2020). On the other hand, the gut commensal microbiome supports the production of secretory IgA by the adaptive immune system, which shapes microbial communities (Huus et al., 2021).

The integrity of the intestinal and lung epithelial barriers is crucial to prevent the translocation of microorganisms between the gut and respiratory tract and to maintain the internal physicochemical characteristics of both anatomical structures. However, the intestinal and lung epithelial barrier can be compromised under specific circumstances such as microaspirations, critical illness, sepsis, or chronic inflammation (Kang et al., 2023). As a result, microorganisms can translocate from the gut to the respiratory tract, potentially leading to the colonization of the respiratory tract by gut-derived microorganisms and contributing to the development or increase of severity of respiratory infections (Dickson et al., 2015; Wheatley et al., 2022). Similarly, respiratory system microorganisms, such as *Streptococcus pneumoniae*, have the potential to colonize the gastrointestinal tract (Floeystad et al., 2020). Furthermore, studies on a mice model, showed that the intratracheal inoculation of Lipopolysaccharides result in lung and gut microbiome perturbations with a parallel increase of bacterial load in the blood (Sze et al., 2014), underscoring the close interaction between these sites.

The dysbiosis and the resulting inflammation in one or both organs may contribute to the development of disease (Fabbrizzi et al., 2019). These interactions are influenced by immune cell migration and microbial metabolites in response to infection or inflammation (McGhee and Fujihashi, 2012; Zhao et al., 2022). Microbial metabolites produced by gut microbiota, such as SCFAs, tryptophan, secondary bile acids and their derivatives, modulate immune and epithelial cells (Agus et al., 2018; Ashique et al., 2022). SCFAs are a preferred energy source for colonocytes; they regulate the integrity of the intestinal barrier by inducing the secretion of IL-18 and antimicrobial peptides and the expression of the tight junctions. SCFAs inhibit macrophage production of

proinflammatory cytokines and regulate T cell differentiation to Th1, Th17, and Tregs, thus are a central component of this interaction (Sun et al., 2017; Sencio et al., 2021).

Overall, the gut-lung axis is a complex and multifaceted system involving interactions between immune cells, microbiota, and signaling molecules from both systems. The response as a whole will depend on the health conditions and comorbidities of the individual and the different disease etiologies, which highlights the importance of understanding these interactions in different pathological conditions. An important factor to consider is the profound effect of antibiotics on the gut microbiome which have been found to have an increased risk for respiratory diseases in human studies as well as animal models (Ichinohe et al., 2011; Metsälä et al., 2015; Anand and Mande, 2018). The role of the gut-lung axis in tuberculosis has gained increasing recognition in recent years, highlighting its significance in the context of this infectious disease (Naidoo et al., 2019). Several studies have revealed that gut microbial dysbiosis can exacerbate lung inflammation and contribute to a dysregulated immune response to *M. tuberculosis* (Sekyere et al., 2020; Comberiati et al., 2021).

Gut-lung axis and the impact of tuberculosis treatment

Tuberculosis is a disease that has co-evolved with humankind for millennia. Infection with MTB can result in a dynamic spectrum of clinical manifestations that range from elimination to asymptomatic latent TB to clinically active TB. Several factors influence these dynamic states, notably the immune response, microbiota, and the interaction between them. The main risk factors for tuberculosis include HIV infection [Relative Risk (RR) 18], alcohol use disorders (RR 3.3), undernourishment (RR 3.2), smoking (RR 1.6), and diabetes (RR 1.5) (World Health Organization [WHO], 2021a), all of which are associated with gut dysbiosis and proinflammatory susceptibility.

Of particular importance is the fact that MTB-infected individuals often have delayed diagnosis or undergo non-tuberculosis antibiotic treatment before a specific TB treatment is prescribed (Shi et al., 2021; Teo et al., 2021); in both instances, the resulting microbiome dysbiosis may increase the severity of the disease (Hogan et al., 2017). Broad-spectrum antibiotics, including cephalosporins and fluoroquinolones, are among the most frequently empirically prescribed antibiotics. Specifically, a decrease in the abundance of *Roseburia*, *Kluyvera*, and *Citrobacter genera*, and a near depletion of SCFA-producing bacteria, have been reported in these TB patients (Shi et al., 2021). Thus, gut microbiome dysbiosis, with a predisposition to inflammatory response, is expected in most patients secondary to the start of empirical antibiotic treatment, even before starting specific anti-TB treatment.

Drug-susceptible MTB infection

Tuberculosis can be caused by MTB strains that are either resistant or susceptible to a variety of drugs. Between 2018 and 2021, 26.3 million TB patients were treated, of which 25.6 million were drug-susceptible (DS) and 649,000 drug-resistant (DR) (World Health Organization [WHO], 2022a). It is important to emphasize that treatments for tuberculosis are among the most prolonged antibiotic treatments approved by WHO; they range from four to 6 months for DS MTB and up to 20 months for DR MTB (World Health Organization [WHO], 2022b,c). These treatments include a combination of broad-spectrum and narrow-spectrum drugs with mycobacterial-specific targets (**Table 2**).

The WHO standard recommended scheme for DS MTB consists of four essential drugs designated as "first-line" anti-TB treatment: isoniazid (H), rifampicin (R), pyrazinamide (Z), and ethambutol (E) for 2 months, followed by 4 months of only HR; recently the WHO added moxifloxacin (Mfx) and rifapentine (Rpt, a synthetic derivative of rifampicin) to primary treatment. Rifampicin, and moxifloxacin are broad-spectrum antibiotics used in other non-mycobacterial infections, whereas isoniazid, pyrazinamide and ethambutol have mycobacterial-specific targets. Two alternative DS treatments have been recently approved by WHO; one includes a 2-month treatment of Rpt, moxifloxacin (Mfx), H and Z followed by 2 months with RptHMfx (Dorman et al., 2021; World Health Organization [WHO], 2022c), and the second one, a 2-month treatment of bedaquiline (Bdq), Linezolid (Lzd) and HZE, which recently proved their effectiveness in clinical trials (Paton et al., 2023). Both of these new alternative treatments significantly decrease the time of treatment but contain broad-spectrum antibiotics (Lzd and Mfx) that result in broader damage to gut microbiota and should be evaluated accordingly (Dorman et al., 2021; Paton et al., 2023).

The effect of each of these antibiotics in the microbiome cannot be evaluated individually on tuberculosis patients. However, several studies of broad-spectrum antibiotics used in anti-TB treatments on healthy individuals have shown drastic and long lasting effects in the gut microbiome. For example, 5 days treatment of ciprofloxacin or Mfx resulted in a drastic reduction in alpha diversity, characterized by a decreased abundance in *Alistipes*, *Bilophila*, *Butyricimonas*, *Coprobacillus*, *Faecalibacterium*, *Odoribacter*, *Oscillibacter*, *Parasutterella*, *Roseburia*, and *Sutterella* genera (De Gunzburg et al., 2018; Burdet et al., 2019). Similarly, studies with Lzd showed an increase of resistant *Enterococci* in the gut and an overall decrease of Gram-positive bacteria cells in the nasal, pharyngeal, and intestinal microbiomes (Bourgeois-Nicolaos et al., 2014). Furthermore, antibiotic therapies of first-line anti-TB medications (R or HZ) in murine models demonstrated changes in taxonomic composition and a decreased alpha and beta diversity; Rifampicin lead to an expansion of *Bacteroides*, *Verrucomicrobiaceae*, and a decrease in *Lachnospiraceae* families. Unexpectedly, the treatment with HZ, mycobacterial specific drugs, resulted in an expansion, although a modest one, of Bacteroidetes, particularly the *Clostridiaceae* family (Khan et al., 2019).

The consequence of initial TB treatment on the microbiome has implications for the overall outcome: relapse, reinfection, and perhaps the severity of the disease (Khan et al., 2016; Hu et al., 2019). Thus it is important to understand its implications in the development of disease as well as in the patient's overall state of health. Several studies have shown a gut microbiome dysbiosis during first-line anti-TB treatment for DS TB that encompasses both bacteria and fungi. A decrease in abundance of the bacterial genera *Ruminococcus*, *Eubacterium*, *Lactobacillus*,

TABLE 2 Drug-resistant anti-TB treatment.

Groups and steps	Medicine	Abbreviation	Antibiotic spectrum	Dysbiosis time	Alteration in the microbiota	Model	References
Group A: Include all three medicines	Levofloxacin or moxifloxacin	Lfx Mfx	Broad	10 months	Decrease abundance of Alistipes, Bilophila, Butyricimonas, Coprobacillus, Faecalibacterium, Odoribacter, Oscillibacter, Parasutterella, Roseburia, Sutterella, Kluyvera, and Citrobacter genera.	Human	Dethlefsen et al., 2007; De Gunzburg et al., 2018; Burdet et al., 2019; Shi et al., 2021
	Bedaquiline	Bdq	Narrow	Unknow	Decrease *Streptococcus mutans*.	*In vitro*	Zhang et al., 2021
	Linezolid	Lzd	Broad	Unknow	Increase abundance of resistant *Enterococci* in the gut and an overall decrease of Gram-positive bacteria.	Human	Bourgeois-Nicolaos et al., 2014
Group B: Add one or both medicines	Clofazimine	Cfz	Narrow	Unknow	Unknow	Unknow	Unknow
	Cycloserine or terizidone	Cs Trd	Broad	Unknow	Decrease *abundance of Bifidobacterium* species and other butyrate producers.	Human	Minichino et al., 2021
Group C: Add to complete the regimen and when medicines from Groups A and B cannot be used	Ethambutol	E	Narrow	Unknow	Unknow	Unknow	Unknow
	Delamanid	Dlm	Narrow	Unknow	Unknow	Unknow	Unknow
	Pyrazinamide	Z	Narrow	Unknow	Decrease abundance of *Clostridia species* and increase *Anaeroplasma*.	Murine	Namasivayam et al., 2017
	Imipenem- cilastatin	Ipm-Cln	Broad	Unknow	Decrease abundance of *Enterobacteria*, *Enterococci*, *Bifidobacteria*, *Eubacteria*, *Lactobacilli*, and *Bacteroides*.	Human	Bhalodi et al., 2019
	Meropenem	Mpm	Broad	Unknow	Decrease abundance of *Enterobacteria*, *Clostridia*, and *Bacteroides* and increase *Enterococci*.	Human	Bhalodi et al., 2019
	Amikacin (or streptomycin)	Am (S)	Broad	Unknow	Decrease abundance of *Bacteroidales*, *Clostridiales* and increases in the *Lachnospiraceae* and *Bacteroidaceae*.	Murine	Lichtman et al., 2016
	Ethionamide or prothionamide	Eto Pto	Narrow	Unknow	Unknow	Unknow	Unknow
	P-aminosalicylic acid	PAS	Narrow	Unknow	Unknow	Unknow	Unknow

Principal regimen options for drug-resistant tuberculosis (Dethlefsen et al., 2007; Bourgeois-Nicolaos et al., 2014; Lichtman et al., 2016; De Gunzburg et al., 2018; Bhalodi et al., 2019; Burdet et al., 2019; Minichino et al., 2021; Shi et al., 2021; Zhang et al., 2021; World Health Organization [WHO], 2022c).

10.3389/fmicb.2023.1237998

Coprococcus, *Dialister*, *Dorea*, *Bacteroides*, and *Oscillospirales*, and simultaneous increase of *Erysipelatoclostridium*, *Veillonella*, *Bifidobacterium*, *Klebsiella*, and *Prevotella* have been reported (Wipperman et al., 2017; Meng et al., 2022; **Figure 2**). Whereas, an increase in the relative abundance of the fungi genera *Purpureocillium*, *Nakaseomyces*, *Rhodotorula*, and *Genolevuria*, with a decrease in *Naganishia* and *Mucor* genera (Cao et al., 2021) was shown.

This dysbiosis results in an overall decrease of microbial SCFAs production, which has been associated with a weakened intestinal epithelial barrier, reduction of mucin and AMP expression with the corresponding exacerbation of systemic inflammatory response (Sun et al., 2017; Zhang et al., 2023). Although studies of respiratory tract microbiome are fewer and harder to compare due to differences in sample and study design, they do confirm disruption of the microbiome affected by MTB infection and treatment; overall an increase abundance of *Bacteroides* and *Oscillospira* and a decrease in *Lactobacillus*, *Prevotella*, and *Veillonella* has been reported (**Figure 2**; Valdez-Palomares et al., 2021; Zhang et al., 2022).

Furthermore, when oral antibiotics cannot be used, patients may require intravenous antibiotics like carbapenems. However, carbapenems for anti-TB treatment are prescribed in conjunction with clavulanic acid, since MTB has a constitutive beta-lactamase BlaC, that has a penicillinase, cephalosporinase, and carbapenemase activity that is inhibited by clavulanic acid (Bhattacharya et al., 2021). Moreover, in México and other Latin American countries, clavulanic acid is administered with amoxicillin, which adds another broad-spectrum antibiotic to the treatment (National Center for Preventive Programs and Disease Control, 2020). The administration of these antibiotics results in further changes in the gut microbiota, including the increase of the *Bacteroidales* order and *Bifidobacterium* species in the gut microbiota (Gaucher et al., 2021). Even monotherapies of carbapenems have shown drastic effects on the gut microbiota. In particular, meropenem administration in healthy volunteers decreased the abundance of *Enterobacteria*, *Clostridia*, and *Bacteroides* and increased *Enterococci*, while genera like *Bifidobacterium* and *Lactobacillus* remain stable. On the other hand, imipenem was shown to reduce all of the species mentioned, with only *Clostridia* remaining stable (Bhalodi et al., 2019).

Drug-resistant MTB infection

Although drug-resistant tuberculosis (DR-TB) corresponds to only 4.2% of total MTB infections in 2021, it has steadily increased in recent decades, from 30,000 cases in 2009 to 450,000 in 2021 (World Health Organization [WHO], 2022a). DR-TB has been divided by the WHO into five categories: rifampicin-resistant (RR), isoniazid-resistant, and rifampicin susceptible (Hr), multidrug-resistant (MDR), defined as H and R resistant; pre-extensively drug-resistant (pre-XDR-TB) which refers to TB that is resistant to R (may also be resistant to H), and any fluoroquinolone; whereas extreme drug-resistant TB (XDR-TB), is resistant to R, (may also be resistant to H), any fluoroquinolone, plus at least one of either Bdq or Lzd (World Health Organization [WHO], 2022b).

Currently, treatment of drug-resistant infection is individualized and includes broad-spectrum as well as mycobacterial-specific antibiotics (see **Table 2**). In 2022, the WHO renewed its recommendations for DR treatment to include three drugs from Group A and at least one from Group B or Group C, depending on the susceptibility pattern and the location of the infection (World Health Organization [WHO], 2022b). Furthermore, newer shorter schemes that include BPaL (Bedaquiline, Pretomanid, Linezolid), or BPaLM (BPaL + Moxifloxacin) for 6 months are being introduced (World Health Organization [WHO], 2022b).

Similar to DS treatment, DR-TB treatment leads to profound changes on the gut microbiome and, thus, impacts the gut-lung axis. Alterations in the gut microbiota of DR-TB-treated patients have been reported in terms of overall decrease in alpha diversity that can last for years after treatment completion (Wang et al., 2020; Shi et al., 2021). In particular, an increase of *Enterobacteriaceae* is seen from healthy to RR and MDR, along with a decrease in members of the phylum Actinobacteria and Firmicutes in MDR patients (Wang et al., 2020; Shi et al., 2022). Furthermore, phylum Verrucomicrobia was found as a predominant component in Pre-XDR-TB, whereas it is almost undetectable in healthy, RR or MDR individuals (Shi et al., 2022). On the other hand, studies on the macaque model have shown an increase in Proteobacteria in RR and MDR but not in Pre-XDR-TB or healthy controls (Namasivayam et al., 2019). Moreover, members of the Bacteroidetes phylum were only found in healthy individuals. Gut-derived metabolites, such as SCFAs, tryptophan and secondary bile acids, decreased from MDR to Pre-XDR and RR to healthy participants, underscoring a complex interaction between the microbiota and immune system (Shi et al., 2022). Studies of monotherapies, although not in TB patients, particularly cycloserine treatment, a group B drug, reduces *Bifidobacterium* species and other butyrate producers in the gut microbiota (Minichino et al., 2021). Overall there are clear changes in the composition and diversity of the microbiota, but inconsistent in terms of specific taxa abundance (**Table 2**).

The latest treatments of TB include the new anti-TB drugs: bedaquiline, delamanid, and pretomanid; the first new anti-TB drugs to be approved in 40 years. Bdq and Dlm/Pto target mycobacterial respiratory chain components, including the ATP-synthase. These drugs are recommended for some forms of RR, MDR, or Pre-XDR and XDR (World Health Organization [WHO], 2022b). Although there is limited information on the effect of either of these drugs on the microbiome recent research showed an inhibition of proliferation and biofilm production of *Streptococcus mutans*, and other oral pathogens after Bdq treatment, which stresses the impact of this antibiotic on the microbiome in general, not only to MTB (Zhang et al., 2021).

Long-term effect of anti-TB treatment on the gut-lung axis

The gut microbiota dysbiosis, consequence of any antibiotic treatment, results in an altered immune response and increased vulnerability to other infections. There is a reduction in the expression and secretion of AMPs, including C-type lectins,

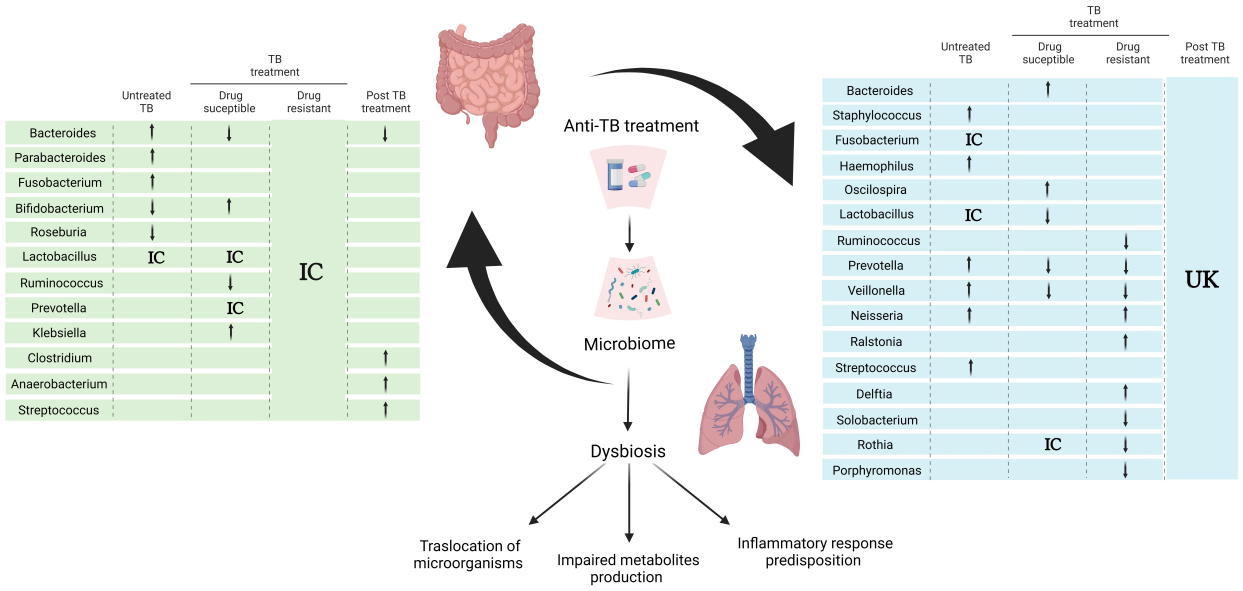


FIGURE 2
Effect of anti-TB treatment on the gut- axis microbiome communication. Alterations of gut and lung microbiota in the course of anti- TB treatment according to selected references using research words "TB treatment," "microbiome," "tuberculosis" "microbiota" in PubMed database NCBI. Arrows indicate genera enrichment or decreased, IC (inconclusive) denotes controversial or non-significant results within references and unknown (UK) and blank spaces for missing data in the references reviewed (Botero et al., 2014; Khan et al., 2016; Krishna et al., 2016; Namasivayam et al., 2017; Wipperman et al., 2017; Vázquez-Pérez et al., 2020; Wang et al., 2020; Cao et al., 2021; Kateete et al., 2021; Lin et al., 2021; Valdez-Palomares et al., 2021; Xiao et al., 2022; Ye et al., 2022; Zhang et al., 2022). Created with BioRender.com.

defensins, and cathelicidins; compromised integrity of the epithelial barrier, as well as reduced production of SCFAs, all of which are part of the first line of defense to incoming pathogens (Schumann et al., 2005; Hill et al., 2010; Willing et al., 2011; Wipperman et al., 2017). Common and recurrent *Clostridium difficile* infections, as well as increased susceptibility to *Salmonella enterica* and *Escherichia coli* infections after antibiotic exposure, have been reported (Croswell et al., 2009; Wang et al., 2020). Furthermore, reduced butyrate has been associated with neutrophil infiltration and T cell anergy (Meijer et al., 2010). Thus it is possible that anti-TB treatment has the side effect of hampering the immune response against the mycobacteria.

After completion of antibiotic therapy, the dysbiotic microbiome will either return to the initial state before treatment or establish a new eubiosis. This process involves cooperation and competition among the microorganisms as well as the changes in the physicochemical properties of the gut tract, which is affected by the length of the treatment and the type of drugs involved. For example, the dysbiosis caused by a 5-day fluoroquinolone treatment is reversed after a 4-week recovery period (Dethlefsen et al., 2007). However, a 6-month DS treatment results in a dysbiosis that lasts at least 1.2 years, and a 20-month MDR treatment may have irreversible consequences for the microbiome (Wipperman et al., 2017; Wang et al., 2020). Furthermore, during anti-TB treatment, some bacteria enter dormancy or a persister state as a result of stressors, including hypoxia. It is possible that disease relapse, result of the activation of these persister bacilli, and increased susceptibility to reinfection is caused by diminished immune control consequence of gut-lung microbiome dysbiosis (Zhang et al., 2012; Quigley and Lewis, 2022).

The intricate relationship between antibiotic treatment, gut-lung microbiome dysbiosis, and tuberculosis outcomes make it evident that it is necessary to consider the microbiome as part of the treatment. For this, it is crucial to understand the impact of different treatments on the microbiome and its potential consequences for disease development. A promising new approach: "Host-directed-therapy" (HDT) aims to improve innate immunity, instead of targeting the pathogen directly. HDT has been used in antitumor therapies, inflammatory bowel disease and infectious diseases, is particularly important in the context of antibiotic resistance (Wei et al., 2015; Langdon et al., 2016; Bergman et al., 2020; Bustamante et al., 2020; Davar et al., 2021; He et al., 2021; Jeong et al., 2023). HDTs include the use of probiotics, prebiotics, symbiotics, microbiota transplants and phage therapy. HDT induces the activation of the endogenous defense mechanisms including antimicrobial peptides, reactive oxygen species, autophagy etc (Bergman et al., 2020; Diallo et al., 2021). For example, a clinical trial in Bangladesh, (Mily et al., 2015), showed improved MTB clearance after use of adjunct therapy of phenylbutyrate (a SCFA) and vitamin D3 in a standard short-course first line TB treatment. Adjunct therapy of Butyrate in Shigellosis also showed early reduction of local inflammation (Raqib et al., 2012). Furthermore, studies suggest that certain probiotic strains of *Lactobacillus* and *Lacticaseibacillus*, may have immunomodulatory effects and could enhance the body's defense mechanisms against infections, including TB (Jiang et al., 2022; Rahim et al., 2022). Probiotics may help regulate inflammation, promote tissue repair, and a better immune

response, all of which are important for patients with TB and post-TB recovery.

In conclusion, the gut microbiome cross talk with the immune response occurs and has an impact in the development of tuberculosis. Furthermore, therapeutic strategies that utilize gut microbiota and their metabolites in combination with the appropriate antibiotic treatment, may provide improved outcomes for patients.

Discussion

There has been a great deal of research on *M. tuberculosis's* long and complex interaction with its host. Many of the factors that contribute to the susceptibility and development of the disease are associated directly or indirectly with immune maintenance, including HIV infection, malnutrition, diabetes, smoking, and substance abuse. All of these conditions result in gut microbiome dysbiosis. In turn, gut dysbiosis has been implicated in disease development locally or distal, including in the respiratory tract. Although we are just beginning to understand the crosstalk in the gut-lung axis that allows passage of microbial and host metabolites, it has become clear that these interactions affect the susceptibility and development of many respiratory diseases, including tuberculosis.

Gut microbiota is altered from the initial lung infection of MTB and increases substantially with the long anti-TB treatments. TB treatment is one of the world's most widely administered antibiotic combinations. The long-term effect of antibiotic treatments is evident; from 6 months of DS TB, treatment that can last up to a year, to potentially irreversible changes after a 20-month DR-TB treatment (Wang et al., 2020). The loss of bacterial diversity as a result of antibiotic treatment can lead to an increased vulnerability to infections, as has been shown for *C. difficile*, *E. coli*, and *S. enterica* (Croswell et al., 2009; Wang et al., 2020), and may be part of the explanation for high relapse or reinfection rates on DR-TB patients. Additionally, even with treatment adherence, 14% of DS and nearly 40% of DR TB patients fail treatment, and 5% of all patients with successful treatment relapse (Getahun et al., 2011; World Health Organization [WHO], 2022a). This suggests that the cure and prevention of relapse in tuberculosis may not depend solely on anti-TB treatment. The respiratory and gut microbiota dysbiosis and its interplay with the immune response play an important part. There is numerous evidence that demonstrates changes in the taxonomic composition as well as the overall diversity of the gut and respiratory microbiome during anti-TB treatment. However, probably due to differences in study design and samples taken, or individual characteristics of each patient, there are inconsistent results in terms of changes of specific organisms. To fully understand the interplay between the microbiome and host defense mechanisms, longitudinal studies that follow patients' respiratory and gut microbiome through their treatments, integrating the immune response, are needed. Furthermore, we need to go beyond the study of only bacteria and include all other microorganisms in the microbiota as well as metabolome and resistome.

Although we have pointed out some of the adverse effects of antibiotic therapies, it is clear that antibiotic therapy for TB and other infectious diseases is a central tool for their treatment. However, strategies that reduce dysbiosis and restore a healthy microbial balance are needed. In tuberculosis management, current efforts include shortened and narrow spectrum antibiotic therapies, together with host-directed-therapies that improve immune response. There are promising results in the use of pre- and probiotic adjunct therapies in TB treatment; however, more clinical studies are needed to establish their effectiveness in this specific context. Patients' individual characteristics, choice of pre or probiotics, dosages, timing need careful consideration.

In sum, the treatment of tuberculosis has broad public health implications, with millions of people being treated with first-line anti-TB medicines for 6 months, resulting in microbiome dysbiosis lasting years after treatment completion (Wipperman et al., 2017). Future research should aim to develop strategies that optimize treatment outcomes by considering the dynamic interplay between the microbiome and host immune responses.

Author contributions

ES-H and NA-P: conceptualization. DG-C, IG-G, and NA-P: methodology. ES-H, DG-C, XM, IG-G, and NA-P: formal analysis, investigation, review and editing, and writing-original draft preparation. IG-G: visualization. ES-H: supervision and funding acquisition. All the authors contributed equally to writing and editing of the document, read, and agreed to the published version of the manuscript.

Funding

This research was supported by the ESH funding from CONACYT Ciencia Frontera 1919178.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Agus, A., Planchais, J., and Sokol, H. (2018). Gut microbiota regulation of tryptophan metabolism in health and disease. *Cell Host Microbe* 23, 716–724. doi: 10.1016/j.chom.2018.05.003

Al Nabhani, Z., Dulauroy, S., Marques, R., Cousu, C., Al Bounny, S., Déjardin, F., et al. (2019). A weaning reaction to microbiota is required for resistance to immunopathologies in the adult. *Immunity* 50, 1276–1288.e5. doi: 10.1016/j.immuni.2019.02.014

Anand, S., and Mande, S. S. (2018). Diet, microbiota and gut-lung connection. *Front. Microbiol.* 9:2147. doi: 10.3389/fmicb.2018.02147

Ashique, S., De Rubis, G., Sirohi, E., Mishra, N., Rihan, M., Garg, A., et al. (2022). Short chain fatty acids: Fundamental mediators of the gut-lung axis and their involvement in pulmonary diseases. *Chem. Biol. Interact.* 11:1209. doi: 10.1016/j.cbi.2022.110231

Atarashi, K., Nishimura, J., Shima, T., Umesaki, Y., Yamamoto, M., Onoue, M., et al. (2008). ATP drives lamina propria TH17 cell differentiation. *Nature* 455, 808–812. doi: 10.1038/nature07240

Bach, L., Ram, A., Ijaz, U. Z., Evans, T. J., Haydon, D. T., and Lindström, J. (2023). The effects of smoking on human pharynx microbiota composition and stability. *Microbiol. Spectr.* 11:e0216621. doi: 10.1128/spectrum.02166-21

Barbosa-Amezcua, M., Galeana-Cadena, D., Alvarado-Peña, N., and Silva-Herzog, E. (2022). The microbiome as part of the contemporary view of tuberculosis disease. *Pathogens* 11:584. doi: 10.3390/pathogens11050584

Belkaid, Y., and Naik, S. (2013). Compartmentalized and systemic control of tissue immunity by commensals. *Nat. Immunol.* 14, 646–653. doi: 10.1038/ni.2604

Bergman, P., Raqib, R., Rekha, R. S., Agerberth, B., and Gudmundsson, G. H. (2020). Host directed therapy against infection by boosting innate immunity. *Front. Immunol.* 11:1209. doi: 10.3389/fimmu.2020.01209

Bhalodi, A. A., Van Engelen, T. S. R., Virk, H. S., and Wiersinga, W. J. (2019). Impact of antimicrobial therapy on the gut microbiome. *J. Antimicrob. Chemother.* 74, I6–I15. doi: 10.1093/jac/dky530

Bhattacharya, S., Junghare, V., Pandey, N. K., Baidya, S., Agarwal, H., Das, N., et al. (2021). Variations in the SDN loop of class a beta-lactamases: A study of the molecular mechanism of BlaC (Mycobacterium tuberculosis) to alter the stability and catalytic activity towards antibiotic resistance of MBIs. *Front. Microbiol.* 12:710291. doi: 10.3389/fmicb.2021.710291

Botero, L. E., Delgado-Serrano, L., Cepeda, M. L., Bustos, J. R., Anzola, J. M., Del Portillo, P., et al. (2014). Respiratory tract clinical sample selection for microbiota analysis in patients with pulmonary tuberculosis. *Microbiome* 2:29. doi: 10.1186/2049-2618-2-29

Bourgeois-Nicolaos, N., Nguyen, T. T., Defrance, G., Massias, L., Alavoine, L., Lefort, A., et al. (2014). The emergence of linezolid resistance among enterococci in intestinal microbiota of treated patients is unrelated to individual pharmacokinetic characteristics. *Antimicrob. Agents Chemother.* 58, 2681–2687. doi: 10.1128/AAC.02251-13

Budden, K. F., Gellatly, S. L., Wood, D. L. A., Cooper, M. A., Morrison, M., Hugenholtz, P., et al. (2017). Emerging pathogenic links between microbiota and the gut-lung axis. *Nat. Rev. Microbiol.* 15, 55–63. doi: 10.1038/nrmicro.2016.142

Burdet, C., Nguyen, T. T., Duval, X., Ferreira, S., Andremont, A., Guedj, J., et al. (2019). Impact of antibiotic gut exposure on the temporal changes in microbiome diversity. *Antimicrob. Agents Chemother.* 63, e00820–19. doi: 10.1128/AAC.00820-19

Bustamante, M., Oomah, B. D., Oliveira, W. P., Burgos-Díaz, C., Rubilar, M., and Shene, C. (2020). Probiotics and prebiotics potential for the care of skin, female urogenital tract, and respiratory tract. *Folia Microbiol.* 65, 245–264. doi: 10.1007/s12223-019-00759-3

Cadena, A. M., Fortune, S. M., and Flynn, J. L. (2017). Heterogeneity in tuberculosis. *Nat. Rev. Immunol.* 17, 691–702. doi: 10.1038/nri.2017.69

Cao, D., Liu, W., Lyu, N., Li, B., Song, W., Yang, Y., et al. (2021). Gut mycobiota dysbiosis in pulmonary tuberculosis patients undergoing anti-tuberculosis treatment. *Microbiol. Spectr.* 9:e0061521.

Cho, I., and Blaser, M. J. (2012). The human microbiome: At the interface of health and disease. *Nat. Rev. Genet.* 13, 260–270. doi: 10.1038/nrg3182

Cho, Y., Abu-Ali, G., Tashiro, H., Kasahara, D. I., Brown, T. A., Brand, J. D., et al. (2018). The microbiome regulates pulmonary responses to ozone in mice. *Am. J. Respir. Cell Molecular Biol.* 59, 346–354. doi: 10.1165/rcmb.2017-0404OC

Chunxi, L., Haiyue, L., Yanxia, L., Jianbing, P., and Jin, S. (2020). The gut microbiota and respiratory diseases: New evidence. *J. Immunol. Res.* 2020:2340670. doi: 10.1155/2020/2340670

Comberiati, P., Di Cicco, M., Paravati, F., Pelosi, U., Di Gangi, A., Arasi, S., et al. (2021). The role of gut and lung microbiota in susceptibility to tuberculosis. *Int. J Environ. Res. Public Health* 18:12220. doi: 10.3390/ijerph182212220

Croswell, A., Amir, E., Teggatz, P., Barman, M., and Salzman, N. H. (2009). Prolonged impact of antibiotics on intestinal microbial ecology and susceptibility to enteric *Salmonella* infection. *Infect. Immun.* 77, 2741–2753. doi: 10.1128/IAI.00006-09

Dang, A. T., and Marsland, B. J. (2019). Microbes, metabolites, and the gut–lung axis. *Mucosal Immunol.* 12, 843–850. doi: 10.1038/s41385-019-0160-6

Davar, D., Dzutsev, A. K., McCulloch, J. A., Rodrigues, R. R., Chauvin, J. M., Morrison, R. M., et al. (2021). Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. *Science* 371, 595–602. doi: 10.1126/science.abf3363

De Gunzburg, J., Ghozlane, A., Ducher, A., Le Chatelier, E., Duval, X., Ruppé, E., et al. (2018). Protection of the human gut microbiome from antibiotics. *J. Infect. Dis.* 217, 628–636. doi: 10.1093/infdis/jix604

De Pessemier, B., Grine, L., Debaere, M., Maes, A., Paetzold, B., and Callewaert, C. (2021). Gut–skin axis: Current knowledge of the interrelationship between microbial dysbiosis and skin conditions. *Microorganisms*. 9:353. doi: 10.3390/microorganisms9020353

Dethlefsen, L., McFall-Ngai, M., and Relman, D. A. (2007). An ecological and evolutionary perspective on humang-microbe mutualism and disease. *Nature* 449, 811–818. doi: 10.1038/nature06245

Diallo, D., Somboro, A. M., Diabate, S., Baya, B., Kone, A., Sarro, Y. S., et al. (2021). Antituberculosis therapy and gut microbiota: Review of potential host microbiota directed-therapies. *Front. Cell. Infect. Microbiol.* 11:673100. doi: 10.3389/fcimb.2021.673100

Dickson, R. P., Erb-Downward, J. R., and Huffnagle, G. B. (2015). Homeostasis and its disruption in the lung microbiome. *Am. J. Physiol. Lung Cell Mol. Physiol.* 309, 1047–1055. doi: 10.1152/ajplung.00279.2015.-The

Dickson, R. P., Erb-Downward, J. R., Martinez, F. J., and Huffnagle, G. B. (2016a). The microbiome and the respiratory tract. *Annu. Rev. Physiol.* 78, 481–504. doi: 10.1146/annurev-physiol-021115-105238

Dickson, R. P., Singer, B. H., Newstead, M. W., Falkowski, N. R., Erb-Downward, J. R., Standiford, T. J., et al. (2016b). Enrichment of the lung microbiome with gut bacteria in sepsis and the acute respiratory distress syndrome. *Nat. Microbiol.* 1:16113. doi: 10.1038/nmicrobiol.2016.113

Dorman, S. E., Nahid, P., Kurbatova, E. V., Phillips, P. P. J., Bryant, K., Dooley, K. E., et al. (2021). Four-month rifapentine regimens with or without moxifloxacin for tuberculosis. *N. Engl. J. Med.* 384, 1705–1718. doi: 10.1056/nejmoa2033400

Duarte, S. M. B., Stefano, J. T., Miele, L., Ponziani, F. R., Souza-Basqueira, M., Okada, L. S. R. R., et al. (2018). Gut microbiome composition in lean patients with NASH is associated with liver damage independent of caloric intake: A prospective pilot study. *Nutr. Metab. Cardiovasc. Dis.* 28, 369–384. doi: 10.1016/j.numecd.2017.10.014

Enaud, R., Prevel, R., Ciarlo, E., Beaufils, F., Wieërs, G., Guery, B., et al. (2020). The gut-lung axis in health and respiratory diseases: A place for inter-organ and inter-kingdom crosstalks. *Front. Cell. Infect. Microbiol.* 10:9. doi: 10.3389/fcimb.2020.00009

Fabbrizzi, A., Amedei, A., Lavorini, F., Renda, T., and Fontana, G. (2019). The lung microbiome: Clinical and therapeutic implications. *Int. Emerg. Med.* 14, 1241–1250. doi: 10.1007/s11739-019-02208-y

Ferrer, M., Méndez-García, C., Rojo, D., Barbas, C., and Moya, A. (2017). Antibiotic use and microbiome function. *Biochem. Pharmacol.* 134, 114–126. doi: 10.1016/j.bcp.2016.09.007

Floeystad, H. K., Berild, J. D., Brandsaeter, B. J., Vestrheim, D. F., Berild, D., and Holm, A. M. (2020). Gastrointestinal symptoms in invasive pneumococcal disease: A cohort study. *BMC Infect. Dis.* 20:479. doi: 10.1186/s12879-020-05211-3

Frankiensztajn, L. M., Elliott, E., and Koren, O. (2020). The microbiota and the hypothalamus-pituitary-adrenocortical (HPA) axis, implications for anxiety and stress disorders. *Curr. Opin. Neurobiol.* 62, 76–82. doi: 10.1016/j.conb.2019.12.003

Gaucher, L., Adda, L., Séjourné, A., Joachim, C., Guillaume, C., Poulet, C., et al. (2021). Associations between dysbiosis-inducing drugs, overall survival and tumor response in patients treated with immune checkpoint inhibitors. *Ther. Adv. Med. Oncol.* 13:17588359211000591. doi: 10.1177/17588359211000591

Getahun, H., Kittikraisak, W., Heilig, C. M., Corbett, E. L., Ayles, H., Cain, K. P., et al. (2011). Development of a standardized screening rule for tuberculosis in people living with HIV in resource-constrained settings: Individual participant data meta-analysis of observational studies. *PLoS Med.* 8:e1000391. doi: 10.1371/journal.pmed.1000391

Giulio, P. (2021). The intestinal microbiota: Towards a multifactorial integrative model. Eubiosis and dysbiosis in morbid physical and psychological conditions. *Arch. Clin. Gastroenterol.* 7:024–035. doi: 10.17352/2455-2283.000094

Gui, X., Yang, Z., and Li, M. D. (2021). Effect of cigarette smoke on gut microbiota: State of knowledge. *Front. Physiol*. 12:673341. doi: 10.3389/fphys.2021.673341

He, L. H., Yao, D. H., Wang, L. Y., Zhang, L., and Bai, X. L. (2021). Gut microbiome-mediated alteration of immunity, inflammation, and metabolism involved in the regulation of non-alcoholic fatty liver disease. *Front. Microbiol*. 12:761836. doi: 10.3389/fmicb.2021.761836

Hill, D. A., Hoffmann, C., Abt, M. C., Du, Y., Kobuley, D., Kirn, T. J., et al. (2010). Metagenomic analyses reveal antibiotic-induced temporal and spatial changes in intestinal microbiota with associated alterations in immune cell homeostasis. *Mucosal Immunol.* 3, 148–158. doi: 10.1038/mi.2009.132

Hillman, E. T., Lu, H., Yao, T., and Nakatsu, C. H. (2017). Microbial ecology along the gastrointestinal tract. *Microbes Environ*. 32, 300–313. doi: 10.1264/jsme2.ME17017

Hogan, C. A., Puri, L., Gore, G., and Pai, M. (2017). Impact of fluoroquinolone treatment on delay of tuberculosis diagnosis: A systematic review and meta-analysis. *J. Clin. Tuberc. Other Mycobacterial Dis.* 6, 1–7. doi: 10.1016/j.jctube.2016.12.001

Hu, Y., Yang, Q., Liu, B., Dong, J., Sun, L., Zhu, Y., et al. (2019). Gut microbiota associated with pulmonary tuberculosis and dysbiosis caused by anti-tuberculosis drugs. *J. Infect.* 78, 317–322. doi: 10.1016/j.jinf.2018.08.006

Huang, Y. J., Nariya, S., Harris, J. M., Lynch, S. V., Choy, D. F., Arron, J. R., et al. (2015). The airway microbiome in patients with severe asthma: Associations with disease features and severity. *J. Allergy Clin. Immunol.* 136, 874–884. doi: 10.1016/j.jaci.2015.05.044

Huus, K. E., Petersen, C., and Finlay, B. B. (2021). Diversity and dynamism of IgA-microbiota interactions. *Nat. Rev. Immunol*. 21, 514–525. doi: 10.1038/s41577-021-00506-1

Ichinohe, T., Pang, I. K., Kumamoto, Y., Peaper, D. R., Ho, J. H., Murray, T. S., et al. (2011). Microbiota regulates immune defense against respiratory tract influenza a virus infection. *Proc. Natl. Acad. Sci. USA*. 108, 5354–5359. doi: 10.1073/pnas.1019378108

Iddrisu, I., Monteagudo-Mera, A., Poveda, C., Pyle, S., Shahzad, M., Andrews, S., et al. (2021). Malnutrition and gut microbiota in children. *Nutrients* 13:2727. doi: 10.3390/nu13082727

Jang, H. M., Lee, K. E., Lee, H. J., and Kim, D. H. (2018). Immobilization stress-induced *Escherichia coli* causes anxiety by inducing NF-κB activation through gut microbiota disturbance. *Sci. Rep*. 8:13897. doi: 10.1038/s41598-018-31764-0

Jeong, J. J., Ganesan, R., Jin, Y. J., Park, H. J., Min, B. H., Jeong, M. K., et al. (2023). Multi-strain probiotics alleviate loperamide-induced constipation by adjusting the microbiome, serotonin, and short-chain fatty acids in rats. *Front. Microbiol.* 14:1174968. doi: 10.3389/fmicb.2023.1174968

Jiang, L., Wang, J., Xu, L., Cai, J., Zhao, S., and Ma, A. (2022). Lactobacillus casei modulates inflammatory cytokines and metabolites during tuberculosis treatment: A post hoc randomized controlled trial. *Asia Pac. J. Clin. Nutr.* 31, 66–77. doi: 10.6133/apjcn.202203_31(1).0008

Jin, S. W., Lee, G. H., Jang, M. J., Hong, G. E., Kim, J. Y., Park, G. D., et al. (2020). Immunomodulatory activity of lactococcus lactis gcwb1176 in cyclophosphamide-induced immunosuppression model. *Microorganisms* 8:1175. doi: 10.3390/microorganisms8081175

Kang, S., Kim, J., Park, A., Koh, M., Shin, W., Park, G., et al. (2023). TRIM40 is a pathogenic driver of inflammatory bowel disease subverting intestinal barrier integrity. *Nat. Commun.* 14:700. doi: 10.1038/s41467-023-36424-0

Kateete, D. P., Mbabazi, M. M., Nakazzi, F., Katabazi, F. A., Kigozi, E., Ssengooba, W., et al. (2021). Sputum microbiota profiles of treatment-naïve TB patients in Uganda before and during first-line therapy. *Sci. Rep*. 11:24486. doi: 10.1038/s41598-021-04271-y

Khan, N., Vidyarthi, A., Nadeem, S., Negi, S., Nair, G., and Agrewala, J. N. (2016). Alteration in the gut microbiota provokes susceptibility to tuberculosis. *Front. Immunol.* 7:529. doi: 10.3389/fimmu.2016.00529

Khan, T. J., Hasan, M. N., Azhar, E. I., and Yasir, M. (2019). Association of gut dysbiosis with intestinal metabolites in response to antibiotic treatment. *Hum. Microbiome J*. 11:100054. doi: 10.1016/j.humic.2018.11.004

Krishna, P., Jain, A., and Bisen, P. S. (2016). Microbiome diversity in the sputum of patients with pulmonary tuberculosis. *Eur. J. Clin. Microbiol. Infect. Dis.* 35, 1205–1210. doi: 10.1007/s10096-016-2654-4

Langdon, A., Crook, N., and Dantas, G. (2016). The effects of antibiotics on the microbiome throughout development and alternative approaches for therapeutic modulation. *Genome Med*. 8:39. doi: 10.1186/s13073-016-0294-z

Lazar, V., Ditu, L. M., Pircalabioru, G. G., Gheorghe, I., Curutiu, C., Holban, A. M., et al. (2018). Aspects of gut microbiota and immune system interactions in infectious diseases, immunopathology, and cancer. *Front. Immunol*. 9:1830. doi: 10.3389/fimmu.2018.01830

Lichtman, J. S., Ferreyra, J. A., Ng, K. M., Smits, S. A., Sonnenburg, J. L., and Elias, J. E. (2016). Host-microbiota interactions in the pathogenesis of antibiotic-associated diseases. *Cell Rep*. 14, 1049–1061. doi: 10.1016/j.celrep.2016.01.009

Lin, D., Wang, X., Li, Y., Wang, W., Li, Y., Yu, X., et al. (2021). Sputum microbiota as a potential diagnostic marker for multidrug-resistant tuberculosis. *Int. J. Med. Sci.* 18, 1935–1945. doi: 10.7150/ijms.53492

Marsland, B. J., Trompette, A., and Gollwitzer, E. S. (2015). The gut-lung axis in respiratory disease. *Ann. Am. Thorac. Soc.* 12(Suppl. 2), S150–S156. doi: 10.1513/AnnalsATS.201503-133AW

Maslowski, K. M., Vieira, A. T., Ng, A., Kranich, J., Sierro, F., Yu, D., et al. (2009). Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* 461, 1282–1286. doi: 10.1038/nature08530

Matsuura, N., Motoshima, H., Uchida, K., and Yamanaka, Y. (2022). Effects of Lactococcus lactis subsp. cremoris YRC3780 daily intake on the HPA axis response to acute psychological stress in healthy Japanese men. *Eur. J. Clin. Nutr.* 76, 574–580. doi: 10.1038/s41430-021-00978-3

McGhee, J. R., and Fujihashi, K. (2012). Inside the mucosal immune system. *PLoS Biol.* 10:e1001397. doi: 10.1371/journal.pbio.1001397

Meijer, K., De Vos, P., and Priebe, M. G. (2010). Butyrate and other short-chain fatty acids as modulators of immunity: What relevance for health? *Curr. Opin. Clin. Nutr. Metab. Care* 13, 715–721. doi: 10.1097/MCO.0b013e32833eebe5

Meng, R., Dong, W., Gao, J., Lu, C., Zhang, C., Liao, Q., et al. (2022). Clostridium, *Bacteroides* and Prevotella associates with increased fecal metabolites Trans-4-Hydroxy-L-proline and Genistein in active pulmonary tuberculosis patients during anti-tuberculosis chemotherapy with isoniazid-rifampin-pyrazinamide-ethambutol (HRZE). *Indian J. Microbiol.* 62, 374–383. doi: 10.1007/s12088-022-01003-2

Metsälä, J., Lundqvist, A., Virta, L. J., Kaila, M., Gissler, M., and Virtanen, S. M. (2015). Prenatal and post-natal exposure to antibiotics and risk of asthma in childhood. *Clin. Exp. Allergy* 45, 137–145. doi: 10.1111/cea.12356

Mily, A., Rekha, R. S., Kamal, S. M. M., Arifuzzaman, A. S. M., Rahim, Z., Khan, L., et al. (2015). Significant effects of oral phenylbutyrate and Vitamin D3 adjunctive therapy in pulmonary tuberculosis: A randomized controlled trial. *PLoS One* 10:e0138340. doi: 10.1371/journal.pone.0138340

Minichino, A., Brondino, N., Solmi, M., Del Giovane, C., Fusar-Poli, P., Burnet, P., et al. (2021). The gut-microbiome as a target for the treatment of schizophrenia: A systematic review and meta-analysis of randomised controlled trials of add-on strategies. *Schizophr. Res.* 234, 58–70. doi: 10.1016/j.schres.2020.02.012

Naidoo, C. C., Nyawo, G. R., Wu, B. G., Walzl, G., Warren, R. M., Segal, L. N., et al. (2019). The microbiome and tuberculosis: State of the art, potential applications, and defining the clinical research agenda. *Lancet Respir. Med*. 7, 892–906. doi: 10.1016/S2213-2600(18)30501-0

Namasivayam, S., Kauffman, K. D., Mcculloch, J. A., Yuan, W., Thovarai, V., Mittereder, L. R., et al. (2019). Correlation between disease severity and the intestinal microbiome in Mycobacterium tuberculosis-infected rhesus macaques. *Mbio* 10:19. doi: 10.1128/mBio

Namasivayam, S., Maiga, M., Yuan, W., Thovarai, V., Costa, D. L., Mittereder, L. R., et al. (2017). Longitudinal profiling reveals a persistent intestinal dysbiosis triggered by conventional anti-tuberculosis therapy. *Microbiome* 5:71. doi: 10.1186/s40168-017-0286-2

Nash, A. K., Auchtung, T. A., Wong, M. C., Smith, D. P., Gesell, J. R., Ross, M. C., et al. (2017). The gut mycobiome of the human microbiome project healthy cohort. *Microbiome* 5:153. doi: 10.1186/s40168-017-0373-4

National Center for Preventive Programs and Disease Control (2020). *Programa nacional para el control y prevención de la tuberculosis*. Available online at: www.gob.mx/salud/cenaprece

Nicholson, J. K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W., et al. (2012). Host-gut microbiota metabolic interactions. *Science* 336, 1262–1267. doi: 10.1126/science.1223813

Onyszkiewicz, M., Gawrys-Kopczynska, M., Konopelski, P., Aleksandrowicz, M., Sawicka, A., Koźniewska, E., et al. (2019). Butyric acid, a gut bacteria metabolite, lowers arterial blood pressure via colon-vagus nerve signaling and GPR41/43 receptors. *Pflugers Arch. Eur. J. Physiol.* 471, 1441–1453. doi: 10.1007/s00424-019-02322-y

Paton, N. I., Cousins, C., Suresh, C., Burhan, E., Chew, K. L., Dalay, V. B., et al. (2023). Treatment strategy for rifampin-susceptible tuberculosis. *N. Engl. J. Med.* 388, 873–887. doi: 10.1056/nejmoa2212537

Quigley, J., and Lewis, K. (2022). Noise in a metabolic pathway leads to persister formation in Mycobacterium tuberculosis. *Microbiol. Spectr.* 10:e0294822. doi: 10.1128/spectrum.02948-22

Rahim, M. A., Seo, H., Kim, S., Tajdozian, H., Barman, I., Lee, Y., et al. (2022). In vitro anti-tuberculosis effect of probiotic Lacticaseibacillus rhamnosus PMC203 isolated from vaginal microbiota. *Sci. Rep.* 12:8290. doi: 10.1038/s41598-022-12413-z

Raqib, R., Sarker, P., Mily, A., Alam, N. H., Arifuzzaman, A. S. M., Rekha, R. S., et al. (2012) Efficacy of sodium butyrate adjunct therapy in shigellosis: a randomized, double-blind, placebo-controlled clinical trial. *BMC Infect. Dis*. 12:111. doi: 10.1186/1471-2334-12-111

Reys, L. G., Ortiz-Pomales, Y. T., Lopez, N., Cheadle, G., De Oliveira, P. G., Eliceiri, B., et al. (2013). Uncovering the neuroenteric-pulmonary axis: Vagal nerve stimulation prevents acute lung injury following hemorrhagic shock. *Life Sci.* 92, 783–792. doi: 10.1016/j.lfs.2013.02.009

Rutten, E. P. A., Lenaerts, K., Buurman, W. A., and Wouters, E. F. M. (2014). Disturbed intestinal integrity in patients with COPD: Effects of activities of daily living. *Chest* 145, 245–252. doi: 10.1378/chest.13-0584

Samuelson, D. R., Gu, M., Shellito, J. E., Molina, P. E., Taylor, C. M., Luo, M., et al. (2021). Pulmonary immune cell trafficking promotes host defense against alcohol-associated Klebsiella pneumonia. *Commun. Biol.* 4:997. doi: 10.1038/s42003-021-02524-0

Schumann, A., Nutten, S., Donnicola, D., Comelli, E. M., Mansourian, R., Cherbut, C., et al. (2005). Neonatal antibiotic treatment alters gastrointestinal tract developmental gene expression and intestinal barrier transcriptome. *Physiol. Genom.* 23, 235–245. doi: 10.1152/physiolgenomics.00057.2005.-The

Sekyere, J. O., Maningi, N. E., and Fourie, P. B. (2020). Mycobacterium tuberculosis, antimicrobials, immunity and the lung-gut microbiota crosstalk: Current updates and emerging advances. *Ann. N. Y. Acad. Sci.* 1467, 21–47. doi: 10.1111/nyas.14300

Sencio, V., Machado, M. G., and Trottein, F. (2021). The lung–gut axis during viral respiratory infections: The impact of gut dysbiosis on secondary disease outcomes. *Mucosal Immunol.* 14, 296–304. doi: 10.1038/s41385-020-00361-8

Shi, J., Gao, G., Yu, Z., Wu, K., Huang, Y., Wu, L. P., et al. (2022). The relevance of host gut microbiome signature alterations on de novo fatty acids synthesis in patients with multi-drug resistant tuberculosis. *Infect. Drug Resist.* 15, 5589–5600. doi: 10.2147/IDR.S372122

Shi, W., Hu, Y., Ning, Z., Xia, F., Wu, M., Hu, Y. O. O., et al. (2021). Alterations of gut microbiota in patients with active pulmonary tuberculosis in China: A pilot study. *Int. J. Infect. Dis.* 111, 313–321. doi: 10.1016/j.ijid.2021.08.064

Shreiner, A. B., Kao, J. Y., and Young, V. B. (2015). The gut microbiome in health and in disease. *Curr. Opin. Gastroenterol.* 31, 69–75. doi: 10.1097/MOG.0000000000000139

Sun, M., Wu, W., Liu, Z., and Cong, Y. (2017). Microbiota metabolite short chain fatty acids, GPCR, and inflammatory bowel diseases. *J. Gastroenterol.* 52, 1–8. doi: 10.1007/s00535-016-1242-9

Sze, M. A., Hogg, J. C., and Sin, D. D. (2014). Bacterial microbiome of lungs in COPD. *Int. J. COPD* 9, 229–238. doi: 10.2147/COPD.S38932

Teo, A. K. J., Singh, S. R., Prem, K., Hsu, L. Y., and Yi, S. (2021). Duration and determinants of delayed tuberculosis diagnosis and treatment in high-burden countries: A mixed-methods systematic review and meta-analysis. *Respir. Res.* 22:251. doi: 10.1186/s12931-021-01841-6

Thaiss, C. A., Zmora, N., Levy, M., and Elinav, E. (2016). The microbiome and innate immunity. *Nature* 535, 65–74. doi: 10.1038/nature18847

Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R., and Gordon, J. I. (2007). The human microbiome project. *Nature* 449, 804–810. doi: 10.1038/nature06244

Usuda, H., Okamoto, T., and Wada, K. (2021). Leaky gut: Effect of dietary fiber and fats on microbiome and intestinal barrier. *Int. J. Mol. Sci.* 22:7613. doi: 10.3390/ijms22147613

Valdez-Palomares, F., Torrico, M. M., Palacios-González, B., Soberón, X., and Silva-Herzog, E. (2021). Altered microbial composition of drug-sensitive and drug-resistant TB patients compared with healthy volunteers. *Microorganisms* 9:1762. doi: 10.3390/microorganisms9081762

Vázquez-Pérez, J. A., Carrillo, C. O., Iñiguez-García, M. A., Romero-Espinoza, I., Márquez-García, J. E., Falcón, L. I, et al. (2020). Alveolar microbiota profile in patients with human pulmonary tuberculosis and interstitial pneumonia. *Microb. Pathog.* 139:103851. doi: 10.1016/j.micpath.2019.103851

Wang, J., Xiong, K., Zhao, S., Zhang, C., Zhang, J., Xu, L., et al. (2020). Long-term effects of multi-drug-resistant tuberculosis treatment on gut microbiota and its health consequences. *Front. Microbiol.* 11:53. doi: 10.3389/fmicb.2020.00053

Wei, Y., Zhu, W., Gong, J., Guo, D., Gu, L., Li, N., et al. (2015). Fecal microbiota transplantation improves the quality of life in patients with inflammatory bowel disease. *Gastroenterol. Res. Pract.* 2015:517597. doi: 10.1155/2015/517597

Weiss, G. A., and Hennet, T. (2017). Mechanisms and consequences of intestinal dysbiosis. *Cell. Mol. Life Sci.* 74, 2959–2977. doi: 10.1007/s00018-017-2509-x

Wheatley, R. M., Caballero, J. D., van der Schalk, T. E., De Winter, F. H. R., Shaw, L. P., Kapel, N., et al. (2022). Gut to lung translocation and antibiotic mediated selection shape the dynamics of *Pseudomonas aeruginosa* in an ICU patient. *Nat. Commun.* 13:6523. doi: 10.1038/s41467-022-34101-2

Willing, B. P., Russell, S. L., and Finlay, B. B. (2011). Shifting the balance: Antibiotic effects on host-microbiota mutualism. *Nat. Rev. Microbiol.* 9, 233–243. doi: 10.1038/nrmicro2536

Wipperman, M. F., Fitzgerald, D. W., Juste, M. A. J., Taur, Y., Namasivayam, S., Sher, A., et al. (2017). Antibiotic treatment for Tuberculosis induces a profound dysbiosis of the microbiome that persists long after therapy is completed. *Sci. Rep.* 7:10767. doi: 10.1038/s41598-017-10346-6

World Health Organization [WHO] (2021a). *Global tuberculosis report 2021*. Geneva: *WHO*.

World Health Organization [WHO] (2021b). *Module 3: Diagnosis WHO consolidated guidelines on tuberculosis Rapid diagnostics for tuberculosis detection*. Geneva: WHO.

World Health Organization [WHO] (2022a). *Global Tuberculosis report 2022*. Geneva: *WHO*.

World Health Organization [WHO] (2022b). *WHO consolidated guidelines on drug-resistant tuberculosis treatment*. Geneva: WHO.

World Health Organization [WHO] (2022c). *WHO consolidated guidelines on tuberculosis. Module 4, Treatment: Drug-resistant tuberculosis treatment*. Geneva: WHO.

Wypych, T. P., Wickramasinghe, L. C., and Marsland, B. J. (2019). The influence of the microbiome on respiratory health. *Nat. Immunol.* 20, 1279–1290. doi: 10.1038/s41590-019-0451-9

Xiao, G., Cai, Z., Guo, Q., Ye, T., Tang, Y., Guan, P., et al. (2022). Insights into the unique lung microbiota profile of pulmonary tuberculosis patients using metagenomic next-generation sequencing. *Microbiol. Spectr.* 10:e0190121.

Ye, S., Wang, L., Li, S., Ding, Q., Wang, Y., Wan, X., et al. (2022). The correlation between dysfunctional intestinal flora and pathology feature of patients with pulmonary tuberculosis. *Front. Cell. Infect. Microbiol.* 12:1090889. doi: 10.3389/fcimb.2022.1090889

Yoo, J. Y., Groer, M., Dutra, S. V. O., Sarkar, A., and McSkimming, D. I. (2020). Gut microbiota and immune system interactions. *Microorganisms* 8:1587. doi: 10.3390/microorganisms8101587

Yuan, H., and Silberstein, S. D. (2016). Vagus nerve and vagus nerve stimulation, a comprehensive review: Part II. *Headache* 56, 259–266. doi: 10.1111/head.12650

Zevin, A. S., McKinnon, L., Burgener, A., and Klatt, N. R. (2016). Microbial translocation and microbiome dysbiosis in HIV-associated immune activation. *Curr. Opin. HIV AIDS* 11, 182–190. doi: 10.1097/COH.0000000000000234

Zhang, D., Jian, Y. P., Zhang, Y. N., Li, Y., Gu, L. T., Sun, H. H., et al. (2023). Short-chain fatty acids in diseases. *Cell Commun. Signal.* 21:212. doi: 10.1186/s12964-023-01219-9

Zhang, M., Shen, L., Zhou, X., and Chen, H. (2022). The microbiota of human lung of pulmonary tuberculosis and the alteration caused by anti-tuberculosis drugs. *Curr. Microbiol.* 79:321. doi: 10.1007/s00284-022-03019-9

Zhang, M., Yu, W., Zhou, S., Zhang, B., Lo, E. C. M., Xu, X., et al. (2021). In vitro antibacterial activity of an FDA-approved H+-ATPase inhibitor, bedaquiline, against streptococcus mutans in acidic milieus. *Front. Microbiol.* 12:647611. doi: 10.3389/fmicb.2021.647611

Zhang, Y., Yew, W. W., and Barer, M. R. (2012). Targeting persisters for tuberculosis control. *Antimicrob. Agents Chemother.* 56, 2223–2230. doi: 10.1128/AAC.06288-11

Zhao, M., Shao, F., Yu, D., Zhang, J., Liu, Z., Ma, J., et al. (2022). Maturation and specialization of group 2 innate lymphoid cells through the lung-gut axis. *Nat. Commun.* 13:7600. doi: 10.1038/s41467-022-35347-6

Zheng, D., Liwinski, T., and Elinav, E. (2020). Interaction between microbiota and immunity in health and disease. *Cell Res.* 30, 492–506. doi: 10.1038/s41422-020-0332-7

frontiers | Frontiers in Microbiology

TYPE Review
PUBLISHED 11 December 2023
DOI 10.3389/fmicb.2023.1295215

OPEN ACCESS

EDITED BY
Chun-Hsing Liao,
National Yang Ming Chiao Tung University, Taiwan

REVIEWED BY
Yen-Chu Huang,
Taichung Veterans General Hospital, Taiwan
Naser Alsharairi,
Griffith University, Australia
Wendy Fonseca,
University of Michigan, United States

*CORRESPONDENCE
Joaquín Zúñiga
joazu@yahoo.com
Angel Camarena
ang_edco@yahoo.com.mx

RECEIVED 15 September 2023
ACCEPTED 15 November 2023
PUBLISHED 11 December 2023

CITATION
Galeana-Cadena D, Gómez-García IA, Lopez-Salinas KG, Irineo-Moreno V, Jiménez-Juárez F, Tapia-García AR, Boyzo-Cortes CA, Matías-Martínez MB, Jiménez-Alvarez L, Zúñiga J and Camarena A (2023) Winds of change a tale of: asthma and microbiome.
Front. Microbiol. 14:1295215.
doi: 10.3389/fmicb.2023.1295215

Winds of change a tale of: asthma and microbiome

David Galeana-Cadena[1], Itzel Alejandra Gómez-García[1,2], Karen Gabriel Lopez-Salinas[1,2], Valeria Irineo-Moreno[1,2], Fabiola Jiménez-Juárez[1,2], Alan Rodrigo Tapia-García[1,3], Carlos Alberto Boyzo-Cortes[1], Melvin Barish Matías-Martínez[1,2], Luis Jiménez-Alvarez[1], Joaquín Zúñiga[1,2]* and Angel Camarena[1]*

[1]Laboratorio de Inmunobiología y Genética, Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas (INER), Mexico City, Mexico, [2]Tecnologico de Monterrey, Escuela de Medicina y Ciencias de la Salud, Mexico City, Mexico, [3]Red de Medicina para la Educación, el Desarrollo y la Investigación Científica de Iztacala, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, Mexico City, Mexico

The role of the microbiome in asthma is highlighted, considering its influence on immune responses and its connection to alterations in asthmatic patients. In this context, we review the variables influencing asthma phenotypes from a microbiome perspective and provide insights into the microbiome's role in asthma pathogenesis. Previous cohort studies in patients with asthma have shown that the presence of genera such as *Bifidobacterium, Lactobacillus, Faecalibacterium*, and *Bacteroides* in the gut microbiome has been associated with protection against the disease. While, the presence of other genera such as *Haemophilus, Streptococcus, Staphylococcus*, and *Moraxella* in the respiratory microbiome has been implicated in asthma pathogenesis, indicating a potential link between microbial dysbiosis and the development of asthma. Furthermore, respiratory infections have been demonstrated to impact the composition of the upper respiratory tract microbiota, increasing susceptibility to bacterial diseases and potentially triggering asthma exacerbations. By understanding the interplay between the microbiome and asthma, valuable insights into disease mechanisms can be gained, potentially leading to the development of novel therapeutic approaches.

KEYWORDS

asthma, microbiota, exacerbations, gut-lung axis, diversity, environmental factors, asthma phenotypes

1 Introduction

Asthma is a common respiratory disease that affects individuals of all ages. It is now recognized as a condition with several phenotypes and as a group of several distinct diseases, known as endotypes. Some asthma phenotypes that have been described include young individuals with allergies, overweight middle-aged individuals, and elderly individuals with unhealthy aging, among many others. However, their similarities give rise to a common syndrome characterized by reversible airway obstruction, nonspecific airway hyperresponsiveness, and chronic airway inflammation (Kuruvilla et al., 2019; Hizawa, 2023).

The underlying pathogenesis of asthma is extremely complex and diverse, with a significant economic impact due to the need for long-term treatment (Nurmagambetov et al., 2018) and a potential decrease in quality of life. Clinically, asthma is a chronic airways disease characterized

by recurrent episodes of wheezing, coughing, thoracic oppression, and dyspnea (GINA Report, 2022). The immune system plays a central role in the pathophysiology of asthma, involving the inflammatory response and sensitivity to allergens (Bush, 2019). Furthermore, recent research has highlighted the importance of the microbiome in the development of the immune response, as it is educated and modified by microorganisms and metabolites of the microbiome (Zheng et al., 2020). On the other hand, respiratory diseases have been associated with decreased microbial diversity, termed dysbiosis, defined as deviation from a normal microbial composition, is associated with a number of adverse biological phenomena, sometimes with clinical consequences (Natalini et al., 2023). Respiratory and gut dysbiosis modifies immune system responses which influences inflammation in the lungs, leading to a potential role in asthma pathophysiology, phenotypes, and clinical outcomes (Ver Heul et al., 2019; Hufnagl et al., 2020). Typically, attention is usually focused on a single point, involving the analysis of microbiota from singular anatomical sites during specific developmental stages or, in certain instances, restricting the focus solely to pediatric and adult cohorts (Zimmermann et al., 2019; Losol et al., 2021; Aldriwesh et al., 2023). However, a noteworthy challenge arises when endeavoring to amalgamate shared findings from diverse studies. While certain commonalities have been identified, their respective implications vary depending upon the contextual framework (Barcik et al., 2020; Lupu et al., 2023; Zhao et al., 2023). Consequently, it has proven to be quite formidable to identify a specific taxonomic group that consistently influences or mitigates the risk factors or clinical presentations of asthma across all scenarios. Thus, we assert the significance of exploring the role of the microbiome within the context of its development, eschewing the presumption that a particular taxonomic group universally assumes an identical role in all circumstances. In this context, we have conducted a comprehensive review to explore the diverse roles of the microbiome in relation to the phenotypes and endotypes of asthma throughout human growth and development, encompassing prenatal factors, birth, childhood, adolescence, adulthood, and the elderly.

2 Microbiome

The microbiome encompasses the microbiota, their genetic material, metabolites, and the surrounding microenvironment (Berg et al., 2020). Each body site has its own distinct composition and complexity of microorganisms. When evaluating the microbiome based on sequencing data, two important terms are often employed: alpha diversity, which measures the number and abundance of microorganisms in a specific sample or site, and beta diversity, which quantifies the variation in microorganisms between different samples or sites (Finotello et al., 2018). Within the microbiome, a multitude of commensal microorganisms have undergone co-evolution with human cells; giving rise to complex, dynamic, interdependent, and context-dependent relationships essential for maintaining ecosystem balance within their respective communities, a state referred to as eubiosis (Iebba et al., 2016). Eubiosis and host-microbiome relationship influence various host functions, including metabolism, immunity, circadian rhythms, nutritional responses, and homeostasis (Zheng et al., 2020). In order to achieve its functions, human cells engage in intricate communication mechanisms through a system-system axis, enabling coordinated responses across different organs such as the gut-brain axis and gut-lung axis (Suganya and Koo, 2020; Ahlawat et al., 2021).

The gut-lung axis plays a significant role in respiratory pathologies as it establishes a bidirectional pathway for the transmission of internal and external factors, creating a signaling network that can influence systemic functions and responses (Enaud et al., 2020). The composition of the microbiome on mucosal surfaces, including the gastrointestinal and respiratory tracts, is highly dynamic. The gut microbiota consists of approximately 3,594 species, primarily classified under the phyla *Actinobacteria, Bacteroidota, Firmicutes*, and *Proteobacteria* (Leviatan et al., 2022). Contrary to previous beliefs, it is now known that the human lung harbors a distinct lung microbiota, mainly composed of genera such as *Prevotella, Veillonella*, and *Streptococcus*, thanks to advancements in sequencing techniques (Dickson et al., 2016; Yagi et al., 2021; Natalini et al., 2023). Molecular signals, including short-chain fatty acids (SCFAs) produced by *Bifidobacterium, Lactobacillus, Faecalibacterium*, and *Ruminococcus* (Tsukuda et al., 2021), facilitate communication between these organs through circulation or via the vagus nerve. Several species have also shown marked effects as neuromodulators and neurotransmitters such as monoamines, serotonin, and brain-derived neurotrophic factor (Suganya and Koo, 2020). *Lactobacillus* is strongly involved in both the gut-lung axis and the brain-gut axis (Rastogi and Singh, 2022), and can produce GABA and activate receptor expression, leading to cognitive enhancement via the vagus nerve (Breit et al., 2018). Oral ingestion of *L. rhamnosus* and *L. murinus* promotes migration of T Regulatory Cell (Treg) to the lungs and blocks the Th2 response (Zhang et al., 2018a; Han et al., 2021), thereby reducing respiratory inflammation (Figure 1). Wang et al. also prove that *L. fermentum* can reduce the expression of Toll-like Receptor 2 and Toll Like Receptor 4 in OVA mice model, with concurrent reduction in inflammatory cell infiltration and alveolar swelling (Wang et al., 2022). The composition and function of the microbiome in both the intestine and the lung are influenced by a variety of factors, including genetics, the immune system, pregnancy, birth conditions, age, dietary habits, pollution, antibiotics, and lifestyle (Martino et al., 2022; Figure 2).

Abbreviations: WHO, World Health Organization; IL, Interleukins; TNF, Tumor Necrosis Factor; IFN, Interferon; Tregs, T Regulatory Cells; Th, T Helper Cells; GM-CSF, Granulocyte-Macrophage Colony-Stimulating Factor; ICAM, Intercellular Adhesion Molecule; NF-kB, Nuclear Factor-kappa B; TAK1, Transforming Growth Factor Beta-Activated Kinase 1; MAPK, Mitogen-Activated Protein Kinase; SCFAs, Short-Chain Fatty Acids; FEV1, Forced Expiratory Volume in 1 s; mbGWAS, microbiome- genome wide association; mbQTL, microbiome quantitative trait loci; PEF, Peak Expiratory Flow; C-section, Cesarean section; RSV, Respiratory Syncytial Virus; HRV, Human Rhinovirus; LPS, Lipopolysaccharide; TLR, Toll-Like Receptors; GABA, Gamma-Aminobutyric Acid; OVA, Ovalbumin; CDKIs, Cyclin-Dependent Kinase Inhibitors; SA- beta gal, Senescence-Associated Beta-Galactosidase; ROS, Reactive Oxygen Species; MetS, metabolic syndrome; ICS, Inhalated corticosteroids; OCS, Oral Corticosteroid.

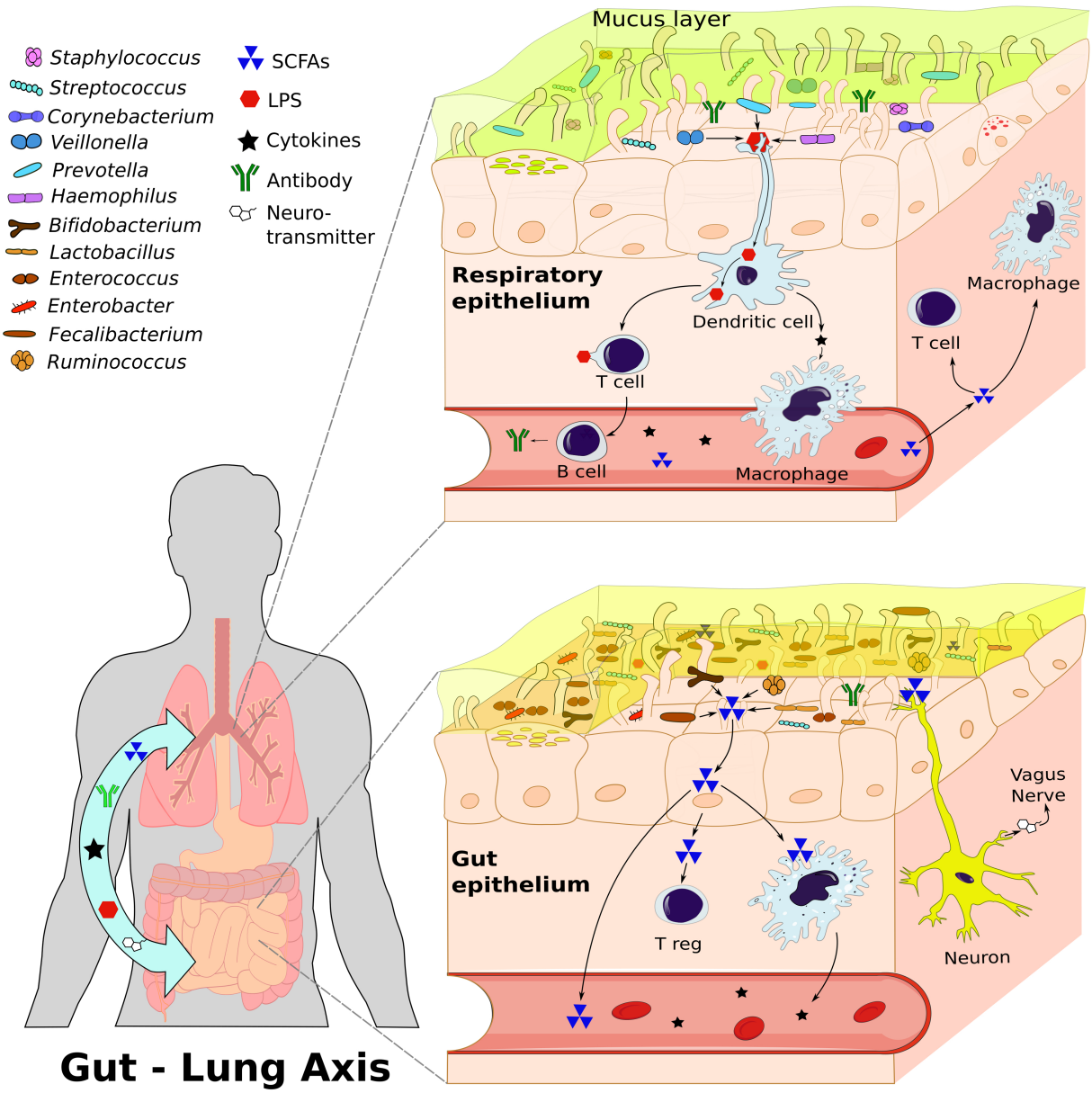


FIGURE 1
The Gut-Lung Axis: A bidirectional communication network between the gastrointestinal tract and the respiratory system involving interactions among immune cells, neurons, microbiota, and signaling molecules. This figure illustrates the composition of both lung and gut microbiota and the dynamic interactions within this axis; highlighting the role of microbiome dependent communication. This communication stimulates the immune cells through signal molecules, such as short-chain fatty acids (SCFAs), lipopolysaccharide (LPS), and cytokines. Additionally, through the vagus nerve, the microbiome stimulates the production of neurotransmitters. All these molecules can travel through the bloodstream to influence both the gut and lung systems. Created with inkscape.

3 Microbiome and asthma: the importance of the beginning

The journey of an individual begins with the intricate process of fertilization and genetic recombination, which sets the foundation for the expression of unique characteristics that will define their existence. However, the conditions of birth, growth, and development that an individual experience will play a crucial role in shaping their traits and creating the environment in which they thrive; shaping an individual's life journey.

3.1 Genetics factors

Asthma is considered a chronic complex disease that is the consequence of an interaction between genetic and environmental factors. Over 100 genes have been associated with asthma and the features of the disease; however, there is marked variability in replication attempts in independent studies. Genetic variants on chromosome 17q21 near to ORMDL3/GSDMB locus have been associated with childhood-onset asthma (Ntontsi et al., 2021; Afzal et al., 2023). Kumar et al. highlighted the association between the

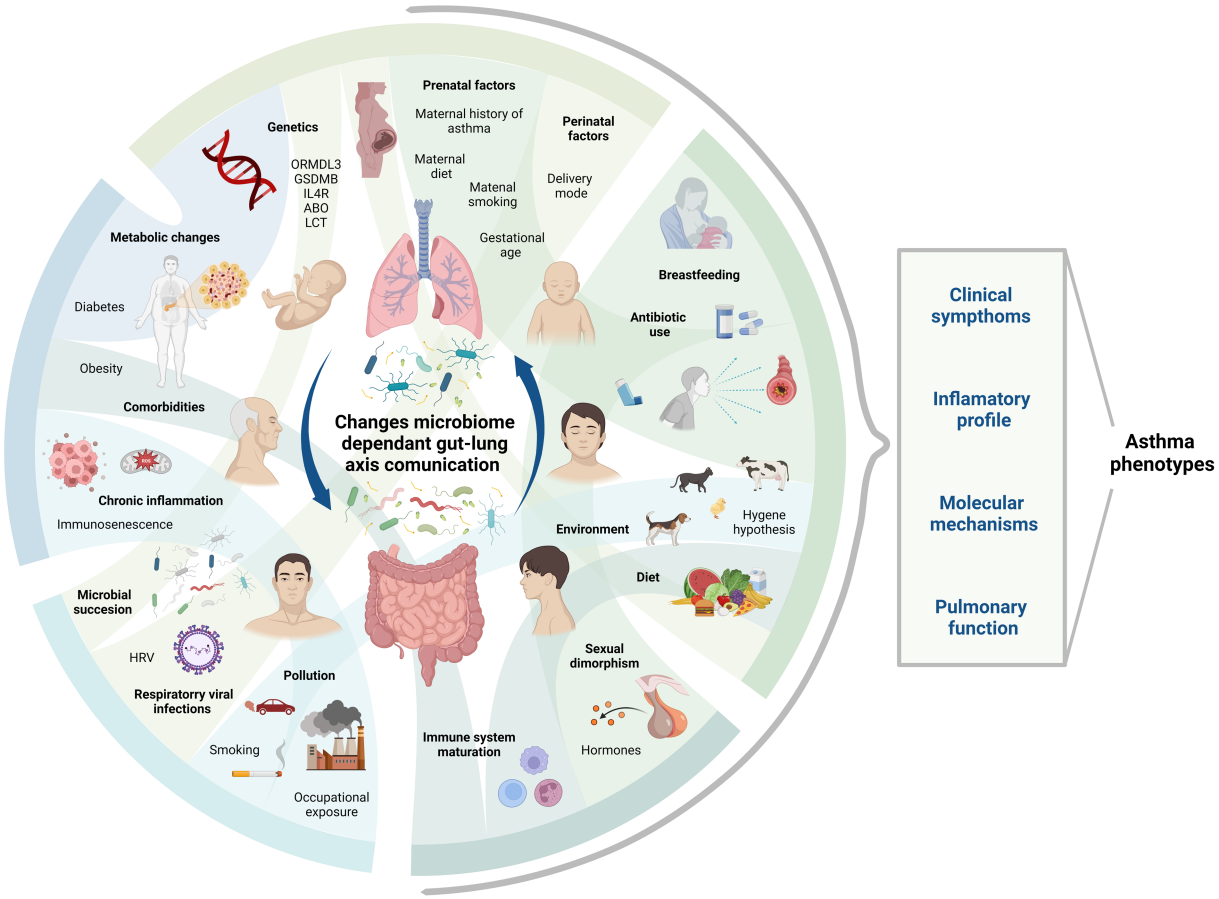


FIGURE 2
Asthma phenotype and factors determining microbiome through life stages. This figure shows some of the factors influencing asthma phenotype and microbiome composition during different life stages. Their complex interactions require study in order to comprehend their role in the context of gut-lung axis communication via microbiome and asthma development. Created with BioRender.com. Bio render agreement number: PT264MFOUQ.

variant rs1805011 in the IL-4 receptor gene and Th1/Th2 differentiation, which increases susceptibility to asthma (Kumar et al., 2015). This finding shed light on the intricate interplay between genetic factors and T-cell responses.

In this context, there has recently been growing interest in understanding the role of host genetics in shaping the gut microbiome, and several studies have shed light on this complex interplay. Boulund et al., identified significant correspondences in microbial taxa that are partly regulated by host genotype, with host genes associated with these taxa being related to secretion-metabolism, signaling-transport, and immunity (Boulund et al., 2022). Similarly, Lopera-Mayá et al., conducted a genome-wide association study to comprehensively characterize the effects of host genetics on the gut microbiome, discovering two study-wide significant signals near the Lactase and ABO genes with the *Bifidobacterium* and *Collinsella* genera, respectively (Lopera-Maya et al., 2022). Rühlemann et al., reported an association between the *Prevotella* genus in asthma and ABO blood groups (Rühlemann et al., 2021). Additionally, Ahluwalia et al. proposed that variations in the Fucosyltransferase 2 (FUT2) and ABO genes, along with epistatic effects, may contribute to an increased risk of early childhood asthma (Ahluwalia et al., 2020). They suggest that the expression of AB antigens in the respiratory epithelium and *Streptococcus pneumoniae* infection may be involved. Kurilshikov et al., conducted a genome-wide association study investigating human host genetic variation's impact on microbial taxa. They identified 31 loci that influence the gut microbiome. Among these loci, the LCT gene stands out as being particularly significant for the Bifidobacterium genus (Kurilshikov et al., 2021). Regarding the gut microbiome, host genetics, and asthma, Li et al., described that, through a two-sample Mendelian randomization analysis, they predicted a positive correlation between the gut species *Barnesiella* and *RuminococcaceaeUCG014* genera and the risk of asthma. Furthermore, they found that *Akkermansia* reduced the risk of adult-onset asthma (Li et al., 2023). Perez-Garcia et al., through a study of microbiome- genome wide association (mbGWAS) and microbiome quantitative trait loci (mbQTL) analysis, reported the identification of polymorphisms in the APOBEC3B-APOBEC3c, TRIM24, and TPST2 genes that are associated with asthma comorbidities. These polymorphisms were found to be mbQTLs related to *Streptococcus, Tannerella*, and *Campylobacter* in the upper airway (Perez-Garcia et al., 2020). Nevertheless, as proposed by Chen et al., it is suggested that risk variants on 17q12-21 and perturbations in the maturation of intestinal microbiota associate independently and exhibit additive effects on the risk of asthma development (Chen et al., 2023). Therefore, more studies are needed to define the role of host genetics in microbial diversity and asthma.

3.2 Prenatal factors

Prenatal factors have been associated with increased susceptibility to asthma, as demonstrated by cross-sectional studies and systematic reviews (Castro-Rodriguez et al., 2016; Arif and Veri, 2019; Esmeraldino et al., 2022). These factors include maternal smoking (Moradzadeh et al., 2018), maternal history of asthma (García-Serna et al., 2021), antibiotic use during pregnancy (Alhasan et al., 2020), maternal diet (Gray et al., 2017), and maternal stress (Van De Loo et al., 2016; Douros et al., 2017), among others.

Furthermore, the human fetal immune system initiates its development during the first four weeks of gestation (Park et al., 2020). Early exposures to metabolites from the maternal microbiota contribute to establishing a functional immune response at birth (Donald and Finlay, 2023). In a murine model, the transfer of antigen-specific IgG during fetal development provides protection to the offspring against allergic airway inflammation (Nakata et al., 2010). This suggests that antibodies play a dual role by not only safeguarding against particular pathogens but also contributing to the establishment of tolerance and recognition of commensal bacteria that will colonize the newborn (Koch et al., 2016; Macpherson et al., 2017; Mimoun et al., 2020). Additionally, the maternal microbiota prepares the newborn for host-microbial mutualism, which results from microbial metabolite transfer. By transiently colonizing pregnant female mice, with *E. coli* HA107, the maternal microbiota shapes the immune system of the offspring. Maternal microbial metabolites increases intestinal group 3 innate lymphoid cells and F4/80 + CD11c (Gomez de Agüero et al., 2016). Regarding the translocation of maternal microorganisms for fetal colonization, Jimenez et al. designed an experiment using a labeled strain of *Enterococcus faecium*. They orally inoculated pregnant mice and successfully recovered the microorganism from the meconium and amniotic fluid of the cesarean-born animals (Jiménez et al., 2008). However, a systematic review reports that the meconium microbiota in humans begins to develop after birth (Turunen et al., 2023).

On the other hand, the administration of antibiotics closer to parturition has been shown to have a significant impact on the diversity of both neonatal and maternal microbiota. Specifically, it has been found to increase the abundance of the phylum Proteobacteria in neonates exposed to antibiotics. In contrast, unexposed neonates tend to have a dominance of phylum Firmicutes with families such as Streptococcaceae and Lactobacillaceae (Stiemsma and Michels, 2017).

Furthermore, antibiotics can disrupt the maternal microbiome in the vagina. For example, the administration of antibiotics to the mother during the intrapartum period before birth, as well as the duration of rupture of membranes (ROM), have been found to be significantly associated with a decreased transmission rate of *Lactobacillus*-dominant mixed flora to neonates (Keski-Nisula et al., 2013). Considering all of the above, we believe it is necessary to continue investigating prenatal factors in the development of the newborn's immune system and microbiome.

3.3 Perinatal factors

The process of birth represents a complex series of changes, including the first interaction between the newborn and the microbiome along with the onset of immune system training. In the beginning, we were born with an immature immune system mainly dependent on the innate immune system. Neonatal dendritic cells (DCs) exhibit adult levels of the immunoregulatory cytokine IL-10 when stimulated with lipopolysaccharides (LPS) from the maternal microbiome, but they are less proficient in promoting T helper 1 (Th1) cell differentiation due to delayed IL-12 production. Instead, the neonatal immune system tends to favor immunoregulatory and Th2 cell responses. This serves as a protective mechanism to prevent excessive inflammatory responses to novel antigens found in the environment and commensal microorganisms of their own microbiota (Donald and Finlay, 2023).

Hygiene hypothesis proposes that during early life, exposure to exogenous determinants such as breastfeeding, environment and microbiome plays a protective role against allergic diseases by facilitating the maturation of the immune system. This critical period of exposure spans from perinatal life until school-age (Liu, 2007; Garn et al., 2021; Pfefferle et al., 2021). In essence, it suggests that a lack of exposure to microorganisms may result in impaired immune tolerance development.

The initial colonization of microorganisms is closely linked to the mode of delivery and gestational age at birth (Castro-Rodriguez et al., 2016). When a baby is born vaginally, they are immediately exposed to microorganisms primarily inhabiting the maternal gut lumen and vagina, such as *Lactobacillus, Bacteroides*, and *Bifidobacterium* genera (Kalbermatter et al., 2021). Consequently, the gut microbiota of newborns born vaginally tends to be similar to that of their mothers (Song et al., 2021; Yao et al., 2021). Within the first days after birth, Clostridiaceae, Enterococcaceae, and Streptococcaceae families are observed, followed by the appearance of *Bacteroides* and *Bifidobacterium* in the guts of 40% of infants after the third day (Yao et al., 2021). In contrast, the colonization of the upper respiratory tract initiates with *Staphylococcus* and *Streptococcus* followed by the proliferation of *Moraxella, Corynebacterium, Dolosigranulum*, and/or *Haemophilus* species (Bosch et al., 2016), which are associated with reduced risk of respiratory symptoms (Biesbroek et al., 2014; Teo et al., 2015).

However, the colonization process can be interrupted for several reasons, mainly Cesarean section (C-section) delivery. Compared to babies born vaginally, infants delivered via C-section share roughly 30% fewer bacterial species with their mothers (Kalbermatter et al., 2021). In the upper respiratory tract Bosh et al., reported a lower abundance of *Corynebacterium and Dolosigranulum,* especially in the first months of life (Bosch et al., 2016). In gut microbiota, these newborns primarily harbor microbes from the maternal skin and antibiotic-resistant bacteria from the hospital environment, including *Staphylococcus, Streptococcus*, and *Clostridium*, which can alter its maturation (Rutayisire et al., 2016).

In fact, evidence suggests that alteration of microbiota by C-section, especially the elective one, is associated with alterations in the immune system, increasing the risk for developing asthma, allergies, type I diabetes mellitus, and celiac disease (Salas Garcia et al., 2018; Ferllini Montealegre et al., 2019; Kumbhare et al., 2019). Słabuszewska-Jóźwiak et al. in a meta-analysis study for C-section delivery and asthma in offspring, reported an odds ratio of 1.23 (95%CI 1.14–1.33, $p < 0.00001$) and a higher frequency of asthma in the C-section delivered children (Słabuszewska-Jóźwiak et al., 2020). Another cohort study revealed a relative risk of 1.11 (95% CI 1.00 to 1.25) in children with partially controlled asthma which increased to

1.8 (95% CI 1.00 to 1.39) in children with uncontrolled asthma (Moore et al., 2023). This risk could be explained because children who maintain a microbiota associated with C-section display a different immune response during respiratory symptom episodes, with lower levels of TNF-a, IL-4, IL-13, or IL-1b. Additionally, infants delivered by C-section to long-term have high levels of IgE making them susceptible to asthma or the development of allergies (Stokholm et al., 2018).

C-section delivery is more common in premature babies (infants born before 37 weeks of gestation) and has been associated with the development of various health problems, including asthma. Also, preterm can lead to delayed and reduced gut colonization by beneficial bacteria like *Bacteroides, Bifidobacterium*, and *Lactobacillus* species. This creates an opportunity for other bacteria, such as family Clostridiaceae, to colonize gut lumen, potentially contributing to the development of asthma and allergic disorders (Zimmermann et al., 2019). Zhang et al., through a meta-analysis reported that prematures have up to a 36% higher risk of asthma compared to infants born at term estimated (Zhang et al., 2018b). Furthermore, formula feeding in premature babies reduces overall gut microbial diversity and reduces *Bifidobacterium* levels (Healy et al., 2022). A Swiss cohort study that followed 4, millions of births up to 46 years of age, described that the risk of developing asthma increases as the age of the infant at birth decreases, with a probability 1.5 to 2.5 times greater than that of a full-term newborn (Crump et al., 2023). Moreover, premature infants often experience reduced lung function and structural alterations in the lungs, leading to airflow problems (Arroyas et al., 2020).

Furthermore, preterm infants exhibit elevated rates of hospitalization related to Respiratory Syncytial Virus (RSV), admissions to the intensive care unit, the need for mechanical ventilation, and extended hospital stays when contrasted with full-term infants (Anderson et al., 2017). In a multicenter prospective cohort study involving 221 infants affected by RSV bronchiolitis, Raita et al. identified an endotype characterized by several distinctive features. This endotype included a high prevalence of parental asthma, IgE sensitization, and concurrent rhinovirus (HRV) infection. Notably, the co-dominance of *Streptococcus pneumoniae* and *Moraxella catarrhalis* in the nasopharynx, along with an elevated IFN-α and -γ response, were also prominent characteristics. This particular endotype was significantly associated with an increased risk of developing childhood asthma. It's worth noting that among these patients, 22.2% were born prematurely (Raita et al., 2021). Importantly, infection with HRV-C has been linked to more severe asthma exacerbations compared to HRV-A and HRV-B (Bizzintino et al., 2011).

These infections reduce Th1 and IFN-γ responses, leading to an increase in Th2 responses, which promote inflammation and bronchoconstriction (Pinto et al., 2006). Additionally, premature infants often have elevated levels of IL-17 from Th17 cells, which further exacerbates inflammation, resulting in characteristics of both eosinophilic asthma (IL-4 and IL-13) and neutrophilic asthma (IL-17), leading to a combined type of asthma (Chesné et al., 2014; Anderson et al., 2023).

While progress has been made in the study of the aforementioned factors, additional long-term longitudinal studies are still needed to understand the effects of changes in the microbiome during the perinatal period.

4 Infancy and childhood, imprint development

Childhood asthma is a prevalent and complex respiratory condition that affects millions of children worldwide, posing significant health challenges and burdens. According to literature review, early-life microbiota disturbances can lead to immune alterations, including T-reg cells proliferation, Th17 response, and IgE response in humans (Lee and Kim, 2017). Furthermore, the gut and airway microbiota in the first year of life has been reported to induce T-reg cells that enhance tolerogenic immunity in infants at high risk for asthma (Busse and Rosenwasser, 2003). Multiple investigations have shown differences between the lung and gut microbiome of individuals with established asthma vs. healthy subjects, being the population with asthma the ones with lower bacterial diversity (Marsland et al., 2015; Carr et al., 2019; Liu et al., 2020). Case–control studies have associated gut dysbiosis with a reduction in the specific genera *Faecalibacterium, Bifidobacterium, Lachnospira, Veillonella*, and *Rothia* (Yap et al., 2014; Arrieta et al., 2015). Therefore, systematic review highlights the importance of establishing a healthy microbiota during the first years of life. Notably, breastfeeding (Doherty et al., 2018) and supplementation with *Lactobacillus* (Durack et al., 2018; Alliet et al., 2022), have long-lasting potential for immune development and reduce the risk of developing asthma and allergic conditions.

4.1 Breastfeeding and changes in diet

Evidence suggests that the microbiome in children is influenced to a greater extent by first feeding method and dietary intake. Human milk, contains immunomodulators and anti-inflammatory agents such as alpha-tocopherol, beta-casomorphins, prolactin, lactoferrin, lysozyme, antioxidants, cytokines, and secretory IgA (Miliku and Azad, 2018), which promote the proper development of both mucosal and systemic immune systems, playing an important role in shaping a more robust immune system compared to the immune system of formula fed infants (Munblit et al., 2017; Domenici and Vierucci, 2022).

The presence of certain bacterial genera, including *Staphylococcus, Streptococcus, Lactobacillus,* and *Bifidobacterium*, has been consistently reported in human milk (Boquien, 2018; Lyons et al., 2020). According to a randomized double-blind trial in Sweden, the richness of bacterial species in breast milk appears to be critical in preventing the overgrowth of potentially harmful species associated with asthma development like *Enterococcus* (Dzidic et al., 2020). Moreover, *Bifidobacterium*, a common genus in breast milk, has demonstrated anti-inflammatory properties by promoting the production of anti-inflammatory cytokines, inhibiting Th2 immune responses, and suppressing IgE production, which further supports the notion that breast milk contributes to reducing asthma risk (Eslami et al., 2020). In contrast, a cohort made in Korea shows that infants who are formula-fed demonstrate early diversification of their gut microbiota, accompanied by decreased levels of *Bifidobacteria* and increased abundance of *Escherichia, Veillonella*, and *Enterococcus* (Lee et al., 2015). Compared with direct breastfeeding, any other mode of infant feeding (including formula) was associated with a higher occurrence

of opportunistic pathogens, antibiotic resistance, and an increased risk of asthma (Klopp et al., 2017; Pärnänen et al., 2022).

While all these suggest breastfeeding is protective against asthma in children (Ahmadizar et al., 2017), others have found no significant association at all (Elliott et al., 2009). These discrepancies may be attributed to several factors, including differences in study design and confounding variables such as breast milk composition, which can vary across populations, potentially explaining the observed variations in breastfeeding effects of breastfeeding across studies (Miliku and Azad, 2018). It is important to continue research efforts to better understand the complex relationship between breastfeeding and asthma, taking into account microbiome immune training.

An important event during infancy that impacts the development of the gut microbiome is the introduction of solid food and the cessation of breastfeeding. This shift results in an increase in Lachnospiraceae and Ruminococcaceae, while causing a decrease in Bifidobacterium, Enterobacteriaceae, Enterococcaceae, Lactobacillaceae, Veillonellaceae, Clostridiaceae, and an overall increase in microbial diversity (Laursen et al., 2017). This transition is both necessary and advantageous. It fosters the development of a microbial community better suited to extract energy and process a diet that is no longer reliant on milk, transitioning to a diet rich in fiber and protein (Dong and Gupta, 2019).

Dietary intake has been shown to influence systemic inflammation. The Western diet is characterized by a lack of antioxidants and high levels of fatty acids. This diet has been associated with the promotion of oxidative stress and the activation of inflammatory cascades through receptors such as Toll-like receptor 4 (TLR4), leading to a pro-inflammatory environment, according to a cohort study which compares asthmatic patients vs. healthy controls (Wood et al., 2015). Additionally, the consumption of high-fat mixed meals has been found to increase sputum neutrophils in patients with asthma, particularly observed 4 h after the meal (Wood et al., 2011). Schroeder et al. conducted a study using a mouse model fed a Western diet, which resulted in a reduction in the populations of *Bifidobacterium, Sutturella*, and *Akkermansia* genus. Simultaneously, there was an increase in the Clostridiales order and the *Lactobacillus* and *Oscillospira* genus. Notably, the decrease in *Bifidobacterium* taxa coincided with the onset of mucus defects in this model (Schroeder et al., 2018). Moreover, a Western diet can lead to endotoxemia, contributing to intestinal barrier impairment and increased levels of bacterial lipopolysaccharides (LPS), consequently leading to heightened inflammatory signaling (Pendyala et al., 2012).

Conversely, the Mediterranean diet characterized by a diverse range of fruits, olive oil, vegetables, and whole grain cereals is believed to create an anti-inflammatory environment. This is attributed to the presence of dietary fiber, unsaturated fatty acids, such as monounsaturated fatty acids and Omega-3, as well as the abundance of antioxidants. In a meta-analysis conducted by Garcia-Marcos et al., it was found that the Mediterranean diet was significantly associated with a reduced prevalence of asthma (OR: 0.86; 95% CI 0.78–0.95; $p = 0.004$) (Garcia-Marcos et al., 2013). Similarly, a meta-analysis by Zhang et al., reported protective effects of a Mediterranean diet on asthma (OR = 0.88; 95% CI: 0.79–0.97; $p = 0.014$) (Zhang et al., 2023). However, further research is required to gain a deeper understanding of the specific role of the microbiome in individuals with asthma who adhere to a Mediterranean diet. Moreover, high-fiber diets have been associated with an increase in the colonic Bacteroidetes and Actinobacteria phyla, while the Firmicutes and Proteobacteria phyla decreased. These shifts in microbial composition provide protection against allergic responses, as demonstrated in mouse models (Zhang et al., 2016). Dietary fibers, such as pectin, are fermented by commensal gut bacteria, which produce metabolites including SCFAs, which mediate anti-inflammatory responses (Blanco-Pérez et al., 2021). Ketogenic diet during pregnancy, lactation, and early childhood have been related to low risk of developing asthma due to changes in epigenetic markers. For instance, Bifidobacterium and Lactobacillus species produce SCFAs, which exert anti-inflammatory effects on immune cells through an epigenetic mechanism that involves the inhibition of histone deacetylases associated with butyrate. This modulation of the immune response leads to a reduced risk of asthma (Alsharairi, 2020).

4.2 Environment influences on children's development and growth

Numerous epidemiological studies have shown that living on a farm during early childhood is associated with a reduced risk of developing asthma in childhood (Stein et al., 2016; House et al., 2017; Depner et al., 2020). Under these circumstances, Bifidobacterium and Lactobacillus species produce SCFAs, which exert anti-inflammatory effects on immune cells through an epigenetic mechanism involving the inhibition of histone deacetylases associated with butyrate. In particular, a study that compares the Amish and Hutterites populations, who had similar genetic backgrounds but different farming practices, found that the Amish, who followed traditional farming practices involving high microbial exposures to animals, had a lower risk of childhood asthma. In contrast, the Hutterites, who practiced industrialized farming, did not show the same protective effect (Stein et al., 2016).

A study conducted across 14 countries compared the effects of farm environments and inner city environments on the development of allergic diseases (Campbell et al., 2017). The findings revealed that exposure to a farm environment was associated with a protective effect against allergic diseases. One of the key factors identified by other authors in this protective effect was the consumption of farm milk, which has been reported to contain higher levels of gram-negative bacteria such as *Escherichia coli, Pseudomonas,* and *Klebsiella*, as well as lipopolysaccharides (LPS) (Garedew et al., 2012; Gehring et al., 2020). Endotoxins as LPS, most commonly studied in combination with dust, induce a Th2 response in mice and exacerbate lung eosinophilia via TLR4 pathways, which can result protective against allergic diseases, such as asthma (Ren et al., 2019). A low dose but continuous exposure to an endotoxin, was protective in a mouse model of asthma. This suggests that by the time children reach school age, they will exhibit a marked suppression of the capacity of a Th2 response as a consequence of long-term exposure to environmental endotoxins (Braun-Fahrländer et al., 2002).

Furthermore, it has been considered that growing up with pets and siblings exerts complex changes in microbiota composition, a longitudinal study comparing fecal microbiota composition in infants reports that subjects exposed to both pets and siblings tended to have low relative abundance of family Bifidobacteriaceae and other bacterias (Azad et al., 2013). These changes in gut microbiota result in the development of a healthy immune system that can end up being

protective against asthma and allergy development. Siblings are another of the most important determinants in the development of microbiota during early childhood, due to its impact on alpha and beta diversity (Christensen et al., 2022). However, there is also a risk of exacerbating asthma if the child has a pet-specific allergic sensitization (Pinot de Moira et al., 2022). Moreover, at least 56 bacterial genera were significantly more abundant in homes with dogs, such as *Prevotella, Porphyromonas, Moraxella,* and *Bacteroides*, which were found in the mouth and feces of the animals and also in their owners (Barberán et al., 2015; Hufnagl et al., 2020).

The relationship between hygiene hypothesis and its effect at immunology level can be explained by different mechanisms. First, exposure to a larger diversity of bacterial species enables the development of a balanced immune response. Second, these exposures might serve as the starting point for the development of the infant gastrointestinal microbiome. Third, dietary transitions that facilitate immune tolerance to food nutrients. Fourth, the environment in which one grows. Lack of appropriate microbial and diet exposure might be related with allergic diseases such as asthma (Penders et al., 2006; Fujimura et al., 2010; Garn et al., 2021; Pfefferle et al., 2021).

4.3 Antibiotics: use and abuse

Nowadays, antibiotics play an important role in modern medicine and have improved the prognosis of an endless number of patients, however, we must not forget they have several adverse effects to take into consideration, such as antibiotic resistance and microbiome alteration (Ni et al., 2019).

Several studies have shown that the use of antibiotics in early childhood increases the risk for developing asthma up to 2.3 times (Kim et al., 2018; Lin et al., 2020; Li et al., 2023), these risks increase even more with the number of antibiotics courses prescribed. In infants (<1 year) antibiotic use is associated with a 24% higher incidence of asthma for every 10% increase in prescriptions (Patrick et al., 2020). Among the most prescribed are B-lactams such as amoxicillin (2-fold), followed by macrolides, second-generation cephalosporins (2.7 fold) and sulfonamides (Marra et al., 2009; Li et al., 2023).

A cohort of 697 children demonstrates that the exposure to 2 or more antibiotics from B-lactam group, during the first year of life was related with an increment in the risk of asthma together with longitudinal changes in the nasal microbiome, being the most significant change *Moraxella* sparsity (Toivonen et al., 2021). In another experimental study where mice were exposed to antibiotic treatment there was an increase in Phylum Proteobacteria, and a decrease in Bacteroidetes and Firmicutes, in the same model, 2 weeks after antibiotic-free period Firmicutes and Bacteroidetes returned to dominance, however Proteobacteria turned up to be relatively increased, compared to controls (Antonopoulos et al., 2009), change that has been found in fecal samples from both allergic and non-allergic asthmatic subjects compared to healthy ones (Zheng et al., 2022).

Specifically, regarding amoxicillin antibiotic, a clinical trial in humans demonstrates that Clostridia and Firmicutes part of gut microbiome after cessation of treatment with this antibiotic were decreased in abundance. In fact, the use of amoxicillin for long periods of time was related with significant depletion of SCFAs bacterial species even after antibiotic therapy completion (Dhariwal et al., 2023). As mentioned before SCFAs mediate anti-inflammatory responses, explaining their protective effects against asthma and other allergic diseases (Blanco-Pérez et al., 2021).

An experimental study using mouse models demonstrated that the disturbance of the microbiota following antibiotic exposure leads to an elevated presence of fungal microbiota, particularly *Candida albicans*. These fungi are known to produce molecules with immunomodulatory functions, such as prostaglandin-like oxilipin protein (Noverr et al., 2004). Furthermore, the same study reported that the disruption of the microbiota resulted in an upregulation of the Th2 immune response to spores and ovalbumin, indicating a consistent allergic reaction (Noverr et al., 2004).

The complex interplay between antibiotics, microbiome changes, and the development of asthma calls for further research. However, it is true that antibiotics are essential for treating bacterial infections and that due to their impact on human microbiome and the risk they represent for the development of asthma and other allergic diseases their excessive or inappropriate use must be approached with caution, especially in children.

5 Adolescence, why does everything change?

Changes are a natural process in the course of life. Adolescence is an important transitioning phase of maturation from childhood to adulthood, comprising the 10th to 19th years according to the WHO. Adolescents experience rapid metabolic, immune, sexual, and psychosocial changes among many others. Adolescent development significantly influences the shift in the prevalence of diseases from childhood to adulthood, including asthma.

5.1 Adolescence, sexual dimorphism, and hormones

In puberty, significant changes occur in the metabolic, immune, and hormonal responses, which have lasting effects into adulthood. This crucial period shapes the pattern of immune reactions and hormonal regulation, setting the foundation for future responses. Analyses of gene expression and epigenetic modifications have revealed interactions between genotype and puberty on the expression of B cell (IGKV1-27 in males) and T cell (TRBV30 in females) antigen-recognition proteins, with the influence of genetic factors on gene expression to tend to diminish as puberty progresses. Genes associated with pulmonary function exhibit an upregulation, suggesting potential improvements in respiratory capacity during this stage. However, in females, changes in gene expression related to puberty demonstrate a positive correlation with asthma symptoms and an inverse correlation with pulmonary function. With a notable shift in the immune response from a predominantly innate to a more adaptive pattern in females (Resztak et al., 2023).

Hormones are involved in growth and development, especially during adolescence. Sex hormones, in turn, play a key role in the maturation of many tissues. Male and female endocrine patterns differ, resulting in what is known as sexual dimorphism, which has been studied in multiple etiologies. Recently, many research groups

have investigated how this phenomenon affects the immune system and its relation with the microbiome (Ucciferri and Dunn, 2022).

The sex-specific prevalence of asthma changes throughout life. Boys have a higher prevalence of asthma than girls (CDC and Prevention. Asthma data, statistics, and Surveillance, 2020) and are twice as likely to be hospitalized for an asthma exacerbation (Kynyk et al., 2011). This pattern can be explained in part by the fact that they have increased allergic inflammation, elevated serum IgE levels (Borish et al., 2005), and dysanapsis, described as a reduction in airway diameter relative to lung volume (Pagtakhan et al., 1984).

In teenagers (around 11 to 16 years), asthma prevalence decreases in males but increases in females (Genuneit, 2014). It has also been observed that adult women are three times more likely to be hospitalized for an exacerbation than men and that this difference decreases after menopause (Troisi et al., 1995). Fu et al., studied this phenomenon and associated symptom progression in girls with the onset of puberty, at the time when it decreases in boys (Fu et al., 2014). During adolescence hormonal differences between males and females modify physiological aspects as well as abiotic (pH, oxygen levels, nutrition) and biotic factors (immune surveillance, signaling molecules), creating numerous niches that allow for the appearance of microbiome differences with further implications for the host. The best-studied example to date is the intestinal microbiome, which has been strongly implicated in numerous sex-specific physiological processes and diseases (Fuseini and Newcomb, 2017). Also, many studies have shown that alpha diversity tends to be higher in adult females than males, with less in older adults (de la Cuesta-Zuluaga et al., 2019; Takagi et al., 2019). Furthermore, studies have provided evidence supporting the presence of sexual dimorphism in the adult gut microbiome (Shin et al., 2019). In a mouse model, it was observed that androgens had a significant impact on the modulation of the gut microbiome and the glutamine/glutamate ratio (Gao et al., 2021). A study conducted on human dizygotic twins revealed that while male and female infant twins displayed conserved beta diversity of the gut microbiome, differentiation between the sexes became more apparent during puberty (Yatsunenko et al., 2012). Additionally, the beta diversity of the gut microbiomes in pubertal males and females became increasingly similar to the adult microbiomes of their respective sexes as they progressed further into puberty (Yuan et al., 2020).

Since both innate and adaptive immunity shapes many of the interactions in the gut microbiome and vice versa, the sexually dimorphic nature of immune systems shows us an association between gut microbiome allergy and autoimmune disorders, as it seems for Intestinal Bowel Disease (Sisk-Hackworth et al., 2023). Currently, it is unclear how immunity and microbiome dimorphism modify the natural history of asthma, but further research may enlighten it.

6 Adult, new adaptation

Asthma incidence is lower in adults compared to children, but adult asthma complications have a higher mortality rate (Dharmage et al., 2019). Adults with early-onset current asthma were more prone to atopic conditions and had a higher occurrence of asthma attacks, adult-onset asthma represents a unique phenotype primarily associated with environmental risk factors (Tan et al., 2015). However, He et al., reported through a prospective cohort study that adult-onset asthma has higher all-cause and cardiovascular mortality (He et al., 2021). Determining asthma prevalence in adults is challenging due to reliance on self-reporting and varied approaches in studies. Globally, doctor-diagnosed asthma, clinical/treated asthma, and wheezing prevalence in adults is 4.3, 4.5, and 8.6%, respectively, (To et al., 2012). Recent prevalence ranges from 5.4 to 17.9% depending on the definition and region (Song et al., 2016).

According to the phenotype, Wang et al. aimed to characterize the inflammatory response in acute and stable asthma in adults and children. They found that paucigranulocytic inflammation was the most common phenotype in children and adults with stable asthma. However, in acute asthma, neutrophilic inflammation was more prevalent in adults, while eosinophilic inflammation was more prevalent in children (Wang et al., 2017).

In healthy adults, the most common bacterial phyla in the lungs are *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*. Dominant genera found in bronchoalveolar lavage (BAL) from healthy adults include *Prevotella, Veillonella, Pseudomonas, Fusobacteria*, and *Streptococcus* (Wang et al., 2017; Leviatan et al., 2022). Wang et al., compared the gut microbiota of 185 controls and 36 asthmatic adults in the UK and found *Faecalibacterium prausnitzii, Sutterella wadsworthensis,* and *Bacteroides stercoris* were depleted in cases, while *Clostridium* with *Eggerthella* were over-represented in individuals with asthma (Wang et al., 2018). Concerning the respiratory microbiome in adults with established asthma has been reported an increased abundance of the genera *Streptococcus, Haemophilus, and Moraxella* while a decrease of *Prevotella* and *Corynebacterium*, which is associated with proinflammatory response, associated with severe airway obstruction and airway neutrophilia, through activation of a Th2 response (Hufnagl et al., 2020).

Overall, changes in the microbiome that take place during adulthood can be more reliably linked to health and disease compared to younger individuals. Specific taxa have been found to have associations with both health and disease, indicating the importance of preserving potentially beneficial symbionts (Ghosh et al., 2022).

6.1 Environment pollution and microbiome in asthma adulthood

Asthma is a complex condition influenced by several factors, including the immune system, allergens, environmental triggers, and epigenetics. Within this intricate interplay, environmental factors play a significant role. These factors encompass a wide range of biomolecules, such as pollutants, household cleaners, microplastics, nanoparticles, and tobacco smoke.

A study conducted by Gehring et al., provided evidence of a notable increase in asthma occurrence among individuals exposed to pollutants during early life, which can have long-term consequences in adulthood (Gehring et al., 2020). In industrialized countries, where people spend most of their day indoors, the composition of indoor air is affected by various factors, including outdoor pollutants, ventilation quality and quantity, indoor allergens, and activities such as smoking, heating, and cooking. Microorganisms present in indoor air, particularly certain fungal species such as *Aspergillus, Penicillium, and Cladosporium*, have been associated with an increased risk of asthma in both children and adults (Sharpe et al., 2015).

Cigarette smoke contains nicotine, aldehydes, and polycyclic aromatic compounds, which can decrease endogenous antioxidants, increase lipid peroxidation, and induce oxidative stress. Furthermore, these substances can contribute to intestinal dysbiosis. Animal models have shown that cigarette smoking significantly reduces the concentrations of organic acids, such as acetic acid, propionic acid, butyric acid, and valeric acid, as well as the population of *Bifidobacterium* in the cecum, indicating the presence of intestinal dysbiosis (Tomoda et al., 2011). In a study by Pfeiffer et al., *Prevotella, Veillonella, Streptococcus*, and *Actinomyces* were found to be the most abundant genera in the respiratory tract of smokers (Pfeiffer et al., 2021). Furthermore, when evaluating smoking patterns, they observed a negative correlation between the prevalence of *Corynebacterium* and *Dolosigranulum* in nasal samples and the maximum number of cigarettes smoked daily. Simpson et al. examined asthma patients and characterized their sputum microbiota. Their findings showed that ex-smokers had a greater occurrence of the Fusobacteria phylum, as well as higher levels of Firmicutes and Bacteroidetes, while they had lower levels of Proteobacteria when compared to individuals who had never smoked. Additionally, they discovered a connection between smoking and increased bacterial diversity (Simpson et al., 2016). In contrast, Munk et al. report no significant changes in the microbiome of smoking asthmatic patients compared to those who have quit smoking (Munck et al., 2016). Hence, further research is needed to elucidate the connection between smoking and the microbiome in individuals with asthma.

6.2 Microbiome and asthma in occupationally exposed workers

Occupational asthma, which accounts for 10 to 25% of asthma cases in adulthood, is the most common form of occupational lung disease. It can be classified into different types based on its etiology, including work-exacerbated asthma, irritant-induced asthma, and immunologic occupational asthma. Low molecular weight isocyanates are particularly prevalent among the compounds responsible for occupational asthma (Kenyon et al., 2012; Maestrelli et al., 2020). Isocyanates have also been linked to dysbiosis observed in other chronic inflammatory diseases such as atopic dermatitis, as they disrupt the symbiotic pathways between *Roseomonas mucosa* and *Staphylococcal* species present on the skin (Zeldin et al., 2023).

Another study conducted by Ahmed et al., focused on ceramics industry workers in a major industrial Egyptian city compared to individuals from a rural village. They found a significant increase in the relative abundance of the Proteobacteria phylum in the industrial group ($p = 0.02$). The industrial group was predominantly populated by *Staphylococcus, Sphingomonas*, and *Moraxella*, leading to the conclusion that environmental pollution may alter the nasal microbiome and disrupt its community structure (Ahmed et al., 2019). While the changes in the microbiota resulting from occupational exposure are well-documented, as are their associations with other inflammatory diseases, establishing a direct link between these changes and the prevalence of asthma remains challenging based on current evidence. However, considering that occupational exposure is part of our life, several occupations could represent a risk factor for the development and exacerbation of asthma through diverse mechanisms.

6.3 Viral infections associated with the development of asthma

Respiratory viral infections play a crucial role in the development of asthma and are significant contributors to asthma exacerbations (Hofstra et al., 2015). Among viral infections, HRV infections are particularly common, as this pathogen circulates widely within the community. In adults, viral infections, especially HRV, are responsible for 50–80% of asthma exacerbations, with HRV being detected in up to 83% of adult cases (Jartti et al., 2020; Ojanguren et al., 2022).

In a study involving 88 adults hospitalized for asthma exacerbation, respiratory viruses were detected in 50% of the patients. HRV was the most frequently identified virus (77%), followed by human coronavirus (16%), parainfluenza virus (5%), and human metapneumovirus (2%). Six of these patients also had bacterial coinfections (Bjerregaard et al., 2017). Voraphani et al., reports that individuals who are active smokers and have a history of respiratory syncytial virus infections during the first 3 years are 1.7 times more likely to have current asthma as adults (Voraphani et al., 2014).

Interestingly, in a study of the virome in the sputum of asthma patients, Choi et al. reported an increase in the abundance of Cytomegalovirus (CMV) and Epstein–Barr virus (EBV). Additionally, there was a decrease in Streptococcus phage in patients who experienced exacerbations, which was correlated with more severe disease (Choi et al., 2021).

The precise mechanisms underlying virus-related asthma are still under investigation. However, deficient interferon-γ and interleukin-10 responses, along with an increase in Th2 cytokines such as interleukin-4, interleukin-5, and interleukin-13, have been strongly associated with poor clinical outcomes in the context of viral infections (Busse et al., 2010).

Moreover, respiratory virus infections have been found to induce changes in the composition of the upper respiratory tract microbiota. Infections with rhinovirus, for instance, can predispose individuals to bacterial diseases such as otitis media, sinusitis, and pneumonia (Hofstra et al., 2015). These infections have been linked to an increase in the relative abundance of bacteria such as *Haemophilus, Neisseria, Streptococcus,* and *Moraxella*. These alterations in bacterial composition have been associated with a neutrophilic airway phenotype and persistent asthma that is resistant to treatment (Ver Heul et al., 2019).

That is why it is necessary to continue investigating the complex interplay between environmental exposures, viral infections, dysbiosis, and asthma. Through this exploration we can gain valuable insights into the mechanisms driving asthma exacerbations and potentially develop new strategies for prevention and treatment.

7 Elderly, everything has changed

Aging refers to all natural and progressive physiological changes that lead to cellular senescence and a gradual decline in the organism's biological functions and metabolic stress adaptability. Certain biomarkers help determine aging, such as bone density, frailty, muscle mass, cognitive function, cardiovascular health, some blood biometrics and chemistry parameters, and telomere length (López-Otín et al., 2013), and the microbiome (Partridge et al., 2018). For this reason, chronic systemic inflammation, immunosenescence, and

microbiome changes are important for understanding aging diseases such as asthma.

7.1 Metabolic changes and comorbidities

Comorbidities are a fundamental factor when studying asthma. They can occur at all ages, however, from adulthood onwards they become more important. Yañez et al., showed that of a total of 152 elderly people with asthma, 36% had three or more comorbidities (Yáñez et al., 2018). Obesity, metabolic syndrome (MetS), and diabetes are among the most common metabolic disorders related to asthma, due to their high prevalence (Park et al., 2018). Furthermore, adipose tissue mass is positively related to high levels of proinflammatory molecules like leptin, IL-6, and TNF-a, and negatively related to anti-inflammatory markers such as adiponectin. Similarly, inflamed adipose tissue releases adipokines that circulate to the lungs and contribute to hyperresponsiveness (Shore, 2010; Park et al., 2018; Palma et al., 2022).

A connection has been established between dysbiosis and obesity, characterized by a decrease in the diversity of bacterial genera that constitute the microbiota (Kim et al., 2021). A study by Fu et al. reported that the richness of bacterial microbiota correlates negatively with body mass index and serum triglyceride levels, while positively correlating with serum High-density lipoprotein levels (Fu et al., 2015). Likewise, it has been reported that individuals with obesity and severe asthma showed an increase in taxa belonging to family Prevotellaceae, Mycoplasmataceae, Lachnospiraceae, and Spirochaetaceae (Huang et al., 2015).

The relationship between intestinal microbiota alterations and the improvement of asthma symptoms remains poorly understood. However, it is speculated that increased production of SCFAs, particularly butyrate and propionate, reduces pro-inflammatory cytokines and/or increased immunoregulatory cytokines (Kim et al., 2021). Nevertheless, these associations have been primarily described within the context of the intestinal microbiota, highlighting the need for further investigation of the relationship between asthma, obesity, and pulmonary microbiota dysbiosis.

7.2 Microbial succession in the final stage

Late succession has become very important as a subject of study because of the direct relationship between it and healthy aging. Over time, the alpha diversity of the microbiota decreases, and beta diversity increases, making older adults more susceptible to infection by opportunistic bacteria (Martino et al., 2022). In 2016, Biagi et al. showed that the gut microbiota of elderly people is dominated by the families Bacteroidaceae, Lachnospiraceae, and Ruminococcaceae (Biagi et al., 2016). However, other families such as Prevotellaceae, Enterococcaceae, Lactobacillaceae, Turicibacteraceae, Christensenellaceae, Clostridiaceae, Enterobacteriaceae, Bifidobacteriaceae, Porphyromonadaceae, Peptostreptococcaceae, and Coriobacteriaceae were also found. It has been described that the elderly generally has a gut microbiota composed predominantly of Firmicutes, Tenericutes, Actinobacteria, Lachnospira, and Proteobacteria.

Nevertheless, a decline in taxa such as *Prevotella, Eubacterium, Bifidobacterium, Faecalibacterium, Coprococcus*, and *Roseburia* is observed with increasing age. In contrast, *Akkermansia, Odoribacter, Butyricimonas, Butyrivibrio, Oscillospira, Christensenellaceae*, and *Barnesiellaceae* have been found to increase in abundance in older adults, which has been associated with healthy aging (Biagi et al., 2010; Claesson et al., 2012; Park et al., 2015; Biagi et al., 2016; Effendi et al., 2022). The gradual decline of some microorganisms directly affects systemic inflammation and disease development in the elderly. Additionally, some conformational changes are also associated with unhealthy aging, such as an increase in pathogenic microorganisms like *Eggerthella, Bacteroides, Desulfovibrio, Enterobacteriaceae, Campylobacter, Streptococcus, Actinomyces,* and *Clostridium species.*

Even though the gut microbiome is the best studied, organisms in the respiratory tract are also important when studying respiratory diseases such as asthma. A study reported by Lee et al., examined the composition of airway microbiota in young adults and elderly individuals, comparing those with and without asthma. The dominant phyla in young adults and elderly groups were Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes, but their relative abundances differed significantly. Additionally, the research noted a higher prevalence of Moraxella in elderly individuals without asthma compared to their asthmatic counterparts (Lee et al., 2019). Recently, centenarian gut microbiota have been found to undergo new compositional changes despite differences or similarities between different populations. This has sparked interest in their study, as an increase in the abundance of genera such as *Akkermansia*, *Bifidobacterium, Christensenellaceae*, and other species associated with healthy aging has been described (Biagi et al., 2016; Kato et al., 2017). However, further studies need to be conducted to understand these relatively recent findings.

7.3 Immunosenescence and chronic inflammation

Immunosenescence is a multifactorial phenomenon in which both innate and acquired immunity are affected over time, impairing the effective immune response against pathogens, pathobionts and antigens (Van Den Munckhof et al., 2020). It is thought to result from a combination of three factors: Autoimmunity, Immunodeficiency, and Immune dysregulation (Chotirmall and Burke, 2015). The increase in pro-inflammatory cells leads to a chronic low-grade inflammatory state known as "inflammaging." This inflammaging is a synergistic process between immunosenescence, chronic disease, and the microbiome in which older adults become vulnerable to potentially dangerous bacteria and increase the risk for diseases such as diabetes, obesity, heart disease, and asthma (Chotirmall and Burke, 2015; Huang et al., 2020).

Chronic inflammation is also mediated by the abundance of certain microorganisms. Short-chain fat-producing genera such as *Faecalibacterium, Roseburia, Lachnospira, Eubacterium, Coprococcus, Butyricimonas*, and *Butyrivibrio* have been studied to maintain immune homeostasis by downregulating proinflammatory mediators (Serrano-Villar et al., 2017; Effendi et al., 2022; Singh et al., 2023). Unfortunately, the progressive decrease of these genera leads to a deficiency of SCFA, which increases the permeability of the intestinal mucosa. For this reason, genera such as *Akkermansia* become more important as they are acetate producers (Bodogai et al., 2018; Wu et al., 2021). In contrast to the above genera, the increase of *Bacteroides*

is associated with low-grade inflammation, as shown in a study conducted in Korea by Lim et al. (2021). This study also showed that increases in *C. hathewayi* positively correlate with activation of proinflammatory Th17 cells. Similarly, some *Campylobacter* strains produce cytolysin toxins that induce hyperinflammatory proteins, and *Desulfovibrio* oxidizes butyrate (Callahan et al., 2021).

Some microorganisms help regulate the immune system. *Enterococcus faecalis* is a ROS-producing species involved in oxidative metabolism. However, its progressive increase contributes to inflammation, increased apoptosis, and contributes to oxidative damage to mitochondrial and nuclear DNA (Hemachandra Reddy, 2011; Bullone and Lavoie, 2017). *Bacteroides fragilis* produces PSA, a polysaccharide that binds to B cells inducing CD4+ and CD8+ regulatory T cells, thereby secreting IL-10 (Ramakrishna et al., 2019). It has also been suggested that this species may stimulate and differentiate Treg cells and thus participate in immune regulation (Troy and Kasper, 2010; Johnson et al., 2015; Wu et al., 2021). A study by Li et al. (2023), showed that species such as *Lactobacillus fermentum* and *Bacteroides fragilis* play a role as probiotic strains. Their combined use in senescent mice improved neuronal cell necrosis, antioxidant capacity, and reduced inflammation levels (Li et al., 2023).

7.4 Microbiome and asthma in elderly people

Asthma is a heterogeneous phenotypic disease that has not been fully characterized in the elderly (Liu et al., 2020). However, it is known that reversible obstruction, hyperresponsiveness, and chronic airway inflammation are representative features of this disease. The deterioration of the immune system, systemic inflammation, impaired lung function, different phenotypes, airway remodeling, comorbidities and late onset of this disease complicate its investigation and treatment (Zhang and Huang, 2021). Allergens, tobacco, pollution, and diet are also directly involved in the development of the disease. With age, microbiome alterations in the elderly lead to opportunistic microorganisms colonizing the lungs as environmental conditions are optimal for their development and the immune system is less effective in eliminating them (Santacroce et al., 2020).

A study by Lee et al. analyzed the composition and functional profile of the microbiota in asthmatic and non-asthmatic elderly in Seoul (Lee et al., 2019). The genera *Burkholderia* and *Psychrobacter* were positively correlated with lower forced expiratory volume (FEV1). Therefore, the authors suggested that the low abundance of these microorganisms might be related to asthmatic features. It was also suggested that the increased abundance of *Corynebacterium* might be related to the development of asthma, as this genus has been described in other respiratory diseases such as rhinosinusitis.

Although asthma directly affects the airways, it has been discovered that the gut microbiota can be associated with lung function and asthma. Begley et al., showed that the gut microbiota of older adults in Michigan is certainly dominated by some Prevotella species and that they are associated with chronic inflammatory diseases (Begley et al., 2018). *Staphylococcus aureus* is a microorganism of the microbiota involved in the pathophysiology of airway diseases, including asthma. A study by Song et al., showed that staphylococcal enterotoxin IgE (SE-IgE) is significantly associated with asthma in the elderly, particularly with late-onset asthma (Song et al., 2016). This species has also been shown to produce staphylococcal enterotoxin B (SEB) which can induce Th2 polarization, inflammation and corticosteroid resistance and can inhibit regulatory T-cell functions in humans (Hauk et al., 2000; Cardona et al., 2006).

The elderly are a highly vulnerable sector of the population, diminished by the effects of aging and various diseases. For this reason, the study of the microbiota in the elderly has become a useful and fundamental tool to understand pathologies, find new treatments, and create an adequate culture of prevention. Research on the microbiota and its relationship with diseases such as asthma has been limited; however, understanding this relationship may lead to useful insights for people of other ages.

8 Effects of asthma treatment on the composition of microbial diversity

International Asthma guidelines define corticosteroids as the key asthma treatment (Reddel et al., 2022). These anti-inflammatory molecules inhibit the recruitment of immune cells in the airway by suppressing the production of IL-1B, IL-6, GM-CSF, ICAM-1, induce eosinophils apoptosis, and diminish the survival of T-lymphocytes and mast cells (Barnes, 2010). Usually inhaled corticosteroids (ICS) are enough to control symptoms and reduce complications, but during asthma exacerbations, higher doses are needed and sometimes Oral Corticosteroids (OCS) therapy is required, with increased adverse reactions because of their systemic effects (Perez-Garcia et al., 2020).

Zhou et al., conducted a longitudinal study to identify changes in nasal microbiota related to the risk of asthma exacerbations despite ICS therapy (Zhou et al., 2019). Nasal swabs were collected among 214 European children with mild–moderate asthma, at the time of well-controlled and during the first loss-asthma-control episode. Patients with nasal microbiome dominated by *Corynebacterium* and *Dolosigranulum* had fewer episodes of exacerbation and longer time between them compared to those with predominant *Staphylococcus, Streptococcus*, or *Moraxella* genera. Bacterial richness increased during exacerbations compared with well-controlled asthma. Furthermore, a higher relative abundance of *Corynebacterium* was associated with a lower risk of asthma exacerbations requiring OCS use, whereas *Moraxella* was associated with a higher risk of requiring OCS (Zhou et al., 2019).

Immune modulatory effects of corticosteroids might change the respiratory microbiome. Huang et al. evaluate the effect of ICS on microbiome composition, they studied asthma patients over 9 months, sampling before dosage, three months later, and nine months after the start of treatment. Genera *Streptococcus, Rothia, Actinomyces, Leptotrichia,* and *Neisseria* were identified as the predominant in all samples without significant differences between them or in alpha diversity during the study. More than two-fold decrease the percentage of Wallemia, Cladosporium, Penicillium and Alternia genera compared with baseline, concurrently with decrease in bacteria-fungus intra and inter-kingdom networks after ICS therapy (Huang et al., 2022). Martin et al., compared low- and high-dose ICS groups in sputum microbiome composition without significant differences in bacterial load or overall community (Martin et al., 2020). However, Streptococcus genera showed significantly higher relative abundance in subjects taking low-dose ICS and *Haemophilus parainfluenzae* was significantly more abundant in subjects on high-dose fluticasone

propionate than those on high-dose budesonide over a 2-week period. Denner et al., studied the bronchial microbiome and correlated OCS use with a decrease in the relative abundance of Bacteroidetes and Fusobacteria with an increase in Proteobacteria phyla, generalized linear models on brush samples demonstrated OCS usage influence the relative abundance of *Pseudomonas, Rickettsia, Lactobacillus* and *Streptococcus* genera, significantly enriched in asthmatic patients sample (Denner et al., 2016). In addition, α-diversity in brush samples from asthmatic subjects was correlated with lowest FEV1 levels, a clinical parameter of airway obstruction.

Goleva et al., showed that asthma patients resistant to corticosteroid treatment occur due to the expansion of specific gram-negative bacteria in the airways, like *Haemophilus parainfluenzae*, the LPS of item interact with Toll-like receptor 4 and activate transforming growth factor-b-associated kinase-1 (TAK1), by MyD88 pathway resulting in the p38 MAPK activation and Nuclear Factor-kappa Beta (NF-kb), increasing the production of proinflammatory cytokines like IL-8, also activation of TAK1 inhibits the production of MKP-1 mediated by glucocorticoid receptor, this results in reduced cellular responses to corticosteroids and reduction of sensitivity to them (Goleva et al., 2013).

These studies show the complex correlation between microbiome and corticosteroids, enlightening the need for more research to better understand the phenomena and its implications for better and more reasonable treatment of asthma patients.

9 New perspective for asthma treatment: probiotics

The adaptive immune system provides versatile defense against infectious agents but faces the challenge of potential autoimmune inflammation due to T cell self-reactivity. Tregs play an important role in preventing autoimmunity. Tregs can reverse fatal autoimmunity, tissue pathology, and offer long-term protection (Hu et al., 2021).

Probiotics have the potential to modulate various types of immune cells, including T helper (Th)-1, Th2, Th17, Treg cells, and B cells, which play an important role in human health and the development of immune-related disorders. The use of probiotics has been associated with the modulation of the severity of allergic inflammation (Dargahi et al., 2019). The beneficial effect induced by probiotics is based on their ability to act as an "on/off" switch to control immune responses in a strain-dependent manner at the mucosal level. In allergic asthma, they protect the immune system's homeostasis by regulating the balance between Th1 and Th2 cells, reducing the inflammatory response, modulating the gut microbiota, and increasing the number of Tregs (Huang et al., 2021).

In a study conducted by Wu et al., a probiotic formulation comprising *Lactobacillus acidophilus, Lactobacillus rhamnosus,* and *Bifidobacterium animalis* demonstrated the ability to regulate peribronchial inflammation and control the expression of the PI3K gene in individuals with allergic asthma (Wu et al., 2022). A systematic review conducted by Lin et al., revealed that supplementation with probiotics may reduce the number of asthma episodes (Lin et al., 2018). However, no significant improvements were observed in terms of symptoms during the daytime or nighttime, as well as pulmonary function measures such as FEV1 and PEF. Nevertheless, the authors emphasize the importance of further well-designed randomized controlled trials with larger sample sizes to fully evaluate the effects of probiotics in children with asthma.

10 Conclusion

The interplay between the host microbiome and asthma exhibits significant variations across diverse contexts, and whether a microbiome is considered healthy or disease-associated depends on the context (Table 1). A comprehensive understanding of these intricate interactions among variables and the microbiome is essential for unveiling the underlying mechanisms of asthma phenotypes and for developing precise interventions for prevention and treatment.

The microbiome has been shown to play a significant role in early-life immune development and modulation. It's crucial to note that this interaction can be influenced by genetic factors, the mode of birth (vaginal delivery or cesarean section), first feeding method (breast or formula), upbringing environment (rural or urban, presence of older siblings or pets), among others. In early-life, it has been reported that certain genus in the gut microbiome, including *Bifidobacterium, Lactobacillus, Faecalibacterium*, and *Bacteroides*, play a protective role against asthma. During adolescence, changes in the microbiome occur, partially influenced by the maturation of the immune system and hormonal changes during this stage. As individuals reach adulthood, the impact of the microbiome on health and disease becomes more apparent, contributing to the development of chronic conditions that can lead to comorbidities and proinflammatory states that predispose individuals to asthma and other diseases in old age. In adults, there is evidence indicating an increase in the presence of *Clostridium* and *Eggerthella* in the gut microbiome of individuals with asthma. Moreover, genus such *as Haemophilus, Streptococcus, Staphylococcus*, and *Moraxella* in the respiratory microbiome have emerged as significant contributors to the pathogenesis of asthma. Additionally, the reduction of the genus *Corynebacterium* in the respiratory tract during early-life and adult stages has been associated with proinflammatory responses in specific contexts. However, this genus may also be linked to the development of asthma, particularly among the elderly population.

To illustrate this concept, let us consider a forest with its plant and animal species. The population and composition of these organisms can vary significantly based on whether the forest is in a temperate, equatorial, or Mediterranean climate. Moreover, the roles these organisms play are influenced by seasonal changes, creating distinct contexts. Much like this ecological example, the microbiome's impact on various stages of human growth and development also demonstrates dynamic variations, including its relevance to asthma.

Our review has some limitations; many studies are confined to cohort designs, lacking long-term longitudinal data on individual changes. Furthermore, obtaining samples from the lower respiratory tract to investigate this microbiome remains challenging, and much of the information is derived from the gut microbiome. Moreover, a substantial portion of these studies relies on the sequencing and analysis of the 16S ribosomal gene, limiting the scope to bacterial aspects and gender-based analyses. Consequently, a comprehensive understanding of the virome and mycobiome is still needed. Therefore, further research and long-term follow-up studies are necessary to fully elucidate the mechanisms underlying these interactions and explore potential interventions, such as probiotics, that can modulate immune

TABLE 1 Microbiota profiling in asthmatic and healthy individuals.

Sample method	Population	Asthmatic subjects	Healthy controls (Ref)
Superior airway			
Nasopharyngeal aspirate[S]	*Infants* (0–12 months) $n = 234$	- Increased *Streptococcus*. Strong asthma predictor. *Streptococcus, Moraxella, or Haemophilus* marked virus-associated with acute respiratory infections in asthma.	- Dominated by *Staphylococcus* (41%) and *Corynebacterium* (22%). Antibiotic usage in the four weeks prior to sampling was associated with higher abundances of *Haemophilus*, *Streptococcus*, and *Moraxella* and lower abundances of *Alloiococcus* and *Corynebacterium* (Teo et al., 2015).
Nasal swab[S]	*Children and adolescents* (6–20 years) $n = 14$	- Increased *Moraxella Catarrhalis, Escherichia* and *Psychrobacter*. Dominated by *M. Catarrahalis* and less diverse.	- *M. catarrhalis* less abundant *Corynebacterium tuberculostearicum*. Found in high abundance (Castro-Nallar et al., 2015).
Nasal swab[S]	*Adults* (35.8 +/− 16) $n = 72$	Increased *Bacteroidetes (Prevotella), Proteobacteria (Alkanindiges), Actinobacteria (Gardnerella).*	Less *Proteobacteria and Bacteroidetes* (Fazlollahi et al., 2018).
Nasopharyngeal swab[S]	*Elderly* (<60 years) $n = 40$	- Higher relative abundance of *Moraxella.* Higher abundance of *Proteobacteria.*	Higher relative abundance of *Corynebacteriales* (Lee et al., 2019).
Oropharyngeal swab[S]	*Elderly* (53.4+/− 17.1//55 +/− 13) $n = 47$	*Proteobacteria* (*Pseudomonas* s) and *Firmicutes* (*Lactobacillus spp*) are the most dominant populations in asthmatic subjects, these microorganisms not detected in healthy subjects.	- *Proteobacteria* (*Pseudomonas*) and *Firmicutes (Lactobacillus* spp) no detected in healthy subjects *Bacteroidetes* (*Streptococcus, Veillonella, Prevotella, and Neisseria*) dominant in healthy oropharynx (Park et al., 2014).
Hypopharyngeal aspirate [C]	*Neonates* (~1 month) $n = 321$	Neonates colonized with *Streptococcus pneumoniae, M. catarrhalis, Haemophilus influenzae* showed increased asthma prevalence at 5 years.	Neonates not colonized with *S. pneumoniae, M. catarrhalis or H. influenzae* show less risk of a first wheezy episode (Bisgaard et al., 2007).
Hypopharyngeal aspirate[S]	*Children* (12–36 months) $n = 68$	Increase abundance of *Moraxella, Haemophilus* and *Streptococcus*, being *Moraxella* the predominant genera with mean relative abundance of 43.63%.	No healthy control was included (Thorsen et al., 2021).
Broncho-alveolar lavage (BAL)[S]	*Children* (11.8 +/− 2.8 years) $n = 20$	Increase *Proteobacteria* (*Haemophilus*) *and Firmicutes* (*Streptococcus*) in asthmatic children.	Increase *Bacteroidetes (Prevotella)* in healthy subjects (Hilty et al., 2010).
Inferior airway			
Broncho-alveolar lavage (BAL)[S]	*Children* (11.8 +/− 2.8 years) $n = 20$	Increase *Proteobacteria (Haemophilus) and Firmicutes (Streptococcus)* in asthmatic children.	Increase *Bacteroidetes (Prevotella)* in healthy subjects (Hilty et al., 2010).
Sputum[S]	*Adults* (39–62 years) $n = 97$	Main species present in airway of healthy and asthmatics patients include *Streptococcus Mitis, Streptococcus Aliviarus* and *Veillonella Dispar.*	Airway microbiota similar to asthmatic patients. No differences in airway diversity between asthmatic patients and healthy controls in the composition of microbiota (Ham et al., 2021).
Bronchial brushing [S]	*Adults* (20–63 years) $n = 40$	- Increased *Bacteroidetes* and *Firmicutes* in severe asthma. Increased *Actinobacteria (Mycobacteria, Streptomyces)* and *proteobacteria (Klebsiella).*	Less abundant in *Proteobacteria (Klebsiella)* (Huang et al., 2015).
Induced sputum[S]	*Adults* (age 56–59) $n = 167$	- Significant decrease of alpha diversity in neutrophilic phenotypes - High abundance of *Moraxella* and *Haemophilus* in neutrophilic phenotypes. Negative correlation of sputum neutrophil percentages with *Gemella*, *Porphyromonas* and *Streptococcus* Taxa.	No healthy control was included (Taylor et al., 2018).
Gut			
Broncho-alveolar lavage (BAL)[S]	*Children* 11.8 +/− 2.8 years) $n = 20$	Increase *Proteobacteria (Haemophilus) and Firmicutes (Streptococcus)* in asthmatic children.	Increase *Bacteroidetes (Prevotella)* in healthy subjects (Hilty et al., 2010).
Fresh stool[S]	*Adults* (18–50 years) $n = 67$	- Lower alpha diversity enrichment of *Ruminococcus gnavus, Clostridium clostridioforme,* and *Bifidobacterium pseudocatenulatum* - Depletion of *Roseburia intestinalis* and *Roseburia inulinivorans.*	- Richer alpha diversity Enrichment of *Roseburia inulinivorans* and *Clostridium disporicum* (Zou et al., 2021).
Fecal stool[S]	*Adults* (39–62 years) $n = 97$	At genus level, the leading bacteria are *Prevotella, Bacteroides, Faecalibacterium,* and *Rominococcus*. The most common species were *Prevotella Copri, Faecalibacterium prausnitzii*, and *Bacteroides Plebeius.*	Similar to asthmatic patients. There were no significant differences between groups or associations between gut microbiota composition and asthma (Ham et al., 2021).

This table shows the microbial diversity in patients with asthma compared to other study groups, considering different life stages and respiratory/gut tract locations. Analysis Method. S: 16S rRNA gene sequencing. C: Culture. Ref.: Reference.

10.3389/fmicb.2023.1295215

responses and improve health outcomes in diverse populations worldwide.

Author contributions

DG-C: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft, Writing – review & editing. IG-G: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. KL-S: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. VI-M: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. FJ-J: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. AT-G: Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. CB-C: Formal analysis, Investigation, Validation, Writing – original draft, Writing – review & editing. MM-M: Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. LJ-A: Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. JZ: Conceptualization, Formal analysis, Funding acquisition, Investigation, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing. AC: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

This paper and the work behind it, would not have been possible without the lectures and support of the UNAM and Red MEDICI program.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Afzal, S., Ramzan, K., Ullah, S., Jamal, A., Basit, S., AlKattan, K. M., et al. (2023). Association between 17q21 variants and asthma predisposition in Pashtun population from Pakistan. *J. Asthma* 60, 63–75. doi: 10.1080/02770903.2021.2025391

Ahlawat, S., Asha, and Sharma, K. K. (2021). Gut–organ axis: a microbial outreach and networking. *Lett. Appl. Microbiol.* 72, 636–668. doi: 10.1111/lam.13333

Ahluwalia, T. S., Eliasen, A. U., Sevelsted, A., Pedersen, C. T., Stokholm, J., Chawes, B., et al. (2020). 'FUT2–ABO epistasis increases the risk of early childhood asthma and *Streptococcus pneumoniae* respiratory illnesses', nature. *Communications* 11, 1–12. doi: 10.1038/s41467-020-19814-6

Ahmadizar, F., Vijverberg, S. J. H., Arets, H. G. M., de Boer, A., Garssen, J., Kraneveld, A. D., et al. (2017). Breastfeeding is associated with a decreased risk of childhood asthma exacerbations later in life. *Pediatr. Allergy Immunol.* 28, 649–654. doi: 10.1111/pai.12760

Ahmed, N., Mahmoud, N. F., Solyman, S., and Hanora, A. (2019). Human nasal microbiome as characterized by metagenomics differs markedly between rural and industrial communities in Egypt. *OMICS J. Integr. Biol.* 23, 573–582. doi: 10.1089/omi.2019.0144

Aldriwesh, M. G., al-Mutairi, A. M., Alharbi, A. S., Aljohani, H. Y., Alzahrani, N. A., Ajina, R., et al. (2023). Paediatric asthma and the microbiome: a systematic review. *Microorganisms* 11, 1–29. doi: 10.3390/microorganisms11040939

Alhasan, M. M., Cait, A. M., Heimesaat, M. M., Blaut, M., Klopfleisch, R., Wedel, A., et al. (2020). 'Antibiotic use during pregnancy increases offspring asthma severity in a dose-dependent manner', allergy: European. *J. Allergy Clin. Immunol.* 75, 1979–1990. doi: 10.1111/all.14234

Alliet, P., Vandenplas, Y., Roggero, P., Jespers, S. N. J., Peeters, S., Stalens, J. P., et al. (2022). Safety and efficacy of a probiotic-containing infant formula supplemented with 2'-fucosyllactose: a double-blind randomized controlled trial. *Nutr. J.* 21, 11–16. doi: 10.1186/s12937-022-00764-2

Alsharairi, N. A. (2020). The role of short-chain fatty acids in the interplay between a very low-calorie ketogenic diet and the infant gut microbiota and its therapeutic implications for reducing asthma. *Int. J. Mol. Sci.* 21, 1–21. doi: 10.3390/ijms21249580

Anderson, E. J., Carbonell-Estrany, X., Blanken, M., Lanari, M., Sheridan-Pereira, M., Rodgers-Gray, B., et al. (2017). Burden of severe respiratory syncytial virus disease among 33-35 weeks' gestational age infants born during multiple respiratory syncytial virus seasons. *Pediatr. Infect. Dis. J.* 36, 160–167. doi: 10.1097/INF.0000000000001377

Anderson, J., do, L. A. H., Wurzel, D., and Licciardi, P. V. (2023). 'Understanding the increased susceptibility to asthma development in preterm infants', allergy: European. *J. Allergy Clin. Immunol.* 78, 928–939. doi: 10.1111/all.15662

Antonopoulos, D. A., Huse, S. M., Morrison, H. G., Schmidt, T. M., Sogin, M. L., and Young, V. B. (2009). Reproducible community dynamics of the gastrointestinal microbiota following antibiotic perturbation. *Infect. Immun.* 77, 2367–2375. doi: 10.1128/IAI.01520-08

Arif, A. A., and Veri, S. D. (2019). The association of prenatal risk factors with childhood asthma. *J. Asthma* 56, 1056–1061. doi: 10.1080/02770903.2018.1515224

Arrieta, M. C., Stiemsma, L. T., Dimitriu, P. A., Thorson, L., Russell, S., Yurist-Doutsch, S., et al. (2015). Early infancy microbial and metabolic alterations affect risk of childhood asthma. *Sci. Transl. Med.* 7:307ra152. doi: 10.1126/scitranslmed.aab2271

Arroyas, M., Calvo, C., Rueda, S., Esquivias, M., Gonzalez-Menchen, C., Gonzalez-Carrasco, E., et al. (2020). Asthma prevalence, lung and cardiovascular function in adolescents born preterm. *Sci. Rep.* 10, 19616–19610. doi: 10.1038/s41598-020-76614-0

Azad, M. B., Konya, T., Maughan, H., Guttman, D. S., Field, C. J., Sears, M. R., et al. (2013). Infant gut microbiota and the hygiene hypothesis of allergic disease: impact of household pets and siblings on microbiota composition and diversity. *Allergy Asthma Clin. Immunol.* 9, 1–9. doi: 10.1186/1710-1492-9-15

Barberán, A., Dunn, R. R., Reich, B. J., Pacifici, K., Laber, E. B., Menninger, H. L., et al. (2015). The ecology of microscopic life in household dust. *Proc. Biol. Sci.* 282:20151139. doi: 10.1098/rspb.2015.1139

Barcik, W., Boutin, R. C. T., Sokolowska, M., and Finlay, B. B. (2020). The role of lung and gut microbiota in the pathology of asthma. *Immunity* 52, 241–255. doi: 10.1016/j.immuni.2020.01.007

Barnes, P. J. (2010). Inhaled corticosteroids. *Pharmaceuticals* 3, 514–540. doi: 10.3390/ph3030514

Begley, L., Madapoosi, S., Opron, K., Ndum, O., Baptist, A., Rysso, K., et al. (2018). 'Gut microbiota relationships to lung function and adult asthma phenotype: a pilot study', BMJ open. *Respir. Res.* 5:e000324. doi: 10.1136/bmjresp-2018-000324

Berg, G., et al. (2020). Microbiome definition re-visited: old concepts and new challenges. Microbiome. 2020;8(1):1–22. Definition re-visited: old concepts and new challenges. *Microbiome* 8, 1–22.

Biagi, E., Franceschi, C., Rampelli, S., Severgnini, M., Ostan, R., Turroni, S., et al. (2016). Gut microbiota and extreme longevity. *Curr. Biol.* 26, 1480–1485. doi: 10.1016/j.cub.2016.04.016

Biagi, E., Nylund, L., Candela, M., Ostan, R., Bucci, L., Pini, E., et al. (2010). Through ageing, and beyond: gut microbiota and inflammatory status in seniors and centenarians. *PLoS One* 5:e10667. doi: 10.1371/journal.pone.0010667

Biesbroek, G., Bosch, A. A. T. M., Wang, X., Keijser, B. J. F., Veenhoven, R. H., Sanders, E. A. M., et al. (2014). The impact of breastfeeding on nasopharyngeal microbial communities in infants. *Am. J. Respir. Crit. Care Med.* 190, 298–308. doi: 10.1164/rccm.201401-0073OC

Bisgaard, H., Hermansen, M. N., Buchvald, F., Loland, L., Halkjaer, L. B., Bønnelykke, K., et al. (2007). Childhood asthma after bacterial colonization of the airway in neonates. *N. Engl. J. Med.* 357, 1487–1495. doi: 10.1056/NEJMoa052632

Bizzintino, J., Lee, W. M., Laing, I. A., Vang, F., Pappas, T., Zhang, G., et al. (2011). Association between human rhinovirus C and severity of acute asthma in children. *Eur. Respir. J.* 37, 1037–1042. doi: 10.1183/09031936.00092410

Bjerregaard, A., Laing, I. A., Poulsen, N., Backer, V., Sverrild, A., Fally, M., et al. (2017). Characteristics associated with clinical severity and inflammatory phenotype of naturally occurring virus-induced exacerbations of asthma in adults. *Respir. Med.* 123, 34–41. doi: 10.1016/j.rmed.2016.12.010

Blanco-Pérez, F., Steigerwald, H., Schülke, S., Vieths, S., Toda, M., and Scheurer, S. (2021). The dietary Fiber pectin: health benefits and potential for the treatment of allergies by modulation of gut microbiota. *Curr Allergy Asthma Rep* 21:43. doi: 10.1007/s11882-021-01020-z

Bodogai, M., O'Connell, J., Kim, K., Kim, Y., Moritoh, K., Chen, C., et al. (2018). Commensal bacteria contribute to insulin resistance in aging by activating innate B1a cells. *Sci. Transl. Med.* 10, 1–26. doi: 10.1126/scitranslmed.aat4271

Boquien, C. Y. (2018). Human milk: an ideal food for nutrition of preterm newborn. *Front. Pediatr.* 6, 1–9. doi: 10.3389/fped.2018.00295

Borish, L., Chipps, B., Deniz, Y., Gujrathi, S., Zheng, B., and Dolan, C. M. (2005). Total serum IgE levels in a large cohort of patients with severe or difficult-to-treat asthma. *Ann. Allergy Asthma Immunol.* 95, 247–253. doi: 10.1016/S1081-1206(10)61221-5

Bosch, A. A. T. M., Levin, E., van Houten, M. A., Hasrat, R., Kalkman, G., Biesbroek, G., et al. (2016). Development of upper respiratory tract microbiota in infancy is affected by mode of delivery. *EBioMedicine* 9, 336–345. doi: 10.1016/j.ebiom.2016.05.031

Boulund, U., Bastos, D. M., Ferwerda, B., van den Born, B. J., Pinto-Sietsma, S. J., Galenkamp, H., et al. (2022). Gut microbiome associations with host genotype vary across ethnicities and potentially influence cardiometabolic traits. *Cell Host Microbe* 30, 1464–1480.e6. doi: 10.1016/j.chom.2022.08.013

Braun-Fahrländer, C., Riedler, J., Herz, U., Eder, W., Waser, M., Grize, L., et al. (2002). Environmental exposure to endotoxin and its relation to asthma in school-aged children. *N. Engl. J. Med.* 347:869. doi: 10.1056/NEJMoa020057

Breit, S., Kupferberg, A., Rogler, G., and Hasler, G. (2018). Vagus nerve as modulator of the brain-gut axis in psychiatric and inflammatory disorders. *Front. Psych.* 9:e0044. doi: 10.3389/fpsyt.2018.00044

Bullone, M., and Lavoie, J. P. (2017). The contribution of oxidative stress and inflamm-aging in human and equine asthma. *Int. J. Mol. Sci.* 18, 1–23. doi: 10.3390/ijms18122612

Bush, A. (2019). Pathophysiological mechanisms of asthma. *Front. Pediatr.* 7, 1–17. doi: 10.3389/fped.2019.00068

Busse, W., Lemanske, R., and Gern, J. (2010). Role of viral respiratory infections in asthma and asthma exacerbations. *Lancet* 376, 826–834. doi: 10.1016/S0140-6736(10)61380-3

Busse, W. W., and Rosenwasser, L. J. (2003). Mechanisms of asthma. *J. Allergy Clin. Immunol.* 111, S799–S804. doi: 10.1067/mai.2003.158

Callahan, S. M., Dolislager, C. G., and Johnson, J. G. (2021). The host cellular immune response to infection by campylobacter s and its role in disease. *Infect. Immun.* 89, e0011621–e0011617. doi: 10.1128/IAI.00116-21

Campbell, B., Raherison, C., Lodge, C. J., Lowe, A. J., Gislason, T., Heinrich, J., et al. (2017). The effects of growing up on a farm on adult lung function and allergic phenotypes: an international population-based study. *Thorax* 72, 236–244. doi: 10.1136/thoraxjnl-2015-208154

Cardona, I. D., Goleva, E., Ou, L. S., and Leung, D. Y. M. (2006). Staphylococcal enterotoxin B inhibits regulatory T cells by inducing glucocorticoid-induced TNF receptor-related protein ligand on monocytes. *J. Allergy Clin. Immunol.* 117, 688–695. doi: 10.1016/j.jaci.2005.11.037

Carr, T. F., Alkatib, R., and Kraft, M. (2019). Microbiome in mechanisms of asthma. *Clin. Chest Med.* 40, 87–96. doi: 10.1016/j.ccm.2018.10.006

Castro-Nallar, E., Shen, Y., Freishtat, R. J., Pérez-Losada, M., Manimaran, S., Liu, G., et al. (2015). Integrating microbial and host transcriptomics to characterize asthma-associated microbial communities. *BMC Med. Genet.* 8, 50–59. doi: 10.1186/s12920-015-0121-1

Castro-Rodriguez, J. A., Forno, E., Rodriguez-Martinez, C. E., and Celedón, J. C. (2016). 'Risk and protective factors for childhood asthma: what is the evidence?', Journal of allergy and clinical immunology. *In Pract.* 4, 1111–1122. doi: 10.1016/j.jaip.2016.05.003

CDC and Prevention. Asthma data, statistics, and Surveillance (2020). Available at: https://www.cdc.gov/asthma/most_recent_national_asthma_data.htm (accessed May 2023).

Chen, Y. C., Chen, Y., Lasky-Su, J., Kelly, R. S., Stokholm, J., Bisgaard, H., et al. (2023). Environmental and genetic associations with aberrant early-life gut microbial maturation in childhood asthma. *J. Allergy Clin. Immunol.* 151, 1494–1502.e14. doi: 10.1016/j.jaci.2023.01.006

Chesné, J., Braza, F., Mahay, G., Brouard, S., Aronica, M., and Magnan, A. (2014). IL-17 in severe asthma: where do we stand? *Am. J. Respir. Crit. Care Med.* 190, 1094–1101. doi: 10.1164/rccm.201405-0859PP

Choi, S., Sohn, K. H., Jung, J. W., Kang, M. G., Yang, M. S., Kim, S., et al. (2021). Lung virome: new potential biomarkers for asthma severity and exacerbation. *J. Allergy Clin. Immunol.* 148, 1007–1015.e9. doi: 10.1016/j.jaci.2021.03.017

Chotirmall, S. H., and Burke, C. M. (2015). Aging and the microbiome: implications for asthma in the elderly? *Expert Rev. Respir. Med.* 9, 125–128. doi: 10.1586/17476348.2015.1002473

Christensen, E. D., Hjelmsø, M. H., Thorsen, J., Shah, S., Redgwell, T., Poulsen, C. E., et al. (2022). The developing airway and gut microbiota in early life is influenced by age of older siblings. *Microbiome* 10, 106–115. doi: 10.1186/s40168-022-01305-z

Claesson, M. J., Jeffery, I. B., Conde, S., Power, S. E., O'Connor, E. M., Cusack, S., et al. (2012). Gut microbiota composition correlates with diet and health in the elderly. *Nature* 488, 178–184. doi: 10.1038/nature11319

Crump, C., Sundquist, J., and Sundquist, K. (2023). Preterm or early term birth and long-term risk of asthma into midadulthood: a national cohort and cosibling study. *Thorax* 78, 653–660. doi: 10.1136/thorax-2022-218931

Dargahi, N., Johnson, J., Donkor, O., Vasiljevic, T., and Apostolopoulos, V. (2019). Immunomodulatory effects of probiotics: can they be used to treat allergies and autoimmune diseases? *Maturitas* 119, 25–38. doi: 10.1016/j.maturitas.2018.11.002

de la Cuesta-Zuluaga, J., Kelley, S. T., Chen, Y., Escobar, J. S., Mueller, N. T., Ley, R. E., et al. (2019). Age- and sex-dependent patterns of gut microbial diversity in human adults. *mSystems* 4:e00261–19. doi: 10.1128/msystems.00261-19

Denner, D. R., Sangwan, N., Becker, J. B., Hogarth, D. K., Oldham, J., Castillo, J., et al. (2016). Corticosteroid therapy and airflow obstruction influence the bronchial microbiome, which is distinct from that of bronchoalveolar lavage in asthmatic airways. *J. Allergy Clin. Immunol.* 137, 1398–1405.e3. doi: 10.1016/j.jaci.2015.10.017

Depner, M., Taft, D. H., Kirjavainen, P. V., Kalanetra, K. M., Karvonen, A. M., Peschel, S., et al. (2020). Maturation of the gut microbiome during the first year of life contributes to the protective farm effect on childhood asthma. *Nat. Med.* 26, 1766–1775. doi: 10.1038/s41591-020-1095-x

Dhariwal, A., Haugli Bråten, L. C., Sturød, K., Salvadori, G., Bargheet, A., Åmdal, H., et al. (2023). Differential response to prolonged amoxicillin treatment: long-term resilience of the microbiome versus long-lasting perturbations in the gut resistome. *Gut Microbes* 15, 1–22. doi: 10.1080/19490976.2022.2157200

Dharmage, S. C., Perret, J. L., and Custovic, A. (2019). 'Epidemiology of asthma in children and adults', Frontiers. *Pediatrics* 7, 1–15. doi: 10.3389/fped.2019.00246

Dickson, R. P., Erb-Downward, J. R., Martinez, F. J., and Huffnagle, G. B. (2016). The microbiome and the respiratory tract. *Annu. Rev. Physiol.* 78, 481–504. doi: 10.1146/annurev-physiol-021115-105238

Doherty, A. M., Lodge, C. J., Dharmage, S. C., Dai, X., Bode, L., and Lowe, A. J. (2018). 'Human milk oligosaccharides and associations with immune-mediated disease and infection in childhood: a systematic review', Frontiers. *Pediatrics* 6, 1–8. doi: 10.3389/fped.2018.00091

Domenici, R., and Vierucci, F. (2022). Exclusive breastfeeding and vitamin D supplementation: a positive synergistic effect on prevention of childhood infections? *Int. J. Environ. Res. Public Health* 19, 1–29. doi: 10.3390/ijerph19052973

Donald, K., and Finlay, B. B. (2023). Early-life interactions between the microbiota and immune system: impact on immune system development and atopic disease. *Nat. Rev. Immunol.* 23, 735–748. doi: 10.1038/s41577-023-00874-w

Dong, T. S., and Gupta, A. (2019). Influence of early life, diet, and the environment on the microbiome. *Clin. Gastroenterol. Hepatol.* 17, 231–242. doi: 10.1016/j.cgh.2018.08.067

Douros, K., Moustaki, M., Tsabouri, S., Papadopoulou, A., Papadopoulos, M., and Priftis, K. N. (2017). Prenatal maternal stress and the risk of asthma in children. *Front. Pediatr.* 5, 1–9. doi: 10.3389/fped.2017.00202

Durack, J., Kimes, N. E., Lin, D. L., Rauch, M., McKean, M., McCauley, K., et al. (2018). 'Delayed gut microbiota development in high-risk for asthma infants is temporarily modifiable by Lactobacillus supplementation', nature. *Communications* 9, 1–9. doi: 10.1038/s41467-018-03157-4

Dzidic, M., Mira, A., Artacho, A., Abrahamsson, T. R., Jenmalm, M. C., and Collado, M. C. (2020). Allergy development is associated with consumption of breastmilk with a reduced microbial richness in the first month of life. *Pediatr. Allergy Immunol.* 31, 250–257. doi: 10.1111/pai.13176

Effendi, R. M. R. A., Anshory, M., Kalim, H., Dwiyana, R. F., Suwarsa, O., Pardo, L. M., et al. (2022). Akkermansia muciniphila and Faecalibacterium prausnitzii in immune-related diseases. *Microorganisms* 10, 1–16. doi: 10.3390/microorganisms10122382

Elliott, L., et al. (2009). Prospective study of breastfeeding in relation to wheeze, atopy, and bronchial hyperresponsiveness in the Avon longitudinal study of parents and children (ALSPAC). *Genomics* 94, 20–31. doi: 10.1016/j.jaci.2008.04.001.Prospective

Enaud, R., Prevel, R., Ciarlo, E., Beaufils, F., Wieërs, G., Guery, B., et al. (2020). The gut-lung Axis in health and respiratory diseases: a place for inter-organ and inter-kingdom Crosstalks. *Front. Cell. Infect. Microbiol.* 10, 1–11. doi: 10.3389/fcimb.2020.00009

Eslami, M., Bahar, A., Keikha, M., Karbalaei, M., Kobyliak, N. M., and Yousefi, B. (2020). Probiotics function and modulation of the immune system in allergic diseases. *Allergol. Immunopathol.* 48, 771–788. doi: 10.1016/j.aller.2020.04.005

Esmeraldino, L., Traebert, E., Nunes, R. D., and Traebert, J. (2022). Association between prenatal and neonatal factors and occurrence of asthma symptoms in six-year-old children. *Ciencia e Saude Coletiva* 27, 545–554. doi: 10.1590/1413-81232022272.44892020

Fazlollahi, M., Lee, T. D., Andrade, J., Oguntuyo, K., Chun, Y., Grishina, G., et al. (2018). The nasal microbiome in asthma. *J. Allergy Clin. Immunol.* 142, 834–843.e2. doi: 10.1016/j.jaci.2018.02.020

Ferllini Montealegre, S. M., Miranda Muñoz, M. F., and Vindas Vargas, J. F. (2019). Revista Medica Sinergia 4, e266. doi: 10.31434/rms.v4i9.266,

Finotello, F., Mastrorilli, E., and Di Camillo, B. (2018). Measuring the diversity of the human microbiota with targeted next-generation sequencing. *Brief. Bioinform.* 19, bbw119–bbw692. doi: 10.1093/bib/bbw119

Fu, J., Bonder, M. J., Cenit, M. C., Tigchelaar, E. F., Maatman, A., Dekens, J. A. M., et al. (2015). The gut microbiome contributes to a substantial proportion of the variation in blood lipids. *Circ. Res.* 117, 817–824. doi: 10.1161/CIRCRESAHA.115.306807

Fu, L., Freishtat, R. J., Gordish-Dressman, H., Teach, S. J., Resca, L., Hoffman, E. P., et al. (2014). Natural progression of childhood asthma symptoms and strong influence of sex and puberty. *Ann. Am. Thorac. Soc.* 11, 939–944. doi: 10.1513/AnnalsATS.201402-084OC

Fujimura, K. E., Johnson, C. C., Ownby, D. R., Cox, M. J., Brodie, E. L., Havstad, S. L., et al. (2010). Man's best friend? The effect of pet ownership on house dust microbial communities. *J. Allergy Clin. Immunol.* 126, 8–13. doi: 10.1016/j.jaci.2010.05.042

Fuseini, H., and Newcomb, D. C. (2017). Mechanisms Driving Gender Differences in Asthma. *Curr Allergy Asthma Rep* 17:19. doi: 10.1007/s11882-017-0686-1

Gao, A., Su, J., Liu, R., Zhao, S., Li, W., Xu, X., et al. (2021). 'Sexual dimorphism in glucose metabolism is shaped by androgen-driven gut microbiome', nature. *Communications* 12, 1–14. doi: 10.1038/s41467-021-27187-7

Garcia-Marcos, L., Castro-Rodriguez, J. A., Weinmayr, G., Panagiotakos, D. B., Priftis, K. N., and Nagel, G. (2013). Influence of Mediterranean diet on asthma in children: a systematic review and meta-analysis. *Pediatr. Allergy Immunol.* 24, 330–338. doi: 10.1111/pai.12071

García-Serna, A. M., Martín-Orozco, E., Hernández-Caselles, T., and Morales, E. (2021). Prenatal and perinatal environmental influences shaping the neonatal immune system: a focus on asthma and allergy origins. *Int. J. Environ. Res. Public Health* 18, 1–24. doi: 10.3390/ijerph18083962

Garedew, L., Berhanu, A., Mengesha, D., and Tsegay, G. (2012). Identification of gram-negative bacteria from critical control points of raw and pasteurized cow milk consumed at Gondar town and its suburbs, Ethiopia. *BMC Public Health* 12:1. doi: 10.1186/1471-2458-12-950

Garn, H., Potaczek, D. P., and Pfefferle, P. I. (2021). The hygiene hypothesis and new perspectives—current challenges meeting an old postulate. *Front. Immunol.* 12, 1–7. doi: 10.3389/fimmu.2021.637087

Gehring, U., Wijga, A. H., Koppelman, G. H., Vonk, J. M., Smit, H. A., and Brunekreef, B. (2020). Air pollution and the development of asthma from birth until young adulthood. *Eur. Respir. J.* 56:2000147. doi: 10.1183/13993003.00147-2020

Genuneit, J. (2014). Sex-specific development of asthma differs between farm and nonfarm children: a cohort study. *Am. J. Respir. Crit. Care Med.* 5, 588–590. doi: 10.1164/rccm.201403-0428LE

Ghosh, T. S., Shanahan, F., and O'Toole, P. W. (2022). Toward an improved definition of a healthy microbiome for healthy aging. *Nat. Aging* 2, 1054–1069. doi: 10.1038/s43587-022-00306-9

Goleva, E., Jackson, L. P., Harris, J. K., Robertson, C. E., Sutherland, E. R., Hall, C. F., et al. (2013). The effects of airway microbiome on corticosteroid responsiveness in asthma. *Am. J. Respir. Crit. Care Med.* 188, 1193–1201. doi: 10.1164/rccm.201304-0775OC

Gomez de Agüero, M., Ganal-Vonarburg, S. C., Fuhrer, T., Rupp, S., Uchimura, Y., Li, H., et al. (2016). The maternal microbiota drives early postnatal innate immune development. *Science* 351, 1296–1302. doi: 10.1126/science.aad2571

Gray, L. E. K., O'Hely, M., Ranganathan, S., Sly, P. D., and Vuillermin, P. (2017). The maternal diet, gut bacteria, and bacterial metabolites during pregnancy influence offspring asthma. *Front. Immunol.* 8, 1–13. doi: 10.3389/fimmu.2017.00365

Ham, J., Kim, J., Choi, S., Park, J., Baek, M. G., Kim, Y. C., et al. (2021). Interactions between ncr+ilc3s and the microbiome in the airways shape asthma severity. *Immune Network* 21, 1–16. doi: 10.4110/in.2021.21.e25

Han, W., Tang, C., Baba, S., Hamada, T., Shimazu, T., and Iwakura, Y. (2021). Ovalbumin-induced airway inflammation is ameliorated in Dectin-1–deficient mice, in which pulmonary regulatory T cells are expanded through modification of intestinal commensal Bacteria. *J. Immunol.* 206, 1991–2000. doi: 10.4049/jimmunol.2001337

Hauk, P. J., Hamid, Q. A., Chrousos, G. P., and Leung, D. Y. M. (2000). Induction of corticosteroid insensitivity in human PBMCS by microbial superantigens. *J. Allergy Clin. Immunol.* 105, 782–787. doi: 10.1067/mai.2000.105807

He, X., Cheng, G. S., He, L., Liao, B., du, Y. J., Xie, X., et al. (2021). Adults with current asthma but not former asthma have higher all-cause and cardiovascular mortality: a population-based prospective cohort study. *Sci. Rep.* 11:1329. doi: 10.1038/s41598-020-79264-4

Healy, D. B., Ryan, C. A., Ross, R. P., Stanton, C., and Dempsey, E. M. (2022). Clinical implications of preterm infant gut microbiome development. *Nat. Microbiol.* 7, 22–33. doi: 10.1038/s41564-021-01025-4

Hemachandra Reddy, P. (2011). Mitochondrial dysfunction and oxidative stress in asthma: implications for mitochondria-targeted antioxidant therapeutics. *Pharmaceuticals* 4, 429–456. doi: 10.3390/ph4030429

Hilty, M., Burke, C., Pedro, H., Cardenas, P., Bush, A., Bossley, C., et al. (2010). Disordered microbial communities in asthmatic airways. *PLoS One* 5:e8578. doi: 10.1371/journal.pone.0008578

Hizawa, N. (2023). The understanding of asthma pathogenesis in the era of precision medicine. *Allergol. Int.* 72, 3–10. doi: 10.1016/j.alit.2022.09.001

Hofstra, J. J., Matamoros, S., van de Pol, M. A., de Wever, B., Tanck, M. W., Wendt-Knol, H., et al. (2015). Changes in microbiota during experimental human rhinovirus infection. *BMC Infect. Dis.* 15, 336–339. doi: 10.1186/s12879-015-1081-y

House, J. S., Wyss, A. B., Hoppin, J. A., Richards, M., Long, S., Umbach, D. M., et al. (2017). Early-life farm exposures and adult asthma and atopy in the agricultural lung health study. *J. Allergy Clin. Immunol.* 140, 249–256.e14. doi: 10.1016/j.jaci.2016.09.036

Hu, W., Wang, Z. M., Feng, Y., Schizas, M., Hoyos, B. E., van der Veeken, J., et al. (2021). Regulatory T cells function in established systemic inflammation and reverse fatal autoimmunity. *Nat. Immunol.* 22, 1163–1174. doi: 10.1038/s41590-021-01001-4

Huang, Y. J., Nariya, S., Harris, J. M., Lynch, S. V., Choy, D. F., Arron, J. R., et al. (2015). The airway microbiome in patients with severe asthma: associations with disease features and severity. *J. Allergy Clin. Immunol.* 136, 874–884. doi: 10.1016/j.jaci.2015.05.044

Huang, C., Ni, Y., du, W., and Shi, G. (2022). Effect of inhaled corticosteroids on microbiome and microbial correlations in asthma over a 9-month period. *Clin. Transl. Sci.* 15, 1723–1736. doi: 10.1111/cts.13288

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395, 497–506. doi: 10.1016/S0140-6736(20)30183-5

Huang, J., Yang, Z., Li, Y., Chai, X., Liang, Y., Lin, B., et al. (2021). *Lactobacillus paracasei* R3 protects against dextran sulfate sodium (DSS)-induced colitis in mice via regulating Th17/Treg cell balance. *J. Transl. Med.* 19, 1–13. doi: 10.1186/s12967-021-02943-x

Hufnagl, K., Pali-Schöll, I., Roth-Walter, F., and Jensen-Jarolim, E. (2020). Dysbiosis of the gut and lung microbiome has a role in asthma. *Semin. Immunopathol.* 42, 75–93. doi: 10.1007/s00281-019-00775-y

Iebba, V., Totino, V., Gagliardi, A., Santangelo, F., Cacciotti, F., Trancassini, M., et al. (2016). Eubiosis and dysbiosis: the two sides of the microbiota. *New Microbiol.* 39, 1–12.

Jartti, T., Bønnelykke, K., Elenius, V., and Feleszko, W. (2020). Role of viruses in asthma. *Semin. Immunopathol.* 42, 61–74. doi: 10.1007/s00281-020-00781-5

Jiménez, E., Marín, M. L., Martín, R., Odriozola, J. M., Olivares, M., Xaus, J., et al. (2008). Is meconium from healthy newborns actually sterile? *Res. Microbiol.* 159, 187–193. doi: 10.1016/j.resmic.2007.12.007

Johnson, J. L., Jones, M. B., and Cobb, B. A. (2015). Polysaccharide a from the capsule of *Bacteroides fragilis* induces clonal CD4+ T cell expansion. *J. Biol. Chem.* 290, 5007–5014. doi: 10.1074/jbc.M114.621771

Kalbermatter, C., Fernandez Trigo, N., Christensen, S., and Ganal-Vonarburg, S. C. (2021). Maternal microbiota, early life colonization and breast Milk drive immune development in the newborn. *Front. Immunol.* 12, 1–22. doi: 10.3389/fimmu.2021.683022

Kato, K., Odamaki, T., Mitsuyama, E., Sugahara, H., Xiao, J. Z., and Osawa, R. (2017). Age-related changes in the composition of gut Bifidobacterium species. *Curr. Microbiol.* 74, 987–995. doi: 10.1007/s00284-017-1272-4

Kenyon, N. J., Morrissey, B. M., Schivo, M., and Albertson, T. E. (2012). Occupational asthma. *Clin. Rev. Allergy Immunol.* 43, 3–13. doi: 10.1007/s12016-011-8272-0

Keski-Nisula, L., Kyynäräinen, H. R., Kärkkäinen, U., Karhukorpi, J., Heinonen, S., and Pekkanen, J. (2013). Maternal intrapartum antibiotics and decreased vertical transmission of Lactobacillus to neonates during birth Acta Paediatrica. *Int. J. Paediatr.* 102, 480–485. doi: 10.1111/apa.12186

Kim, D. H., Han, K., and Kim, S. W. (2018). Effects of antibiotics on the development of asthma and other allergic diseases in children and adolescents. *Allergy Asthma Immunol. Res.* 10, 457–465. doi: 10.4168/aair.2018.10.5.457

Kim, Y., Womble, J. T., Gunsch, C. K., and Ingram, J. L. (2021). The gut/lung microbiome Axis in obesity, asthma and bariatric surgery: a literature review. Yeon. *Obesity* 29, 636–644. doi: 10.1002/oby.23107

Klopp, A., Vehling, L., Becker, A. B., Subbarao, P., Mandhane, P. J., Turvey, S. E., et al. (2017). Modes of infant feeding and the risk of childhood asthma: a prospective birth cohort study. *J. Pediatr.* 190:e2, 192–199.e2. doi: 10.1016/j.jpeds.2017.07.012.

Koch, M. A., Reiner, G. L., Lugo, K. A., Kreuk, L. S. M., Stanbery, A. G., Ansaldo, E., et al. (2016). Maternal IgG and IgA antibodies dampen mucosal T helper cell responses in early life. *Cells* 165, 827–841. doi: 10.1016/j.cell.2016.04.055

Kumar, A., das, S., Agrawal, A., Mukhopadhyay, I., and Ghosh, B. (2015). Genetic association of key Th1/Th2 pathway candidate genes, IRF2, IL6, IFNGR2, STAT4 and IL4RA, with atopic asthma in the Indian population. *J. Hum. Genet.* 60, 443–448. doi: 10.1038/jhg.2015.45

Kumbhare, S. V., Patangia, D. V., Patil, R. H., Shouche, Y. S., and Patil, N. P. (2019). Factors influencing the gut microbiome in children: from infancy to childhood. *J. Biosci.* 44, 1–19. doi: 10.1007/s12038-019-9860-z

Kurilshikov, A., Medina-Gomez, C., Bacigalupe, R., Radjabzadeh, D., Wang, J., Demirkan, A., et al. (2021). Large-scale association analyses identify host factors influencing human gut microbiome composition. *Nat. Genet.* 53, 156–165. doi: 10.1038/s41588-020-00763-1

Kuruvilla, M. E., Lee, F. E. H., and Lee, G. B. (2019). Understanding asthma phenotypes, Endotypes, and mechanisms of disease. *Clin. Rev. Allergy Immunol.* 56, 219–233. doi: 10.1007/s12016-018-8712-1

Kynyk, J. A., Mastronarde, J. G., and McCallister, J. W. (2011). Asthma, the sex difference. *Curr. Opin. Pulm. Med.* 17, 6–11. doi: 10.1097/MCP.0b013e3283410038

Laursen, M. F., Bahl, M. I., Michaelsen, K. F., and Licht, T. R. (2017). First foods and gut microbes. *Front. Microbiol.* 8, 1–8. doi: 10.3389/fmicb.2017.00356

Lee, N., and Kim, W. U. (2017). Microbiota in T-cell homeostasis and inflammatory diseases. *Exp. Mol. Med.* 49:e340. doi: 10.1038/emm.2017.36

Lee, J. J., Kim, S. H., Lee, M. J., Kim, B. K., Song, W. J., Park, H. W., et al. (2019). 'Different upper airway microbiome and their functional genes associated with asthma in young adults and elderly individuals', allergy: European. *J. Allergy Clin. Immunol.* 74, 709–719. doi: 10.1111/all.13608

Lee, S. A., Lim, J. Y., Kim, B. S., Cho, S. J., Kim, N. Y., Kim, O. B., et al. (2015). Comparison of the gut microbiota profile in breast-fed and formula-fed Korean infants using pyrosequencing. *Nutr. Res. Pract.* 9, 242–248. doi: 10.4162/nrp.2015.9.3.242

Leviatan, S., Shoer, S., Rothschild, D., Gorodetski, M., and Segal, E. (2022). An expanded reference map of the human gut microbiome reveals hundreds of previously unknown species. *Nat. Commun.* 13, 3863–3814. doi: 10.1038/s41467-022-31502-1

Li, R., Guo, Q., Zhao, J., Kang, W., Lu, R., Long, Z., et al. (2023). Assessing causal relationships between gut microbiota and asthma: evidence from two sample Mendelian randomization analysis. *Front. Immunol.* 14:684. doi: 10.3389/fimmu.2023.1148684

Li, R., Zheng, W. X., Zhang, Q. R., Song, Y., Liao, Y. T., Shi, F. C., et al. (2023). Longevity-associated Core gut microbiota mining and effect of mediated probiotic combinations on aging mice: case study of a long-lived population in Guangxi, China. *Nutrients* 15, 1–22. doi: 10.3390/nu15071609

Lim, M. Y., Hong, S., Kim, J. H., and Nam, Y. D. (2021). Association between gut microbiome and frailty in the older adult population in Korea. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* 76, 1362–1368. doi: 10.1093/gerona/glaa319

Lin, Y. C., Chen, Y. C., Kuo, C. H., Chang, Y. H., Huang, H. Y., Yeh, W. J., et al. (2020). Antibiotic exposure and asthma development in children with allergic rhinitis. *J. Microbiol. Immunol. Infect.* 53, 803–811. doi: 10.1016/j.jmii.2019.02.003

Lin, J., Zhang, Y., He, C., and Dai, J. (2018). Probiotics supplementation in children with asthma: a systematic review and meta-analysis. *J. Paediatr. Child Health* 54, 953–961. doi: 10.1111/jpc.14126

Liu, A. H. (2007). Hygiene theory and allergy and asthma prevention. *Paediatr. Perinat. Epidemiol.* 21, 2–7. doi: 10.1111/j.1365-3016.2007.00878.x

Liu, Q. H., Kan, X., Wang, Y. B., Liu, K. X., and Zeng, D. (2020). Differences in the clinical characteristics of early- and late-onset asthma in elderly patients. *Biomed. Res. Int.* 2020, 1–7. doi: 10.1155/2020/2940296

Liu, C., Makrinioti, H., Saglani, S., Bowman, M., Lin, L. L., and Camargo, C. A., et al. (2022). Microbial dysbiosis and childhood asthma development: Integrated role of the airway and gut microbiome, environmental exposures, and host metabolism and immune response. *Front. Immunol.* 12, 1–9. doi: 10.3389/fimmu.2022.1028209

Lopera-Maya, E. A., Kurilshikov, A., van der Graaf, A., Hu, S., Andreu-Sánchez, S., Chen, L., et al. (2022). Effect of host genetics on the gut microbiome in 7,738 participants of the Dutch microbiome project. *Nat. Genet.* 54, 143–151. doi: 10.1038/s41588-021-00992-y

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., and Kroemer, G. (2013). The hallmarks of aging Europe PMC funders group. *Cells* 153, 1194–1217. doi: 10.1016/j.cell.2013.05.039

Losol, P., Choi, J. P., Kim, S. H., and Chang, Y. S. (2021). The role of upper airway microbiome in the development of adult asthma. *Immune Network* 21, 1–18. doi: 10.4110/in.2021.21.e19

Lu, Y., Wang, Y., Wang, J., Lowe, A. J., Grzeskowiak, L. E., and Hu, Y. J. (2023). Early-life antibiotic exposure and childhood asthma trajectories: a National Population-Based Birth Cohort. *Antibiotics* 12, 1–15. doi: 10.3390/antibiotics12020314

Lupu, A., Jechel, E., Mihai, C. M., Mitrofan, E. C., Fotea, S., Starcea, I. M., et al. (2023). The footprint of microbiome in pediatric asthma-a complex puzzle for a balanced development. *Nutrients* 15, 1–18. doi: 10.3390/nu15143278

Lyons, K. E., Ryan, C. A., Dempsey, E. M., Ross, R. P., and Stanton, C. (2020). Breast milk, a source of beneficial microbes and associated benefits for infant health. *Nutrients* 12, 1–30. doi: 10.3390/nu12041039

Macpherson, A. J., De Agüero, M. G., and Ganal-Vonarburg, S. C. (2017). How nutrition and the maternal microbiota shape the neonatal immune system. *Nat. Rev. Immunol.* 17, 508–517. doi: 10.1038/nri.2017.58

Maestrelli, P., Henneberger, P. K., Tarlo, S., Mason, P., and Boschetto, P. (2020). Causes and phenotypes of work-related asthma. *Int. J. Environ. Res. Public Health* 17, 1–10. doi: 10.3390/ijerph17134713

Marra, F., Marra, C. A., Richardson, K., Lynd, L. D., Kozyrskyj, A., Patrick, D. M., et al. (2009). Antibiotic use in children is associated with increased risk of asthma. *Pediatrics* 123, 1003–1010. doi: 10.1542/peds.2008-1146

Marsland, B. J., Trompette, A., and Gollwitzer, E. S. (2015). The gut-lung axis in respiratory disease. *Ann. Am. Thorac. Soc.* 12, S150–S156. doi: 10.1513/AnnalsATS.201503-133AW

Martin, M. J., Zain, N. M. M., Hearson, G., Rivett, D. W., Koller, G., Wooldridge, D. J., et al. (2020). The airways microbiome of individuals with asthma treated with high and low doses of inhaled corticosteroids. *PLoS One* 15, e0244681–e0244614. doi: 10.1371/journal.pone.0244681

Martino, C., Dilmore, A. H., Burcham, Z. M., Metcalf, J. L., Jeste, D., and Knight, R. (2022). Microbiota succession throughout life from the cradle to the grave. *Nat. Rev. Microbiol.* 20, 707–720. doi: 10.1038/s41579-022-00768-z

Miliku, K., and Azad, M. B. (2018). Breastfeeding and the developmental origins of asthma: current evidence, possible mechanisms, and future research priorities. *Nutrients* 10, 1–15. doi: 10.3390/nu10080995

Mimoun, A., Delignat, S., Peyron, I., Daventure, V., Lecerf, M., Dimitrov, J. D., et al. (2020). Relevance of the Materno-fetal Interface for the induction of antigen-specific immune tolerance. *Front. Immunol.* 11, 1–16. doi: 10.3389/fimmu.2020.00810

Moore, L. E., Serrano-Lomelin, J., Rosychuk, R. J., Kozyrskyj, A. L., Chari, R., Crawford, S., et al. (2023). Perinatal and early life factors and asthma control among preschoolers: a population-based retrospective cohort study. *BMJ Open Respir. Res.* 10, e001928–e001929. doi: 10.1136/bmjresp-2023-001928

Moradzadeh, R., Mansournia, M. A., Baghfalaki, T., Nadrian, H., Gustafson, P., and McCandless, L. C. (2018). The impact of maternal smoking during pregnancy on childhood asthma: adjusted for exposure misclassification; results from the National Health and nutrition examination survey, 2011–2012. *Ann. Epidemiol.* 28, 697–703. doi: 10.1016/j.annepidem.2018.07.011

Munblit, D., Peroni, D., Boix-Amorós, A., Hsu, P., Land, B., Gay, M., et al. (2017). Human milk and allergic diseases: an unsolved puzzle. *Nutrients* 9, 1–43. doi: 10.3390/nu9080894

Munck, C., Helby, J., Westergaard, C. G., Porsbjerg, C., Backer, V., and Hansen, L. H. (2016). Smoking cessation and the microbiome in induced sputum samples from cigarette smoking asthma patients. *PLoS One* 11, e0158622–e0158611. doi: 10.1371/journal.pone.0158622

Nakata, K., Kobayashi, K., Ishikawa, Y., Yamamoto, M., Funada, Y., Kotani, Y., et al. (2010). The transfer of maternal antigen-specific IgG regulates the development of allergic airway inflammation early in life in an FcRn-dependent manner. *Biochem. Biophys. Res. Commun.* 395, 238–243. doi: 10.1016/j.bbrc.2010.03.170

Natalini, J. G., Singh, S., and Segal, L. N. (2023). The dynamic lung microbiome in health and disease. *Nat. Rev. Microbiol.* 21, 222–235. doi: 10.1038/s41579-022-00821-x

Ni, J., Friedman, H., Boyd, B. C., McGurn, A., Babinski, P., Markossian, T., et al. (2019). Early antibiotic exposure and development of asthma and allergic rhinitis in childhood. *BMC Pediatr.* 19, 225–228. doi: 10.1186/s12887-019-1594-4

Noverr, M. C., Noggle, R. M., Toews, G. B., and Huffnagle, G. B. (2004). Role of antibiotics and fungal microbiota in driving pulmonary allergic responses. *Infect. Immun.* 72, 4996–5003. doi: 10.1128/IAI.72.9.4996-5003.2004

Ntontsi, P., Photiades, A., Zervas, E., Xanthou, G., and Samitas, K. (2021). Genetics and epigenetics in asthma. *Int. J. Mol. Sci.* 22, 1–14. doi: 10.3390/ijms22052412

Nurmagambetov, T., Kuwahara, R., and Garbe, P. (2018). The economic burden of asthma in the United States, 2008-2013. *Ann. Am. Thorac. Soc.* 15, 348–356. doi: 10.1513/AnnalsATS.201703-259OC

Ojanguren, I., Satia, I., and Usmani, O. S. (2022). The role of viral infections on severe asthma exacerbations: present and future. *Archivos de Bronconeumologia* 58, 632–634. doi: 10.1016/j.arbres.2021.10.007

Pagtakhan, R. D., Bjelland, J. C., Landau, L. I., Loughlin, G., Kaltenborn, W., Seeley, G., et al. (1984). Sex differences in growth patterns of the airways and lung parenchyma in children. *J. Appl. Physiol. Res. Environ. Exerc. Physiol.* 56, 1204–1210. doi: 10.1152/jappl.1984.56.5.1204

Palma, G., Sorice, G. P., Genchi, V. A., Giordano, F., Caccioppoli, C., D'Oria, R., et al. (2022). Adipose tissue inflammation and pulmonary dysfunction in obesity. *Int. J. Mol. Sci.* 23, 10–12. doi: 10.3390/ijms23137349

Park, S., Choi, N. K., Kim, S., and Lee, C. H. (2018). The relationship between metabolic syndrome and asthma in the elderly. *Sci. Rep.* 8:9378. doi: 10.1038/s41598-018-26621-z

Park, J. E., Jardine, L., Gottgens, B., Teichmann, S. A., and Haniffa, M. (2020). Prenatal development of human immunity. *Science* 368, 600–603. doi: 10.1126/science.aaz9330

Park, S. H., Kim, K. A., Ahn, Y. T., Jeong, J. J., Huh, C. S., and Kim, D. H. (2015). Comparative analysis of gut microbiota in elderly people of urbanized towns and longevity villages. *BMC Microbiol.* 15:49. doi: 10.1186/s12866-015-0386-8

Park, H. K., Shin, J. W., Park, S. G., and Kim, W. (2014). Microbial communities in the upper respiratory tract of patients with asthma and chronic obstructive pulmonary disease. *PLoS One* 9:e109710. doi: 10.1371/journal.pone.0109710

Pärnänen, K. M. M., Hultman, J., Markkanen, M., Satokari, R., Rautava, S., Lamendella, R., et al. (2022). Early-life formula feeding is associated with infant gut microbiota alterations and an increased antibiotic resistance load. *Am. J. Clin. Nutr.* 115, 407–421. doi: 10.1093/ajcn/nqab353

Partridge, L., Deelen, J., and Slagboom, P. E. (2018). Facing up to the global challenges of ageing. *Nature* 561, 45–56. doi: 10.1038/s41586-018-0457-8

Patrick, D. M., Sbihi, H., Dai, D. L. Y., al Mamun, A., Rasali, D., Rose, C., et al. (2020). Decreasing antibiotic use, the gut microbiota, and asthma incidence in children: evidence from population-based and prospective cohort studies. *Lancet Respir. Med.* 8, 1094–1105. doi: 10.1016/S2213-2600(20)30052-7

Penders, J., Stobberingh, E. E., Thijs, C., Adams, H., Vink, C., van Ree, R., et al. (2006). Molecular fingerprinting of the intestinal microbiota of infants in whom atopic eczema was or was not developing. *Clin. Exp. Allergy* 36, 1602–1608. doi: 10.1111/j.1365-2222.2006.02599.x

Pendyala, S., Walker, J. M., and Holt, P. R. (2012). A high-fat diet is associated with endotoxemia that originates from the gut. *Gastroenterology* 142, 1100–1101.e2. doi: 10.1053/j.gastro.2012.01.034

Perez-Garcia, J., Herrera-Luis, E., Lorenzo-Diaz, F., González, M., Sardón, O., Villar, J., et al. (2020). Precision medicine in childhood asthma: Omic studies of treatment response. *Int. J. Mol. Sci.* 21, 1–15. doi: 10.3390/ijms21082908

Pfefferle, P. I., Keber, C. U., Cohen, R. M., and Garn, H. (2021). The hygiene hypothesis – learning from but not living in the past. *Front. Immunol.* 12, 1–6. doi: 10.3389/fimmu.2021.635935

Pfeiffer, S., Herzmann, C., Gaede, K. I., Kovacevic, D., Krauss-Etschmann, S., and Schloter, M. (2021). Different responses of the oral, nasal and lung microbiomes to cigarette smoke. *Thorax* 77, 191–195. doi: 10.1136/thoraxjnl-2020-216153

Pinot de Moira, A., Strandberg-Larsen, K., Bishop, T., Pedersen, M., Avraam, D., Cadman, T., et al. (2022). Associations of early-life pet ownership with asthma and allergic sensitization: a meta-analysis of more than 77,000 children from the EU child cohort network. *J. Allergy Clin. Immunol.* 150, 82–92. doi: 10.1016/j.jaci.2022.01.023

Pinto, R. A., Arredondo, S. M., Bono, M. R., Gaggero, A. A., and Díaz, P. V. (2006). T helper 1/T helper 2 cytokine imbalance in respiratory syncytial virus infection is associated with increased endogenous plasma cortisol. *Pediatrics* 117, e878–e886. doi: 10.1542/peds.2005-2119

Raita, Y., Pérez-Losada, M., Freishtat, R. J., Harmon, B., Mansbach, J. M., Piedra, P. A., et al. (2021). 'Integrated omics endotyping of infants with respiratory syncytial virus bronchiolitis and risk of childhood asthma', nature. *Communications* 12, 1–13. doi: 10.1038/s41467-021-23859-6

Ramakrishna, C., Kujawski, M., Chu, H., Li, L., Mazmanian, S. K., and Cantin, E. M. (2019). *Bacteroides fragilis* polysaccharide a induces IL-10 secreting B and T cells that prevent viral encephalitis. *Nat. Commun.* 10, 1–13. doi: 10.1038/s41467-019-09884-6

Rastogi, S., and Singh, A. (2022). Gut microbiome and human health: exploring how the probiotic genus Lactobacillus modulate immune responses. *Front. Pharmacol.* 13, 1–17. doi: 10.3389/fphar.2022.1042189

Reddel, H. K., Bacharier, L. B., Bateman, E. D., Brightling, C. E., Brusselle, G. G., Buhl, R., et al. (2022). Global initiative for asthma strategy 2021: executive summary and rationale for key changes. *EurRespirJ* 59:2102730. doi: 10.1183/13993003.02730-2021

Ren, Y., Ichinose, T., He, M., Youshida, S., Nishikawa, M., and Sun, G. (2019). Co-exposure to lipopolysaccharide and desert dust causes exacerbation of ovalbumin-induced allergic lung inflammation in mice via TLR4/MyD88-dependent and -independent pathways. *Allergy Asthma Clin. Immunol.* 15, 1–12. doi: 10.1186/s13223-019-0396-4

GINA Report (2022). Global strategy for asthma management and prevention. Available at: https://ginasthma.org/gina-reports/

Resztak, J. A., Choe, J., Nirmalan, S., Wei, J., Bruinsma, J., Houpt, R., et al. (2023). Analysis of transcriptional changes in the immune system associated with pubertal development in a longitudinal cohort of children with asthma. *Nat. Commun.* 14, 230–214. doi: 10.1038/s41467-022-35742-z

Rühlemann, M. C., Hermes, B. M., Bang, C., Doms, S., Moitinho-Silva, L., Thingholm, L. B., et al. (2021). Genome-wide association study in 8,956 German individuals identifies influence of ABO histo-blood groups on gut microbiome. *Nat. Genet.* 53, 147–155. doi: 10.1038/s41588-020-00747-1

Rutayisire, E., Huang, K., Liu, Y., and Tao, F. (2016). The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year of infants' life: a systematic review. *BMC Gastroenterol.* 16, 1–12. doi: 10.1186/s12876-016-0498-0

Salas Garcia, M. C., Yee, A. L., Gilbert, J. A., and Dsouza, M. (2018). Dysbiosis in children born by caesarean section. *Ann. Nutr. Metab.* 73, 24–32. doi: 10.1159/000492168

Santacroce, L., Charitos, I. A., Ballini, A., Inchingolo, F., Luperto, P., de Nitto, E., et al. (2020). The human respiratory system and its microbiome at a glimpse. *Biology* 9, 1–16. doi: 10.3390/biology9100318

Schroeder, B. O., Birchenough, G. M. H., Ståhlman, M., Arike, L., Johansson, M. E. V., Hansson, G. C., et al. (2018). Bifidobacteria or Fiber protects against diet-induced microbiota-mediated colonic mucus deterioration. *Cell Host and Microbe* 23, 27–40.e7. doi: 10.1016/j.chom.2017.11.004

Serrano-Villar, S., Vázquez-Castellanos, J. F., Vallejo, A., Latorre, A., Sainz, T., Ferrando-Martínez, S., et al. (2017). The effects of prebiotics on microbial dysbiosis, butyrate production and immunity in HIV-infected subjects. *Mucosal Immunol.* 10, 1279–1293. doi: 10.1038/mi.2016.122

Sharpe, R. A., Bearman, N., Thornton, C. R., Husk, K., and Osborne, N. J. (2015). Indoor fungal diversity and asthma: a meta-analysis and systematic review of risk factors. *J. Allergy Clin. Immunol.* 135, 110–122. doi: 10.1016/j.jaci.2014.07.002

Shin, J. H., Park, Y. H., Sim, M., Kim, S. A., Joung, H., and Shin, D. M. (2019). Serum level of sex steroid hormone is associated with diversity and profiles of human gut microbiome. *Res. Microbiol.* 170, 192–201. doi: 10.1016/j.resmic.2019.03.003

Shore, S. A. (2010). Obesity, airway hyperresponsiveness, and inflammation. *J. Appl. Physiol.* 108, 735–743. doi: 10.1152/japplphysiol.00749.2009

Simpson, J. L., Daly, J., Baines, K. J., Yang, I. A., Upham, J. W., Reynolds, P. N., et al. (2016). Airway dysbiosis: Haemophilus influenza and Tropheryma in poorly controlled asthma. *Eur. Respir. J.* 47, 792–800. doi: 10.1183/13993003.00405-2015

Singh, V., Lee, G. D., Son, H. W., Koh, H., Kim, E. S., Unno, T., et al. (2023). Butyrate producers, "the sentinel of gut": their intestinal significance with and beyond butyrate, and prospective use as microbial therapeutics. *Front. Microbiol.* 13, 1–16. doi: 10.3389/fmicb.2022.1103836

Sisk-Hackworth, L., Kelley, S. T., and Thackray, V. G. (2023). Sex, puberty, and the gut microbiome. *Soc. Reprod. Fertility* 165, R61–R74. doi: 10.1530/REP-22-0303

Słabuszewska-Jóźwiak, A., Szymański, J. K., Ciebiera, M., Sarecka-Hujar, B., and Jakiel, G. (2020). Pediatrics consequences of caesarean section—a systematic review and meta-analysis. *Int. J. Environ. Res. Public Health* 17, 1–17. doi: 10.3390/ijerph17218031

Song, W. J., Sintobin, I., Sohn, K. H., Kang, M. G., Park, H. K., Jo, E. J., et al. (2016). Staphylococcal enterotoxin IgE sensitization in late-onset severe eosinophilic asthma in the elderly. *Clin. Exp. Allergy* 46, 411–421. doi: 10.1111/cea.12652

Song, S. J., Wang, J., Martino, C., Jiang, L., Thompson, W. K., Shenhav, L., et al. (2021). Naturalization of the microbiota developmental trajectory of cesarean-born neonates after vaginal seeding. *Med* 2, 951–964.e5. doi: 10.1016/j.medj.2021.05.003

Stein, M., Hrusch, C. L., Gozdz, J., Igartua, C., Pivniouk, V., Murray, S. E., et al. (2016). Innate immunity and asthma risk in amish and hutterite farm children. *N. Engl. J. Med.* 375, 411–421. doi: 10.1056/nejmoa1508749

Stiemsma, L. T., and Michels, K. B. (2017). The role of the microbiome in the developmental origins of health and disease. *Pediatrics* 141:e20172437

Stokholm, J., Blaser, M. J., Thorsen, J., Rasmussen, M. A., Waage, J., Vinding, R. K., et al. (2018). 'Maturation of the gut microbiome and risk of asthma in childhood', nature. *Communications* 9, 1–10. doi: 10.1038/s41467-017-02573-2

Suganya, K., and Koo, B. S. (2020). Gut–brain axis: role of gut microbiota on neurological disorders and how probiotics/prebiotics beneficially modulate microbial and immune pathways to improve brain functions. *Int. J. Mol. Sci.* 21, 1–29. doi: 10.3390/ijms21207551

Takagi, T., Naito, Y., Inoue, R., Kashiwagi, S., Uchiyama, K., Mizushima, K., et al. (2019). Differences in gut microbiota associated with age, sex, and stool consistency in healthy Japanese subjects. *J. Gastroenterol.* 54, 53–63. doi: 10.1007/s00535-018-1488-5

Tan, D. J., Walters, E. H., Perret, J. L., Lodge, C. J., Lowe, A. J., Matheson, M. C., et al. (2015). Age-of-asthma onset as a determinant of different asthma phenotypes in adults: a systematic review and meta-analysis of the literature. *Expert Rev. Respir. Med.* 9, 109–123. doi: 10.1586/17476348.2015.1000311

Taylor, S. L., Leong, L. E. X., Choo, J. M., Wesselingh, S., Yang, I. A., Upham, J. W., et al. (2018). Inflammatory phenotypes in patients with severe asthma are associated with distinct airway microbiology. *J. Allergy Clin. Immunol.* 141, 94–103.e15. doi: 10.1016/j.jaci.2017.03.044

Teo, S. M., Mok, D., Pham, K., Kusel, M., Serralha, M., Troy, N., et al. (2015). The infant nasopharyngeal microbiome impacts severity of lower respiratory infection and risk of asthma development. *Cell Host Microbe* 17, 704–715. doi: 10.1016/j.chom.2015.03.008

Thorsen, J., Stokholm, J., Rasmussen, M. A., Mortensen, M. S., Brejnrod, A. D., Hjelmsø, M., et al. (2021). The airway microbiota modulates effect of azithromycin treatment for episodes of recurrent asthma-like symptoms in preschool children a randomized clinical trial. *Am. J. Respir. Crit. Care Med.* 204, 149–158. doi: 10.1164/rccm.202008-3226OC

To, T., Stanojevic, S., Moores, G., Gershon, A. S., Bateman, E. D., Cruz, A. A., et al. (2012). Global asthma prevalence in adults: findings from the cross-sectional world health survey. *BMC Public Health* 12:204. doi: 10.1186/1471-2458-12-204

Toivonen, L., Schuez-Havupalo, L., Karppinen, S., Waris, M., Hoffman, K. L., Camargo, C. A., et al. (2021). Antibiotic treatments during infancy, changes in nasal microbiota, and asthma development: population-based cohort study. *Clin. Infect. Dis.* 72, 1546–1554. doi: 10.1093/cid/ciaa262

Tomoda, K., Kubo, K., Asahara, T., Andoh, A., Nomoto, K., Nishii, Y., et al. (2011). Cigarette smoke decreases organic acids levels and population of bifidobacterium in the caecum of rats. *J. Toxicol. Sci.* 36, 261–266. doi: 10.2131/jts.36.261

Troisi, R. J., Speizer, F. E., Willett, W. C., Trichopoulos, D., and Rosner, B. (1995). Menopause, postmenopausal estrogen preparations, and the risk of adult-onset asthma. *Am. J. Respir. Crit. Care Med.* 152, 1183–1188. doi: 10.1164/ajrccm.152.4.7551368

Troy, E. B., and Kasper, D. L. (2010). Beneficial effects of *Bacteroides fragilis* polysaccharides on the immune system. *Front. Biosci.* 15, 25–34. doi: 10.2741/3603

Tsukuda, N., Yahagi, K., Hara, T., Watanabe, Y., Matsumoto, H., Mori, H., et al. (2021). Key bacterial taxa and metabolic pathways affecting gut short-chain fatty acid profiles in early life. *ISME J.* 15, 2574–2590. doi: 10.1038/s41396-021-00937-7

Turunen, J., Tejesvi, M. V., Paalanne, N., Pokka, T., Amatya, S. B., Mishra, S., et al. (2023). Investigating prenatal and perinatal factors on meconium microbiota: a systematic review and cohort study. *Pediatr. Res.* 14, 1–11. doi: 10.1038/s41390-023-02783-z

Ucciferri, C. C., and Dunn, S. E. (2022). 'Effect of puberty on the immune system: relevance to multiple sclerosis', Frontiers. *Pediatrics* 10, 1–20. doi: 10.3389/fped.2022.1059083

van de Loo, K., van Gelder, M., Roukema, J., Roeleveld, N., Merkus, P. J., and Verhaak, C. M. (2016). Prenatal maternal psychological stress and childhood asthma and wheezing: a meta-analysis. *Eur. Respir. J.* 47, 133–146. doi: 10.1183/13993003.00299-2015

van den Munckhof, E. H. A., Hafkamp, H. C., de Kluijver, J., Kuijper, E. J., de Koning, M. N. C., Quint, W. G. V., et al. (2020). Nasal microbiota dominated by Moraxella s is associated with respiratory health in the elderly population: a case control study. *Respir. Res.* 21, 181–189. doi: 10.1186/s12931-020-01443-8

Ver Heul, A., Planer, J., and Kau, A. (2019). The human microbiota and asthma. *Clin. Rev. Allergy Immunol.* 57, 350–363. doi: 10.1007/s12016-018-8719-7

Voraphani, N., Stern, D. A., Wright, A. L., Guerra, S., Morgan, W. J., and Martinez, F. D. (2014). Risk of current asthma among adult smokers with respiratory syncytial virus illnesses in early life. *Am. J. Respir. Crit. Care Med.* 190, 392–398. doi: 10.1164/rccm.201311-2095OC

Wang, W., Li, Y., Han, G., Li, A., and Kong, X. (2022). *Lactobacillus fermentum* CECT5716 alleviates the inflammatory response in asthma by regulating TLR2/TLR4 expression. *Front. Nutr.* 9, 1–12. doi: 10.3389/fnut.2022.931427

Wang, Q., Li, F., Liang, B., Liang, Y., Chen, S., Mo, X., et al. (2018). A metagenome-wide association study of gut microbiota in asthma in UK adults. *BMC Microbiol.* 18, 114–117. doi: 10.1186/s12866-018-1257-x

Wang, J., Li, F., and Tian, Z. (2017). Role of microbiota on lung homeostasis and diseases. *Sci. China Life Sci.* 60, 1407–1415. doi: 10.1007/s11427-017-9151-1

Wood, L. G., Garg, M. L., and Gibson, P. G. (2011). A high-fat challenge increases airway inflammation and impairs bronchodilator recovery in asthma. *J. Allergy Clin. Immunol.* 127, 1133–1140. doi: 10.1016/j.jaci.2011.01.036

Wood, L. G., Shivappa, N., Berthon, B. S., Gibson, P. G., and Hebert, J. R. (2015). Dietary inflammatory index is related to asthma risk, lung function and systemic inflammation in asthma. *Clin. Exp. Allergy* 45, 177–183. doi: 10.1111/cea.12323

Wu, Z., Mehrabi Nasab, E., Arora, P., and Athari, S. S. (2022). Study effect of probiotics and prebiotics on treatment of OVA-LPS-induced of allergic asthma inflammation and pneumonia by regulating the TLR4/NF-kB signaling pathway. *J. Transl. Med.* 20, 130–114. doi: 10.1186/s12967-022-03337-3

Wu, Y. L., Xu, J., Rong, X. Y., Wang, F., Wang, H. J., and Zhao, C. (2021). Gut microbiota alterations and health status in aging adults: from correlation to causation. *Aging Med.* 4, 206–213. doi: 10.1002/agm2.12167

Yagi, K., Huffnagle, G. B., Lukacs, N. W., and Asai, N. (2021). The lung microbiome during health and disease. *Int. J. Mol. Sci.* 22, 1–13. doi: 10.3390/ijms221910872

Yáñez, A., Soria, M., de Barayazarra, S., Recuero, N., Rovira, F., Jares, E., et al. (2018). Clinical characteristics and comorbidities of elderly asthmatics who attend allergy clinics. *Asthma Res. Pract.* 4, 1–7. doi: 10.1186/s40733-018-0041-x

Yao, Y., Cai, X., Ye, Y., Wang, F., Chen, F., and Zheng, C. (2021). The role of microbiota in infant health: from early life to adulthood. *Front. Immunol.* 12, 1–21. doi: 10.3389/fimmu.2021.708472

Yap, G. C., Loo, E. X., Aw, M., Lu, Q., Shek, L. P., and Lee, B. W. (2014). Molecular analysis of infant fecal microbiota in an Asian at-risk cohort-correlates with infant and childhood eczema. *BMC. Res. Notes* 7, 1–6. doi: 10.1186/1756-0500-7-166

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., et al. (2012). Human gut microbiome viewed across age and geography. *Nature* 486, 222–227. doi: 10.1038/nature11053

Yuan, X., Chen, R., Zhang, Y., Lin, X., and Yang, X. (2020). Sexual dimorphism of gut microbiota at different pubertal status. *Microb. Cell Factories* 19, 152–116. doi: 10.1186/s12934-020-01412-2

Zeldin, J., Chaudhary, P. P., Spathies, J., Yadav, M., D'Souza, B. N., Alishahedani, M. E., et al. (2023). Exposure to isocyanates predicts atopic dermatitis prevalence and disrupts therapeutic pathways in commensal bacteria. *Sci. Adv.* 9, eade8898–eade8820. doi: 10.1126/sciadv.ade8898

Zhang, J., He, M., Yu, Q., Xiao, F., Zhang, Y., and Liang, C. (2023). The effects of a healthy diet on asthma and wheezing in children and adolescents: a systematic review and Meta-analysis. *J. Asthma Aller.* 6, 1007–1024. doi: 10.2147/JAA.S423884

Zhang, Y., and Huang, L. (2021). Characteristics of older adult hospitalized patients with bronchial asthma: a retrospective study. *Allergy Asthma Clin. Immunol.* 17, 122–127. doi: 10.1186/s13223-021-00628-0

Zhang, J., Ma, J.-y., Li, Q.-h., Su, H., and Sun, X. (2018a). *Lactobacillus rhamnosus* GG induced protective effect on allergic airway inflammation is associated with gut microbiota. *Cell. Immunol.* 332, 77–84. doi: 10.1016/j.cellimm.2018.08.002

Zhang, J., Ma, C., Yang, A., Zhang, R., Gong, J., and Mo, F. (2018b). Is preterm birth associated with asthma among children from birth to 17 years old? -a study based on 2011-2012 US National Survey of Children's health. *Ital. J. Pediatr.* 44, 151–159. doi: 10.1186/s13052-018-0583-9

Zhang, Z., Shi, L., Pang, W., Liu, W., Li, J., Wang, H., et al. (2016). Dietary fiber intake regulates intestinal microflora and inhibits ovalbumin-induced allergic airway inflammation in a mouse model. *PLoS One* 11, 1–16. doi: 10.1371/journal.pone.0147778

Zhao, X., Hu, M., Zhou, H., Yang, Y., Shen, S., You, Y., et al. (2023). The role of gut microbiome in the complex relationship between respiratory tract infection and asthma. *Front. Microbiol.* 14, 1–13. doi: 10.3389/fmicb.2023.1219942

Zheng, D., Liwinski, T., and Elinav, E. (2020). Interaction between microbiota and immunity in health and disease. *Cell Res.* 30, 492–506. doi: 10.1038/s41422-020-0332-7

Zheng, P., Zhang, K., Lv, X., Liu, C., Wang, Q., and Bai, X. (2022). Gut microbiome and metabolomics profiles of allergic and non-allergic childhood asthma. *J. Asthma Aller.* 15, 419–435. doi: 10.2147/JAA.S354870

Zhou, Y., Jackson, D., Bacharier, L. B., Mauger, D., Boushey, H., Castro, M., et al. (2019). The upper-airway microbiota and loss of asthma control among asthmatic children. *Nat. Commun.* 10, 5714–5710. doi: 10.1038/s41467-019-13698-x

Zimmermann, P., Messina, N., Mohn, W. W., Finlay, B. B., and Curtis, N. (2019). Association between the intestinal microbiota and allergic sensitization, eczema, and asthma: a systematic review. *J. Allergy Clin. Immunol.* 143, 467–485. doi: 10.1016/j.jaci.2018.09.025

Zou, X. L., Wu, J. J., Ye, H. X., Feng, D. Y., Meng, P., Yang, H. L., et al. (2021). Associations between gut microbiota and asthma endotypes: a cross-sectional study in South China based on patients with newly diagnosed asthma. *J. Asthma Aller.* 14, 981–992. doi: 10.2147/JAA.S320088

pathogens

MDPI

Review

The Microbiome as Part of the Contemporary View of Tuberculosis Disease

Martín Barbosa-Amezcua [1], David Galeana-Cadena [2], Néstor Alvarado-Peña [3] and Eugenia Silva-Herzog [3,4,*]

[1] Laboratorio de Farmacogenómica, Instituto Nacional de Medicina Genomica (INMEGEN), Mexico City 14610, Mexico; mvz.mba@gmail.com
[2] Laboratorio de Inmunobiología y Genética, Instituto Nacional de Enfermedades Respiratorias (INER), Mexico City 14080, Mexico; david.microbiomics@gmail.com
[3] Coordinación de Infectología y Microbiología, Instituto Nacional de Enfermedades Respiratorias (INER), Mexico City 14080, Mexico; nestor321.alvarado@gmail.com
[4] Laboratorio de Vinculación Científica, Facultad de Medicina-Universidad Nacional Autonoma de Mexico-Instituto Nacional de Medicina Genomica (UNAM-INMEGEN), Mexico City 14610, Mexico
* Correspondence: esilvaherzog@inmegen.gob.mx

Abstract: The study of the microbiome has changed our overall perspective on health and disease. Although studies of the lung microbiome have lagged behind those on the gastrointestinal microbiome, there is now evidence that the lung microbiome is a rich, dynamic ecosystem. Tuberculosis is one of the oldest human diseases, it is primarily a respiratory infectious disease caused by strains from the *Mycobacterium tuberculosis* Complex. Even today, during the COVID-19 pandemic, it remains one of the principal causes of morbidity and mortality worldwide. Tuberculosis disease manifests itself as a dynamic spectrum that ranges from asymptomatic latent infection to life-threatening active disease. The review aims to provide an overview of the microbiome in the tuberculosis setting, both in patients' and animal models. We discuss the relevance of the microbiome and its dysbiosis, and how, probably through its interaction with the immune system, it is a significant factor in tuberculosis's susceptibility, establishment, and severity.

Keywords: tuberculosis; microbiome; dysbiosis; disease dynamics; disease severity

Citation: Barbosa-Amezcua, M.; Galeana-Cadena, D.; Alvarado-Peña, N.; Silva-Herzog, E. The Microbiome as Part of the Contemporary View of Tuberculosis Disease. *Pathogens* **2022**, *11*, 584. https://doi.org/10.3390/pathogens11050584

Academic Editors: Katalin Burián and Dóra Paróczai

Received: 7 April 2022
Accepted: 7 May 2022
Published: 16 May 2022

1. Introduction

Tuberculosis (TB) is a disease that has accompanied humankind for thousands of years [1,2]. Signs of the disease have been found in Egyptian mummies from 2400 BC, and detailed descriptions of it exist in Chinese and Greek literature, including Hippocrates in 400 BC and Galen in 200 AD [3].

TB, caused by the organisms of the *Mycobacterium tuberculosis* Complex (MTBC), includes *Mycobacterium tuberculosis*, *M. africanum*, *M. orygis*, *M. bovis*, *M. microti*, *M. canetti*, *M. caprae*, *M. pinnipedi*, *M. suricattae*, and *M. mungi*, has been responsible for over one billion deaths in the last 200 years [4]. Pre-COVID-19 pandemic, TB was globally the deadliest infectious disease, claiming 1.4 million lives in 2019 and causing illness in close to 10 million. It ranks even now among the top thirteen causes of death worldwide [5]. Furthermore, the health care disruptions caused by the pandemic in 2020 led, for the first time in years, to an increase in deaths by TB with millions of undiagnosed and untreated cases [5].

Infection with *M. tuberculosis* (MTB) occurs when the aerosol droplets carrying the bacillus are inhaled. However, not everyone infected becomes sick. Only a small proportion (5–10%) of immunocompetent individuals will develop active TB (ATB); many will clear the pathogen, and others will resolve in a latent tuberculosis infection (LTBI). LTBI individuals have no symptoms, are unable to transmit the disease, but can revert to active TB at any point in their lives. The heterogeneous manifestation of MTB infection suggests a decisive role of the host in the progression of the disease. The host's innate and adaptive

immunological mechanisms, and their interaction with the microbiome, influence the balance between pathogenesis and host clearance [6–10].

The advent of Next Generation Sequencing (NGS) has revealed the significant role of the microbiome in the balance between health and disease; it has been proposed that changes in the microbiome may become a powerful biomarker for many pathological conditions in the near future [11–14]. Although the study of the microbiome of the respiratory tract has lagged behind that of other body sites, mainly due to the invasiveness and difficulty in obtaining reliable samples, it has become clear that: (1) the lower respiratory tract (LRT) is not sterile; (2) acute and chronic respiratory diseases change the ecological conditions of the respiratory tract, thus affecting the resulting microbial communities; (3) the microbiome trains the immune system; and (4) the immune system modulates the microbiome [15–19]. This interaction of the immune response and the microbiome is critical to the balance between health and disease, including the response to pathogens and other challenges such as allergies, asthma, cystic fibrosis, and cancer [18,20–23]. This review aims to provide a brief overview of the lung microbiome and its relation to TB with its clinical manifestations.

2. The Clinical Course of Tuberculosis

Clinically, TB presents as a disease with a subacute to chronic evolution caused by infection with MTBC. Although MTBC can infect many parts of the body, the vast majority of infections (84%) reside in the lungs as pulmonary TB [5]. The primary infection takes place mainly in the alveoli, where alveolar macrophages phagocytose MTB. It then either crosses the alveolar barrier by diapedesis to settle in the interstice, or spreads directly by migration through the alveolar barrier into circulation, leading to a systemic spread [24]. This primary infection can have at least three outcomes: First, clearance of MTB by the immune system, either by innate or acquired immune response without memory of T cells; although some individuals will clear the pathogen and preserve a robust memory T cell response. In a second outcome, MTB is not cleared but persists in a latent state (LTBI), defined as the state of continuous immune response to MTB antigens but with no evidence of clinical manifestations or bacterial replication [4,25]. The third outcome involves the progression to active disease (ATB) or subclinical TB, characterized by bacterial growth, rapid host deterioration, and leads to different degrees of clinical manifestations [26].

MTB bacilli are cloistered in a granuloma, the histopathology stamp of TB; it is composed of macrophages, lymphocytes, and other immune cells in response to lingering stimuli. The granuloma is very important for containing the infection; there is a constant clash of pro-inflammatory and anti-inflammatory signals. The result of this either promotes or limits the spread of MTB. If there is a strong pro-inflammatory response in this process, then a remodeling of the granuloma with liquefaction and softening of the caseum, as well as the destruction of the lung parenchyma, may signal the beginning of ATB [27]. On the other hand, a predominantly anti-inflammatory response within the granuloma is associated with a decreased risk of reactivation and better clinical outcomes [28,29].

The World Health Organization (WHO) estimates that about a quarter of the world's population is infected with MTB; however, only 127 new cases per 100,000 population were reported in 2020, which suggests that there are millions of people with LTBI functioning as a reservoir for the disease [5]. If left untreated, approximately 5–10% of these LTB infections will progress to active TB during their lifetime. Therefore, the diagnosis and treatment of LTBI are paramount for controlling and eliminating TB. Individuals with LTBI can progress to active TB disease, or it remains as latent tuberculosis infection, depending on the changes in host immunity, host microbiome, and other risk factors that include HIV infection (Relative Risk (RR) 18), undernourishment (RR 3.2), alcohol abuse disorders (RR 3.3), diabetes (RR 1.6), and smoking (RR 1.6) [4,5].

2.1. Definitions and Clinical Manifestations

Clinically, weight loss and night sweats have the most relevant association with active TB, with an odds ratio of 4.47 and 3.29, respectively [30]. However, common symptoms include cough, fever, anorexia, and chest pain [31], all common to many respiratory illnesses, and thus cannot be used for TB diagnostics. This is why TB diagnosis must be confirmed by culture and molecular diagnostic tests [4]. Although a persistent cough is not a definite diagnosis, it is one of the most common symptoms of advanced pulmonary ATB. As the disease progresses, increased inflammation is followed by tissue necrosis that can progress into the tubercular caverns, which are regions with a high bacillary load. The inflammation of the lung parenchyma close to the pleura can cause pleuritic pain [32]. Dyspnoea can be a significant clinical component after a substantial amount of the lung is destroyed or there is a significant pleural effusion [30]. Physical examination of the chest in pulmonary TB is unrevealing [33]. However, the changes are more pronounced in the upper lobes because MTBC is strictly aerobic, and these areas are more ventilated, leading to greater growth of the bacilli [34].

Extrapulmonary Tuberculosis (EPTB) refers to any bacteriologically confirmed case of TB involving organs other than the lungs, e.g., pleura, lymph nodes, abdomen, genitourinary tract, skin, joints, bones, or meninges [35]. It represents 16% of all tuberculosis cases. Its development depends on age, presence, or absence of underlying disease, the MTB strain, immune status, and ethnic background, and, possibly, the microbiome [5,36]. About 10–50% of EPTB patients have associated pulmonary TB [37].

Without treatment, TB is a life-threatening disease. Studies in patients with pulmonary TB, and positive smear microscopy, prior to the advent of anti-TB drugs, were followed up for five years: 50–60% died; 20–25% were cured spontaneously; and 10–25% continued with symptoms of TB [38].

2.2. Tuberculosis Treatment

The objective of any TB therapy is, first, to reduce the number of actively growing bacilli in the patient, thereby decreasing the severity of the disease, and halting transmission of MTB; second, to eradicate populations of persisting bacilli to achieve a long-lasting cure and prevent relapse, and third to prevent the acquisition of drug resistance during therapy [39].

The treatment of ATB relies on multidrug regimens. In the case of drug-susceptible TB (DS-TB), the treatment includes six months of four first-line anti-TB drugs: isoniazid (H), rifampicin (R), ethambutol (E), and pyrazinamide (Z) [40]. This treatment is divided into two phases: an intensive or bactericidal phase with the four drugs H, R, E, Z, administered for two months, with the objective of reducing the bacillary load and the transmission, as well as avoiding the selection of resistant strains associated with these four drugs. The second, or sterilization, phase includes R and H administered for four months, this phase aims to continue with the sterilization of the tissue, including intracellular bacilli, prevent relapses, and therefore have a cure [39]. This regimen has proven to be very successful, with an 85% success rate, and has been widely adopted worldwide for decades [5]. Currently, it is possible to shorten the treatment from six to four months with a scheme with similar efficacy and safety, that includes Rifapentine (P), Moxifloxacin (Mfx), H, and Z [41].

Antibiotic resistance is a great concern for all infectious diseases, including TB. Drug-resistant TB (DR-TB) has increased from 30,000 cases in 2009 to 157,903 in 2020 worldwide [5,42]. There are several types of DR-TB: Rifampicin-Resistant (RR), bacteria resistant to Rifampicin; Multidrug-Resistant TB (MDR-TB), those resistant to at least isoniazid and rifampicin; Pre-Extreme Drug-Resistant (Pre-XDR) are MDR, as well as to any fluoroquinolone; and XDR-TB are strains that fulfill the definition of Pre-XDR for at least one drug of the WHO's Group A list [43] (see below).

The treatment of DR-TB (MDR, Pre-XDR, XDR) can be either with standardized regimens recommended by WHO or individualized plans that are tailored to the pattern of resistance and the patient's particular characteristics, in which specific drugs can be modified according to the pattern of resistance [44]. Anti-tubercular drugs have been classified based on efficacy into Group A: Levofloxacin (Lfx), Moxifloxacin (Mfx), Bedaquiline (Bdq) and Linezolid (Lzd); Group B: Clofazimine (CFZ), Cycloserine (Cs) or Terizidone (Trd); and Group C: Ethambutol (E), Delamanid (Dlm), Pyrazinamide (Z), Imipenem-cilastatin (Imp-Cln) or Meropenem (Mpm), Amikacin (Am) or Streptomycin (S), Ethionamide (Eto) or Prothionamide (Pto) or P-aminosalicylic acid (PAS) [45]. On the other hand, treatment of LTBI has several options: these include six to nine months of daily H or one month of daily Rifapentine plus Isoniazid and four months of daily Rifampicin, just to mention the more common options [25,46].

In sum, all TB treatment involves long multidrug regimens that undoubtedly will have profound effects on the microbiome and the host's overall wellbeing.

3. Human Microbiome and Its Importance in Health and Disease

The term host-associated microbiome refers to the microbial communities occupying a discrete habitat as well as their 'theater of activity', which result in the formation of individual ecological niches. The microbiome forms a dynamic ecosystem that is integrated with its eukaryotic host [47,48]. To fully understand this interaction in the balance between health and disease, a systems approach, that includes proteomic, metabolomic, and genomic data of the distinct microbiomes, will be necessary.

The factors that have been proposed to contribute to the formation of this host-associated microbiome include evolutionary conserved relationships between the host and the colonizing microorganisms [49], interactions between members of the microbial community [50], and with the immune system [51]. Furthermore, structure and distinct physicochemical properties may develop ecological niches with recognizable functional profiles [52]. When all these factors are balanced, or in homeostasis in a particular niche, the microbiome is said to be in eubiosis [53,54], which is a state that reflects a microbiome resilient to changes and thus benefits both the host and the microbial communities (Figure 1a). Given the confusion that the terms balance and unbalance can cause, in the present review we propose to define dysbiosis as 'the reduction of adaptive capacity of a eubiotic microbiome to changes in physicochemical conditions, immune response, microbial diversity, keystone taxa (taxa that are highly connected with other microorganisms and can significantly influence the structure and function of the microbiome) [55,56], dominance or function, increase in pathobionts (commensal microorganisms that have the potential to cause disease), that cause unfavorable alterations for the host or contributes to disease' (Figure 1b). It should be clarified that an infection is only one example of dysbiosis, since any significant change in the microbiome that affects its function is a dysbiotic state, including those due to metabolic alterations [57].

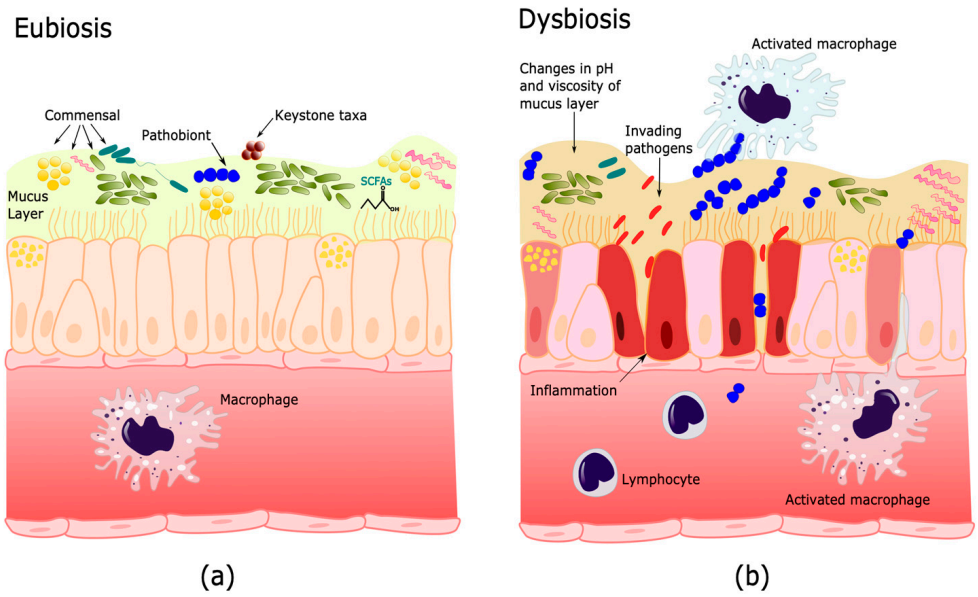


Figure 1. Microbiome dynamics. In eubiosis (**a**), the factors that conform to the microbiome are in homeostasis and produce metabolites that favor the host's wellbeing. While in dysbiosis, (**b**), microorganisms can decrease their adaptive capacity to changes produced by an invading pathogenic agent and microenvironments that promote an increase in pathobionts, changes in the inflammatory response, and the immune system. Elaborated with Inkscape.

3.1. Microbiome Functions

After millions of years of coevolution, the microbiome is able to perform the critical functions of many biological processes of the host, including modulating the metabolic phenotype, regulating epithelial development, and modulation of the immune response. In metabolism, it facilitates the digestion of complex macromolecules [58] and vitamin synthesis [59]. The commensal microbiome has been proposed to prevent the establishment of new microorganisms by competitive exclusion [60], changing the physicochemical factors of the microenvironment [61,62], producing antibiotics and secondary metabolites [63], or modulating the expression of virulence factors [64]. Indirectly, through its metabolites, the microbiome may stimulate the development and function of the immune system [19,65]. Furthermore, both in the gastrointestinal and respiratory tract, the microbiome induces secretion of mucins by Goblet cells that protect the epithelia, and Paneth cells in the gut produce antimicrobial molecules [21]. The microbiome may also promote resistance to future infections in the gastrointestinal tract through the production of metabolites that promote inflammation, which in turn contributes to the protection against future pathogen invasions, which has been called meta-organism memory [66].

3.2. Gut–Lung Axis

Human bodies are made up of different systems that are in constant communication to maintain homeostasis, despite physical barriers. Similarly, microbiomes of different niches have long-distance effects on other body organs, including the skin, gut, brain, and lung [67–70]. This review will focus on the relationship between the gastrointestinal (GI) and respiratory systems. The gut has the most concentration of microorganisms in the human body; it is for this reason, and the fact that the samples are more easily accessible, that the gut is the most studied site regarding the host microbiome. Gut microorganisms come from food and water intake [71,72] and are seeded at birth [73]. In healthy individuals, gut microbiota are dominated by Firmicutes (e.g., *Lactobacillus*, *Bacillus*, and *Clostridium*), as well as Bacteroidetes (e.g., *Bacteroides*), and to a lesser extent, Proteobacteria (e.g., *Escherichia*), and Actinobacteria (e.g., *Bifidobacterium*) [74]. As mentioned above, the effect of the gut microbiome is not limited to the GI tract. It can extend to other organs, including the lung,

in what is known as the "gut–lung axis" [8]. Similarly, the lung microbiome impacts the gut microbiome, and presumably establishes a truly bidirectional network of communication [74]. This communication is achieved through the microorganisms' metabolic products, including small chain fatty acids (SCFAs), which modulate the immune response in both gut and lung systems [70]. Quorum sensing, which allows intraspecies, interspecies, and interkingdom cell-to-cell communication, has been associated with colonization, regulation of virulence factors, resistance to antibiotics, and the adaptive capacity to changes in the microenvironment for the communities that comprise the microbiome [75].

3.3. The Lower Respiratory Tract (LRT) Microbiome

The LRT microbiome changes over time, as well as between individuals [76]. In healthy lungs, microbial communities are primarily determined by immigration, elimination, and reproductive rates, whereas in advanced lung disease, membership is primarily determined by regional growth conditions and reproduction rates [15]. Nevertheless, there is individual compositional microbiome stability and possibly an individual core LRT-commensal microbiome [77]. Similar to the gut microbiota, healthy lungs are predominantly comprised of the phylum Bacteroidetes, Firmicutes, and Proteobacteria, followed by lesser proportions of Actinobacteria [16], but at the genus level, the most abundant are *Streptococcus*, *Prevotella*, *Fusobacterium*, *Haemophilus*, *Pseudomonas*, *Veillonella*, and *Porphyromonas* [78].

The lung microbiome has been reported to change in different conditions including metabolic diseases [79], asthma [80], COPD [81], pulmonary cystic fibrosis [82], and cancer [83]. During infections, these changes can be produced by the entry of a pathogen, an increase of pathobionts, loss of commensals or keystone taxa [55,56]. In the case of the entry of a pathogen, the microbiome may, together with the host's immune response, eliminate the pathogen and maintain the eubiosis [15], or go into a state of dysbiosis which can result in disease [84]. The microbiome can protect from secondary infections inducing IgA and IgG specific responses and adaptive immune response [85]. Nevertheless, dysbiotic microbiomes can also favor co-infections, as in the case of Respiratory Syncytial Virus (RSV), where the modified microenvironment allows the expansion of pathobionts [86]. Thus, dysbiosis of the respiratory microbiome is a critical element in systemic inflammatory responses and the clinical outcome of patients [87].

Recasting the system's approach, where we consider that all microbiomes are interconnected, LRT infections affect the gastrointestinal tract. Influenza, a primary respiratory infection, may cause digestive tract manifestations through hematogenous dissemination of infected lymphocytes from the respiratory tract [88]; and a decreased Bacteroides/Firmicutes ratio in the GI tract has been observed during RSV respiratory tract infection [89]. On the other hand, gut dysbiosis has been associated with both decreased levels of butyrate and exacerbated bacterial pneumonia, which supports the critical role of SCFAs in pulmonary host defense [90] and increases susceptibility to infections [91,92].

4. Microbiome Changes during Tuberculosis

Although dysbiosis has been reported to have negative health effects [93], and was associated with the pathogenesis of various diseases: gastrointestinal diseases, obesity, diabetes, allergies, asthma, colorectal cancer, etc. [13,94], its influence on MTB infection in the lungs is still a subject of study [95].

4.1. Microbiome and Mycobacterium Tuberculosis Infection

As discussed above, MTB infection can have a spectrum of clinical manifestations, ranging from clearance of the bacillus to active establishment of the infection. What determines these outcomes is poorly understood, but has been primarily associated with host factors, such as the immune system response [96] and, more recently, the microbiome [9,97,98].

Although some authors have reported differences in the microbiota between healthy individuals and patients with active TB [99–102], the primary pulmonary response to MTB

colonization is very difficult to assess directly on humans, which is why the use of animal models has been employed. These models have provided valuable information, increasing our knowledge of the disease.

Studies on aerosolized MTB-infected mice, showed a rapid loss (6 days) of intestinal microbial diversity followed by a gradual recovery of beta-diversity, probably because of crosstalk between the microbiome and the host immune system during TB infection [103]. However, similar studies observed slower (12 weeks) and less evident alterations in the intestinal microbiota of mice after the infection with MTB, probably due to differences in the MTB strain used (CDC1551 vs. H37Rv) and/or genetic factors between the animal models (Balb/c vs. C57BL/6 mice) [104].

Parallel studies using murine models of gastrointestinal dysbiosis induced by broad-spectrum antibiotics prior to MTB inoculation, show increased bacilli colonization and dissemination (liver and spleen). This dysbiosis was associated with a reduction in the number of mucosal-associated invariant T cells (MAIT), less expression of IL-17A, IFN-γ, and TNF-α (associated with protection against TB) and increased regulatory T cells (associated with susceptibility to TB); additionally more and larger pulmonary granulomas were observed in these mice, suggesting that antibiotic-induced dysbiosis increases the spread of the disease [9,97]. Furthermore, the restoration of the microbiome through fecal transplant reversed these effects: it increased the number of MAIT cells, the expression of IFN-γ and TNF-α (produced by MAIT cells Th1), and reduced the regulatory T cells, supporting a key role for the microbiome in the colonization of the lungs, the response to MTB, and the severity of the infection in mice [9,97].

Taken together, these findings demonstrate that microbial communities are essential for the modulation of host immunity and that changes in the microbiome, even at distal sites, can determine TB outcomes and prognosis. However, the precise role of dysbiosis in the balance between health and disease is just beginning to be understood.

4.2. The Microbiome during Latent and Active TB

As mentioned earlier, the immune system controls the infection of approximately 90% of people exposed to MTB; these individuals either completely clear the bacilli or remain asymptomatic throughout their lives as LTBI [80]. In LTBI, the immune response restrains MTB within granulomas, where the bacteria may persist, but not spread. It is possible that the lung microbiome is involved in the formation and dynamics of the granuloma, probably through the stimulation of the Th1 response through IL-17, and it is a dysbiotic state that influences the progression of the disease [105]. The influence of the microbiome on the host's adaptive immune response has been reported in other respiratory infections such as influenza, where an intact gut, and/or nasal microbiome is necessary to induce Th1 cytotoxic T lymphocytes (CTL) and IgA responses during viral infection [19].

The role of the GI or LRT microbiome in TB progression is not yet fully understood. However, Perry et al. [106] reported that patients with LTBI and *H. pylori* infection, (one of the most prevalent pathogenic gastric bacteria in the world) had a better Th-1 cytokine response (INF-γ, IL2, TNF-α, CXCL-10) to TB antigens, compared to LTBI individuals with no *H. pylori* infection. In addition, non-human primates exposed to TB as well as individuals with LTBI are less likely to develop active TB when they have a prior *H. pylori* co-infection. This suggests that *H. pylori* infection generates a pro-inflammatory state that enhances the host's innate immune response against MTB and other infectious diseases. Conversely, MTB inoculation after natural colonization of the intestine of mice by *H. hepaticus*, in combination with an intestinal dysbiosis characterized by a greater abundance of *Bacteroidaceae* and reduction of *Clostridiales*, *Ruminococcaceae*, *Lachnospiraceae*, and *Prevotellaceae*, cause an overstimulation of the innate immune response and excessive inflammation (increased pro-inflammatory cytokines) that increased the susceptibility to MTB, and severe lung damage [107].

Other studies, working with a non-human primate model and a combination of 16S rRNA and metagenomics, found an enrichment of the families *Lachnospiraceae* and *Clostridi-*

aceae, even before infection, in the gut microbiome of monkeys that developed severe TB. The prevalence of these bacteria continued after MTB infection with an added reduction of *Streptococcaceae*, *Bacteroidales* RF16, and *Clostridiales vadin* B660 [14]. Furthermore, studies in West Africa where both *M. africanum* (MAF) and MTB are endemic, showed that patients with TB due to MAF had lower alpha diversity, increased *Enterobacteriaceae* in the GI tract, and higher expression of inflammatory genes prior to antibiotic treatment, when compared to the MTB patients and healthy controls. In addition, the MAF patients had a reduction in *Actinobacteria* and *Verrucomicrobia* when compared to the MTB patients. The authors speculate that in this region, where an individual can encounter both bacilli, which bacteria (MTB or MAF) will establish an infection is determined by the host's immune system and its microbiome [108]. This further supports the hypothesis that the gastrointestinal microbiome modulates the susceptibility and development of TB.

On the other hand, studies on the LRT microbiome of TB patients have shown variable results when compared to healthy individuals, perhaps due to differences in samples (BAL vs. sputum), populations analyzed, experimental design, and the definition of healthy. However, several authors have reported an increased microbial diversity in the lower respiratory tract of ATB patients [100–102,109,110]. Other studies have shown increased diversity in DR-TB vs. DS-TB patients [102,111].

This increased microbial diversity during ATB may be due to tissue damage reduction of lung commensal bacterial and a higher susceptibility to opportunistic microorganisms such as members of the *Leptotrichia*, *Granulicatella*, *Campylobacter*, *Delfitia* or *Kingella genus*; or pathogens such as *Klebsiella*, *Pseudomonas* and *Acinetobacter*, which have been associated with other respiratory tract pathologies [109,111], and may contribute to additional damage and aggravated symptoms. In fact, epidemiological studies have shown a correlation between opportunistic infections and increased risk of DR-TB development [112], probably due to an indiscriminate use of antibiotics.

Thus, in addition to multiple risk factors (diabetes, malnutrition, co-infections, parasites, etc.) [113], there is clear evidence that supports the crosstalk between the microbiome and the immune system in the establishment of MTB infection, and between microbiome dysbiosis and progression of MTB infection.

4.3. Microbiome Changes during and after Antituberculosis Treatment

As aforementioned, the standard treatment for drug-susceptible TB requires the use of broad-spectrum and specific antibiotics (H, R, Z, and E) against mycobacteria for at least six months, causing intestinal dysbiosis that persists in patients for more than a year after finishing the treatment [104]. In fact, rifampicin, a broad-spectrum bactericide, causes the greatest alterations in the microbiome [114].

As mentioned previously, there is an increase in the incidence of antibiotic-resistant TB (DR-TB) [5], whose treatment can be up to 20 months and involves the use of combinations of antibiotics that induce intestinal dysbiosis during and for up to eight years after treatment [93]. Fecal transplantation and the use of probiotics have been proposed for the restoration of microbiome eubiosis after DR-TB treatment to reduce the development of comorbidities and poor outcomes [93].

Oral administration of *Lactobacillus rhamnosus* NK210 and *Bifidobacterium longum* NK219 partially help to restore the populations of Firmicutes, Bacteroidetes, Proteobacteria, and Verrucomicrobia in a murine model of gut dysbiosis caused by the use of ampicillin, and during a state of LPS-induced systemic inflammation. In both cases, the administration of NK210 and NK219 decreased the expression of IFN-γ, TNF-α, Tbet; it increased the expression of IL-10 and Foxp3 (both involved in the reduction of the inflammatory response), improving gut dysbiosis and decreasing inflammation [115]. However, the inoculation of a single microorganism was not enough to restore the normal microbial community or prevent recurrent infections in patients with other diseases, such as intractable bacterial vaginosis, but a microbiome transplant from healthy donors was effective in improving symptoms and the laboratory features of the disease [116].

4.4. Influence of the Microbiome in Post-TB Patients

Lung damage, reduced pulmonary function, and proinflammatory lung microenvironment in post-TB patients make them more susceptible to develop recurrent respiratory infections by bacteria (*P. aeruginosa*, *H. influenzae*, *M. catarrhalis*, and *S. aureus*) and fungi (*A. fumigatus*, *A. niger* and *A. flavus*) [111,117].

Furthermore, approximately 6% of patients who complete the standard treatment for drug-susceptible TB, relapse [118]. The persistent dysbiosis of the lung microbiome of TB patients has been associated with treatment failure and relapse [80,119]. Relapsing patients show differences in alpha diversity with an increase in the phyla Proteobacteria and Actinobacteria (rich in pathogenic species) and a reduction in Bacteroidetes (mainly beneficial commensal organisms) in the gut microbiome [80]. Notably, a higher *Pseudomonas*/*Mycobacterium* and lower *Treponema*/*Mycobacterium* ratio in the lung microbiome may be a risk factor associated with relapse [119].

These data suggest that maintaining microbiome eubiosis could be beneficial for TB recovery, as well as to avoid relapse [80]. However, more studies are needed to establish the connection between the microbiome and poor TB outcome [120].

5. Conclusions and Perspectives

The study of the microbiome has changed the perspective of the interactions between microorganisms and their host, as well as our understanding of health and disease. As we have stressed in this review, the microbiome has a central role in the normal physiology of the host, as well as in the immune response before and during infections. An important point to consider is that this interaction is dynamic. The elements that surround and form any particular microbiome are constantly changing and it is the adaptive capacity of an eubiotic microbiome that maintains the balance and wellbeing of the host.

Studies of the microbiome in respiratory disease are recent but have shown that the microbiome has an important role in the establishment and progression of the disease. In particular, TB and microbiome studies are only starting to understand this relationship. TB is an ancient disease that is still now, in the XXI century with new diagnostics and treatments, having a devastating impact on millions of people. The COVID-19 pandemic exposed the fragility of our healthcare systems and left TB patients without diagnosis and treatment. It made clear that new strategies for diagnosis and treatment are desperately needed; we think the microbiome study may provide new insights.

Although further studies are required to fully understand the interaction between the microbiome, the immune response, and MTB pathogenesis, preliminary studies show a possible association between dysbiosis, susceptibility to MTB infection, and TB progression. Dysbiosis generated by changes in the lung environment of TB patients, including loss of commensal and keystone taxa, allows the colonization and proliferation of oral, upper respiratory tract, and environmental microorganisms, resulting in opportunistic infections that aggravate the disease and maybe a risk for relapse (Figure 2).

Furthermore, increased severity of the disease was shown in animal models that were previously treated with antibiotics, and the susceptibility of individuals to different members of the MBTC was associated with distinct gut microbiome.

As in any other infectious disease, antibiotics induce a rapid loss of bacterial populations, generating a dysbiosis that persists even after treatment ends. Restoration of the microbiome at the end of antibiotic treatment could benefit the patient. In this sense, the use of probiotics capable of modulating the immune response and reducing inflammation could help restore eubiosis, avoiding reinfections and relapses.

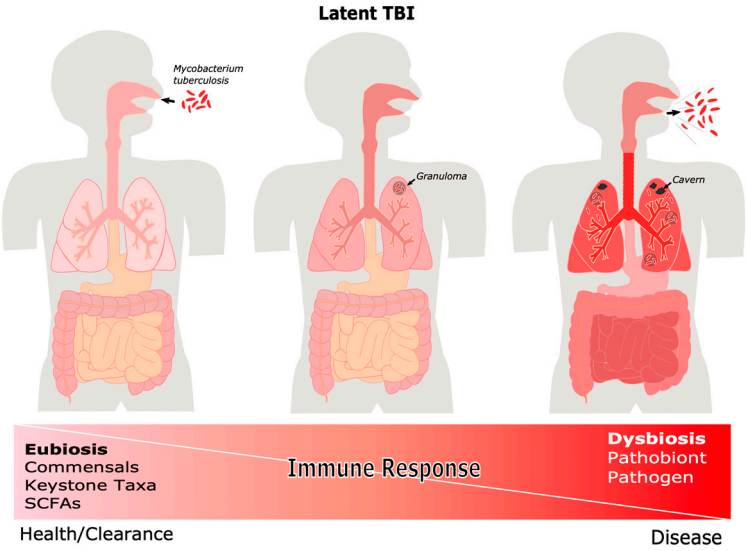


Figure 2. Host-associated microbiome factors in the TB spectrum of disease. TB presents itself as a spectrum of disease, after infection the individual may clear the bacilli, become LTBI, or develop ATB. The outcome of TB infection is modulated by the microbiome as well as the host. (SCFAs, Short Chain Fatty Acids).

It is tempting to think that the microbiome, with its interaction with the immune response, determines the clinical spectrum of the disease, as was suggested for other respiratory infections. The role in immune modulation of fungi, viruses, and parasites in the pathogenesis of TB must also be analyzed. Future, longitudinal studies on the interaction of the respiratory and gastrointestinal microbiome of tuberculosis patients and their close contacts can identify biomarkers to better understand the establishment and progression of tuberculosis and improve patient prognosis.

Author Contributions: Conceptualization, E.S.-H.; methodology, M.B.-A., D.G.-C. and N.A.-P.; formal analysis, M.B.-A., D.G.-C., N.A.-P. and E.S.-H.; investigation, M.B.-A., D.G.-C., N.A.-P. and E.S.-H.; data curation, M.B.-A., D.G.-C., N.A.-P. and E.S.-H.; writing—original draft preparation, M.B.-A., D.G.-C., N.A.-P. and E.S.-H.; writing—review and editing, M.B.-A., D.G.-C. and E.S.-H.; visualization, D.G.-C.; supervision, E.S.-H.; funding acquisition, E.S.-H. All the authors contributed equally to writing and editing of the document. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no extra funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Comas, I.; Coscolla, M.; Luo, T.; Borrell, S.; Holt, K.E.; Kato-Maeda, M.; Parkhill, J.; Malla, B.; Berg, S.; Thwaites, G.; et al. Out-of-Africa migration and Neolithic coexpansion of Mycobacterium tuberculosis with modern humans. *Nat. Genet.* **2013**, *45*, 1176–1182. [CrossRef]
2. Kay, G.L.; Sergeant, M.J.; Zhou, Z.; Chan, J.Z.M.; Millard, A.; Quick, J.; Szikossy, I.; Pap, I.; Spigelman, M.; Loman, N.J.; et al. Eighteenth-century genomes show that mixed infections were common at time of peak tuberculosis in Europe. *Nat. Commun.* **2015**, *6*, 6717. [CrossRef] [PubMed]
3. Barberis, I.; Bragazzi, N.L.; Galluzzo, L.; Martini, M. The history of tuberculosis: From the first historical records to the isolation of Koch's bacillus. *J. Prev. Med. Hyg.* **2017**, *58*, E9. [PubMed]
4. Pai, M.; Behr, M.A.; Dowdy, D.; Dheda, K.; Divangahi, M.; Boehme, C.C.; Ginsberg, A.; Swaminathan, S.; Spigelman, M.; Getahun, H.; et al. Tuberculosis. *Nat. Rev. Dis. Primers* **2016**, *2*, 16076. [CrossRef] [PubMed]

5. World Health Organization. *Global Tuberculosis Report 2021*; World Health Organization: Geneva, Switzerland, 2021.
6. Adami, A.J.; Cervantes, J.L. The microbiome at the pulmonary alveolar niche and its role in Mycobacterium tuberculosis infection. *Tuberculosis* **2015**, *95*, 651–658. [CrossRef] [PubMed]
7. Hong, B.-Y.; Maulén, N.P.; Adami, A.J.; Granados, H.; Balcells, M.E.; Cervantes, J. Microbiome Changes during Tuberculosis and Antituberculous Therapy. *Clin. Microbiol. Rev.* **2016**, *29*, 915–926. [CrossRef]
8. Dumas, A.; Bernard, L.; Poquet, Y.; Lugo-Villarino, G.; Neyrolles, O. The role of the lung microbiota and the gut-lung axis in respiratory infectious diseases. *Cell. Microbiol.* **2018**, *20*, e12966. [CrossRef]
9. Khan, N.; Vidyarthi, A.; Nadeem, S.; Negi, S.; Nair, G.; Agrewala, J.N. Alteration in the Gut Microbiota Provokes Susceptibility to Tuberculosis. *Front. Immunol.* **2016**, *7*, 529. [CrossRef]
10. Negi, S.; Pahari, S.; Bashir, H.; Agrewala, J.N. Gut Microbiota Regulates Mincle Mediated Activation of Lung Dendritic Cells to Protect Against Mycobacterium tuberculosis. *Front. Immunol.* **2019**, *10*, 1142. [CrossRef]
11. Cho, I.; Blaser, M.J. The human microbiome: At the interface of health and disease. *Nat. Rev. Genet.* **2012**, *13*, 260–270. [CrossRef]
12. Dethlefsen, L.; McFall-Ngai, M.; Relman, D.A. An ecological and evolutionary perspective on human–microbe mutualism and disease. *Nature* **2007**, *449*, 811–818. [CrossRef]
13. Carding, S.; Verbeke, K.; Vipond, D.T.; Corfe, B.M.; Owen, L.J. Dysbiosis of the gut microbiota in disease. *Microb. Ecol. Health Dis.* **2015**, *26*, 26191. [CrossRef]
14. Namasivayam, S.; Kauffman, K.D.; McCulloch, J.A.; Yuan, W.; Thovarai, V.; Mittereder, L.R.; Trinchieri, G.; Barber, D.L.; Sher, A. Correlation between Disease Severity and the Intestinal Microbiome in Mycobacterium tuberculosis-Infected Rhesus Macaques. *mBio* **2019**, *10*, e01018-19. [CrossRef]
15. Dickson, R.P.; Erb-Downward, J.R.; Huffnagle, G.B. Homeostasis and its disruption in the lung microbiome. *Am. J. Physiol.-Lung Cell. Mol. Physiol.* **2015**, *309*, L1047–L1055. [CrossRef]
16. Shukla, S.D.; Budden, K.F.; Neal, R.; Hansbro, P.M. Microbiome effects on immunity, health and disease in the lung. *Clin. Transl. Immunol.* **2017**, *6*, e133. [CrossRef]
17. Huffnagle, G.B.; Dickson, R.P.; Lukacs, N.W. The respiratory tract microbiome and lung inflammation: A two-way street. *Mucosal Immunol.* **2016**, *10*, 299–306. [CrossRef]
18. Coburn, B.; Wang, P.W.; Diaz Caballero, J.; Clark, S.T.; Brahma, V.; Donaldson, S.; Zhang, Y.; Surendra, A.; Gong, Y.; Tullis, D.E.; et al. Lung microbiota across age and disease stage in cystic fibrosis. *Sci. Rep.* **2015**, *5*, 10241. [CrossRef]
19. Ichinohe, T.; Pang, I.K.; Kumamoto, Y.; Peaper, D.R.; Ho, J.H.; Murray, T.S.; Iwasaki, A. Microbiota regulates immune defense against respiratory tract influenza A virus infection. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 5354–5359. [CrossRef]
20. Frati, F.; Salvatori, C.; Incorvaia, C.; Bellucci, A.; Di Cara, G.; Marcucci, F.; Esposito, S. The Role of the Microbiome in Asthma: The Gut–Lung Axis. *Int. J. Mol. Sci.* **2018**, *20*, 123. [CrossRef]
21. Mezouar, S.; Chantran, Y.; Michel, J.; Fabre, A.; Dubus, J.-C.; Leone, M.; Sereme, Y.; Mège, J.-L.; Ranque, S.; Desnues, B.; et al. Microbiome and the immune system: From a healthy steady-state to allergy associated disruption. *Hum. Microbiome J.* **2018**, *10*, 11–20. [CrossRef]
22. Lazar, V.; Ditu, L.-M.; Pircalabioru, G.G.; Gheorghe, I.; Curutiu, C.; Holban, A.M.; Picu, A.; Petcu, L.; Chifiriuc, M.C. Aspects of Gut Microbiota and Immune System Interactions in Infectious Diseases, Immunopathology, and Cancer. *Front. Immunol.* **2018**, *9*, 1830. [CrossRef]
23. Pushalkar, S.; Hundeyin, M.; Daley, D.; Zambirinis, C.P.; Kurz, E.; Mishra, A.; Mohan, N.; Aykut, B.; Usyk, M.; Torres, L.E.; et al. The Pancreatic Cancer Microbiome Promotes Oncogenesis by Induction of Innate and Adaptive Immune Suppression. *Cancer Discov.* **2018**, *8*, 403–416. [CrossRef]
24. Ryndak, M.B.; Laal, S. Mycobacterium tuberculosis Primary Infection and Dissemination: A Critical Role for Alveolar Epithelial Cells. *Front. Cell. Infect. Microbiol.* **2019**, *9*, 299. [CrossRef]
25. World Health Organization. WHO consolidated guidelines on tuberculosis. In *Module 1: Prevention—Tuberculosis Preventive Treatment*; World Health Organization: Geneva, Switzerland, 2020.
26. World Health Organization. *Systematic Screening for Active Tuberculosis: An Operational Guide*; World Health Organization: Geneva, Switzerland, 2015.
27. Cadena, A.M.; Fortune, S.M.; Flynn, J.L. Heterogeneity in tuberculosis. *Nat. Rev. Immunol.* **2017**, *17*, 691–702. [CrossRef]
28. Coleman, M.T.; Maiello, P.; Tomko, J.; Frye, L.J.; Fillmore, D.; Janssen, C.; Klein, E.; Lin, P.L. Early Changes by 18Fluorodeoxyglucose Positron Emission Tomography Coregistered with Computed Tomography Predict Outcome after Mycobacterium tuberculosis Infection in Cynomolgus Macaques. *Infect. Immun.* **2014**, *82*, 2400–2404. [CrossRef]
29. Lin, P.L.; Maiello, P.; Gideon, H.P.; Coleman, M.T.; Cadena, A.M.; Rodgers, M.A.; Gregg, R.; O'Malley, M.; Tomko, J.; Fillmore, D.; et al. PET CT Identifies Reactivation Risk in Cynomolgus Macaques with Latent M. tuberculosis. *PLoS Pathog.* **2016**, *12*, e1005739. [CrossRef]
30. Newsom, S.W.B. Tuberculosis. Clinical diagnosis and management of tuberculosis and measures for its prevention and control. *J. Hosp. Infect.* **2006**, *64*, 309–310. [CrossRef]
31. Rathman, G.; Sillah, J.; Hill, P.C.; Murray, J.F.; Adegbola, R.; Corrah, T.; Lienhardt, C.; McAdam, K.P. Clinical and radiological presentation of 340 adults with smear-positive tuberculosis in The Gambia. *Int. J. Tuberc. Lung Dis.* **2003**, *7*, 942–947.
32. Murray, H.W.; Tuazon, C.U.; Kirmani, N.; Sheagren, J.N. The Adult Respiratory Distress Syndrome Associated with Miliary Tuberculosis. *Chest* **1978**, *73*, 37–43. [CrossRef] [PubMed]

33. Brändli, O. The clinical presentation of tuberculosis. *Respiration* **1998**, *65*, 97–105. [CrossRef]
34. Loddenkemper, R.; Lipman, M.; Zumla, A. Clinical Aspects of Adult Tuberculosis. *Cold Spring Harb. Perspect. Med.* **2015**, *6*, a017848. [CrossRef] [PubMed]
35. World Health Organization. *Definitions and Reporting Framework for Tuberculosis—2013 Revision: Updated December 2014 and January 2020*; World Health Organization: Geneva, Switzerland, 2013.
36. Caws, M.; Thwaites, G.; Dunstan, S.; Hawn, T.R.; Thi Ngoc Lan, N.; Thuong, N.T.T.; Stepniewska, K.; Huyen, M.N.T.; Bang, N.D.; Huu Loc, T.; et al. The Influence of Host and Bacterial Genotype on the Development of Disseminated Disease with Mycobacterium tuberculosis. *PLoS Pathog.* **2008**, *4*, e1000034. [CrossRef] [PubMed]
37. Lee, J.Y. Diagnosis and treatment of extrapulmonary tuberculosis. *Tuberc. Respir. Dis.* **2015**, *78*, 47. [CrossRef] [PubMed]
38. Tiemersma, E.W.; van der Werf, M.J.; Borgdorff, M.W.; Williams, B.G.; Nagelkerke, N.J.D. Natural History of Tuberculosis: Duration and Fatality of Untreated Pulmonary Tuberculosis in HIV Negative Patients: A Systematic Review. *PLoS ONE* **2011**, *6*, e17601. [CrossRef]
39. Nahid, P.; Dorman, S.E.; Alipanah, N.; Barry, P.M.; Brozek, J.L.; Cattamanchi, A.; Chaisson, L.H.; Chaisson, R.E.; Daley, C.L.; Grzemska, M.; et al. Official American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America Clinical Practice Guidelines: Treatment of Drug-Susceptible Tuberculosis. *Clin. Infect. Dis.* **2016**, *63*, e147–e195. [CrossRef]
40. World Health Organization. *Guidelines for Treatment of Drug-Susceptible Tuberculosis and Patient Care, 2017 Update*; World Health Organization: Geneva, Switzerland, 2017.
41. Dorman, S.E.; Nahid, P.; Kurbatova, E.V.; Phillips, P.P.J.; Bryant, K.; Dooley, K.E.; Engle, M.; Goldberg, S.V.; Phan, H.T.T.; Hakim, J.; et al. Four-Month Rifapentine Regimens with or without Moxifloxacin for Tuberculosis. *N. Engl. J. Med.* **2021**, *384*, 1705–1718. [CrossRef]
42. World Health Organization. *Global Tuberculosis Control: WHO Report 2010*; World Health Organization: Geneva, Switzerland, 2010.
43. World Health Organization. *Meeting Report of the WHO Expert Consultation on the Definition of Extensively Drug-Resistant Tuberculosis, 27–29 October 2020*; World Health Organization: Geneva, Switzerland, 2021.
44. World Health Organization. WHO consolidated guidelines on tuberculosis. In *Module 4: Treatment—Drug-Resistant Tuberculosis Treatment*; World Health Organization: Geneva, Switzerland, 2020.
45. World Health Organization. *WHO Consolidated Guidelines on Drug-Resistant Tuberculosis Treatment*; World Health Organization: Geneva, Switzerland, 2019.
46. Sterling, T.R.; Njie, G.; Zenner, D.; Cohn, D.L.; Reves, R.; Ahmed, A.; Menzies, D.; Horsburgh, C.R.; Crane, C.M.; Burgos, M.; et al. Guidelines for the Treatment of Latent Tuberculosis Infection: Recommendations from the National Tuberculosis Controllers Association and CDC, 2020. *MMWR. Recomm. Rep.* **2020**, *69*, 1196–1206. [CrossRef]
47. Berg, G.; Rybakova, D.; Fischer, D.; Cernava, T.; Vergès, M.-C.C.; Charles, T.; Chen, X.; Cocolin, L.; Eversole, K.; Corral, G.H.; et al. Microbiome definition re-visited: Old concepts and new challenges. *Microbiome* **2020**, *8*, 103. [CrossRef]
48. Bauer, M.A.; Kainz, K.; Carmona-Gutierrez, D.; Madeo, F. Microbial wars: Competition in ecological niches and within the microbiome. *Microb. Cell* **2018**, *5*, 215–219. [CrossRef]
49. Kumar, P.S. Microbial dysbiosis: The root cause of periodontal disease. *J. Periodontol.* **2021**, *92*, 1079–1087. [CrossRef]
50. Tshikantwa, T.S.; Ullah, M.W.; He, F.; Yang, G. Current trends and potential applications of microbial interactions for human welfare. *Front. Microbiol.* **2018**, *9*, 1156. [CrossRef]
51. Perry, F.; Arsenault, R.J. The study of Microbe–Host Two-way communication. *Microorganisms* **2022**, *10*, 408. [CrossRef]
52. Shaiber, A.; Willis, A.D.; Delmont, T.O.; Roux, S.; Chen, L.-X.; Schmid, A.C.; Yousef, M.; Watson, A.R.; Lolans, K.; Esen, Ö.C.; et al. Functional and genetic markers of niche partitioning among enigmatic members of the human oral microbiome. *Genome Biol.* **2020**, *21*, 292. [CrossRef]
53. Giulio, P. The intestinal microbiota: Towards a multifactorial integrative model. eubiosis and dysbiosis in morbid physical and psychological conditions. *Arch. Clin. Gastroenterol.* **2021**, *7*, 24–35. [CrossRef]
54. Greenbaum, S.; Greenbaum, G.; Moran-Gilad, J.; Weintraub, A.Y. Ecological Dynamics of the vaginal microbiome in relation to health and disease. *Am. J. Obstet. Gynecol.* **2019**, *220*, 324–335. [CrossRef]
55. Banerjee, S.; Schlaeppi, K.; van der Heijden, M.G. Keystone taxa as drivers of microbiome structure and functioning. *Nat. Rev. Microbiol.* **2018**, *16*, 567–576. [CrossRef]
56. Brüssow, H. Problems with the concept of gut microbiota dysbiosis. *Microb. Biotechnol.* **2019**, *13*, 423–434. [CrossRef]
57. Wang, J.; Zheng, J.; Shi, W.; Du, N.; Xu, X.; Zhang, Y.; Ji, P.; Zhang, F.; Jia, Z.; Wang, Y.; et al. Dysbiosis of maternal and neonatal microbiota associated with gestational diabetes mellitus. *Gut* **2018**, *67*, 1614–1625. [CrossRef]
58. Gutierrez Lopez, D.E.; Lashinger, L.M.; Weinstock, G.M.; Bray, M.S. Circadian rhythms and the gut microbiome synchronize the host's metabolic response to Diet. *Cell Metab.* **2021**, *33*, 873–887. [CrossRef]
59. Das, P.; Babaei, P.; Nielsen, J. Metagenomic analysis of microbe-mediated vitamin metabolism in the human gut microbiome. *BMC Genom.* **2019**, *20*, 208. [CrossRef]
60. Segura Munoz, R.R.; Mantz, S.; Martínez, I.; Li, F.; Schmaltz, R.J.; Pudlo, N.A.; Urs, K.; Martens, E.C.; Walter, J.; Ramer-Tait, A.E. Experimental evaluation of ecological principles to understand and modulate the outcome of bacterial strain competition in gut microbiomes. *ISME J.* **2022**, *16*, 1646–1654. [CrossRef]

61. Pickard, J.M.; Núñez, G. Pathogen colonization resistance in the gut and its manipulation for improved health. *Am. J. Pathol.* **2019**, *189*, 1300–1310. [CrossRef]
62. Duar, R.M.; Henrick, B.M.; Casaburi, G.; Frese, S.A. Integrating the ecosystem services framework to define dysbiosis of the breastfed infant gut: The role of B. Infantis and human milk oligosaccharides. *Front. Nutr.* **2020**, *7*, 33. [CrossRef]
63. Netzker, T.; Flak, M.; Krespach, M.K.C.; Stroe, M.C.; Weber, J.; Schroeckh, V.; Brakhage, A.A. Microbial interactions trigger the production of antibiotics. *Curr. Opin. Microbiol.* **2018**, *45*, 117–123. [CrossRef]
64. Federici, S.; Nobs, S.P.; Elinav, E. Phages and their potential to modulate the microbiome and immunity. *Cell. Mol. Immunol.* **2020**, *18*, 889–904. [CrossRef]
65. Laursen, M.F.; Sakanaka, M.; von Burg, N.; Mörbe, U.; Andersen, D.; Moll, J.M.; Pekmez, C.T.; Rivollier, A.; Michaelsen, K.F.; Mølgaard, C.; et al. Bifidobacterium species associated with breastfeeding produce aromatic lactic acids in the infant gut. *Nat. Microbiol.* **2021**, *6*, 1367–1382. [CrossRef]
66. Stacy, A.; Andrade-Oliveira, V.; McCulloch, J.A.; Hild, B.; Oh, J.H.; Perez-Chaparro, P.J.; Sim, C.K.; Lim, A.I.; Link, V.M.; Enamorado, M.; et al. Infection trains the host for microbiota-enhanced resistance to pathogens. *Cell* **2021**, *184*, 615–627.e17. [CrossRef]
67. Salem, I.; Ramser, A.; Isham, N.; Ghannoum, M.A. The gut microbiome as a major regulator of the gut-skin axis. *Front. Microbiol.* **2018**, *9*, 1459. [CrossRef]
68. Adolph, T.E.; Grander, C.; Moschen, A.R.; Tilg, H. Liver–microbiome axis in health and disease. *Trends Immunol.* **2018**, *39*, 712–723. [CrossRef]
69. Bajinka, O.; Simbilyabo, L.; Tan, Y.; Jabang, J.; Saleem, S.A. Lung-Brain Axis. *Crit. Rev. Microbiol.* **2022**, *48*, 257–269. [CrossRef]
70. Dang, A.T.; Marsland, B.J. Microbes, metabolites, and the gut–lung axis. *Mucosal Immunol.* **2019**, *12*, 843–850. [CrossRef] [PubMed]
71. Lassalle, F.; Spagnoletti, M.; Fumagalli, M.; Shaw, L.; Dyble, M.; Walker, C.; Thomas, M.G.; Bamberg Migliano, A.; Balloux, F. Oral microbiomes from hunter-gatherers and traditional farmers reveal shifts in commensal balance and pathogen load linked to Diet. *Mol. Ecol.* **2017**, *27*, 182–195. [CrossRef] [PubMed]
72. Willis, J.R.; González-Torres, P.; Pittis, A.A.; Bejarano, L.A.; Cozzuto, L.; Andreu-Somavilla, N.; Alloza-Trabado, M.; Valentín, A.; Ksiezopolska, E.; Company, C.; et al. Citizen science charts two major "stomatotypes" in the oral microbiome of adolescents and reveals links with habits and drinking water composition. *Microbiome* **2018**, *6*, 218. [CrossRef] [PubMed]
73. Wampach, L.; Heintz-Buschart, A.; Fritz, J.V.; Ramiro-Garcia, J.; Habier, J.; Herold, M.; Narayanasamy, S.; Kaysen, A.; Hogan, A.H.; Bindl, L.; et al. Birth mode is associated with earliest strain-conferred gut microbiome functions and immunostimulatory potential. *Nat. Commun.* **2018**, *9*, 5091. [CrossRef]
74. Sencio, V.; Machado, M.G.; Trottein, F. The lung–gut axis during viral respiratory infections: The impact of gut dysbiosis on secondary disease outcomes. *Mucosal Immunol.* **2021**, *14*, 296–304. [CrossRef]
75. Fan, Q.; Wang, H.; Mao, C.; Li, J.; Zhang, X.; Grenier, D.; Yi, L.; Wang, Y. Structure and signal regulation mechanism of interspecies and Interkingdom quorum sensing system receptors. *J. Agric. Food Chem.* **2022**, *70*, 429–445. [CrossRef]
76. Mayhew, D.; Devos, N.; Lambert, C.; Brown, J.R.; Clarke, S.C.; Kim, V.L.; Magid-Slav, M.; Miller, B.E.; Ostridge, K.K.; Patel, R.; et al. Longitudinal profiling of the lung microbiome in the Aeris study demonstrates repeatability of bacterial and eosinophilic COPD exacerbations. *Thorax* **2018**, *73*, 422–430. [CrossRef]
77. Nielsen, R.; Xue, Y.; Jonassen, I.; Haaland, I.; Kommedal, Ø.; Wiker, H.G.; Drengenes, C.; Bakke, P.S.; Eagan, T.M. Repeated bronchoscopy in health and obstructive lung disease: Is the airway microbiome stable? *BMC Pulm. Med.* **2021**, *21*, 342. [CrossRef]
78. Marimón, J.M. The lung microbiome in health and respiratory diseases. *Clin. Pulm. Med.* **2018**, *25*, 131–137. [CrossRef]
79. Wang, G.; Hu, Y.-X.; He, M.-Y.; Xie, Y.-H.; Su, W.; Long, D.; Zhao, R.; Wang, J.; Dai, C.; Li, H.; et al. Gut-lung dysbiosis accompanied by diabetes mellitus leads to pulmonary fibrotic change through the NF-KB signaling pathway. *Am. J. Pathol.* **2021**, *191*, 838–856. [CrossRef]
80. Luo, M.; Liu, Y.; Wu, P.; Luo, D.-X.; Sun, Q.; Zheng, H.; Hu, R.; Pandol, S.J.; Li, Q.-F.; Han, Y.-P.; et al. Alternation of gut microbiota in patients with pulmonary tuberculosis. *Front. Physiol.* **2017**, *8*, 822. [CrossRef]
81. Wang, Z.; Singh, R.; Miller, B.E.; Tal-Singer, R.; Van Horn, S.; Tomsho, L.; Mackay, A.; Allinson, J.P.; Webb, A.J.; Brookes, A.J.; et al. Sputum microbiome temporal variability and dysbiosis in chronic obstructive pulmonary disease exacerbations: An analysis of the COPDMAP study. *Thorax* **2017**, *73*, 331–338. [CrossRef]
82. Frey, D.L.; Boutin, S.; Dittrich, S.A.; Graeber, S.Y.; Stahl, M.; Wege, S.; Herth, F.J.F.; Sommerburg, O.; Schultz, C.; Mall, M.A.; et al. Relationship between airway dysbiosis, inflammation and lung function in adults with cystic fibrosis. *J. Cyst. Fibros.* **2021**, *20*, 754–760. [CrossRef]
83. Dong, H.; Huang, D.; Yuan, M.; Cai, S. The characterization of lung microbiome in sputum of lung cancer patients with different clinicopathology. *Am. J. Respir. Crit. Care Med.* **2019**, *199*, A5553. [CrossRef]
84. Roquilly, A.; Torres, A.; Villadangos, J.A.; Netea, M.G.; Dickson, R.; Becher, B.; Asehnoune, K. Pathophysiological role of respiratory dysbiosis in hospital-acquired pneumonia. *Lancet Respir. Med.* **2019**, *7*, 710–720. [CrossRef]
85. Klomp, M.; Ghosh, S.; Mohammed, S.; Nadeem Khan, M. From virus to inflammation, how influenza promotes lung damage. *J. Leukoc. Biol.* **2020**, *110*, 115–122. [CrossRef]
86. Pacheco, G.A.; Gálvez, N.M.; Soto, J.A.; Andrade, C.A.; Kalergis, A.M. Bacterial and viral coinfections with the human respiratory syncytial virus. *Microorganisms* **2021**, *9*, 1293. [CrossRef]

87. Kitsios, G.D.; Yang, H.; Yang, L.; Qin, S.; Fitch, A.; Wang, X.-H.; Fair, K.; Evankovich, J.; Bain, W.; Shah, F.; et al. Respiratory tract dysbiosis is associated with worse outcomes in mechanically ventilated patients. *Am. J. Respir. Crit. Care Med.* **2020**, *202*, 1666–1677. [CrossRef]
88. Minodier, L.; Charrel, R.N.; Ceccaldi, P.-E.; van der Werf, S.; Blanchon, T.; Hanslik, T.; Falchi, A. Prevalence of gastrointestinal symptoms in patients with influenza, clinical significance, and pathophysiology of human influenza viruses in faecal samples: What do we know? *Virol. J.* **2015**, *12*, 215. [CrossRef]
89. Groves, H.T.; Cuthbertson, L.; James, P.; Moffatt, M.F.; Cox, M.J.; Tregoning, J.S. Respiratory disease following viral lung infection alters the murine gut microbiota. *Front. Immunol.* **2018**, *9*, 182. [CrossRef]
90. Samuelson, D.R.; Smith, D.R.; Cunningham, K.C.; Wyatt, T.A.; Hall, S.C.; Murry, D.J.; Chhonker, Y.S.; Knoell, D.L. Zip8-mediated intestinal dysbiosis impairs pulmonary host defense against bacterial pneumonia. *Int. J. Mol. Sci.* **2022**, *23*, 1022. [CrossRef]
91. Tsang, T.K.; Lee, K.H.; Foxman, B.; Balmaseda, A.; Gresh, L.; Sanchez, N.; Ojeda, S.; Lopez, R.; Yang, Y.; Kuan, G.; et al. Association between the respiratory microbiome and susceptibility to influenza virus infection. *Clin. Infect. Dis.* **2019**, *71*, 1195–1203. [CrossRef]
92. Hakim, H.; Dallas, R.; Wolf, J.; Tang, L.; Schultz-Cherry, S.; Darling, V.; Johnson, C.; Karlsson, E.A.; Chang, T.-C.; Jeha, S.; et al. Gut microbiome composition predicts infection risk during chemotherapy in children with acute lymphoblastic leukemia. *Clin. Infect. Dis.* **2018**, *67*, 541–548. [CrossRef]
93. Wang, J.; Xiong, K.; Zhao, S.; Zhang, C.; Zhang, J.; Xu, L.; Ma, A. Long-term effects of multi-drug-resistant tuberculosis treatment on gut microbiota and its health consequences. *Front. Microbiol.* **2020**, *11*, 53. [CrossRef]
94. Zheng, D.; Liwinski, T.; Elinav, E. Interaction between microbiota and immunity in health and disease. *Cell Res.* **2020**, *30*, 492–506. [CrossRef]
95. Sala, C.; Benjak, A.; Goletti, D.; Banu, S.; Mazza-Stadler, J.; Jaton, K.; Busso, P.; Remm, S.; Leleu, M.; Rougemont, J.; et al. Multicenter analysis of sputum microbiota in tuberculosis patients. *PLoS ONE* **2020**, *15*, e0240250. [CrossRef] [PubMed]
96. Harishankar, M.; Selvaraj, P.; Bethunaickan, R. Influence of genetic polymorphism towards pulmonary tuberculosis susceptibility. *Front. Med.* **2018**, *5*, 213. [CrossRef] [PubMed]
97. Dumas, A.; Corral, D.; Colom, A.; Levillain, F.; Peixoto, A.; Hudrisier, D.; Poquet, Y.; Neyrolles, O. The host microbiota contributes to early protection against lung colonization by mycobacterium tuberculosis. *Front. Immunol.* **2018**, *9*, 2656. [CrossRef]
98. Namasivayam, S.; Sher, A.; Glickman, M.S.; Wipperman, M.F. The microbiome and tuberculosis: Early evidence for cross talk. *mBio* **2018**, *9*, e01420-18. [CrossRef] [PubMed]
99. Krishna, P.; Jain, A.; Bisen, P.S. Microbiome diversity in the sputum of patients with pulmonary tuberculosis. *Eur. J. Clin. Microbiol. Infect. Dis.* **2016**, *35*, 1205–1210. [CrossRef] [PubMed]
100. Hong, B.-y.; Paulson, J.N.; Stine, O.C.; Weinstock, G.M.; Cervantes, J.L. Meta-analysis of the lung microbiota in pulmonary tuberculosis. *Tuberculosis* **2018**, *109*, 102–108. [CrossRef]
101. Vázquez-Pérez, J.A.; Carrillo, C.O.; Iñiguez-García, M.A.; Romero-Espinoza, I.; Márquez-García, J.E.; Falcón, L.I.; Torres, M.; Herrera, M.T. Alveolar microbiota profile in patients with human pulmonary tuberculosis and interstitial pneumonia. *Microb. Pathog.* **2020**, *139*, 103851. [CrossRef]
102. Valdez-Palomares, F.; Muñoz Torrico, M.; Palacios-González, B.; Soberón, X.; Silva-Herzog, E. Altered microbial composition of drug-sensitive and drug-resistant TB patients compared with healthy volunteers. *Microorganisms* **2021**, *9*, 1762. [CrossRef]
103. Winglee, K.; Eloe-Fadrosh, E.; Gupta, S.; Guo, H.; Fraser, C.; Bishai, W. Aerosol mycobacterium tuberculosis infection causes rapid loss of diversity in gut microbiota. *PLoS ONE* **2014**, *9*, e97048. [CrossRef]
104. Wipperman, M.F.; Fitzgerald, D.W.; Juste, M.A.; Taur, Y.; Namasivayam, S.; Sher, A.; Bean, J.M.; Bucci, V.; Glickman, M.S. Antibiotic treatment for tuberculosis induces a profound dysbiosis of the microbiome that persists long after therapy is completed. *Sci. Rep.* **2017**, *7*, 10767. [CrossRef]
105. Balcells, M.E.; Yokobori, N.; Hong, B.-y.; Corbett, J.; Cervantes, J. The lung microbiome, vitamin D, and the tuberculous granuloma: A balance triangle. *Microb. Pathog.* **2019**, *131*, 158–163. [CrossRef]
106. Perry, S.; de Jong, B.C.; Solnick, J.V.; de la Luz Sanchez, M.; Yang, S.; Lin, P.L.; Hansen, L.M.; Talat, N.; Hill, P.C.; Hussain, R.; et al. Infection with helicobacter pylori is associated with protection against tuberculosis. *PLoS ONE* **2010**, *5*, e8804. [CrossRef]
107. Majlessi, L.; Sayes, F.; Bureau, J.-F.; Pawlik, A.; Michel, V.; Jouvion, G.; Huerre, M.; Severgnini, M.; Consolandi, C.; Peano, C.; et al. Colonization with helicobacter is concomitant with modified gut microbiota and drastic failure of the immune control of mycobacterium tuberculosis. *Mucosal Immunol.* **2017**, *10*, 1178–1189. [CrossRef]
108. Namasivayam, S.; Diarra, B.; Diabate, S.; Sarro, Y.D.S.; Kone, A.; Kone, B.; Tolofoudie, M.; Baya, B.; Diakite, M.T.; Kodio, O.; et al. Patients infected with mycobacterium africanum versus mycobacterium tuberculosis possess distinct intestinal microbiota. *PLoS Negl. Trop. Dis.* **2020**, *14*, e0008230. [CrossRef]
109. Cui, Z.; Zhou, Y.; Li, H.; Zhang, Y.; Zhang, S.; Tang, S.; Guo, X. Complex sputum microbial composition in patients with pulmonary tuberculosis. *BMC Microbiol.* **2012**, *12*, 276. [CrossRef]
110. Maji, A.; Misra, R.; Dhakan, D.B.; Gupta, V.; Mahato, N.K.; Saxena, R.; Mittal, P.; Thukral, N.; Sharma, E.; Singh, A.; et al. Gut microbiome contributes to impairment of immunity in pulmonary tuberculosis patients by alteration of butyrate and propionate producers. *Environ. Microbiol.* **2017**, *20*, 402–419. [CrossRef]
111. Lin, D.; Wang, X.; Li, Y.; Wang, W.; Li, Y.; Yu, X.; Lin, B.; Chen, Y.; Lei, C.; Zhang, X.; et al. Sputum microbiota as a potential diagnostic marker for multidrug-resistant tuberculosis. *Int. J. Med. Sci.* **2021**, *18*, 1935–1945. [CrossRef]

112. Zhang, C.; Wang, Y.; Shi, G.; Han, W.; Zhao, H.; Zhang, H.; Xi, X. Determinants of multidrug-resistant tuberculosis in Henan province in China: A case control study. *BMC Public Health* **2015**, *16*, 42. [CrossRef]
113. Cervantes, J.; Hong, B.-Y. The gut–lung axis in tuberculosis. *Pathog. Dis.* **2017**, *75*, ftx097. [CrossRef]
114. Namasivayam, S.; Maiga, M.; Yuan, W.; Thovarai, V.; Costa, D.L.; Mittereder, L.R.; Wipperman, M.F.; Glickman, M.S.; Dzutsev, A.; Trinchieri, G.; et al. Longitudinal profiling reveals a persistent intestinal dysbiosis triggered by conventional anti-tuberculosis therapy. *Microbiome* **2017**, *5*, 71. [CrossRef]
115. Ma, X.; Shin, Y.-J.; Jang, H.-M.; Joo, M.-K.; Yoo, J.-W.; Kim, D.-H. Lactobacillus rhamnosus and Bifidobacterium Longum alleviate colitis and cognitive impairment in mice by regulating IFN-γ to IL-10 and TNF-α to IL-10 expression ratios. *Sci. Rep.* **2021**, *11*, 20659. [CrossRef]
116. Lev-Sagie, A.; Goldman-Wohl, D.; Cohen, Y.; Dori-Bachash, M.; Leshem, A.; Mor, U.; Strahilevitz, J.; Moses, A.E.; Shapiro, H.; Yagel, S.; et al. Vaginal microbiome transplantation in women with intractable bacterial vaginosis. *Nat. Med.* **2019**, *25*, 1500–1504. [CrossRef]
117. Hsu, D.; Irfan, M.; Jabeen, K.; Iqbal, N.; Hasan, R.; Migliori, G.B.; Zumla, A.; Visca, D.; Centis, R.; Tiberi, S. Post tuberculosis treatment infectious complications. *Int. J. Infect. Dis.* **2020**, *92*, S41–S45. [CrossRef]
118. Romanowski, K.; Balshaw, R.F.; Benedetti, A.; Campbell, J.R.; Menzies, D.; Ahmad Khan, F.; Johnston, J.C. Predicting tuberculosis relapse in patients treated with the standard 6-month regimen: An individual patient data meta-analysis. *Thorax* **2018**, *74*, 291–297. [CrossRef]
119. Wu, J.; Liu, W.; He, L.; Huang, F.; Chen, J.; Cui, P.; Shen, Y.; Zhao, J.; Wang, W.; Zhang, Y.; et al. Sputum microbiota associated with new, recurrent and treatment failure tuberculosis. *PLoS ONE* **2013**, *8*, e83445. [CrossRef]
120. Naidoo, C.C.; Nyawo, G.R.; Wu, B.G.; Walzl, G.; Warren, R.M.; Segal, L.N.; Theron, G. The microbiome and tuberculosis: State of the art, potential applications, and defining the Clinical Research Agenda. *Lancet Respir. Med.* **2019**, *7*, 892–906. [CrossRef]