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MICROBIOTA DO TRATO RESPIRATÓRIO SUPERIOR DE PACIENTES COM INFECÇÃO RESPIRATÓRIA E SUA ASSOCIAÇÃO COM SARS-CoV-2

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“Acolher os micróbios que viajaram conosco por milhões de anos é o primeiro passo para aprendermos a valorizar quem realmente somos e, no fim, nos tornamos 100% humanos.”

Alanna Collen — 10% Humano.

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SUMÁRIO

LISTA DE ABREVIATURAS

(+)ssRNA: Do inglês *positive-sense single-stranded RNA*

16S rRNA: Do inglês *16S ribosomal ribonucleic acid* (Subunidade do gene 16S do RNA ribossomal)

18S rRNA: Do inglês *18S ribosomal ribonucleic acid* (Subunidade do gene 18S do RNA ribossomal)

ACE2: Do inglês *Angiotensin-converting enzyme 2* (Enzima conversora da angiotensina 2)

ARI: Do inglês *Acute Respiratory Infection* (Infecção Respiratória Aguda)

SARI: Do inglês *Severe Acute Respiratory Infection* (Infecção Respiratória Aguda Grave)

COVID-19: Do inglês *Coronavírus Disease 2019* (Doença do Coronavírus 2019)

HAdV: Do inglês *human Adenovirus* (Adenovirus humano)

HBoV: Do inglês *human bocavirus* (Bocavirus humano)

HCoVs: Do inglês *human coronaviruses* (Coronavirus humano)

HMPV: Do inglês *human metapneumovirus* (Metapneumovírus humano)

HPIV: Do inglês *human parainfluenza virus* (Vírus parainfluenza humano)

IAV: Do inglês *Influenza A virus* (Vírus Influenza do tipo A)

IBV: Do inglês *Influenza B virus*(Vírus Influenza do tipo B)

ICTV: Do inglês *International Committee on Taxonomy of Viruses*

IgA: Imunoglobulina A

IL-6: Interleucina 6

MERS-CoV: Do inglês *Middle East Respiratory Syndrome-CoronaVirus*

NSPs: Do inglês *non structural proteins*

OMS: Organização Mundial da Saúde

RSV: Do inglês *Respiratory Syncytial Virus* (Vírus sincicial respiratório)

RV: Rinovírus

SARS-CoV: Do inglês *Severe Acute Respiratory Syndrome-CoronaVirus*

SARS-CoV-2: Do inglês *Severe Acute Respiratory Syndrome-CoronaVirus-2*

SG: Síndrome Gripal

SRAG: Síndrome Respiratória Aguda Grave

TMPRSS2: Do inglês *Transmembrane Serine Protease 2*

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RESUMO

O coronavírus SARS-CoV-2 emergiu no final de 2019, causando a COVID-19, uma doença respiratória aguda declarada pandemia pela Organização Mundial de Saúde em março de 2020. Os fatores associados à suscetibilidade e à gravidade da infecção causada pelo SARS-CoV-2 ainda não estão bem definidos, e perfis bastante diferentes são observados nos pacientes variando de assintomáticos a morte. A microbiota do trato respiratório superior funciona como uma primeira barreira contra o ataque de infecções de qualquer origem. A microbiota compreende uma comunidade de microrganismos, como fungos, bactérias e vírus, os quais podem ser comensais ou potencialmente patogênicos. Esses microrganismos formam interações que influenciam perfis de saúde ou doença do hospedeiro. Desta forma, a caracterização das comunidades bacterianas no trato respiratório superior de pacientes com diferentes níveis de gravidade de COVID-19 pode fornecer evidências sobre potenciais associações entre a microbiota e o risco de condições clínicas mais graves da doença. Sendo assim, o objetivo deste estudo foi caracterizar a comunidade bacteriana presente em amostras de nasofaringe de pacientes com Síndrome Gripal (SG) ou com Síndrome Respiratória Aguda Grave (SRAG), infectados (+) e não infectados (-) por SARS-CoV-2. Amostras de 96 pacientes foram avaliadas utilizando sequenciamento massivo de amplicons da região V4 do gene 16S RNA ribossomal. A seleção das amostras foi baseada em uma amostragem estratificada aleatória, a partir de variáveis estratificadoras estabelecidas previamente como síndromes, diagnóstico para SARS-CoV-2, e idade. Segundo os critérios anteriores, as amostras foram divididas em 4 grupos: pacientes com sintomas de SG, positivos e negativos para SARS-CoV-2 (grupos SG+, e SG-); e pacientes apresentando SRAG, positivos e negativos para SARS-CoV-2 (grupos SRAG+, e SRAG-), considerando dois grupos etários 20 – 40 anos e ≥ 60 anos. Em comparações de beta diversidade, considerando primeiramente a idade dos pacientes (20 – 40 anos e + 60 anos), foram encontradas diferenças significativas entre os grupos SRAG+ e SRAG-. Considerando os sintomas respiratórios (SG ou SRAG) e diagnóstico para SARS-CoV-2, também foram encontradas diferenças significativas de beta diversidade entre o grupo SG+ e o grupo SRAG+; e entre os grupos SRAG+ e SRAG-. Em comparações de alfa diversidade, medida pelo índice de Shannon, houve diferenças significativas entre os grupos SG+ e SRAG+, sendo que o grupo SRAG+ apresentou diminuição na diversidade bacteriana no nível de gênero ($p < 0,05$). Além disso, a

composição da microbiota da nasofaringe dos grupos de estudo foi caracterizada por uma abundância relativa de alguns táxons, incluindo os gêneros *Staphylococcus* spp., *Corynebacterium* spp., *Shigella* spp., *Acinetobacter* spp., *Enterococcus* spp., e *Caloramator* spp. Verificamos, ainda, que o grupo SG+ apresentou como táxon diferencialmente abundante o gênero *Bacillus* spp., enquanto no grupo SRAG+ esse gênero não foi encontrado. Em contrapartida, o grupo SRAG+ apresentou *Streptococcus* spp., *Veillonella* spp. e *Staphylococcus* spp. como gêneros diferencialmente abundantes quando comparados ao grupo SRAG-, que teve o gênero *Bacillus* spp. como o mais abundante. Tendo em vista os resultados, nosso estudo identificou diferenças significativas nos perfis da microbiota de pacientes SG em comparação com SRAG, infectados com SARS-CoV-2. Esses dados contribuem para a compreensão do papel da microbiota como indicador de suscetibilidade à infecção por SARS-CoV-2 e gravidade da doença, além de ajudar a estabelecer associações entre o perfil de microbiota e os tipos de desfechos clínicos da COVID-19.

Palavras-chave: microbiota bacteriana, nasofaringe, SARS-CoV-2, síndrome gripal, síndrome respiratória aguda grave.

ABSTRACT

The SARS-CoV-2 coronavirus emerged at the end of 2019, causing COVID-19, an acute respiratory disease declared a pandemic by the World Health Organization in March 2020. The factors associated with the susceptibility and severity of infection caused by SARS-CoV-2 are not yet well-defined. What is known, however, is that the upper respiratory tract microbiota works as a first barrier against the attack of infections from any source. The microbiota comprises a community of microorganisms, such as fungi, bacteria, and viruses, which can be commensal or potentially pathogenic. These microorganisms form interactions that influence the health or disease profiles of the host. In this way, the characterization of bacterial communities in the upper respiratory tract of patients with different levels of COVID-19 severity, may provide evidence about potential associations between the microbiota and the risk of more severe clinical conditions. In this context, this study aimed to characterize the microbiota of the bacterial community present in nasopharyngeal samples from patients with Acute Respiratory Infection (ARI) or Severe Acute Respiratory Infection (SARI), both infected (+) and uninfected (-) by SARS-CoV-2, considering two age groups 20 – 40 years and ≥ 60. Samples from the nasopharynx of 96 patients were evaluated using massive sequencing of amplicons from the V4 region of the 16S rRNA gene. Sample selection was based on random stratified sampling, based on previously established stratifying variables such as syndromes, diagnosis for SARS-CoV-2, and age. According to the above criteria, the samples were divided into 4 groups: patients with ARI, SARS-CoV-2 positive and negative (groups ARI+, and ARI-); and patients with SARI, SARS-CoV-2 positive and negative (groups SARI+, and SARI-), considering two age groups 20 – 40 years and ≥ 60 years. In beta diversity comparisons, firstly considering the age of the patients (20 – 40 years and ≥ 60 years), significant differences were found between the SARI+ and SARI- groups. Considering respiratory symptoms (ARI or SARI) and diagnosis for SARS-CoV-2, significant beta diversity differences were also found between the ARI+ group and the SARI+ group; and between the SARI+ and SARI- groups. In comparisons of alpha diversity, measured by the Shannon index, there were significant differences between the ARI+ and SARI+ groups, with the SARI+ group showing a decrease in bacterial diversity at the gender level ($p < 0.05$). Furthermore, the nasopharyngeal microbiota composition of the study groups was characterized by a relative abundance of some taxa, including the genera *Staphylococcus* spp., *Corynebacterium* spp.,

Shigella spp., *Acinetobacter* spp., *Enterococcus* spp., and *Caloramator* spp. We also verified that the ARI+ group presented the genus *Bacillus* spp. as a differentially abundant taxon, while in the SARI+ group this genus was not found. In contrast, the SARI+ group had *Streptococcus* spp., *Veillonella* spp., and *Staphylococcus* spp. as differentially abundant genera when compared to the SARI- group, which had the genus *Bacillus* spp. as the most abundant. Given the results, our study identified significant differences in the microbiota profiles of ARI compared to SARI patients, infected with SARS-CoV-2. These data contribute to understanding the role of the microbiota as an indicator of susceptibility to SARS-CoV-2 infection and disease severity, in addition to helping to establish associations between the microbiota profile and the types of clinical outcomes of COVID-19.

Keywords: bacterial microbiota, nasopharynx, SARS-CoV-2, acute respiratory infection, severe acute respiratory infection.

1. INTRODUÇÃO

O corpo humano é habitado por comunidades de microrganismos, incluindo procariotos (bactérias e arqueas), eucariotos microbianos (fungos e vermes) e vírus (TURNBAUGH et al., 2007) (Figura 1). Estima-se que esses organismos microscópicos estejam distribuídos em um número compatível ou mesmo superior ao das células existentes no ser humano (SENDER, 2016 a b). Da associação ser humano e microrganismos é estabelecida uma relação ecológica de simbiose. O ser humano fornece um ambiente estável, rico em nutrientes para os microrganismos estabilizarem e formarem redes de interação, tornando a saúde do hospedeiro essencial para a comunidade microbiana formada (LEE; MAZMANIAN, 2010). Em troca, o hospedeiro tem como benefícios uma rede de microrganismos comensais diversificados, degradadores de moléculas complexas e fornecedores de nutrientes essenciais, atuantes no funcionamento do organismo humano (LEE; MAZMANIAN, 2010).

Os microrganismos são necessários para diferentes funções no corpo humano, como contribuir para a homeostase e equilíbrio do sistema imune, proporcionando ao hospedeiro uma defesa eficiente contra o ataque de possíveis patógenos e estabelecendo perfis de saúde ou doença no ser humano (KUMPITSCH et al., 2019; HOU et al., 2022). A constituição de perfis saudáveis e enfermos no ser humano é determinada a partir do conjunto de microrganismos presentes e das interações específicas estabelecidas com o hospedeiro (DAVENPORT et al., 2017). Esses microrganismos apresentam distribuições corporais diversificadas que variam conforme a anatomia e as condições biológicas às quais são expostos (como muco, pH, temperatura, glândulas sebáceas, cílios/pelos). Esses fatores influenciam na densidade, riqueza e na uniformidade de espécies microbianas (PROCTOR; RELMAN, 2017). Devido à grande importância que desempenham, os microrganismos constituintes do microbioma humano são propostos como formadores de um “novo órgão” (BAQUERO; NOMBELA, 2012) (Figura 1).

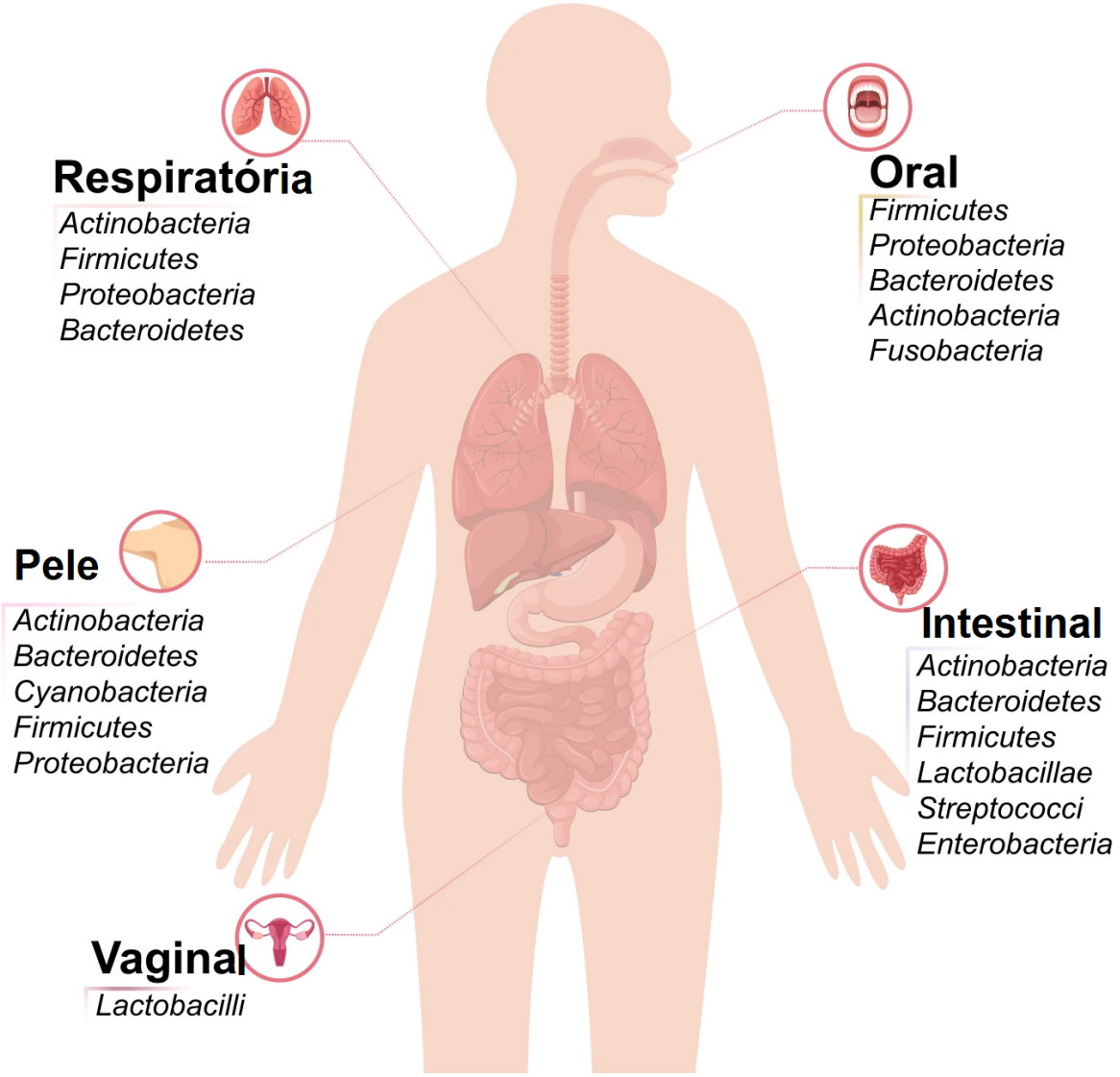

FIGURA 1: A composição da microbiota humana em diferentes localidades do corpo. Adaptado de: HOU et al., 2022 (DOI: https://doi.org/10.1038/s41392-022-00974-4)

1.1 Microbioma e microbiota

A área de pesquisa sobre microbioma progrediu rapidamente nos últimos anos, tornando-se um dos tópicos de maior interesse científico e público (TURNBAUGH et al., 2007; RACKAITYTE; LYNCH, 2020). O campo de análise das comunidades microbianas avançou graças a expansão de tecnologias de sequenciamento de DNA e RNA, analises de proteínas e metabólitos combinado ao aumento de ferramentas computacionais, o que permitiu estudar diferentes aspectos desses microrganismos e seus habitats (TURNBAUGH et al., 2007; RACKAITYTE; LYNCH, 2020). Entretanto, o crescimento rápido em diferentes áreas da microbiologia é acompanhado por confusão de vocabulário e

falta de definição clara para os termos microbioma e microbiota (MARCHESI; RAVEL, 2015). Apesar de os termos serem muitas vezes usados como sinônimos, tratam de definições distintas no campo de pesquisa (MARCHESI; RAVEL, 2015; BERG et al., 2020).

Considerando os aspectos apresentados, o microbioma pode ser definido como a comunidade de microrganismos como fungos, bactérias, arqueas combinado às "atividades funcionais" que desempenham como a produção de moléculas pelos microrganismos, incluindo elementos estruturais (ácidos nucleicos, proteínas, peptídeos, polissacarídeos); metabólitos (moléculas sinalizadoras, toxinas, moléculas orgânicas e inorgânicas), elementos genéticos móveis como fagos, vírus e "DNA relíquia" (DNA extracelular derivado de células mortas) somado a condições ambientais que circundam o organismo que habitam (MARCHESI; RAVEL, 2015; BERG et al., 2020) (Figura 2). Por outro lado, a microbiota é um termo mais direcionado, se refere à comunidade de microrganismos vivos (como bactérias, fungos, arqueas, entre outros) e ao ambiente que colonizam (um ecossistema ou uma região anatômica) (MARCHESI; RAVEL, 2015). O termo microbiota é muito usado em estudos de caracterização da comunidade microbiana de uma amostra (MARCHESI; RAVEL, 2015). A composição da microbiota é estabelecida usando métodos moleculares baseados na análise de genes marcadores como 16S RNA ribossomal (*16S rRNA*) em bactérias, 18S RNA ribossomal (*18S rRNA*) em fungos ou outros genes marcadores e regiões genômicas amplificadas e sequenciadas de amostras biológicas (MARCHESI; RAVEL, 2015) (Figura 2).

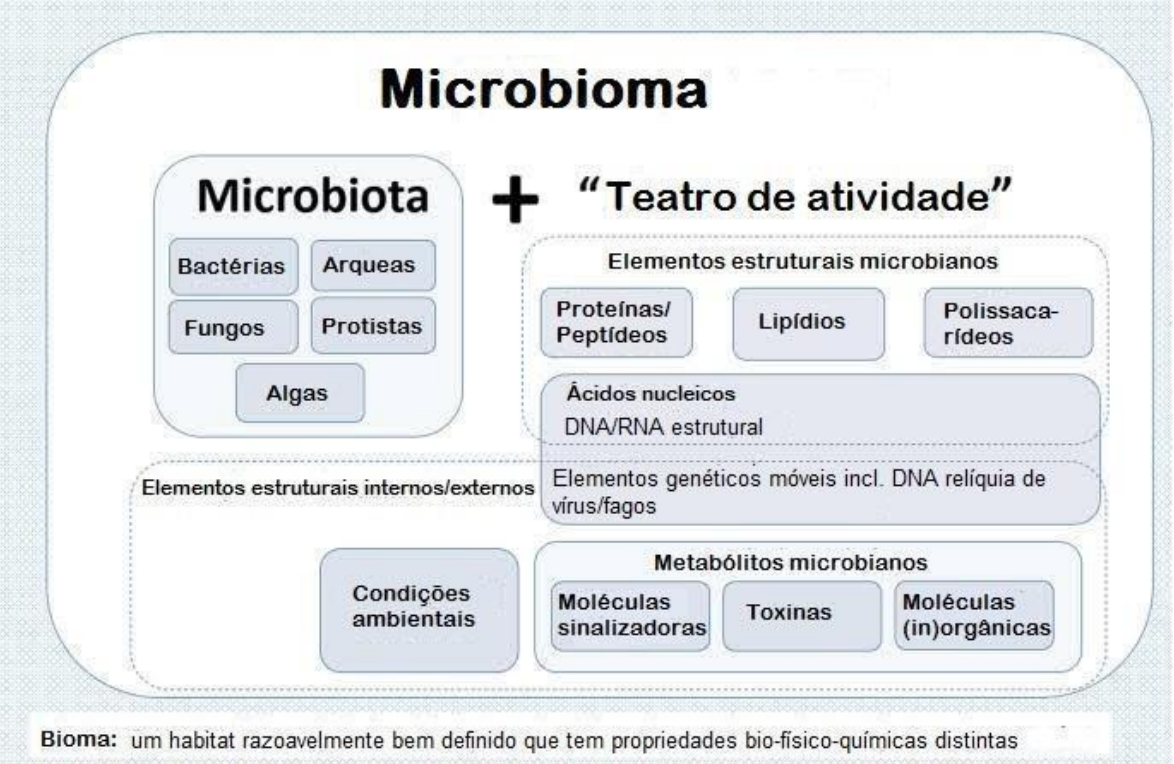

FIGURA 2: Proposta de definição para os termos microbiota e microbioma. Adaptado de: BERG et al., 2020 (DOI: https://doi.org/10.1186/s40168-020-00875-0)

1.2 Técnicas de sequenciamento

O sequenciamento de nova geração (*NGS*) é a tecnologia que descreve todos os métodos de sequenciamento para DNA e RNA em larga escala disponíveis (GOODWIN et al., 2016). A tecnologia NGS permite sequenciar genomas completos mais rapidamente, analisar a expressão gênica e fatores epigenéticos, aplicação oncológica, identificar novos patógenos, investigar o microbioma humano, entre outras funcionalidades (BUERMANS; DUNNEN, 2014; WENSEL et al., 2022). Essa abordagem conta com várias etapas para a realização do sequenciamento: como escolha do tipo de amostra (suabe de nasofaringe, solo, água, etc.); extração de DNA (por método tradicional ou kits comerciais); amplificação por PCR; preparo das bibliotecas; plataforma/tecnologia de sequenciamento; análise de bioinformática, anotação e interpretação dos dados (GOODWIN et al., 2016; WENSEL et al., 2022). Atualmente, o NGS tem como principais métodos o sequenciamento de amplicons que utiliza genes marcadores como o gene *16S rRNA* para bactérias, 18S *rRNA* e ITS para fungos; sequenciamento de genoma completo shotgun e sequenciamento de RNA (WENSEL et al., 2022).

O sequenciamento metagenômico *shotgun* é um método de sequenciamento, não direcionado, de todo o DNA presente em uma determinada amostra (QUINCE et al., 2017, WENSEL et al., 2022). Essa abordagem de sequenciamento permite identificar o perfil da composição taxonômica e potencial funcional das comunidades de microrganismos presentes em uma amostra (QUINCE et al., 2017). A técnica de metagenômica *shotgun* compreende algumas etapas como, coleta das amostras e extração do DNA; fragmentação do DNA; preparo e sequenciamento da biblioteca; análise pós-processamento dos dados e validação (QUINCE et al., 2017, WENSEL et al., 2022). Esse método de sequenciamento apresenta como vantagens a possibilidade de ser aplicado diretamente ao DNA ambiental extraído, uma resolução taxonômica ao nível de espécie ou de cepa, e prever o potencial funcional dos microrganismos. Entretanto, o sequenciamento *shotgun* tem como limitações de sua aplicação um risco de alta presença de DNA do hospedeiro, a exigência um volume maior de dados de sequenciamento, resultando em custos mais altos da técnica (QUINCE et al., 2017; LIU et al., 2021 a; WENSEL et al., 2022).

O sequenciamento de amplicons é um dos métodos NGS mais utilizados para a identificação, caracterização e quantificação de microrganismos em uma amostra (WENSEL et al., 2022). No sequenciamento de amplicons os genes marcadores usados incluem o gene *16S rRNA* (bactérias), o *18S rRNA* ou espaçador interno transcrito (*ITS*) em fungos (LIU et al., 2021 a; WENSEL et al., 2022). O *16S rRNA* é um gene bacteriano com cerca de 1.500 pares de bases de comprimento, com nove regiões hipervariáveis (V1-V9) intercaladas entre regiões conservadas (WENSEL et al., 2022). Para o sequenciamento de amplicons é primeiramente realizada a amplificação por PCR da região hipervariável de interesse, em seguida, os amplicons gerados são sequenciados e após é realizada uma limpeza dos dados brutos gerados, para então realizar a análise taxonômica dos dados (WENSEL et al., 2022). A análise taxonômica de dados do sequenciamento de *16S rRNA* pode ser estimada por duas abordagens computacionais: o método de unidades taxonômicas operacionais (*OTUs*) ou variantes de sequência de amplicons (*ASVs*) (WENSEL et al., 2022).

As *OTUs* são *clusters* de sequências baseados em distâncias, uma *OTU* com identidade de sequência superior a 97% (ou com até 3% de dissimilaridade) é estimada para definir ao nível de filo a gênero. (WENSEL et al., 2022). Já a *ASV* é uma abordagem analítica baseada na correspondência exata de nucleotídeos e as atribuições de táxons

dependem da qualidade dos bancos de dados de referência (WENSEL et al., 2022). Esses métodos computacionais fazem atribuições taxonômicas para cada sequência gerada a um táxon microbiano (bactérias, arqueas, fungos) em diferentes níveis taxonômicos de filo a gênero (WENSEL et al., 2022). Portanto, o sequenciamento de amplicons é uma ferramenta comprovadamente eficiente e econômica que apresenta como vantagens análises mais rápidas; baixo risco de contaminação humana no sequenciamento porque o gene sendo amplificado é específico de bactérias e um volume menor de dados sequenciados (LIU et al., 2021 a; WENSEL et al., 2022). Contudo, a técnica apresenta algumas limitações também como viés de primer e PCR, uma menor resolução e sensibilidade para detectar mudanças no nível de espécies e de cepa (WENSEL et al., 2022).

1.3 Formação da microbiota

É proposto que o desenvolvimento da microbiota de um organismo se inicie logo ao nascimento. Bactérias são transmitidas pela mãe para o recém-nascido via transmissão vertical, por intermédio do parto (normal ou cesariana), da amamentação (leite materno), ou pelo tipo de ambiente ao qual é primeiramente exposto (hospitalar, externo) (CHARBONNEAU et al., 2016; DAVENPORT et al., 2017). Essa microbiota inicial é necessária para o desenvolvimento saudável do bebê, pois bactérias neonatais estimulam a formação dos sistemas imunológico, metabólico, hormonal e nervoso do recém-nascido (CHARBONNEAU et al., 2016; DAVENPORT et al., 2017; DOMINGUEZ-BELO et al., 2019).

A microbiota é altamente dinâmica e instável durante os primeiros anos de vida do ser humano, moldada por acontecimentos ao longo da trajetória de vida do hospedeiro como estação do ano, vacinação, presença de irmãos, frequência a creche, tabagismo, e infecções virais anteriores (MAN et al., 2017; LI et al., 2019) (Figura 3). À medida que os anos passam, a microbiota expande sua diversidade microbiana e capacidade funcional dos microrganismos, conferindo ao hospedeiro vantagens metabólicas de sua colonização (LYNCH; PEDERSEN, 2016; MAN et al., 2017). Entretanto, a microbiota estável não apresenta um estabelecimento exato, visto que as proporções de microrganismos variam de uma pessoa para outra, tornando a composição das comunidades microbianas única a cada indivíduo (LYNCH; PEDERSEN, 2016; MAN et al., 2017; BANA; CABREIRO, 2019).

Ainda, oscilações na composição da microbiota ocorrem a todo momento ocasionadas por conta da localização geográfica do indivíduo, do estilo de vida (interações sociais, exercício físico, animais de estimação, ocupação), e uso de antibióticos considerado um dos principais fatores a perturbar o equilíbrio da microbiota (LYNCH; PEDERSEN, 2016; SCHWARTZ et al., 2020).

Já na fase adulta do desenvolvimento, a microbiota nos mais diferentes locais do corpo se encontra bem estabelecida, e permanece estável por um longo período da vida, sofrendo mudanças novamente com o envelhecimento e senescência do hospedeiro (BANA; CABREIRO, 2019).

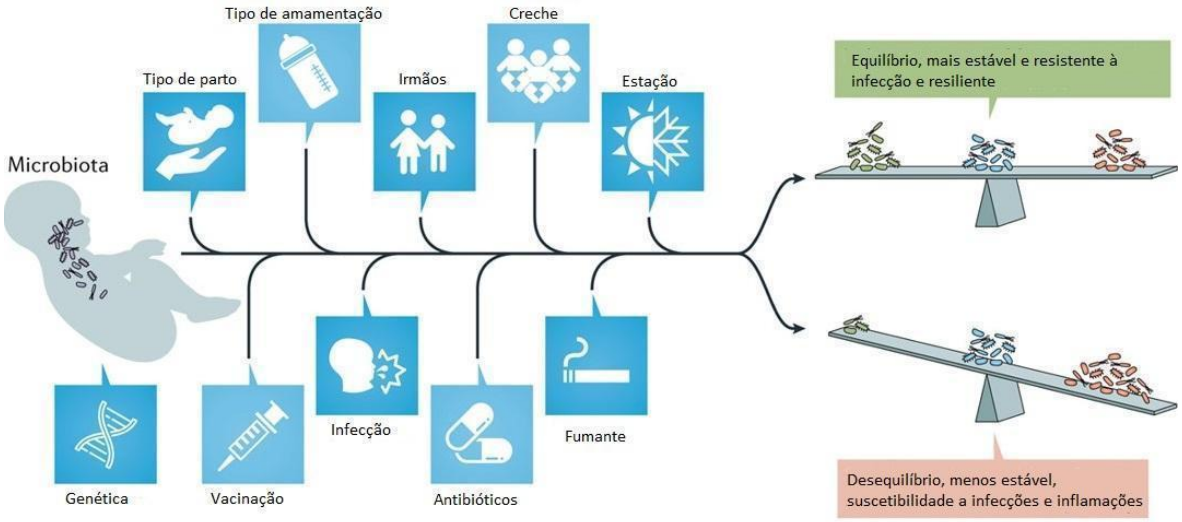

FIGURA 3: Fatores que podem influenciar a formação da microbiota respiratória. Adaptado de: MAN et al., 2017 (DOI: https://doi.org/10.1038/nrmicro.2017.14)

1.4 Microbiota e sistema imune

A microbiota e o sistema imune são formados simultaneamente ao longo do desenvolvimento humano (BELKAID; HAND, 2014; ZHENG et al., 2020). A microbiota primária formada a partir de bactérias transferidas da mãe para o recém-nascido ajuda na formação e modulação do sistema imunológico do hospedeiro (BELKAID; HAND, 2014; CHARBONNEAU et al., 2016; IDRIS et al., 2017; DOMINGUEZ-BELO et al., 2019; ZHENG et al., 2020). Já o sistema imune é um dos principais fatores atuantes na manutenção da microbiota saudável no hospedeiro (THAISS et al., 2016; LEVY et al.,

2017; ZHENG et al., 2020). Tanto o sistema imune inato quanto o sistema imune adaptativo são de extrema importância na regulação e controle da composição da microbiota, proporcionando um habitat imunotolerante a um conjunto de microrganismos no corpo (GENSOLLEN et al., 2016; THAISS et al., 2016; LEVY et al., 2017; ZHENG et al., 2020).

O sistema imune e a microbiota em estado de equilíbrio se encontram em constante interação, atuando de maneira complementar para dirigir a fisiologia de todo o organismo (BELKAID; HAND, 2014; ZHENG et al., 2020). A microbiota e o sistema imune estabelecem uma comunicação bidirecional, limitada pela mucosa epitelial, que impede o contato direto entre os microrganismos e a células imunes, que ocorre através da produção e transporte de fatores imunes e metabólitos microbianos (THAISS et al., 2016; MAYNARD, 2019; ZHENG et al., 2020). Como já documentado, ao longo da vida de um indivíduo a microbiota sofre mudanças ocasionadas, em sua maioria, por fatores externos (BELKAID; HAND, 2014; GENSOLLEN et al., 2016; MAYNARD, 2019), tais como infecções; medicamentos (por exemplo, uso de antibióticos); e mudanças na alimentação (MAYNARD, 2019). O tempo de ação, a relevância e os alvos dessas oscilações podem resultar em respostas do sistema imune destinadas a restabelecer o equilíbrio das funções e diminuir os danos colaterais ocasionados à microbiota do hospedeiro (MAYNARD, 2019; ZHENG et al., 2020). Dessa maneira, a composição da microbiota adquirida no nascimento e moldada por acontecimentos ao longo do desenvolvimento pode influenciar na suscetibilidade, gravidade de doenças e no tipo de resposta imune no curso da vida (BELKAID; HAND, 2014; GENSOLLEN et al., 2016).

Uma microbiota em disbiose é definida como uma estrutura anormal da comunidade microbiana (modificações na composição e funcionalidade de microrganismos) associada ao desequilíbrio do sistema imune (LEVY et al., 2017; BAGHBANI et al., 2020). A desregulação da comunicação entre a comunidade microbiana e o sistema imune é considerado um dos principais mecanismos que contribuem para o desenvolvimento e manutenção de uma microbiota em estado disbiótico (BELKAID; HAND, 2014; LEVY et al., 2017). Uma comunidade microbiana disbiótica estabelecida afeta ativamente o sistema imunológico por meio de sinalizações de componentes celulares e metabólicos microbianos, criando um ciclo no qual o sistema imunológico do hospedeiro e a microbiota regulam cruzadamente um ao outro de maneira desordenada (BELKAID;

HAND, 2014; LEVY et al., 2017). Doenças inflamatórias, autoimunes e metabólicas podem ser exacerbadas por conta das interações entre o sistema imunológico e a microbiota em estado de disbiose (LEVY et al., 2017; BAGHBANI et al., 2020) ocasionando inflamação crônica no indivíduo (BELKAID; HAND, 2014; ZHENG et al., 2020). Além disso, pacientes com doenças inflamatórias podem exibir um perfil microbiano reduzido em abundância e diversidade de táxons quando comparado com o perfil de microbiota de indivíduos saudáveis (BELKAID; HAND, 2014; MAYNARD, 2019).

Apesar das alterações na composição da comunidade microbiana ocasionadas pela condição de disbiose, a microbiota por apresentar plasticidade de fácil modelação permite que após um intervalo de tempo em desequilíbrio dos seus constituintes microbianos, assim que os níveis normais da comunidade de microrganismos é restabelecida, a estabilidade seja retomada e perdura por longos períodos, mantendo a microbiota estável e resistente ao ataque de patógenos (LOZUPONE et al., 2012; URSELL et al., 2012; GILBERT et al., 2018).

1.5 Microbiota respiratória

O trato respiratório superior é formado por uma variedade de cavidades anatômicas, que incluem a cavidade nasal; seios da face; nasofaringe; orofaringe e laringofaringe, contando com uma microbiota distinta em cada uma dessas localidades distintas (ELGAMAL et al., 2021). O primeiro contato com microrganismos ao nascimento marca o estabelecimento da microbiota respiratória. E a formação da microbiota respiratória tem efeito no desenvolvimento do trato respiratório (MAN et al., 2017; FLYNN; DOOLEY, 2021).

A nasofaringe, alvo de estudo do presente trabalho, é um dos subcomponentes do trato respiratório superior (FLYNN; DOOLEY, 2021). Uma ampla variedade de microrganismos habita a nasofaringe, que apresenta um ambiente com características fisiológicas e celulares especificas, com tensão de oxigênio e dióxido de carbono, pH, umidade e temperatura de aproximadamente 34 °C (CLEARY; CLARKE, 2017; MAN et al., 2017, FLYNN; DOOLEY, 2021). A microbiota da nasofaringe em adultos saudáveis é predominantemente colonizada por bactérias dos gêneros *Corynebacterium*, *Dolosigranulum*, *Staphylococcus*, *Streptococcus* e *Lactobacillus.* Além disso, fungos dos

filos Ascomycota e Basidiomycota são também descritos como presentes na composição da microbiota do trato respiratório. Entretanto, a composição da microbiota da nasofaringe pode variar de pessoa para pessoa e conforme a idade do indivíduo (CLEARY; CLARKE, 2017; DUBOURG et al., 2019; FLYNN; DOOLEY, 2021; BELVONCIKOVA et al., 2022).

1.6 Microbiota e doenças infecciosas

Infecções respiratórias de origem viral são acompanhadas por desregulação da microbiota (MARSLAND et al., 2015; LI et al., 2019). A interação estabelecida entre hospedeiro, patógeno e microbiota residente durante quadros de infecção podem induzir perfis de disbiose no hospedeiro (MARSLAND et al., 2015; LI et al., 2019). A composição da microbiota é determinante para a interação patógeno infeccioso-hospedeiro, influenciando os diferentes perfis de suscetibilidade ou não as doenças infecciosas apresentadas por diferentes indivíduos (LI et al., 2019) assim como, na definição de quadros de complicação ou piora sintomatológica, e definição de desfechos clínicos da doença (ZHANG et al., 2020).

Em um estudo de Zhang e colaboradores (2020), foram identificadas associações entre a microbiota do trato respiratório superior, a expressão de genes resistentes a certas classes de antibióticos e a resposta produzida pelo hospedeiro frente à infecção causada pelo vírus influenza. Os dados desse estudo indicaram que a resposta do hospedeiro à infecção por influenza pode afetar indiretamente a expressão de genes de resistência a antibióticos no trato respiratório, impactando a estrutura da comunidade microbiana e a expressão geral dos genes microbianos e esses fatores se mostram contribuintes na severidade da doença respiratória causada pelo vírus influenza (ZHANG et al., 2020).

A microbiota influencia significativamente a imunidade do hospedeiro, desenvolvendo papel crucial no combate a invasões de origem viral. Uma microbiota saudável ajuda a manter uma imunidade robusta contra o ataque de patógenos, ao passo que uma microbiota desregulada pode aumentar a suscetibilidade a uma infecção viral (LI et al., 2019). As bactérias comensais do trato respiratório superior melhoram a resistência à infecção via mecanismos exclusivos, reduzindo a suscetibilidade à aquisição de patógenos oportunistas por intermédio de regulação da mucosa nasal (CLARK, 2020). Sendo assim, alterações na estrutura da comunidade microbiana do hospedeiro resultam em uma maior suscetibilidade a infecções de origem viral, uma vez que essas mudanças podem tornar o

sistema imune incapaz de limitar e frear as infecções causadas por patógenos virais (LI et al., 2019; LIBERTUCCI; YOUNG, 2019).

1.7 Infecções respiratórias agudas

Infecções do trato respiratório são a maior causa de doenças em seres humanos, principalmente em crianças e idosos (ECCLES, 2005). As infecções respiratórias estão associadas a uma elevada taxa de morbidade e mortalidade em diversos países ao redor do mundo todos os anos (MAYOR, 2010), e o combate a essas infecções representa um grande fardo de gastos dos recursos econômicos para a saúde pública (MEIER et al., 2020). Muitas infecções respiratórias são causadas por vírus, sendo os mais comuns o rinovírus (RV); vírus Influenza tipo A e B (IAV e IBV); parainfluenza humano (hPIV) dos tipos 1, 2, 3 e 4; bocavirus humano (HBoV); vírus sincicial respiratório (RSV); metapneumovírus humano (hMPV), adenovírus humano (HAdV); e coronavírus humanos (hCoVs), incluindo alphacoronavirus (HCoV-229E e HCoV-NL63) e betacoronavirus (HCoV-OC43 e HCoV-HKU1) (HAWKES et al., 2021), abrangendo o SARS-CoV-2 predominante durante o período de pandemia (NEHER et al., 2020).

O aspecto clínico das infecções respiratórias virais é bastante variável, influenciado pelo tipo de vírus causador da infecção, assim como por fatores como a idade do paciente, fisiologia, sistema imune e até mesmo pela classe social, etnia e gênero (ECCLES, 2005; PATHAK et al., 2022). Desta maneira, os sintomas apresentados por indivíduos infectados variam de assintomáticos a sintomas de Síndrome Gripal (SG) e ao desenvolvimento da Síndrome Respiratória Aguda Grave (SRAG). A SG é definida como um conjunto de sintomas causados em decorrência de uma infecção no trato respiratório superior, sendo caracterizada como uma síndrome leve e curta, com tempo de duração em torno de 7 dias de infecção. Os sintomas incluem febre, dor de cabeça, calafrios, espirros, obstrução nasal, tosse e dor de garganta (ECCLES, 2005).

A Síndrome Respiratória Aguda Grave (SRAG) é definida como uma causa comum de insuficiência respiratória, podendo ser incapacitante e até mesmo letal (THOMPSON et al., 2017; MATTHAY et al., 2019). Os casos de SRAG são caracterizados pelos sintomas de SG acompanhados por um início agudo de edema pulmonar e sintomas graves como hipoxemia, saturação de O_2 abaixo de 95%, dispneia e aumento da frequência respiratória, resultando na hospitalização do indivíduo e, muitas vezes, necessidade de ventilação

mecânica (FORCE et al., 2012; THOMPSON et al., 2017; MATTHAY et al., 2019). Após os sintomas iniciais de SRAG, a síndrome progride para mais severa em cerca de 7 dias com uma piora dos sintomas respiratórios, tendo como fatores de risco associados pneumonia, cardiopatias, obesidade e sepse (THOMPSON et al., 2017). Com o estabelecimento do vírus SARS-CoV-2 houve um aumento no número de casos de SRAG (GIBSON et al., 2020).

1.8 SARS-CoV-2 e COVID-19

No final do ano de 2019, relatos de uma pneumonia de origem desconhecida começaram a circular em Wuhan, capital da província de Hubei, China (ZHOU et al., 2020 a; ZHU et al., 2020; WU et al., 2020). Rapidamente o sistema de vigilância epidemiológica do país foi ativado para investigar os casos relatados. Posteriormente, o agente etiológico responsável pela doença foi associado ao mercado de frutos do mar na província de Hubei, o qual era conhecido por comercializar animais selvagens vivos (ZHOU et al., 2020 a; ZHU et al., 2020; WU et al., 2020). A partir do sequenciamento do genoma, o agente causador do surto de pneumonia foi identificado como sendo um coronavírus e, por intermédio de análises filogenéticas, foi constatado que se tratava de um novo betacoronavírus pertencente ao subgênero *Sarbecovirus* (LU et al., 2020; ZHOU et al., 2020 a; ZHU et al., 2020; WU et al., 2020). O vírus foi nomeado como SARS-CoV-2 (CSG, 2020) pelo grupo de estudos do Comitê Internacional de Taxonomia de Vírus (*ICTV*), e a doença por ele causada foi denominada *Coronavirus Disease*, ou COVID-19 (WHO, 2020 a, c). Em um curto espaço de tempo, o vírus SARS-CoV-2, o qual é muito contagioso, se disseminou ao redor do mundo devido à transmissão de contato humano com pessoas infectadas (LI et al., 2021). Em 11 de março de 2020, a Organização Mundial da Saúde (OMS) declarou a pandemia causada pelo vírus SARS-CoV-2 (WHO, 2020 b).

Novas evidências apontam o mercado de frutos do mar de Huanan como o epicentro inicial da pandemia de COVID-19 (WOROBEY et al., 2022). O vírus SARS-CoV-2 teria emergido do comércio de vida selvagem na China (PEKAR et al., 2022; WOROBEY et al., 2022), e infectado a população humana como resultado de vários eventos zoonóticos decorrentes do contato com um hospedeiro intermediário (PEKAR et al., 2022) não identificado até o momento, mas podendo ser morcegos, por exemplo (ZHOU et al., 2020 a). As sequências genômicas do SARS-CoV-2 foram relacionadas com

dois coronavírus derivados de morcegos (com 88% de identidade), compartilhando também identidade de sequências com os coronavírus causadores da Síndrome Respiratória Aguda Grave (SARS-CoV) e Síndrome Respiratória do Oriente Médio (MERS-CoV) (79% e 50% de identidade, respectivamente) (LU et al., 2020; ZHOU et al., 2020 a). Deste modo, grande parte das proteínas codificadas pelo SARS-CoV-2 apresentam similaridade com as proteínas correspondentes desses coronavírus (LU et al., 2020; ZHOU et al., 2020 a).

O SARS-CoV-2 é um vírus envelopado de RNA fita simples, sentido positivo (+ssRNA), de aproximadamente 30 mil bases (LU et al., 2020; HU et al., 2021). O vírus SARS-CoV-2 pertence à família Coronaviridae e ao gênero *Betacoronavirus* (CSG, 2020; LU et al., 2020; HU et al., 2021). A estrutura do genoma consiste em quatro proteínas estruturais: proteína spike (S), proteína da membrana (M), proteína do envelope (E), e proteína do nucleocapsídeo (N), além de proteínas não estruturais (*nsps*) (CHAN et al. 2020 a; LU et al., 2020). As proteínas M, E, N são fundamentais para a organização e liberação das novas partículas virais, e a proteína S é responsável pela ligação e entrada do vírus nas células hospedeiras (BOHN et al., 2020). Já as *nsps* são responsáveis pela replicação e invasão viral no hospedeiro (CHAN et al. 2020 a; LU et al., 2020).

O SARS-CoV-2 é transmitido de pessoa para pessoa por aerossóis e gotículas de secreções de indivíduos contaminados (CHAN et al., 2020 b; CHEN et al., 2020). Para entrar nas células humanas, o vírus precisa romper primeiramente a barreira da mucosa epitelial e, posteriormente, da microbiota do trato respiratório superior (BAGHBANI et al., 2020). Nesse contexto, a enzima conversora da angiotensina 2 (ACE2) foi identificada como um dos receptores de entrada do vírus SARS-CoV-2 nas células humanas (LU et al., 2020), sendo altamente expressa nas células epiteliais nasais (SUNGNAK et al., 2020; AHN et al., 2021). A entrada do vírus SARS-CoV-2 nas células da via respiratória superior é mediada a partir da interação estabelecida entre a proteína Spike do vírus e a ligação ao receptor ACE2 (SUNGNAK et al., 2020; AHN et al., 2021). O processamento proteolítico subsequente pela serina protease transmembrana 2 (*TMPRSS2*) e outras proteases desencadeia a fusão da membrana viral e celular do hospedeiro (AHN et al., 2021), em seguida, é iniciada a replicação e liberação de novas partículas virais, as quais podem estar presentes em gotículas e aerossóis respiratórios (SALZBERGER et al., 2021). O SARS-CoV-2 consegue se deslocar e replicar no trato respiratório inferior; quando isso

acontece, a COVID-19 progride para pneumonia e SRAG resultando em casos mais graves e hospitalização do paciente (BATTAGLINI et al., 2021; SALZBERGER et al., 2021).

A COVID-19 pode ser assintomática ou sintomática, manifestando-se com sintomas leves, moderados ou graves (SALZBERGER et al., 2020). Os sintomas clínicos relatados da doença são: febre, dor de garganta, tosse, dor muscular/fadiga, e alguns sintomas menos comuns, como produção de escarro, dor de cabeça e diarreia (HUANG et al., 2020; ZHOU et al., 2020 b). A progressão da COVID-19 de forma sistêmica é acompanhada pela presença de pneumonia e SRAG, podendo levar a falência múltipla dos órgãos em parte dos casos (SALZBERGER et al., 2020). É relatado que pacientes que desenvolveram os sintomas mais graves da COVID-19 tinham idade avançada, eram do sexo masculino e possuíam algum tipo de comorbidade como diabetes, hipertensão ou doenças cardiovasculares (HUANG et al., 2020; ZHOU et al., 2020 b). Também foi visto que pacientes internados com COVID-19 e SRAG apresentavam maiores índices de massa corporal e níveis mais baixos de interleucina — 6 (IL-6), esses pacientes tinham duração prolongada do uso de ventilação mecânica comparados a pacientes com SRAG negativos para SARS-CoV-2 (BAIN et al., 2021).

1.9 Microbiota e a COVID-19

A microbiota coloniza as áreas por onde o vírus se liga para infectar o hospedeiro; assim, é especulado que a microbiota e o vírus invasor interajam, alterando o ambiente do trato respiratório. Por meio de diferentes mecanismos moleculares, a microbiota pode desempenhar papéis chaves que impactam na progressão ou prevenção de condições patológicas infecciosas de maneira direta por meio de competição com o patógeno e indireta via modulação imunológica (BAGHBANI et al., 2020; CLARK, 2020). Por exemplo, a microbiota contribui com a produção de moléculas sinalizadoras que influenciam na ação das células imunes frente a uma resposta imunológica contra infecções causadas no ser humano (LI et al., 2019).

Na COVID-19, as narinas são uma das principais portas de entrada do SARS-CoV-2 (GALLO et al., 2020), pois a enzima ACE2 é altamente expressa nas células do epitélio nasal e trato respiratório superior, consolidando um importante indicador para entender a patogênese da COVID-19 (SUNGNAK et al., 2020). Dados de Koester e colaboradores (2021) indicam que o microbioma está associado com aumento ou

diminuição da expressão da ACE2, sendo decisivo com relação à suscetibilidade à doença e a severidade da infecção por SARS-CoV-2 (KOESTER et al., 2021). Dessa maneira, a microbiota pode funcionar protegendo o hospedeiro contra uma infecção de origem viral, induzindo proteção contra a doença infecciosa, ou ainda a microbiota pode desempenhar um papel inverso, promovendo a propagação e infecção viral por meio de diferentes mecanismos (BAGHBANI et al., 2020). Entretanto, a relação entre a microbiota da via respiratória e a infecção por SARS-CoV-2 ainda não foi bem estabelecida. O papel que a diversidade em componentes da comunidade microbiana do trato respiratório desempenha em relação à patogenicidade e severidade da COVID-19 ainda é muito discutido (LIU et al., 2021 b; YAMAMOTO et al., 2021).

Sendo assim, a microbiota deve ser considerada uma variável importante na determinação de suscetibilidade à COVID-19, uma vez que influência de maneira significativa o sistema imune, podendo regular a resposta imunológica frente à infecção por SARS-CoV-2 (PETERSEN et al., 2020, SHAH, 2021). Com base na possível ligação estabelecida entre a infecção por SARS-CoV-2 e a microbiota presente no sistema respiratório, a prevalência da COVID-19 em pessoas adultas mais velhas e idosas pode ser explicada por meio de duas possíveis hipóteses: a) a população adulta mais velha e idosa não apresenta uma comunidade de microrganismos necessários para combater o vírus, ou b) a microbiota dos adultos mais velhos e idosos é composta por bactérias que facilitam a infecção viral (SHAH, 2021). Portanto, uma microbiota rica e em equilíbrio ajuda a manter uma imunidade robusta contra o ataque de patógenos, ao passo que uma microbiota desregulada aumenta a suscetibilidade a uma infecção viral, e isso se deve ao fato de que o sistema imune nesse caso se encontra incapaz de limitar a infecção viral (LI et al., 2019).

Sendo assim, este estudo visa elucidar as comunidades bacterianas do trato respiratório superior de pacientes com COVID-19 com diferentes níveis de severidade da doença a fim de verificar uma possível associação entre a microbiota e o desenvolvimento de condições clínicas graves da COVID-19. A hipótese é que existam diferenças na composição da microbiota da nasofaringe em pacientes positivos e negativos para COVID-19 e que essas diferenças estejam associadas ao grau de severidade da doença.

2. OBJETIVOS

2.1 Objetivo geral

O objetivo geral deste estudo foi caracterizar a microbiota bacteriana do trato respiratório superior de pacientes com SG e SRAG, infectados e não infectados pelo SARS-CoV-2, a fim de identificar possível associação entre comunidades bacterianas e a COVID-19.

2.2 Objetivos específicos

1 - Analisar dados de pacientes com SG e SRAG do litoral do Rio Grande do Sul (RS) infectados e não infectados com SARS-CoV-2 quanto ao sexo, idade, etnia/raça, data de início dos primeiros sintomas, sintomas, comorbidades, data de hospitalização (para casos de SRAG), desfecho (recuperado ou óbito) e data do desfecho.

2 - Organizar uma coorte de pacientes com SG e SRAG positivos e negativos para SARS-CoV-2, considerando sexo e idade, para análise da microbiota da nasofaringe.

3 - Caracterizar a microbiota das amostras de nasofaringe dos pacientes selecionados quanto à composição, diversidade e abundância.

4 - Verificar possíveis associações entre a comunidade bacteriana identificada, a sintomatologia dos pacientes e o desfecho dos casos estudados em relação à COVID-19.

3. CAPÍTULO I — Artigo Científico de Revisão Sistemática

O referencial teórico está apresentado nesta dissertação na forma de uma revisão sistemática intitulada "**Nasopharyngeal microbiota in COVID-19 patients: a systematic review**", abrangendo o período dezembro de 2019 a abril de 2022.

Essa revisão será submetida a revista *Molecular Microbiology*, com fator de impacto (2019): **3.418**

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Nasopharyngeal microbiota in COVID-19 patients: a systematic review

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Abstract

The microbiota of the human respiratory tract can be impacted by viral infections that deregulate its composition and diversity, causing dysbiosis and possible co-infections. With the emergence of SARS-CoV-2 and the COVID-19 pandemic, studies have been conducted to understand the possible role of the nasopharynx microbiota in individual susceptibility to infection and disease severity. The aim of this systematic review was to evaluate evidence of such associations. This systematic review was conducted through searching studies published between December 2019 and April 30 in five database including PubMed, Embase, Web of Science, Scopus, and ScienceDirect . The search terms were: “SARS-CoV-2” OR “COVID-19” AND “Microbiota" OR “Microbiome”. From 338 articles identified by searching the databases, 31 were in line with the research criteria. Twenty-nine (93%) studies found associations between nasopharynx microbiota and COVID-19, characterized by differences in diversity and composition, dysbiosis, and increase in opportunistic pathogens such as *Rothia, Acinetobacter* and *Pseudomonas*. Ten (32%) studies found associations between the nasopharynx microbiota and COVID-19 severity. Additionally, patients recovered from COVID-19 also present a microbiota with alterations.

Keywords: COVID-19, SARS-CoV-2, nasopharynx microbiota, opportunistic pathogens, co-infections.

1. Introduction

The respiratory tract microbiota harbors a range of commensal and potentially pathogenic microorganisms that vary from specialized communities of bacteria, fungi, and viruses that differ from person to person (Man et al., 2017). Many environmental and genetic factors are known to modify the microbiota composition and diversity throughout life, such as antibiotics, nutrition habits, lifestyle (smoking, exercises), variation in specific host genes and infections. These factors can affect the structure of the microbiota and consequently the role it plays, influencing the human health and disease status (El-Sayed et al., 2021; Man et al., 2017). For viruses to enter a host body it is necessary to cross mucosal epithelial cells and interact with the microbiota that is present at the anatomical structure; the responses that the microbiota will have against the viral infection or vice versa can influence disease outcome (Baghbani et al., 2020).

SARS-CoV-2 is an enveloped, positive single-stranded RNA virus belonging to the genus *Betacoronavirus,* which causes the coronavirus disease (COVID-19) (Hu et al., 2021). The clinical symptoms presented by patients infected with SARS-CoV-2 can range from asymptomatic, mild (fever, cough, sore throat) to severe symptoms (Hu et al., 2021; Huang et al., 2020). Patients that develop severe or critical symptoms present pneumonia, with complications such as acute respiratory distress syndrome and organ failure (Hu et al., 2021; Huang et al., 2020). The main route of replication and transmission of SARS-CoV-2 is through the upper airways (Sungnak et al., 2020), which classifies this anatomical system as an important starting point for understanding the relationship between the local microbiota and susceptibility to SARS-CoV-2 infection and severity.

The commensal microbiota of the respiratory tract can play dual roles, promoting defenses against pathogenic viruses or contributing to susceptibility to viral infections (Mizutani et al., 2022). In COVID-19 cases, evidence showed that alterations in nasopharyngeal microbiota can affect outcomes and be correlated with disease severity in these patients (Ventero et al., 2022; Ventero et al., 2021). However, the molecular mechanisms involved in these features are not clearly understood (Mizutani et al., 2022). This systematic literature review aimed to analyze the evidence on the association between the nasopharyngeal microbiota and COVID-19, also evaluating the association between the microbiota and the COVID-19 severity.

2. Methods

This systematic review was conducted according to the criteria of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis statement (Page et al., 2021). The Rayyan tool was used to assess and filter the articles (Ouzzani et al., 2016).

2.1. Selection criteria and search strategy

In this systematic review, articles that were original clinical studies of any design and addressed the nasopharynx microbiota in patients with COVID-19 were considered. Articles review for eligibility was based on title and abstract selection criteria: must be in English and focused on humans. Articles that do not fit these criteria were excluded. A database search was conducted on MEDLINE/PubMed, EMBASE, Web of Science, ScienceDirect, and Scopus on articles published from December 2019 to April 2022. Examples of search terms used are listed in **Supplemental File 1 (S1 File)**; each search terms strategy was adapted for databases as appropriate. The author (JANR) independently reviewed titles and abstracts of the identified articles, and posterior results were resolved via a second opinion of authors (ABGV and AS).

2.2. Data synthesis

Data about authors, country of study, type and site of sample collection, population characteristics and number of participants, microbiome approach methods (sequencing platform), and principal key findings (alpha and beta-diversity) were collected from all studies. The main findings of the included articles were related and discussed in order to answer our questions about the relationship between nasopharyngeal microbiota and COVID-19 based on the evidence brought by the studies. The studies were organized according to: A) Characteristics of included studies (**Table 1**), and B) Nasopharynx microbiota components in patients with COVID-19 and controls (**Table 2**).

3. Results

A total of 338 articles were retrieved after the screening with the descriptors in the five databases. Of these, 107 were identified as duplicates. After screening the remaining 231 by titles and abstract 179 were additionally excluded. Finally, 52 articles were full-text examined for eligibility, of which 28 were identified as meeting the inclusion criteria. Posteriorly, three articles were identified by searching in references and added to the study, leaving a total of 31 papers eligible for inclusion in this review (**Figure 1**). The thirty-one articles and the main characteristics of included studies are shown in **Table 1**. Eight studies were conducted in the United States of America (Hurst et al., 2022; Shilts et al., 2022; Engen et al., 2021; Kolhe et al., 2021; Merenstein et al., 2021; Rhoades et al., 2021; Rosas-Salazar et al., 2021; Mostafa et al., 2020); four in China (Liu et al., 2021; Miao et al., 2021, Zhang et al., 2021; Wang et al., 2020); four in Italy (Giugliano et al., 2022; Nardelli et al., 2021; Rueca et al., 2021; De Maio et al., 2020); three in Bangladesh (Hoque et al., 2021 a; Hoque et al., 2021 b; Rahaman et al., 2021); two in Spain (Ventero et al., 2022; Ventero et al., 2021); two in India (Gupta et al., 2021; Mahapatra et al., 2021); one in Thailand (Rattanaburi et al., 2021); one in Mexico (Hernández-Terán et al., 2021); one in Israel (Braun et al., 2021); one in Belgium (Lloréns-Rico et al., 2021); one in Turkey (Hursitoglu et al., 2022); one in Russia (Babenko et al., 2021); one in France (Smith et al., 2021); and one in Sweden (Bai et al., 2022). The majority of the studies recruited participants who were either non-hospitalized (mild-moderate symptoms) or hospitalized (severe, admission to ICU to fatal cases) or recovered from COVID-19.

Clinical samples used to analyze the microbiota community varied among studies, including nasopharyngeal swab samples (Hurst et al., 2022; Ventero et al., 2022; Giugliano et al., 2022; Hursitoglu et al., 2022; Bai et al., 2022; Braun et al., 2021; Engen et al., 2021; Gupta et al., 2021; Hoque et al., 2021 a; Hoque et al., 2021 b; Kolhe et al., 2021; Liu et al., 2021; Mahapatra et al., 2021; Nardelli et al., 2021; Rahaman et al., 2021; Rattanaburi et al., 2021; Smith et al., 2021; Ventero et al., 2021; De Maio et al., 2020; Mostafa et al., 2020; Wang et al., 2020); nasal/oropharyngeal swab samples (Rueca et al., 2021); nasal and nasopharyngeal swab samples (Shilts et al., 2022); oropharyngeal swabs, nasopharyngeal swabs, and tracheal aspirates samples (Hernández-Terán et al., 2021; Merenstein et al., 2021); nasopharyngeal swab samples and bronchoalveolar fluid (BALF) (Lloréns-Rico et al., 2021); nasopharyngeal swab or sputum samples (Zhang et al., 2021); nasopharyngeal smears (Babenko et al., 2021); nasal samples (Rhoades et al., 2021; Rosas-Salazar et al., 2021); airways samples (Miao et al., 2021). Six articles (Bai et al., 2022; Shilts et al., 2022; Ventero et al., 2022; Hernández-Terán et al., 2021; Llorens-Rico et al., 2021; Miao et al., 2021) report patients usage of antibiotics, antivirals, or other medications prior to sample collection (before or during COVID-19 disease), which could have an effect on disease progression and on the microbiota.

The time point at sample collection also varied, and most samples were collected as part of clinical management for diagnosis of suspected SARS-CoV-2 infection. Some samples were collected at hospital admission, while others were collected during or after hospitalization (recovered COVID-19 patients). Five studies (Bai et al., 2022; Liu et al., 2021; Miao et al., 2021; Mostafa et al., 2020; Wang et al., 2020) used shotgun whole-genome sequencing to analyze the microbiome. Other studies used 16S rRNA gene amplicon sequencing; among these, nine studies amplified the V4 region of 16S rRNA gene (Hurst et al., 2022; Shilts et al., 2022; Braun et al., 2021; Engen et al., 2021; Gupta et al., 2021; Lloréns-Rico et al., 2021; Rattanaburi et al., 2021; Rhoades et al., 2021; Rosas-Salazar et al., 2021); five amplified the V3-V4 regions (Hursitoglu et al., 2022; Ventero et al., 2022; Hernández-Terán et al., 2021; Smith et al., 2021; Ventero et al., 2021), and some studies amplified different regions such as V5-V6 (De Maio et al., 2020), V4-V5 (Kolhe et al., 2021), V2–V2–V8 and V3-V6 and V7-V9 (Rueca et al., 2021), V1-V2 (Merenstein et al., 2021), V1-V2-V3 (Nardelli et al., 2021), V1-V9 (long-read sequencing) (Mahapatra et al., 2022); one study did not mention the region amplified of 16S rRNA gene (Babenko et al., 2021). Finally, three studies use the metatranscriptomics approach (Giugliano et al., 2022; Rahaman et al., 2021; Zang et al., 2021), and two studies used RNA-seq for the microbiome analysis (Hoque et al., 2021 a; Hoque et al., 2021 b).

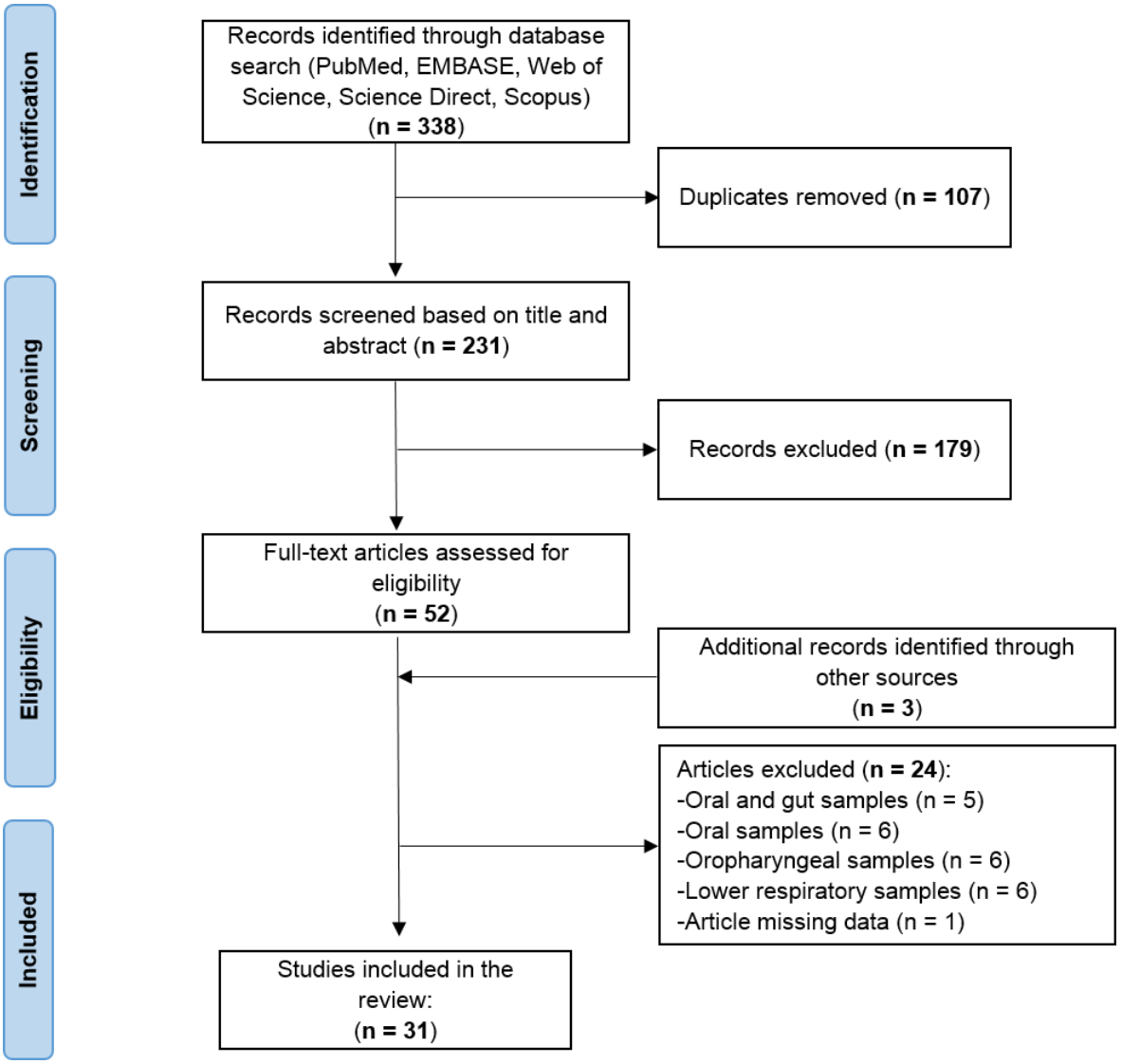

Figure 1: Prisma flow diagram of the study conduction.

Table 1: Characteristics of articles included in the study.

Authors	Location (country)	Participants		Type of sample	Population characteristics	Methods of microbiome sequencing	Key findings
		SARS-CoV-2 patients	Controls subjects				
De Maio et al., (2020)	Italy	n=18	n=22	Nasopharyngeal swab samples	COVID-19 patients: mild symptoms; Control: non-COVID-19 patients	16S rRNA (V5-V6)	**Alpha-diversity and beta-diversity:** no significant differences in either bacterial richness, diversity, or composition between the groups. No significant differences in the relative abundance of bacterial taxa most represented in both patient groups.
Kolhe et al., (2021)	USA	n=30 (PA), n=27 (PSY)	n=27 (NegA)	Nasopharyngeal swab samples	COVID-19 patients: positive asymptomatic (PA), and positive symptomatic (PSY-mild to severe symptoms); Control patients: negative asymptomatic (NegA).	16S rRNA (V4-V5)	**Alpha-diversity:** Shannon, Observed Species, or Bacterial Richness indices showed no significant differences between groups. **Beta-diversity**: microbial community differences found between PA and PSY groups from NegA group.
Rueca et al., (2021)	Italy	ICU n=10, Pauci n=11	Neg Controls n=10, HCoVs n=8	Nasal/ oropharingeal swab samples	COVID-19 patients: paucisymptomatic (Pauci), or in an Intensive Care Unit (ICU); Control patients: negative for COVID-19 (Neg), or affected by a different Human Coronavirus (HKU, NL63 or OC43).	16S (V2–V4–V8 and V3–V6 and V7–V9)	**Alpha-diversity:** Chao1 and Shannon indices resulted in a significant decrease in COVID-19 ICU as compared to COVID-19 Pauci patients, other HCoVs and neg controls. **Beta-diversity:** distinct clustering pattern among samples from neg. control, other HCoVs, COVID-19 Pauci and ICU.
Rattanaburi et al., (2021)	Thailand	n=24	Flu A n=24, Flu B n=24, Non-Flu & COVID-19 n=24	Nasopharyngeal swab samples	Patients infected with influenza A virus (Flu A,), influenza B virus (Flu B), SARS-CoV-2 virus (COVID-19), and patients without the influenza viruses and SARS-CoV-2 virus (Non-Flu & COVID-19).	16S rDNA (V4)	**Alpha-diversity**: Chao1 index showed that bacterial richness was not different among groups. Shannon diversity was significantly lower in Flu A and Flu B groups than in Non-Flu & COVID-19 group. The Flu A group diversity was significantly decreased compared to the COVID-19 group. **Beta-diversity**: microbial communities of Flu A and Flu B groups were highly similar but significantly distinct from those of COVID-19, and Non-Flu & COVID-19 groups.
Hernández-Terán et al., (2021)	Mexico	Mild n=37, severe n=27, and fatal n=19	Healthy controls n=7; non-COVID-19 pneumonia n=5	Oropharyngeal swabs, nasopharyngeal swabs, and tracheal aspirates samples	COVID-19 patients: mild (did not require hospitalization), severe (required hospitalization), and fatal (deceased). Control patients: healthy (without respiratory symptoms and negative for SARS-CoV-2), non-COVID-19 pneumonia (patients with pneumonia that were hospitalized but negative for SARS-CoV-2).	16S rRNA (V3-V4)	**Alpha-diversity**: Shannon index showed health controls as the most diverse in microbiota group and the non-COVID-19-pneumonia group as the least diverse. Considering severity groups, significant differences were observed between the severe and fatal COVID-19 groups. **Beta-diversity**: Indicate significant differences in the microbiota composition between COVID-19 groups (different severity levels) and controls. COVID-19 associated microbiota exhibited significantly higher levels of dysbiosis compared to the healthy controls.
Rosas-Salazar et al., (2021)	USA	n=38	n=21	Nasal samples (mid turbinate swabs)	COVID-19: non-hospitalized patients mild to moderate SARS-CoV-2 infection). Controls: uninfected asymptomatic adults.	16S rRNA (V4)	**Alpha-diversity**: Observed species index was significantly different, with microbiota higher diversity in SARS-CoV-2 patients than at the control group. **Beta-diversity**: no significant differences between groups (Bray-Curtis and Jaccard indices).

Mostafa et al., (2020)	USA	n=40	n=10	Nasopharyngeal swab samples	COVID-19 severity index: not admitted to hospital; admitted to hospital; admitted to Intensive Care Unit (ICU); and required ventilator. Controls: negative for SARS-CoV-2.	Whole-genome sequencing	**Alpha-diversity:** Shannon, Chao richness and Simpson indices found that COVID-19 patients have a significant reduction in the diversity of the bacterial communities at the species level. **Beta-diversity:** significant differences between the communities in SARS-CoV-2-positive and negative patients. Also observed a difference when comparing patients samples grouped by severity index at the species level.
Engen et al., (2021)	USA	n=9	n=10	Nasopharyngeal swab samples	SARS-CoV-2 patients had mild COVID-19 with no hospitalizations or deaths reported.	16S rRNA (V4)	**Alpha-diversity:** no significant differences between groups, but richness was lower in COVID-19 patients compared to negative controls. **Beta-diversity:** significant differences in nasopharyngeal microbial community structure between COVID-19 patients and negative controls.
Ventero et al., (2021)	Spain	n=56	n=18	Nasopharyngeal swab samples	COVID-19 patients: Group1- mild symptomatic without hospitalization, Group 2- moderate hospitalization, and Group 3- severe admission to ICU. Group 0- SARS-CoV-2 negative patients.	16S rRNA (V3-V4)	**Beta-diversity:** samples did not cluster according to the severity either by hierarchical clustering or by NMDS (Non-Metric Multidimensional Scaling). There were significant differences in OTUs composition among severity groups.
Gupta et al., (2021)	India	n=63	n=26	Nasopharyngeal swab samples	COVID-19 Suspected patients and their family contacts. A total of 11 families (n=46 subjects). Classified in groups (0-15 years) n=14; (16-30 years) n=13; (31-46 years) n=8; (46 & above) n=11; symptomatic and asymptomatic patients.	16S rRNA (V4)	**Alpha-diversity**: Simpson and Shannon indices did not show significant differences between infected and non-infected individuals. Observed OTUs and Chao1 values showed significantly decreased in the infected individuals. **Beta-diversity**: no significant differences were observed in the microbial community composition between SARS-CoV-2 infected and non-infected individuals.
Braun et al., (2021)	Israel	n=26	n=29	Nasopharyngeal swab samples	SARS-CoV-2 positive and negative samples were randomly included without specific selection.	16S rRNA (V4)	**Alpha-diversity:** Faith's phylogenetic diversity, Shannon and Evenness indices did not show significant differences between SARS-CoV-2 positive and negative samples. **Beta-diversity**: no significant differences observed within or between SARS-CoV-2 positive and negative samples. Samples of the same patient clustered close to each other, regardless of COVID-19 test results.
Liu et al., (2021)	China	n=9	n=6	Nasopharyngeal swab samples	Patients groups with matched age and gender. Similar clinical characteristics between groups include fever, cough, and ground-glass opacity in the lungs. One of the COVID-19 patients was severe, while two of the controls were severe. None of them was admitted to a critical care unit or on the ventilator.	Whole-genome sequencing	**Alpha-diversity**: No significant differences between COVID-19 and non-COVID-19 samples. **Beta-diversity**: variance in the nasopharyngeal microbiota between COVID-19 and non-COVID-19 groups of patients.

Merenstein et al., (2021)	USA	n=83	n=13	Oropharyngeal, nasopharyngeal swabs, and endotracheal aspirates samples	COVID-19: all hospitalized patients positive for SARS-CoV-2. Non-COVID-19: patients hospitalized in the intensive care unit with other disorders, and healthy controls.	16S rRNA (V1-V2)	**Alpha-diversity:** in oropharyngeal samples, lower diversity was correlated with COVID-19 severity. **Beta-diversity**: oropharyngeal and nasopharyngeal communities of COVID-19 patients differed from those of healthy subjects.
Lloréns-Rico et al., (2021)	Belgium	Upper n=58; and lower respiratory tract n=22	n=13	Nasopharyngeal swab samples and bronchoalveolar fluid (BALF)	COVID-19: All patients included were hospitalized, and admitted to ICU. Controls: pneumonitis patients negative for SARS-CoV-2, with varying disease severity.	16S rRNA (V4)	**Alpha-diversity**: Shannon index was significantly different across sampling moments, suggesting an effect of disease progression and/or treatment. The Shannon index correlated that the microbiome diversity is linked to the length of ICU stay, SARS-CoV-2 viral load, and calprotectin levels.
Nardelli et al., (2021)	Italy	n=18	Negative for SARS-CoV-2 n=12; recovery n=8.	Nasopharyngeal swab samples	Individuals divided in three groups: control-negative for SARS-CoV-2, COVID-19 symptomatic, and recovery patients.	16S rRNA (V1-V2-V3)	**Alpha-diversity**: Chao 1, Shannon, and Simpson diversity indices did not show significant differences in comparisons between COVID-19 patients and negative controls. **Beta-diversity**: significant differences between COVID-19 patients compared to negative controls. The distance between groups was dependent on the relative abundance of taxa rather than on the type.
Rhoades et al., (2021)	USA	n=68	non-COVID-19 outpatients n=21; non-COVID-19 healthcare workers n=45	Nasal swabs samples	COVID-19: SARS-CoV-2 positive patients at the time of diagnosis. Controls: healthy outpatients seeking elective procedures, and healthy healthcare workers (HCWs).	16S (V4)	**Alpha-diversity**: Observed ASVs present an increased richness in nasal community compared to COVID-19 patients and HCWs compared to negative controls. Shannon index did not find significant differences between groups compared. **Beta-diversity:** nasal microbial communities in COVID-19 patients showed the highest intra-group variability followed by communities from negative HCWs and outpatients.
Rahaman et al., (2021)	Bangladesh	n=17	n=2	Nasopharyngeal swab samples	COVID-19 vaccinated patients, COVID-19 unvaccinated patients and COVID-19 negative patients. None of the patients needed hospitalization.	Whole-genome sequencing	**Alpha-diversity**: Chao 1, Shannon, and Simpson indices showed no significant differences between sex and among vaccinated, nonvaccinateed and controls. **Beta-diversity**: no significant differences between male and females, and no significant differences among vaccinated, non-vaccinated, and controls.
Hurst et al., (2022)	USA	n=211	n=74	Nasopharyngeal swab samples	COVID-19 patients: children, adolescents, and young adults (< 21 years) with or without respiratory symptoms. Controls: SARS-CoV-2 exposed but uninfected.	16S rRNA (V4)	**Alpha-diversity:** Shannon index found that diversity increased with age and nasopharyngeal samples were similar between COVID-19 and control patients. Observed richness was not associated with the patient's age, but higher in SARS-CoV-2 patients. **Beta-diversity:** no significant differences between SARS-CoV-2 and controls. Differences in composition between SARS-CoV-2 with respiratory symptoms compared to SARS-CoV-2 without respiratory symptoms.
Shilts et al., (2022)	USA	n=83	n=20	Nasal and Nasopharyngeal swab samples	COVID-19 patients: mild-to-moderate, no hospitalization. Severe, were hospitalized. Controls: asymptomatic SARS-CoV-2 negative patients.	16S rRNA (V4)	**Alpha-diversity**: Shannon and Simpson indices were not significant. Richness was nearly significantly associated with COVID-19 severity. **Beta-diversity**: dissimilarity between samples increased as COVID-19 severity increased.

Ventero et al., (2022)	Spain	n=177	Not mentioned	Nasopharyngeal swab samples	COVID-19 all hospitalized patients.	16S rRNA (V3-V4)	**Alpha-diversity:** Shannon, Pielou and Simpson indices were lower in patients with a fatal outcome. **Beta-diversity**: significant difference grouping the fatal outcome patients, with the lower indexes. In invasive mechanical ventilation (IMV) patients, neither the alpha-diversity indexes nor beta-diversity analyses showed any significant differences.
Giugliano et al., (2022)	Italy	n=89	n=25	Nasopharyngeal swab samples	Samples were selected during the three main COVID-19 waves in Italy. The clinical outcome observed ranged from asymptomatic to severe infection and death. Control: meta-transcriptomic data generated from another study with SARS-CoV-2 negative patients with no other viral infections.	Meta-transcriptomic	The SARS-CoV-2 infection was associated with dysbiosis in the nasopharyngeal microbiota and also with a higher risk of bacterial co-infection that could affect COVID-19 disease progression and outcome.
Hursitoglu et al., (2022)	Turkey	n=49	n=51	Nasopharyngeal swab samples	COVID-19 patients: mild or severe symptoms. Control: healthy controls and recovery COVID-19 patients.	16S rRNA (V3-V4)	**Alpha-diversity:** Observed species, Chao 1, Simpson, and PD-WT indices were lower in the severe COVID-19 group, and significantly different when compared to the other groups. Shannon index- no significant differences between groups. **Beta-diversity:** no significant differences between samples of the groups analyzed.
Babenko et al., (2021) (*Preprint)	Russia	n=336	n=18	Nasopharyngeal smears	COVID-19 Inpatients and outpatients selected during the first and second waves of the epidemic in Russia. Controls: SARS-CoV-2 negative sequences obtained from a similar study were included in the analysis.	16S rRNA	**Alpha-diversity:** community type 4 (*Streptococcus*, *Prevotella* and *Veillonella* genera) had the highest level, and community type 5 (*Staphylococcus*, *Pseudomonas*, *Streptococcus*) showed the lower indice.
Mahapatra et al., (2021) (*Preprint)	India	n=46	n=12	Nasopharyngeal swab samples	COVID-19 patients were asymptomatic or symptomatic (mild symptoms). The control group was negative for SARS-CoV-2 and did not present any flu-like symptoms.	16S rRNA (V1-V9)	**Alpha-diversity**: Shannon and Simpson indices between control and SARS-CoV-2 patients differed significantly. **Beta-diversity**: unweighted and weighted methods found that control, SARS-CoV-2 asymptomatic and SARS-CoV-2 symptomatic patients differed significantly.
Smith et al., (2021)	France	n=49	n=12	Nasopharyngeal swab samples	The severity of Covid-19 disease was mild, moderate, severe and critical symptoms. Healthy controls were asymptomatic adults, negative to SARS-CoV-2.	16S rRNA (V3-V4)	**Alpha-diversity:** Simpson and Shannon indices showed a decrease in 16S rRNA sequences in patients with severe and critical COVID-19. **Beta-diversity**: richness of microbiota communities decreased with disease severity, and profiles of patients with critical disease were different from other patients.

Hoque et al., (2021) b	Bangladesh	n=8	recovered n=7, healthy n=7	Nasopharyngeal swab samples	COVID-19: SARS-CoV-2 positive through RT-qPCR. Recovered: After SARS-CoV-2, infection these patients were tested negative for COVID-19. Healthy control subjects did not show any signs and symptoms of respiratory illness.	RNA-seq	**Alpha-diversity**: Shannon and Simpson indices found significant differences in microbial species richness across three groups. Higher diversity in the microbial niche in recovered patients followed by Healthy and COVID-19 patients. **Beta-diversity**: distinct discrimination across the metagenomes and separated samples by microbial population structure. Significant variation in microbiome diversity and composition across the three groups.
Hoque et al., (2021) a	Bangladesh	n=11	n=10	Nasopharyngeal swab samples	COVID-19 sequences: 5 RNA seq from Bangladesh and 6 retrieved from Chinese RNA-seq database). Non-COVID: 4 retrieved metagenome sequences from USA and 6 metagenome sequences of Chronic obstructive pulmonary disease from UK databases.	RNA-seq	**Alpha diversity:** Shannon index found significant differences in diversity. With the number of identified microbial taxa and Shannon index significantly higher in COVID-19 samples than in non-COVID. **Beta-diversity:** significant differences in composition showing distinct separations among the four groups analyzed. In addition, the COVID-19 samples remained more similar than the non-COVID.
Miao et al., (2021)	China	n=50	n=74	Airway samples	COVID-19 patients stratified as mild, severe and critically severe. Controls: intubated for non-COVID-19 diseases or non-intubated viral pneumonia, and non-incubation non-infectious diseases	Whole-genome sequencing	**Alpha-diversity**: Shannon index was significantly lower in critically severe COVID-19 patients than in non-COVID-19 patients (non-intubation) but similar to that of intubated non-COVID patients. **Beta-diversity**: significant difference between the three groups, critically severe COVID-19 and non-COVID-19 (intubated and non-intubated).
Zhang et al., (2021)	China	n=62	n=125	Nasopharyngeal swab (NS) or sputum (SP)	COVID-19 and non-COVID patients all with pneumonia diagnostic.	Meta-transcriptomic	**Alpha-diversity**: Shannon index showed no significant changes in diversity for the NS samples. However, Shannon index was significantly lower in SP of COVID-19 patients compared to non-COVID patients.
Wang et al., (2020)	China	n=44	Not mentioned	Nasopharyngeal test paper	COVID-19 patients classified as severe/critical or non-severe.	Whole-genome sequencing	**Alpha-diversity** and **beta-diversity** metrics were not analyzed. Were found some differences in microbial composition in upper airways between COVID-19 severe and non-severe patients.
Bai et al., (2022)	Sweden	n=37	n=20	Nasopharyngeal swab samples	COVID-19 patients defined as critically ill, all hospitalized. Negative controls tested negative for SARS-CoV-2.	Shotgun whole metagenome sequencing	**Alpha-diversity**: Richness, Shannon and Simpson indices showed significant reduction of bacterial microbiota at genus and species levels in samples from COVID-19 patients compared to negative controls. **Beta-diversity**: no significant differences were found between samples from COVID-19 patients and negative controls at genus and species levels.

3.1. Microbiome characteristics

The relative abundance of taxa present in nasopharyngeal microbiota in COVID-19 patients and controls is shown in **Supplemental File2 (S2 File)**, organized by phyla, order, families, genera, species, and other key finds. Collectively, 31 microbiota data sets were considered for microbiome extraction and further analysis. There are differences in the nasopharynx bacteria of

patients with COVID-19 reported as increased, decreased compared to controls or predominant in both groups (**Table 2**).

The prevalent phyla identified in nasopharyngeal microbiota composition of COVID-19 patients varied when comparing differently. The main phyla found in samples from nasopharyngeal microbiota are shown in **Figure 2**. Seven studies identified as predominant in COVID-19 patients the phylum Firmicutes, six the Actinobacteria, five the Bacteroidetes, another five the Proteobacteria, and one Fusobacterium (**Figure 2)**. It was also verified in the studies of some phyla, which decreased in abundance in patients with COVID-19. Two studies had the phylum Bacteroidetes, three Fusobacteria, three Proteobacteria, and two Spirochaetes decreased in their composition. It was also verified in the composition of the microbiota that had phyla in common between SARS-CoV-2 patients and controls. It was composed of nine articles with Actinobacteria, nine Bacteroidetes, eleven Firmicutes, four Fusobacteria and nine Proteobacteria not differing between conditions (**Figure 2)**.

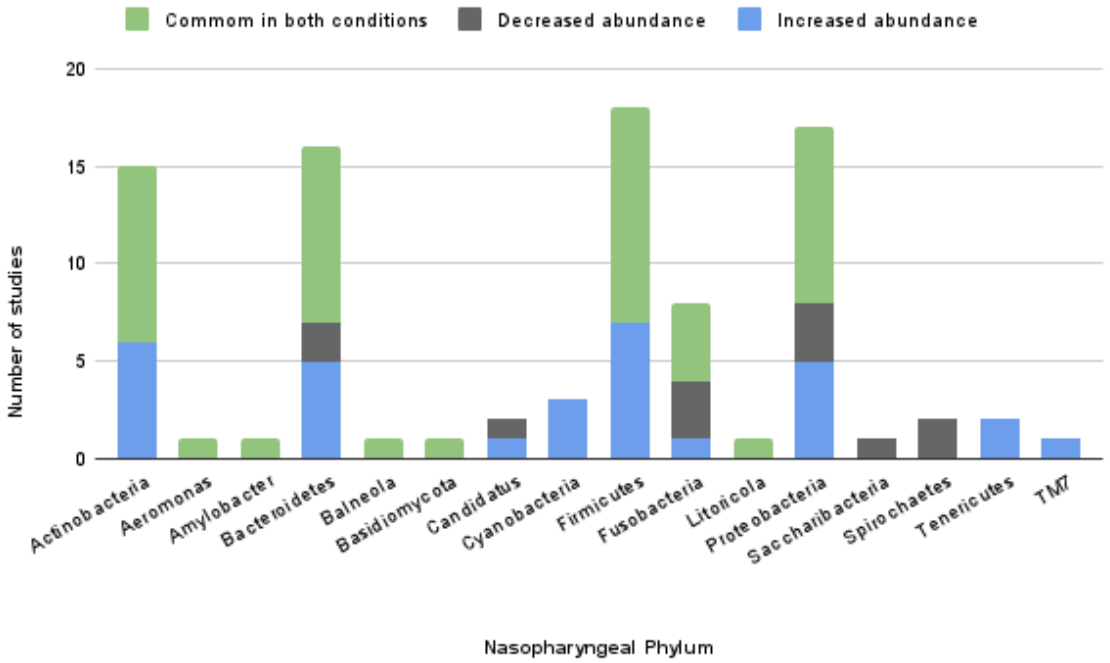

Figure 2: Comparison of nasopharyngeal microbiota composition of patients with COVID-19 compared to controls at phyla level. See supplementary information for further explanation of reported abundance (S2 File and S2 Table)

Further, taking into account the main phyla that compose the natural nasopharynx microbiota, some genera stood out with increased, decreased or not changing abundance between patients with COVID-19 and controls (**Figure 3).** The Actinobacteria phylum presented one article with the *Bifidobacterium* genus with increased abundance, and two with reduced abundance. The genus *Corynebacterium* presented eight articles with increased abundance, 2 with reduced abundance and 10 being considered common in both conditions. In *Actinomyces*, three articles showed increased abundance and four-decreased abundance in patients with SARS-CoV-2 when compared to controls (**Figure 3A**). Four articles reported increased abundance of *Prevotella* (phylum Bacteroidetes) (**Figure 3B**) whereas in two studies both observed an increase and depletion in *Leptotrichia* genus (phylum Fusobacterium) (**Figure 3D**). In addition, some studies

reported increased and common abundance in both conditions of Firmicutes phylum, including the genera *Streptococcus, Staphylococcus, Veillonella* and *Dolosigranulum* (**Figura 3C**). Furthermore, the phylum Proteobacteria highlighted the genera *Pseudomonas, Neisseria, Actinobacilulus, Haemophilus* and *Moraxella* as the most increased and decreased in abundance in patients with COVID-19 (**Figura 3E**).

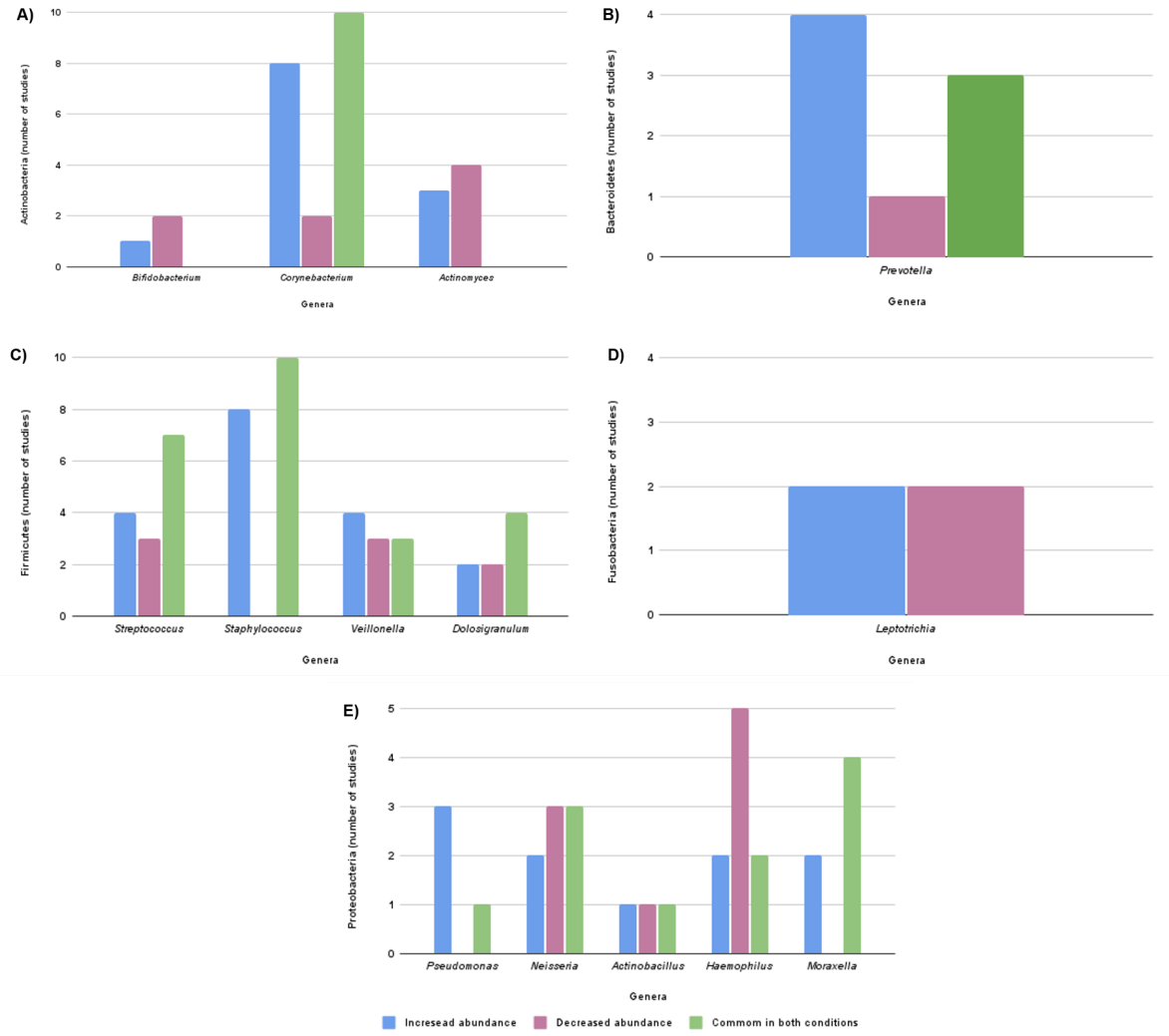

Figure 3: Number of studies presenting genera in nasopharyngeal microbiota in patients with COVID-19 compared to controls. A) Phylum Actinobacteria and genera *Actinomyces, Bifidobacterium* and *Corynebacterium*; B) phylum Bacteroidetes with genus *Prevotella*; C, D and E) Phylum Firmicutes, Fusobacteria and Proteobacteria respectively, and their representatives. See supplementary information for further explanation of reported abundance (S2 and S3 Files)

Table 2: Nasopharyngeal microbiota components in COVID-19 patients compared to negative controls subjects.

Authors	Variation abundance	Respiratory bacterial taxa reported in SARS-CoV-2 patients compared to controls				differentially enriched bacteria or biomarkers	Other key finds
		Phylum	**Family**	**Genus**	**Species**		
	Increase						
	Decrease						
De Maio et al., 2020	Most common	Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria					
	Increase	**COVID-19** (PA and PSY): Cyanobacteria		**COVID-19** (PSY): *Cutibacterium Lentimonas*			
	Decrease		**COVID-19** (PSY)*:* Prevotellaceae	**COVID-19** (PSY)*: Limnephilus Flectobacillus Comamonas, Jannaschia*			
Kolhe et al., (2021)	Most common	**COVID-19** (PA and PSY): Bacteroidota, Litoricola, Amylobacter, Balneola, Aeromonas.					
	Increase	**Neg controls**: Deinococcus Thermus. **Neg controls, SARS-CoV-2 Pauci**: Candidatus Saccharibacteria	**Neg controls**: Alicyclobacillaceae Chromobacteriaceae Deinococcacaee Hydrogenophilaceae Thermoanaerobacteraceae Sporomusaceae Thermoanaerobacterales FamilyIII.Incertae Sedis. **SARS-CoV-2 ICU**: Pectobacteriaceae	**Neg controls**: *Johnsonella*, *Tepidiphilus*, *Thermoanaerobacter Thermoanaerobacterium*, *Thermosinus, Variovorax* **SARS-CoV-2 ICU:** *Salmonella*, *Scardovia*, *Serratia,unk_Pseudomonadaceae.* **SARS-CoV- Pauci:** *Bulleidia,Halanaerobium Streptobacillus, unkn_Epsilonproteobacteria, unkn_Moraxellaceae, unkn_Mycoplasmataceaeunkn_Tenericutes*			
	Decrease			**SARS-CoV-2 ICU**: *Bifidobacterium Clostridium*			
Rueca et al. (2021)	Most common						
Rattanaburi et al., (2021)	Increase	**Flu A, Flu B**: Proteobacteria. **COVID-19**: Firmicutes Bacteroidetes. **Non-Flu & COVID-19:** Firmicutes Bacteroidetes Proteobacteria.	**Flu A and Flu B**: Enterobacteriaceae **COVID-19**: Enterobacteriaceae	**COVID-19**: *Staphylococcus*, *Lautropia*, *Pseudomonas*, *Corynebacterium.* **Non-Flu & COVID-19**: *Prevotella*, *Veillonella*, *Capnocytophaga*, *Fusobacterium*		**Flu A, Flu B**: Enterobacteriaceae. **COVID-19**: *Staphylococcus Pseudomonas.* **Non-Flu & COVID-19**: *Streptococcus*, *Prevotella*, *Veillonella*, *Fusobacterium*	
	Decrease						

	Most common			**COVID-19 and Non-Flu & COVID-19**: *Streptococcus*			
	Upper respiratory tract analysis						
Hernández-Terán et al., (2021)	Increase	**COVID-19 groups**: Firmicutes, Actinobacteria, TM7		**COVID-19:** *Veillonella*, *Staphylococcus*, *Corynebacterium*, *Neisseria*, *Actinobacillus*, *Selenomonas* **Health groups:** *Haemophilus Alloiococcus* **mild to fatal COVID-19**: *Streptococcus Staphylococcus*. **mild COVID-19**: *Haemophilus Actinomyces*. **severe COVID-19**: *Corynebacterium*. **fatal and mild COVID-19**: *Actinobacillus*		**mild COVID-19**: *Prevotella melaninogenica, P. pallens, Veillonella parvula, Neisseria subflava, Fusobacterium, Actinomyces.* **severe COVID-19**: *Megasphaera*, CW040. **fatal COVID-19**: *Rothia dentocariosa*, *Streptococcus infantis*, *Veillonella dispar.* **healthy controls**: *Streptococcus*, *Flavobacterium*, *Oribacterium*, f_*Veillonellaceae.* **non-COVID-19-pneumonia**: *Corynebacterium*, *Prevotella nigrescens*, *Capnocytophaga*, *Enterobacteriaceae*.	
	Decrease	**COVID-19 groups**: Bacteroidetes Proteobacteria		**Health groups**: *Veillonella*, *Staphylococcus*, *Corynebacterium*, *Neisseria*, *Actinobacillus*, *Selenomonas.* **COVID-19 groups**: *Haemophilus Alloiococcus.* **fatal COVID-19**: *Haemophilus Actinomyces*		**fatal COVID-19**: *Haemophilus Actinomyces*	
	Most common	Firmicutes, Bacteroidetes, and Proteobacteria		**COVID-19 groups**: *Veillonella*, *Staphylococcus*, *Corynebacterium*, *Neisseria*, *Actinobacillus*, *Selenomonas*. **Health groups**: *Haemophilus Alloiococcus*.			
	Lower respiratory tract analysis						
	Increase	**fatal COVID-19**: Proteobacteria Bacteroidetes		**fatal COVID-19**: *Prevotella*, *Staphylococcus*, *Haemophilus*, *Enterococcus.* **severe COVID-19**: *Streptococcus Abiotrophia.*		**severe COVID-19**: *Streptococcus*, *Neisseria*, *Abiotrophia*, *Actinobacillus*	
	Decrease					**fatal COVID-19**: *Veillonella parvula V. dispar.*	

	Most common						
	Increase			**SARS-CoV-2–uninfected**: *Staphylococcus*, *Corynebacterium_1*, *Moraxella*, *Dolosigranulum*, *Neisseria* unclassified. **SARS-CoV-2**: *Corynebacterium_1*, *Staphylococcus, Dolosigranulum*, *Peptoniphilus*, *Lawsonella* **SARS-CoV-2** with high viral load: *Corynebacterium_1*, *Staphylococcus*, *Peptoniphilus*, *Anaerococcus Bacteroides*. **SARS-CoV-2** with low viral load: *Corynebacterium_1*, *Staphylococcus*, *Dolosigranulum*, *Lawsonella Peptoniphilus.*		**SARS-CoV-2**: *Brevundimonas*, *Corynebacterium*, *Granulicatella*, *Anaerococcus*, *Peptoniphilus*. **SARS-CoV-2** with high viral load: *Neisseriaceae*, *Anaerococcus*, *Peptoniphilus*, *Campylobacter Enterococcus*. **SARS-CoV-2** and those with high viral load: *Peptoniphilus lacrimalis*, *Campylobacter hominis*, *Prevotella* 9 *copri*, *Anaerococcus* unclassified. **SARS-CoV-2-uninfected** and those with low viral load: *Corynebacterium* unclassified, *Staphylococcus haemolyticus, Prevotella disiens*, *Corynebacterium_1 unclassified.*	
	Decrease					**SARS-CoV-2**: *Corynebacterium_1*, *Prevotella*, *Staphylococcus*, *Anaerostipes*, *Neisseria* **SARS-CoV-2** with high viral load: *Corynebacterium_1*, *Staphylococcus*, *Granulicatella*, *Neisseria*, *Prevotella.*	
Rosas-Salazar et al., (2021)	Most common						
	Increase		**COVID-19**: Propionibacteriaceae				
	Decrease				**COVID-19**: *Corynebacterium accolens*		
Mostafa et al., (2020)	Most common						
	Increase	**COVID-19:** Proteobacteria Actinobacteria				**COVID-19** (Boruta): *Anaerococcus*, *Streptococcus*, *Enterococcus*, *Bacillus.*	
Engen et al., (2021)	Decrease					**COVID-19** (ANCOM): *Streptococcus*. **COVID-19** (DESeq2): *Rothia, Prevotella.*	

	Most common	Proteobacteria, Actinobacteria Firmicutes		*Corynebacterium, Morganella, Moraxella, Escherichia-Shigella, Proteus, Staphylococcus*			
	Increase	**COVID-19**: Bacteroidota Firmicutes		**COVID-19**: *Prevotella, Leptotrichia, Streptococcus.* **group 1**: *Veillonella.* g3. *Prevotella*			
	Decrease						
Ventero et al., (2021)	Most common	Firmicutes, Bacteroidota, Proteobacteria, Actinobacteria		*Streptococcus*, *Prevotella*, *Veillonella*, *Haemophilus*, *Moraxella*			
Gupta et al., (2021)	Increase	**non-infected**: Firmicutes, Proteobacteria, Bacteroidetes. **COVID-19**: Proteobacteria, Firmicutes, Bacteroidetes		**COVID-19:** *Haemophilus*, *Stenotrophomonas*, *Acinetobacter*, *Moraxella Corynebacterium* I, *Gemella*, *Ralstonia*, *Pseudomonas.* **Non-infected**: *Prevotella7*, *Veillonella*, *Neisseria*, *Rothia*, *Leptotrichia*, *Fusobacterium*, *Alloprevotella*, *Megasphaera*, *Dolosigranulum* **COVID-19**: Age Group 1-*Prevotella 7 Haemophilus, Leptotrichia, Alloprevotella, Gemella, Granulicatellata Moraxella*, *Lautropia*. Age Group 2- *Streptococcus, Haemophilus, Corynebacterium 1, Gemella,Granulicatel lata Prevotella* 2. Age Group 3- *Haemophilus, Stenotrophomonas, Leptotrichia, Acinetobacter, Fusobacterium, Prevotella, Pseudomonas, Staphylococcus, Lachnoaerobaculum*. Age Group 4- *Stenotrophomonas, Leptotrichia, Acinetobacter, Neisseria, Alloprevotella, Pseudomonas, Granulicatellata, Ochrobactrum.* symptomatic			

				individuals: *Streptococcus, Neisseria, Acinetobacter, Rothia*.			
	Decrease		**COVID-19:** Prevotellaceae Veillonellaceae Neisseriaceae, Leptotrichiaceae, Fusobacteriaceae	**COVID-19**: symptomatic females- *Streptococcus, Prevotella 7, Haemophilus, Rothia, Corynebacterium 1, Porphyromonas, Gemmella, Actinobacillus* symptomatic males- *Streptococcus, Veillonella Stenotrophomonas, Leptotrichia, Neisseria, Rothia, Actinomyces, Prevotella 6 Actinobacillus*, *Granulicatellata*			
	Most common	Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria	Streptococcaceae, Prevotellaceae, Pasteurellaceae, Veillonellaceae, Xanthomonadaceae, Neisseriaceae, Micrococcaceae, Leptotrichiaceae, Burkholderiaceae, Fusobacteriaceae, Actinomycetaceae, Porphyromonadaceae				
	Increase						
	Decrease						
Braun et al., (2021)	Most common	Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Proteobacteria					
	Increase			**COVID-19**: *Prevotella*, *Veillonella*, *Neisseria*, *Actinomyces*	**COVID-19**: *Prevotella histicola, Megasphaera micronuciformis, Lautropia mirabilis, Streptococcus sanguinis, Veillonella dispar*	**Non-COVID-19**: *Gemella morbillorum, Gemella haemolysans, Campylobacter gracilis, Stenotrophomonas maltophilia, Leptotrichia hofstadii*	
	Decrease						
Liu et al., (2021)	Most common	Firmicutes, Bacteroidetes					
Merenstein et al., (2021)	Increase						

	Decrease	**COVID-19 OP:** Proteobacteria		**COVID-19 OP**: *Haemophilus*, *Actinomyces*, *Neisseria.* **COVID-19 LUNG***:* *Staphylococcus*, *Klebsiella*, *Stenotrophomonas Corynebacterium*, *Prevotella*			
	Most common						
Lloréns-Rico et al., (2021)	Increase					Mechanically ventilated **COVID-19**: *Prevotella, Fusobacterium, Porphyromonas, Lactobacillus, Mycoplasma, Megasphaera Prevotella oris, P. salivae, P. denticola, P. buccalis, P. oralis, Mycoplasma salivarium.*	
	Decrease						
	Most common			*Staphylococcus, Corynebacterium*			
Nardelli et al., (2021)	Increase						
	Decrease	**COVID-19**: Proteobacteria, Fusobacteria		**COVID-19**: *Leptotrichia, Fusobacterium, Haemophilus*	**COVID-19**: *Fusobacterium periodonticum*		
	Most common	Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria					
Rhoades et al., (2021)	Increase					**CoV-**: *Anaerococcus* **HCWs**: *Lawsonella, Burkholderia.* **COVID-19**: *Rothia, Acinetobacter, Pseudomonas.* <u>high Ct value:</u> *Streptococcus* <u>middle and low Ct groups:</u> *Corynebacterium* <u>low Ct group:</u> *Neisseria Pseudomonas*	**Cov- and HCW**: *Anoxybacillus.* **HCW:** *Burkholderia cepacia.* **COVID-19**: *Acinetobacter, Pseudomonas. Pseudomonas aeruginosa* <u>high Ct value</u>: *Streptococcus* <u>middle Ct value:</u> *Corynebacterium*, <u>low Ct value:</u> *Cutibacterium, Neisseria Pseudomonas.*
	Decrease						
	Most common	Actinobacteria, Firmicutes, Proteobacteria		*Corynebacterium, Staphylococcus, Streptococcus, Dolosigranulum Neisseria*		*Corynebacterium, Staphylococcus*	

	Increase				Coinfections **COVID-19** *Streptococcus agalactiae, Neisseria meningitidis, Elizabethkingia anophelis, Stenotrophomonas maltophilia, Klebsiella pneumoniae, Pseudomonas plecoglossicida*	**Vaccinated**: *Enterobacter cloacae*, *Brevundimonas diminuta,S. maltophilia*, uncultured bacterium, *Cutibacterium acnes*. **Nonvaccinated:** *E. cloacae,* uncultured bacterium *B. diminuta Sphingobacterium multivorum S. maltophili*a **Non-COVID-19**: *Streptococcus salivarius*, *Rothia mucilaginosa*, *Streptococcus* sp. oral taxon 431, *Streptococcus mitis, Veillonella atypica* **Males**: *E. cloacae, B. diminuta, C. acnes S.salivarius, S.maltophilia.* **Female**: *E. cloacae B.diminuta, S.maltophilia,* uncultured bacterium*, V. atypica*	Virus- **Vaccinated**: Siphoviridae, Picornaviridae Inoviridae, Adenoviridae Siphoviridae, Herpesviridae Parvoviridae. **Nonvaccinated:** Siphoviridae, Adenoviridae, Podoviridae. **Control**: Streptococcus phage Javan269
	Decrease				Coinfections **non-COVID-19**: S. agalactiae, Neisseria mucosa, N. meningitidis, S. maltophilia, K. pneumoniae		
Rahaman et al., (2021)	Most common	Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Basidiomycota				**Vaccinated and non-vaccinated**: *S.maltophilia, B.diminuta, E. cloacae*, uncultured bacterium	
	Increase			Increasing age: *Corynebacterium, Staphylococcus.* 12 years of age or older: *Lawsonella, Peptoniphilus*		.	
	Decrease			increasing age: *Moraxella Dolosigranulum*			
Hurst et al., (2021)	Most common	*Corynebacterium, Staphylococcus, Moraxella, Dolosigranulu mStreptococcus*					
Shilts et al. (2022)	Increase						
	Decrease						

	Most common			*Staphylococcus*_unclassified.ASV0001, *Corynebacterium*_unclassified.ASV0002, *Corynebacterium*_unclassified.ASV0003, *Dolosigranulum*_pigrum.ASV0006, *Corynebacterium*_unclassified.ASV0004.			
Ventero et al., (2022)	Increase				non-IMV patients: *Selenomonas* spp., *Filifactor* spp., *Actinobacillus* spp Chroococcidiopsis spp. non-exitus patients: *Actinobacillus* spp., *Citrobacter* spp., *Craurococcus* spp., *Moheibacter* spp.		
	Decrease						
	Most common			*Streptococcus* spp., *Staphylococcus* spp., *Corynebacterium* spp			
Giugliano et al., (2022)	Increase	**COVID-19**: Proteobacteria, Firmicutes, Actinobacteria. **Control**: Proteobacteria		**COVID-19**: *Streptococcus, Staphylococcus*	**COVID-19**: *Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Neisseria gonorrhoeae*	**SARS-CoV-2**: Tenericutes, Candidatus Saccharibacteria, Fusobacteria, Bacteroidetes, Firmicutes. **Control:** Proteobacteria, Planctomycetes, Verrucomicrobia, Deinococcus-Thermus **First cluster**: *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa Moraxella catarrhalis Finegoldia magna Acinetobacter baumannii Enterococcus faecium, Lactobacilli, Corynebacterium propinquum, Dolosigranulum pigrum* **Second cluster**: *Streptococcus salivarius, Streptococcus oralis Prevotella* spp., *Schaalia odontolitica, Veillonella parvula, Capnocytophaga gingivalis, Rothia dentocariosa*	

	Decrease	**COVID-19**: Bacteroidetes, Fusobacteria, Candidatus Saccharibacteria Spirochaetes. **Control**: Firmicutes		**COVID-19**: *Bifidobacterium Dolosigranulum* **High SARS-CoV-2**: *Streptococcus, Veillonella, Proteus, Treponema, Brevudimonas*, *Bifidobacterium Lactiscaseinbacillus*	**COVID-19**: *Pseudomonas aeruginosa, Acinetobacter baumannii*		
	Most common	Actinobacteria					
Hursitoglu et al., (2022)	Increase	**Recovered COVID-19:** Fusobacteria Saccharibacteria **Healthy control**: Spirochaete					
	Decrease	**Severe COVID-19:** Fusobacteria Saccharibacteria Spirochaete					
	Most common						
Babenko et al., (2021)	Increase						
	Decrease						
	Most common			**<u>Types 1 and 4</u>**: *Streptococcus, Prevotella Veillonella*. **<u>Type 2</u>**: *Prevotella, Streptococcus Veillonella*. **<u>Type 3</u>**: *Staphylococcus, Pseudomonas*, *Corynebacterium.* **<u>Type 5</u>**: *Staphylococcus, Pseudomonas, Streptococcus*. **<u>Type 6</u>**: *Pseudomonas Stentotraphomonas*			
Mahapatra et al., (2021) (Preprint)	Increase	**COVID-19**: Firmicutes, Actinobacteria. **Control**: Bacteroidetes, Proteobacteria	**COVID-19**: Mycobacteriaceae, Propionibacteriaceae Streptomycetaceae	**COVID-19**: *Mycobacterium Mycolicibacterium, Mycobacteroides, Halothiobacillus, Flavobacterium Bifidobacterium Streptomyces, Rothia, Mycoplasma*. **Control**: *Thermomicrobium, Kingella, Enterobacter, Bacteroides, Prevotella*	**SARS-CoV-2 groups**: *Mycobacterium tuberculosis, Mycobacterium avium, Mycoplasma pneumonia*	**Contro**l: *Gallibacterium Orientia, Acidocella, Citrobacter*. **COVID-19 symptomatic**: *Mycoplasma, Streptosporangium, Mycobacterium, Mycolicibacterium, Mycolicibacillus, Mycobacteroides*. **COVID-19 asymptomatic**: *Oerskovia, Cellulosimicrobium*	
	Decrease						
	Most common						

	Increase				**Critical COVID-19**: *Staphylococcus, Peptostreptococcus, Prevotella*		
	Decrease				**COVID-19** severities: *Corynebacterium, Dolosigranulum*		
Smith et al., (2021)	Most common						
	Increase	**COVID-19**: Bacteroidetes, Proteobacteria, Fusobacteria, Actinobacteria, Cyanobacteria. **Recovered**: Proteobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria. **Healthy**: Proteobacteria, Bacteroidetes, Firmicutes.		**COVID-19**: *Streptococcus, Veillonella, Prevotella, Staphylococcus, Fusobacterium, Clostridium, Leptotrichia, Coprobacillus*. **Recovered**: *Staphylococcus, Streptomyces, Acinetobacter, Corynebacterium, Streptococcus, Helicobacter*. **Healthy**: *Pedobacter, Sphingobacterium, Pseudomonas, Enterobacter, Flavobacterium Pseudoalteromonas, Escherichia, Exiguobacterium, Shewanella, Chryseobacterium, Aeromonas, Klebsiella, Vibrio.*	**COVID-19**: *Streptococcus salivarius* K12, *S. mitis*, *Neisseria subflava*, *Veillonella dispar*, *Acinetobacter junii*, *V. parvula*, *Prevotella melaninogenica, S. parasanguinis, Streptococcus* sp. LPB0220, *N. flavescens, V. atypica.* **Recovered**: *Pseudomonas stutzeri* DSM 4166, *Staphylococcus capitis*, *S. epidermidis* RP62A, *P. mendocina* NK-01, *Moraxella osloensis* A1920, *A. indicus* A648, *Escherichia coli*, *Sphingobacterium* sp. G1-14, *A. junii* 64.5, *S. pneumoniae*, *Ralstonia pickettii, Micrococcus luteus, Rheinheime*ra sp. D18, *Corynebacterium segmentosum, Elizabethkingia anopheles, Cutibacterium acnes*.		Virus: **COVID-19**: *Betacoronavirus,* **Healthy control**: *Betacoronavirus,* **Recovered**: *Alphacoronavirus* Human coronavirus NL63(HCoV-NL6) Archaea: **COVID-19-** *Halogeometricum, Haloquadratum, Natrialba*, *Methanosarcina, Halorhabdus, Methanocaldococ cus Haloterrigena, Methanobrevibact erHalorubrum, Methanococcoides , Methanococcus, Methanocorpuscul um* **Recovered**: *Haloterrigena, Methanocaldococ cus Halogeometricum, Thermococcus Haloquadratum Methanosarcina Pyrococcus*. **Healthy controls**: *Methanospirillum, Methanoregula, Methanocaldococ cus Methanosarcina, Thermococcus, Haloterrigena, Methanococcus, Methanoculleus, Methanosphaera, Euryarchaeota, Methanobrevibact erSulfolobus Haloquadratum*
	Decrease						
Hoque et al., (2021) b	Most common	**COVID-19** and **recovered**: Firmicutes					

	Increase	**COVID-19**: Proteobacteria, Tenericutes, Actinobacteria, Cyanobacteria, Firmicutes, Bacteroidetes. **Non-Covid-19** : Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria		**COVID-19**: *Staphylococcus, Nostoc, Anabaena, Mycobacterium, Cyanothece, Bradyrhizobium Actinomyces, Pseudomonas, Propionibacterium, Corynebacterium Rhodopseudomonas, Nodularia, Burkholderia, Micrococcus, Acinetobacter, Methylobacterium, Streptomyces, Rhodococcus, Rhodobacter.* **Non-Covid-19**: *Prevotella, Streptococcus, Veillonella, Rothia, Actinomyces, Neisseria, Fusobacterium, Lactobacillus, Atopobium, Megasphaera, Porphyromonas, Bacteroides, Peptostreptococcus, Clostridium, Bifidobacterium*			Archaeae- **COVID-19**: *Methanosarcina Methanocaldococcus Thermococcus Methanothermobacter Haloarcula Staphylothermus Natronomonas Ferroglobus, Caldivirga, Halobacterium Natrialba Methanosphaerula Picrophilus* **non-COVID**: *Methanobrevibacter Methanococcus Methanocorpusculum Pyrococcus Methanosphaera Methanococcoides Methanosaeta Archaeoglobus Methanospirillum Methanoculleus.* Virus- **COVID-19:** *Betacoronavirus Tombusvirus, Victorivirus, Partitivirus, Chrysovirus Totivirus.* **non-COVID:** *Betacoronavirus Siphovirus Alphapapillomavirus Myovirus*
	Decrease						
Hoque et al., (2021) a	Most common						
	Increase			**COVID-19**: *Acinetobacter, Klebsiella, Pelomonas, Ralstonia, Sphingomonas*			
	Decrease			**COVID-19:** *Actinomyces, Haemophilus, Neisseria, Prevotella, Streptococcus, Veillonella*			
Miao et al.,(2021)	Most common						

	Increase						Coinfections- **COVID-19:** *Candida albicans*, human alphaherpesvirus 1, human influenza virus, respiratory syncytial viruses. **Non-COVID**: *Haemophilus parainfluenzae, rhinovirus* C.
	Decrease						
Zhang et al., (2021)	Most common						
	Increase			**non-severe**: *Corynebacterium*			
	Decrease						**Non-severe**: *Human gamma herpesvirus 4*
Wang et al., (2020)	Most common			*Haemophilus, Corynebacterium, Prevotella, Staphylococcus, Moraxella, Neisseria, Streptococcus, Megasphaera, Pediococcus, Dolosigranulum*			Virus: *Human herpes virus*, *Torque teno virus. Human gamma herpesvirus 4 Human beta herpesvirus 7.* Fungi: *Candida Aspergillus Mycoplasma*
	Increase			**COVID-19:** *Corynebacterium*		**COVID-19**: *Corynebacterium*	*Staphylococcus aureus, Haemophilus influenzae, Moraxella catarrhalis*
	Decrease					**COVID-19**: *Ralstonia, Lactobacillus, Atopobium, Dialister, Porphyromonas, Slackia, Neisseria, Rothia*	
Bai et al., (2022)	Most common			*Cutibacterium, Corynebacterium, Staphylococcus*	*Cutibacterium acnes, Corynebacterium accolens, Corynebacterium pseudodiphtheriticum, Staphylococcus aureus*		

3.2. Are there alterations in the nasopharyngeal microbiota in COVID-19 patients?

Among the 31 studies that investigated associations between nasopharyngeal microbiota and COVID-19, 29 studies found that there was an association between COVID-19 infection and alteration of nasopharynx microbiota composition, related with an increase in opportunistic pathogens and the presence of co-infections, as well as other factors (functional pathways, cytokines, gene families) (Bai et al., 2022; Hursitoglu et al., 2022; Rahaman et al., 2022;

Rattanaburi et al., 2022; Ventero et al., 2022; Babenko et al., 2021; Engen et al., 2021; Gupta et al., 2021; Giugliano et al., 2021; Hernández-Terán et al., 2021; Hoque et al., 2021 a; Hoque et al., 2021 b; Hurst et al., 2021; Kolhe et al., 2021; Liu et al., 2021; Llórens-Rico et al., 2021; Mahapatra et al., 2021; Merenstein et al., 2021; Miao et al., 2021; Nardelli et al., 2021; Rhoades et al., 2021; Rosas-Salazar et al., 2021; Rueca et al., 2021; Shilts et al., 2021; Smith et al., 2021; Ventero et al., 2021; Wang et al., 2021; Zhang et al., 2021; Mostafa et al., 2020). However, 2 studies suggest that there was no significant difference in the composition of the nasopharyngeal microbiota between COVID-19 patients and controls, suggesting that in patients with milder symptoms the microbiota remains resilient against SARS-CoV-2 infection (Braun et al., 2021; De Maio et al., 2020) (**Table 1**).

Our search identified 21 studies that investigated the association between nasopharyngeal microbiota and COVID-19 through 16S rRNA sequencing. One study analyzed the V4-V5 region of 16S rRNA gene in the nasopharyngeal samples, in a cohort of 84 patients (27 negative asymptomatic (NegA), 30 positive COVID-19 asymptomatic (PA), and 27 positive COVID-19 symptomatic patients (PSY- mild to severe symptoms)). The Shannon, Observed Species or Bacterial Richness indexes of nasopharyngeal microbiota did not show significant differences between groups. In this study, bacteria associated with SARS-CoV-2 infection in COVID-19 positive patients (PA and PSY) showed at the phylum level a high abundance in Cyanobacteria, and at the genus level a higher abundance in the population of *Bacteroidota*, *Litoricola*, *Amylibacter*, *Balneola* and *Aeromonas* (Kolhe et al., 2021). One nasal/oropharyngeal microbiota study based in 16S rRNA in COVID-19 patients (10 patients admitted to Intensive Care (Unit-SARS-CoV-2 ICU), and 11 patients paucisymptomatic- mild to moderate symptoms (SARS-CoV-2 Pauci)) compared to negative controls (10 healthy subjects (Neg. Controls)), and 8 subjects affected by human coronavirus (HCoVs)) (Rueca et al., 2021) found that the alpha diversity in SARS-CoV-2 ICU patients was lower than that of Neg. Controls, Other HCoVs and SARS-CoV-2 Pauci. The family Pectobacteriaceae were exclusively present in SARS-CoV-2 ICU patients. The abundance of three genera, *unkn_Campylobacterales*, *unkn_Clostridiales.Family.XIII_Incertae.Sedis* and *unkn_Enterococcaceae* were common between SARS-CoV-2 Pauci patients and SARS-CoV-2 ICU patients. Furthermore, four genera such as *Salmonella*, *Scardovia*, *Serratia* and *unk_Psudomonadaceae* were increased in SARS-CoV-2 ICU patients. While, two other genera, *Bifidobacterium* and *Clostridium* were depleted in SARS-CoV-2 ICU patients (these microorganisms are involved in short-chain fatty acid production) when compared to Neg. Controls, Other HCoVs and SARS-CoV-2 Pauci. Notably, Other HCoVs were more abundant in richness, with exclusively 24 and 42 families and genera, respectively (Rueca et al., 2021). One study based on V4 amplification of 16S rDNA sequencing of nasopharyngeal swab samples of

COVID-19 patients, Influenza A and B patients (Flu A and Flu B), and patients without influenza viruses and SARS-CoV-2 virus (Non-Flu & COVID-19) found that the microbiota in Flu A and Flu B patients was significantly different from that in COVID-19 and Non-Flu & COVID-19 patients. However, comparing the relative abundance of nasopharyngeal microbiota demonstrated that COVID-19 group were significantly different from Non-Flu & COVID-19. COVID-19 group was increased in Enterobacteriaceae, *Staphylococcus*, *Lautropia*, *Pseudomonas* and *Corynebacterium*, whereas Non-Flu & COVID-19 group as slightly increased in *Prevotella*, *Veillonella*, *Capnocytophaga* and *Fusobacteria*. The results revealed that the COVID-19 group was differentially enriched with bacteria such as *Staphylococcus* and *Pseudomonas,* which could be related to a dysbiotic microbiota, characterized with lower bacterial diversity and increase of these specific bacteria (Rattanaburi et al., 2021). Another study performed a V3-V4 amplification of the 16S rRNA gene in a cohort of 95 patients (37 mild COVID-19, 27 severe COVID-19, 19 fatal COVID-19, 7 healthy controls and 5 non-COVID-19 pneumonia). The diversity and composition of the microbiota in the lower respiratory tract of patients with severe and fatal COVID-19 did not show significant differences between groups. In contrast, the microbiota of the upper respiratory tract showed significant differences between severity levels of COVID-19 groups and controls groups. All severe COVID-19 groups and controls groups showed differently abundant taxa or biomarkers, in mild COVID-19 identified as *Prevotella melaninogenica* and *P. pallens*, *Veillonella parvula*, *Neisseria subflava*, *Fusobacterium* and *Actinomyces* were highly abundant; in severe COVID-19 *Megasphaera* and *CW040* were the most prevalent, and in fatal COVID-19 *Rothia dentocariosa*, *Streptococcus infantis*, and *Veillonella dispar* as the most significant (Hernández-Terán et al., 2021). In another study, in patients with COVID-19 there were no significant associations between high vs. low SARS-CoV-2 viral load and the diversity and composition of the upper respiratory microbiota. However, differential abundance taxa were significantly different with a consistent direction of association between groups with and without SARS-CoV-2 infection, with *Peptoniphilus lacrimalis, Campylobacter hominis*, *Prevotella 9 copri*, and an *Anaerococcus unclassified* being more abundant in those with SARS-CoV-2 infection and in those with high viral load during COVID-19, whereas *Corynebacterium unclassified, Staphylococcus haemolyticus, Prevotella disiens*, and 2 *Corynebacterium_1 unclassified* amplicon sequence variants (ASVs) were more abundant in those without SARS-CoV-2 infection and in those with low viral loads during COVID-19 (Rosas-Salazar et al., 2021). In another cohort, differences in bacterial community structure were described in nasopharyngeal samples from patients with COVID-19 compared to negative controls by analyzing the V4 region of the 16S rRNA gene (Engen et al., 2021). The study also revealed that the microbial community in COVID-19 patients could be characterized as pro-inflammatory with significantly higher

Proteobacteria to Actinobacteria ratio. Moreover, some taxa were identified driving differences between COVID-19 patients and negative controls, such as *Anaerococcus*, *Streptococcus*, *Enterococcus* and *Bacillus* showing that COVID-19 patients have a dysbiotic nasopharyngeal microbiota community (Engen et al., 2021). Another study based on the 16S rRNA method found that *Prevotella* was the most common bacterial genus, followed by *Leptotrichia* and *Streptococcus* in nasopharyngeal swab samples of COVID-19 patients. Moreover, the study also identified members of the phylum Bacteroidetes, Firmicutes, Fusobacteria, and Proteobacteria, that were responsible for differences in community composition between severity groups. Eleven OTUs were more abundant in the mild severity COVID-19 group, which included 3 OTUs classified as members of *Veillonella* genus, as for severe COVID-19 group, fourteen OTUs were more abundant, among which four classified as *Prevotella* (Ventero et al., 2021). In another study, performing a V4 amplification of 16S rRNA analysis in a cohort of 89 patients (63 SARS-CoV-2 infected and 26 non-infected), of which 46 individuals were part of 11 distinct families with SARS-CoV-2 infected and non-infected members. The observed OTUs and Chao 1 indexes found significant decrease in the SARS-CoV-2 infected patients. In this study, core microbiome analysis identified 37 OTUs as part of the core microbiome, and only 7 OTUs were significantly decreased in SARS-CoV-2 infected individuals. The analysis of contribution of the taxa in nasopharyngeal microbiota in SARS-CoV-2 infected and non-infected individuals detected in a higher abundance of opportunistic pathogens such as *Haemophilus*, *Stenotrophomonas*, *Acinetobacter*, *Moraxella*, *Corynebacterium* I, *Gemella*, *Ralstonia*, and *Pseudomonas* contributed in nasal microbiome of SARS-CoV-2 infected individuals inflammatory environment prevalence of bacterial pathogens. However, there were no significant differences in the microbial community composition between SARS-CoV-2 infected and non-infected patients. Differences between microbial abundance of taxa in SARS-CoV-2 infected and non-infected individuals were observed within and across families. It was noted that an abundance of *Streptococcus* increased while abundance of *Veillonella*, *Rothia* and *Prevotella* 7 decreased in SARS-CoV-2 infected individuals. On microbial differences across the families based on age and gender despite having been found changes in the major taxa abundance pattern, not distinct clustering of age based on the microbial community composition and no significant differences were observed between SARS-CoV-2 infected and non-infected individuals of different age groups. Moreover, a shift in the microbiota of male and female SARS-CoV-2 infected and non-infected individuals was observed across all the age groups categories. The microbiota of SARS-CoV-2 infected asymptomatic and symptomatic individuals within the families show that among the most abundant taxa, *Streptococcus*, *Neisseria*, *Acinetobacter*, and *Rothia* increased in symptomatic individuals across all age groups. A decrease in abundance of *Streptococcus, Prevotella* 7, *Haemophilus, Rothia, Corynebacterium* I, *Porphyromonas*, *Gemmella* and

Actinobacillus in symptomatic females. In addition, an abundance decreased of *Streptococcus, Veillonella, Stenotrophomonas, Leptotrichia, Neisseria, Rothia, Actinomyces, Prevotella* 6*, Actinobacillus* and *Granulicatellata* in symptomatic males (Gupta et al., 2021). Another study analyzed the V1-V2 regions of the 16S rRNA gene in upper and lower respiratory tract samples in a cohort of 83 hospitalized COVID-19 patients. Oropharyngeal and nasopharyngeal communities of COVID-19 patients differed from control samples. The lung communities in intubated COVID-19 patients had lower diversity than control samples. Moreover, viral pathogens Anelloviridae and Redonviridae in oropharyngeal samples were positively associated with intubation during hospitalization (Merenstein et al., 2021). Another study based on 16S rRNA sequences found that *Staphylococcus* and *Corynebacterium* were the dominant genera in the microbiome of the entire cohort of COVID-19 patients. The Shannon index of nasopharyngeal swab samples showed significant differences across sampling moments (upon patient ICU admission and later time points), and posterior analysis suggested that the respiratory microbiome diversity is linked to the length of ICU stay, SARS-CoV-2 viral load and inflammatory marker calprotectin levels (Lloréns-Rico et al., 2021). In that report, 20 covariates showed a significant correlation to microbiota composition. These covariates were related to disease and measures of severity, for example, the administration of specific antibiotics showed a significant association with microbiome composition. Besides that, 28 genera were more abundant in samples from COVID-19 mechanically ventilated patients, while 1 genus were more abundant in non-invasively ventilated patients. COVID-19 mechanically ventilated patients reported higher abundances of genera such as *Prevotella*, *Fusobacterium*, *Porphyromonas* or *Lactobacillus,* and the presence of *Mycoplasma* and *Megasphaera* compared to non-mechanically ventilated COVID-19 patients (Lloréns-Rico et al., 2021). In another study, reduction in nasopharyngeal microbiota composition was described in nasopharyngeal swab samples from patients with COVID-19 compared to that in negative controls or recovery patients by analyzing the V1-V2-V3 region of the 16S rRNA gene (Nardelli et al., 2021). The study revealed a significantly reduced abundance of *Leptotrichia, Fusobacterium*, *Haemophilus* and *Fusobacterium periodonticum* among COVID-19 patients. In addition, a negative correlation was observed between *Fusobacterium periodonticum* and the severity of the patients' symptoms (Nardelli et al., 2021). The other study performed a 16S rRNA analysis in a cohort of 134 patients (68 COVID-19, 21 non-COVID-19 outpatients and 45 non-COVID-19 healthcare workers). The Shannon diversity index of nasal specimens showed that all three groups had a comparable low community, which could be an indicator of a low complexity community dominated by few microbes. The study also revealed the enrichment of several pathogenic bacteria such as *Rothia*, *Acinetobacter*, and *Pseudomonas* as the most abundant among COVID-19 patients. In particular, *Pseudomonas aeruginosa* was highly enriched in COVID-19

patients. The report also analyzed the association between SARS-CoV-2 vRNA load and the nasal microbiota. The results showed that in nasal samples there were increases in specific opportunistic pathogens such as *Streptococcus* in vRNA high Ct group, while *Corynebacterium* was more abundant in the middle and low Ct groups, and *Neisseria* and *Pseudomonas* were more abundant in the low Ct group, and *P. aeruginosa* was positively associated with viral loads. Moreover, 662 differentially expressed genes (DEGs) were found in the COVID-19 group relative to HCWs. Among them, 377 upregulated DEGs enriched to multiple Gene Ontology (GO) terms associated with innate and adaptive host defense pathways. Many interferon-stimulated (ISGs) and type I interferon signaling genes mapped to the GO term "response to virus". Genes with roles in nuclear factor kB (NF-kB) and JUN-ATP-1 signaling mapped to GO terms "inflammatory response". Expression of genes involved in leukocyte chemotaxis, hematopoiesis, and B cell activation were upregulated in COVID-19 patients. Genes that encoded inhibitory receptors as well as genes involved in cell death were also highly upregulated. Additional, upregulated DEGs belonged to "neuron death". In addition, the study identified 315 downregulated DEGs mapped to GO terms related to tissue homeostasis, cellular organization, and neuronal processes. Notable, downregulated DEGs encoded integrin, laminins, and microtubules comprising intracellular and extracellular structures such as cilia and cell-cell adhesion junctions. Genes associated with mucin production in nasal passages were downregulated. Finally, genes encoding ion channels important for chloride ion balance and neuron homeostasis were downregulated. This transcriptional profiling indicated a robust local immune response to SARS-CoV-2 infection and provided support for neural damage in the URT leading to anosmia (Rhodes et al., 2021). One study on 16S rRNA sequencing of nasopharyngeal samples of children, adolescents and young adults with COVID-19 and negative controls found that the nasopharyngeal microbiota diversity differed based on age and SARS-CoV-2 infection status. In that report, nasopharyngeal microbiome profile analysis found that among SARS-CoV-2 infected patients, individuals with a *Corynebacterium/Dolosigranulum*-dominant microbiota profile were less likely to have respiratory symptoms than SARS-CoV-2 infected patients with other nasopharyngeal microbiome profiles. Furthermore, the study found nine ASVs associated with SARS-CoV-2 infection, being eight of these ASVs the association varied by participant age. Among them, six ASVs were associated with respiratory symptoms between SARS-CoV-2 infected patients, and for eight of these ASVs, the magnitude of the association varied by participant age (Hurst et al., 2022). One nasopharyngeal microbiota study based on 16S rRNA in COVID-19 prespecified severity groups (27 with mild COVID-19, 28 with moderate COVID-19, 15 hospitalized with severe COVID-19, and 13 hospitalized in ICU with very severe COVID-19) found that alpha-diversity dropped in patients with very severe COVID-19, and bacterial community composition and dispersion differed among

the severity groups. Furthermore, the study showed that increased age, female sex, and presence of comorbidities were associated with increased disease severity, and current smoking was associated with a reduced risk. Only race was significantly associated with COVID-19 severity (Shilts et al., 2022). One nasopharyngeal microbiota study based on 16S rRNA in hospitalized COVID-19 patients found that the alpha-diversity in patients with fatal outcomes was lower. The more abundant genera in COVID-19 patients were *Streptococcus* spp., *Staphylococcus* spp., and *Corynebacterium* spp., without differences regarding patients with invasive mechanical ventilation (IMV) or a fatal outcome. Conversely, there were 34.20% (483/1412) taxa shared between IMV/non-IMV subpopulation, 4.67% (66/1412) taxa exclusively found in IMV patients and 61.12% (863/1412) taxa only detected in non-IMV patients. In fatal outcomes, shared taxa comprised 41.57% (587/1412), taxa exclusively found in the exitus subpopulation were 6.8% (96/1412) and in survivors 51.2% (729/1412) (Ventero et al., 2022). Another study based on 16S rRNA sequencing of nasopharyngeal swab samples of 100 patients (26 recovered COVID-19 patients, 24 mild COVID-19 patients, 25 severe COVID-19 patients and 25 healthy controls) found that the alpha-diversity in severe COVID-19 patients has decreased species richness than that in mild COVID-19, recovered COVID-19 and healthy control patients. The abundance of three phyla, Fusobacteria, Saccharibacteria, and Spirochaete tended to decrease in severe COVID-19 patients. Conversely, twenty strains were identified as taxa whose distribution were significantly different among groups. Among them, 12 strains were detected in recovered COVID-19, 5 strains in mild COVID-19, and three strains in severe COVID-19 patients. The study also showed that the levels of IL-1 Mitogen and Mitogen-Nil concentration in recovered COVID-19 patients were higher; Mitogen, Mitogen-Nil IL-10, IL-17 Mitogen, and Mitogen-Nil lowest in severe COVID-19 patients, *in vitro* cytokines levels were significantly different from the other groups. Furthermore, correlation analysis identified that IL-1β Mitogen-Nil and IL-1β Mitogen levels were negatively correlated with *Actinobacteria*, *Actinobacteria*, *Micrococcales*, *Micrococcaceae* and *Rothia*. Moreover, positively correlated with *Fusobacteria*, *Fusobacteriia*, and *Fusobacteriale*. Meanwhile, IL-10 Mitogen, and IL-10 Mitogen-Nil levels were positively correlated with Bacteroidetes, Bacteroidia, Bacteroidales, and Porphyromonadaceae (Hursitoglu et al., 2022). One nasopharyngeal microbiota study based on 16S rRNA in COVID-19 patients found that the nasopharynx microbiota of patients with COVID-19 (moderate, severe and critical) was different from that of health controls. The abundance of *Corynebacterium* and *Dolosigranulum* tend to decrease in COVID-19 patients (with different severities). Conversely, *Staphylococcus*, *Peptostreptococcus* and *Prevotella* increased in critical COVID-19 patients. Furthermore, the study showed that cytokines that decrease (IL-33, IFN-λ3 and IFN-γ) or increase (EGF) with SARS-CoV-2 infection were associated to microbial alpha-diversity and to presence of

Corynebacterium, which suggest genus-specific and community-driven regulation of mucosal cytokine production. However, nasopharyngeal viral load was associated with *Staphylococcus* abundance, whereas *Prevotella*, *Streptococcus*, *Peptostreptococcus* and *Clostridial* were associated to disease severity-associated nasopharyngeal cytokines (CCL2 and VEGF) (Smith et al., 2021). Two preprint articles studies were identified (non-peer-reviewed) examining the nasopharyngeal microbiota in COVID-19 patients through 16S rRNA sequencing. (Babenko et al., 2021, Mahapatra et al., 2021). Using nasopharyngeal swab samples, one study found 6 types of bacterial communities between 336 COVID-19 patients (196 inpatients and 140 outpatients with COVID-19). Two of these community types were associated with different courses of the disease, in patients with community type dominated with genera *Pseudomonas* and *Stentotraphomonas* presented the most stable composition, and had low lung damage and no need for additional O_2 supply. Whereas, patients with community type dominated by *Staphylococcus*, *Pseudomonas*, and *Streptococcus* as top genera, had a lower alpha-diversity and demonstrated the highest lung damage. The authors also observed factors associated with community type, such as geographic location, month of sample, and patients age (Babenko et al., 2021). The other study observed a significant dysbiosis between control vs. SARS-CoV-2 asymptomatic and symptomatic patients. The main microbiota composition in COVID-19 patients included several opportunistic pathogens such as *Mycobacterium tuberculosis*, *Mycobacterium avium*, and *Mycoplasma pneumonia.* Further*,* correlation analysis identified that chest pain in COVID-19 symptomatic patients was positively correlated with *Mycoplasma, Mycobacterium, Mycolicibacterium*, *Mycolicibacillus*, and *Mycobacteroides* genera. However, *Mycoplasma* also showed positive correlation with chest pain and fever, which showed associations between pathogens and COVID-19 disease (Mahapatra et al., 2021). Finally, two studies (Braun et al., 2021; De Maio et al., 2020) analyzing the V4 (Braun et al., 2021) and V5-V6 regions (De Maio et al., 2020) of 16S rRNA gene, found no significant differences between the nasopharyngeal microbiota in COVID-19 patients when compared to negative controls (Braun et al., 2021; De Maio et al., 2020).

Five studies analyzed the nasopharyngeal microbiome in COVID-19 patients by shotgun whole-genome sequencing (Bai et al., 2022; Liu et al., 2021; Miao et al., 2021; Mostafa et al., 2020Wang et al., 2020). Using samples from nasopharyngeal test papers, one study describes the main microbiome composition of 44 COVID-19 patients (eight severe/critical, and 36 non-severe). The upper respiratory tract composition in COVID-19 patients included *Haemophilus, Corynebacterium, Prevotella, Staphylococcus, Moraxella, Neisseria, Streptococcus, Megasphaera, Pediococcus*, and *Dolosigranulum* as the main bacteria genera. In addition, it was detected opportunistic pathogens such as Human herpes virus and Torque teno virus, and fungal presence of *Candida, Aspergillus, and Mycoplasma* in some cases. Further analysis found some differences in

the microbial composition in upper airways between COVID-19 severe and non-severe patients. For instance, non-severe patients had a higher proportion of *Corynebacterium* and lower of the *Human gamma herpesvirus 4* compared to severe cases. While, in severe COVID-19 patients was noted a decline of certain types of bacteria and an increase of other types of virus, which could explain the differences in severity of COVID-19 cases (Wang et al., 2020). In another study, performing whole genome sequencing in a cohort of 50 patients (40 SARS-CoV-2 positive and 10 SARS-CoV-2 negative). There was found a significant difference in diversity and composition of the nasopharyngeal microbiota between the groups compared. Notably, was also observed a difference in composition when comparing patient's samples grouped by severity at the species level. Co-infection analysis identified potentially pathogenic microorganisms in five of the 40 (12.5 %) COVID-19 patients. Among them, *Haemophilus influenzae*, *Moraxella catarrhalis*, human metapneumovirus (hMPV) and human alphaherpesvirus were in high abundance (Mostafa et al., 2020). In another paper, reported that COVID-19 patients have reduced metabolites including isodesmosine, lactic acid, L-proline, and chlorogenic acid methyl ester (CME) in the serum compared with non-COVID-19 (Liu et al., 2021). In analysis of gene families and functional pathways enriched in the nasopharyngeal microbiota of COVID-19 patients, it was identified that 29 gene families were significantly different, which were all lower in the COVID-19 group. The relative enrichment of 13 KEGG pathways varied significantly between the two groups, been pathways involved in super pathway of L-serine and glycine biosynthesis I, NAD biosynthesis I (from aspartate), super pathway of menaquinol-8 biosynthesis I, super pathway of demethylmenaquinol-8 biosynthesis, and super pathway of menaquinol-8 biosynthesis II were significantly increased in COVID-19 patients. Association analysis of the microbial abundance and serum metabolome found that *Gemella haemolysans* and *Leptotrichia hofstadii* that were depleted in COVID-19 patients were significantly positively associated with CME serum. These two species were also correlated with beta-hydroxy butyric acid. The CME might be an anti-SARS-CoV-2 bacterial metabolite (Liu et al., 2021). In another study, it performed a metagenomic sequencing analysis in a cohort of 323 COVID-19 samples (mild, severe and critically severe). The diversity and composition of airways microbiome showed significant differences between critically severe COVID-19 patients compared to non-COVID-19 patients. In this report, co-infections were detected in 17 of the 323 (5.3%) COVID-19 pneumonia patients. Among them, for bacterial infection were detected *Klebsiella pneumonia* as the most frequently detected, followed by *Enterococcus*, Coagulase-negative *Staphylococcus*, *Scardovia wiggsiae*, and *Mycoplasma hominis.* Moreover, the remain microorganisms were fungal pathogens such as *Candida, Aspergillus*, and *Cryptococcus* that were associated with mortality rate, and viral co-infections including the Cytomegalovirus, Herpes simplex virus, Epstein Barr virus, Torque teno virus, Human Parvovirus

B19, and JC polyomavirus (Miao et al., 2021). One nasopharyngeal microbiome study based on shotgun whole-genome sequencing in critical ill COVID-19 patients (Bai et al., 2022) found that the alpha-diversity in these patients was significantly reduced compared to negative controls. The report also observed a high abundance of respiratory bacteria that commonly cause pneumonia in critically ill COVID-19 patients (e.g., *Staphylococcus aureus, Haemophilus influenzae*, and *Moraxella catarrhalis*) in SARS-CoV-2 patients compared to negative controls. Furthermore, the study showed that bacteria species *Moraxella lincolnii* and *Propionibacterium namnetense* were correlated to better respiratory status and low inflammation level (Bai et al., 2022).

Two studies characterized the nasopharyngeal microbiome in COVID-19 patients through meta-transcriptomic analysis. In a cohort of 19 patients (10 COVID-19 vaccinated patients, 7 COVID-19 nonvaccinated patients and two negative control patients), no significant differences were found in diversity or composition among microbial communities between or across the samples. In that report, coinfection analysis identified that among the 17 COVID-19 samples from vaccinated and nonvaccinated patients, 16 (94.12%) of COVID-19, at least one clinical relevant microorganism coinfected cases. Among them, different pathogenic/opportunistic bacterial coinfections were detected which included *Streptococcus agalactiae*, *Neisseria meningitidis*, *Elizabethkingia anophelis*, *Stenotrophomonas maltophilia*, *Klebsiella pneumoniae*, and *Pseudomonas plecoglossicida.* Furthermore, in COVID-19 patients were found to have higher antibiotic resistance genes (ARGs) compared with control samples. A total of 20 antimicrobial resistance genes (AMRs) were observed in COVID-19 nonvaccinated patients whereas the number increased to 22 in COVID-19 vaccinated patients. In non-COVID-19 samples, there were only eight AMR genes observed. Among them, nine genes are unique to vaccinated samples, followed by five genes to nonvaccinated samples and four for non-COVID-19-control. Among male and female 16 AMR are common to both, 16 only found in males and two found in females (Rahaman et al., 2021). One study reported the meta-transcriptomic in COVID-19 patients during the three main COVID-19 waves in Italy. It showed that the composition of the nasopharyngeal microbiota in 89 COVID-19 patients was predominated by potential respiratory pathogens, such as *Streptococcus*, *Prevotella*, *Rothia*, *Staphylococcus*, and *Veillonella* when compared with non-infected patients. Further analysis based in correlation between SARS-CoV-2 viral load and microbiota composition found that in high-SARS-CoV-2 patients, the microbiota showed less abundance of genera involved in the immunological homeostasis of nasopharyngeal microbiota such as *Streptococcus, Veillonella, Proteus, Treponema, Brevudimonas, Bifidobacterium* and *Lacticaseibacillus.* In contrast, genera such as *Staphylococcus, Corynebacterium, Dolosigranulum, Pseudomonas, Pasteurella*, and *Stenotrophomonas* were more abundant in high-SARS-CoV-2 patients when compared to low-SARS-CoV-2 patients, which suggest that SARS-CoV-2 infection

is associated with a dysbiosis of the microbiota. In addition, the study also detected coinfections in COVID-19 patients, with the presence of pathogens of clinical importance such as *Staphylococcus aureus* in 21 of the 89 patients*, Klebsiella pneumoniae* in 12 patients*, Streptococcus pneumoniae* in 5 patients*, Neisseria gonorrhoeae, Pseudomonas aeruginosa* and *Acinetobacter baumannii* in 1 patient. Furthermore, most abundant bacterial species analysis revealed 55 abundant bacterial species in two cluster groups. The first group was noted for the presence of *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Moraxella catarrhalis, Finegoldia magna, Acinetobacter baumannii, Enterococcus faecium, Lactobacilli, Corynebacterium propinquum, Dolosigranulum pigrum*. The second cluster group was dominated by *Streptococcus salivarius, Streptococcus oralis, Prevotella* spp., *Schaalia odontolitica, Veillonella parvula, Capnocytophaga gingivalis, Rothia dentocariosa*, which indicated a dysbiotic and pro-inflammatory microbiota in COVID-19 patients with the presence of opportunistic pathogens that could be risk to disease severity (Giugliano et al., 2022). The other cohort study was based on metatranscriptome analysis in 187 patients (62 COVID-19 and 125 non-COVID-19 pneumonia). The study detected co-infections in 18 of the 38 (47.4%) COVID-19 patients, which include pathogens such as *Candida albicans* and human alphaherpesvirus 1 as the most frequently detected, and also include viral opportunistic pathogens such as human influenza virus and respiratory syncytial viruses. Furthermore, host gene analysis identified a transcriptional signature of 16 differential pathways, with the majority related to immune signaling. Among them, cytokine signaling was the most deregulated, followed by the innate immune system and neutrophil degranulation pathways in COVID-19 patients. In addition, the host gene classifier was tested to discern disease severity. In COVID-19 patients, a segregated clustering of severe and mild cases was found. Further intergroup comparisons revealed differences between COVID-19 and non-COVID-19, as well as between COVID-19 mild and severe cases (Zhang et al., 2021).

Two studies by Hoque et al., (2021 a,b) using RNA-seq metagenomic investigation of nasopharyngeal samples describe dysbiosis in the bacterial microbiome in COVID-19 when compared to controls. The first study (Hoque et al., 2021 a) included 21 sequences, 11 COVID-19 (6 RNA-seq Bangladesh, 5 retrieved RNA-seq data from China) and 10 non-COVID (retrieved metagenome sequences of human URTI from USA n=4 and 6 COPD from UK). The study found that the COVID-19 disease significantly affected the diversity and composition of the nasopharyngeal microbiome. In addition, the study also showed that COVID-19 associated microbiomes harbored relative higher abundances of genes coding for amino acid metabolism, energy metabolism, membrane transport, ABC transporters, replication and repair, flagellar assembly, and primary immunodeficiency diseases compared to non-COVID related microbiomes. Further, the COVID-19 related microbiomes also presented a relative higher abundance of genes

encoding for pathogenicity islands, clustering-based subsystems, regulation of virulence, biofilm adhesion biosynthesis, adhesion, programmed cell death, membrane transport, gene transfer agent and virulence, disease and defense. Resistome analysis of COVID-19 and non-COVID associated microbiomes identified 30 functional gene groups/classes associated with resistance to antibiotic and toxic compounds (RATC) in both. In contrast, higher abundances of cobalt-zinc-cadmium resistance, beta lactamase, copper homeostasis, multidrug resistance to efflux pumps, multidrug resistance cluster, arsenic resistance and multidrug-efflux-pump in *Campylobacter jejuni* operon genes were found in COVID-19 metagenomes compared to non-COVID metagenomes (Hoque et al. 2021 a). In the other study, 22 patients were analyzed (eight COVID-19 patients, 7 recovered patients, and seven healthy controls) and they found that the composition and relative abundance of nasopharyngeal commensal bacteria differed significantly among COVID-19, recovered and healthy controls. Specifically, healthy controls had a higher number of bacterial genera compared to COVID-19 and recovered patients (Hoque et al., 2021 b). The genera in COVID-19 patients included *Streptococcus, Veillonella, Prevotella, Staphylococcus, Fusobacterium, Clostridium, Leptotrichia*, and *Coprobacillus*. Further, COVID-19 patients had association of 461 opportunistic bacterial species, of them the top abundant were *Streptococcus salivarius* K12, *S. mitis*, *Neisseria subflava, Veillonella dispar, Acinetobacter junii* 64.5, *V. parvula, Prevotella melaninogenica, S. parasanguinis, Streptococcus* sp. LPB0220, *N. flavescens*, and *V. atypica*. The report also showed that COVID-19 and recovered patients had a higher number of viral genera compared to healthy controls, in COVID-19 the viruses detected were *Betacoronavirus*, *Alphacoronavirus* and *Siphovirus*. Further analysis detected 49 resistance to antibiotics and toxic components (RATCs) distributed between the microbial genomes of the three groups, in the COVID-19 group 40 different RATCs were found with genes associated with biofilm formation in *Staphylococcus*, acriflavin resistance, and quorum sensing. Additionally, in COVID-19 patients different gene families found genes coding for pyruvate carboxylase (pyc), genes encoding for adherent junction, tight junction, environmental information processing, carbohydrate metabolism and oxidative phosphorylation in higher relative abundances. Finally, 37 statistical different protein functions were identified in COVID-19, recovered and healthy controls samples. The COVID-19 and recovered patients associated microbiomes showed a higher relative abundance of these functions compared to those of healthy controls (Hoque et al., 2021 b).

3.3. Severity of COVID-19 and the nasopharynx microbiota

The relationship between the severity of COVID-19 and the composition of the nasopharyngeal microbiota were examined in 10 studies (Bai et al., 2022; Ventero et al., 2022; Babenko et al., 2021; Hernández-Terán et al., 2021; Kolhe et al., 2021; Lloréns-Rico et al., 2021;

Merenstein et al., 2021; Shilts et al., 2021; Smith et al., 2021; Ventero et al., 2021;). One study found a potential importance of *Cutibacterium* and *Lentimonas* in the severity of SARS-CoV-2 infection (Kolhe et al., 2021). To understand the relationship between the nasopharyngeal microbiota composition and severity of COVID-19, the study compared the nasal microbiota between positive COVID-19 asymptomatic (PA) and positive COVID-19 symptomatic (PSY-mild to severe symptoms) patients. PSY patients are enriched by *Cutibacterium* and *Lentimonas* and decreased in Prevotellaceae, *Luminiphilus*, *Flectobacillus*, *Comamonas*, and *Jannaschia*. This dysbiosis of the nasal microbiota might be the reason for the increased susceptibility and severity of COVID-19 infection in these patients (Kolhe et al., 2021). Another study reported important associations between some health/demographic characteristics and severity, in which patients with fatal COVID-19 were predominantly male, significantly older, with higher body mass index (BMI) and most of them received prior antibiotic treatment. Another clinical variables were associated with mortality risk and correlated with specific microbial groups, APACHE scores above eight points, Blood Urea Nitrogen (BUN) levels below 40 mg/dl, lymphocytes below 1.25×10^3/µl, myoglobin above 110 ng/ml, troponin above 3.5 ng/m, and urea above 80 mg/dl represent a high risk by negatively affecting the probability of survival. The analysis detect enriched or depleted bacteria in the different risk factor groups for the variables analyzed such as depleted *Neisseria subflava* in the high-risk samples for troponin and APACHE scores, *Veillonella dispar* depleted in the low-risk samples for APACHE, BUN, myoglobin, and urea. On the other hand, *Corynebacterium* was enriched in the high-risk samples for lymphocytes count and urea, while *Actinomyces* was enriched for BUN and urea. Additionally, four ASV´s of the genus *Prevotella* spp., were significantly enriched in the high-risk samples for myoglobin, BUN, troponin, and lymphocyte count. Network analysis for the microbiota associated with the severity levels for COVID-19 revealed differences at the structural level. A different arrangement was found for each and a continuum of loss of complexity across COVID-19 severity groups (from mild to fatal) and the topology associated with each COVID-19 severity level is different (Hernández-Terán et al., 2021). One study showed the potential association of *Prevotella* spp., in the severity of SARS-CoV-2 infection (Ventero et al., 2021). The study assessed the association between the nasopharyngeal microbiota composition and the severity of the COVID-19 disease (mild, moderate, severe) in fifty-six antibiotic-free COVID-19 patients compared to negative controls. A total of 25 OTUs were responsible for approximately 70% of the differences in community composition between severity groups. Of these, 10 were significantly associated between bacterial OTUs and patient severity. Nine were positively associated (eight in-group 2 and 1 in-group 3) and one negatively associated (in-group 3). Of these OTUs positively associated with severity, three were classified as members of the genus *Prevotella*. In particular, *Prevotella* sp. was associated with

patients' severity in COVID-19 disease. In addition, co-abundance networks showed that the complexity of the network decreased with the increase of severity (Ventero et al., 2021). In this work, samples are not clustered according to the severity group nor by hierarchical clustering. Differences in OTUs composition among severity groups were significant (Ventero et al., 2021). The other paper reported that COVID-19 patients have lower lymphocytes-to-neutrophil ratio (LNR) associated with both lower diversity and composition on the oropharyngeal microbiome compared with negative controls (Merenstein et al., 2021). In their study, it was found that the oropharyngeal microbiome composition is globally correlated with systemic immune cell composition. Moreover, their data define signatures of microbial activity associated with intubation and COVID-19 severity (Merenstein et al., 2021). To understand the functional consequences of lung microbiome disturbances, the study assessed Single-cell RNA-seq of bronchoalveolar fluid samples (BAL) of 35 patients (22 COVID-19 pneumonia patients and 13 non-COVID-19 pneumonia patients). A total of 15 top species detected included *Mycoplasma salivarium* as the dominant taxon in five COVID-19 patients in ICU, as well as different *Prevotella* members. The study found that 4% of the COVID-19 patients' cells were associated with bacterial cells. Being in COVID-19 patients, neutrophils, monocytes and monocytes-derived macrophages are associated with bacteria. The study also found that in COVID-19 patients different bacteria are associated with distinct host cells, such as bacteria from *Mycoplasma* genus associated to monocyte-derived macrophages, while *Rothia, Enterobacter* or *Klebsiella* are enriched in monocytes. The results found in this study suggest that bacteria detected in these cell subsets via scRNA-seq analysis may contribute to inflammatory response in the host (Lloréns-Rico et al., 2021). The other paper reported that among those infected with SARS-CoV-2, bacterial load was significantly associated with disease severity. In their study, within-group dissimilarities in the very severe COVID-19 group were among the highest, similar to the dissimilarities between different COVID-19 severity groups. In particular, *Corynebacterium* ASV relative abundance was significantly different between severity groups, decreasing as disease severity increased (Shilts et al., 2022). One study showed the potential importance of differential represented genera that could be used as a severity biomarker in COVID-19 hospitalized patients. To understand the association between the nasopharyngeal microbiota and COVID-19 severity, the study assessed the relationship between the microbiota and SARS-CoV-2 infection clinical outcomes (invasive mechanical ventilation (IMV), non-invasive mechanical ventilation, and mortality) in 177 COVID-19 patients. The presence of *Selenomonas* spp., *Filifactor* spp., *Actinobacillus* spp., and *Chroococcidiopsis* spp., genera was associated with a reduced risk of IMV. In particular, the presence of *Actinobacillus* spp., *Citrobacter* spp., *Craurococcus* spp., or *Moheibacter* spp., was associated with a reduced risk of a fatal outcome (Ventero et al., 2022). Another paper reported that in analysis of differentially abundant taxonomic

units in COVID-19 patients identified *Rothia* and *Solobacterium* genera and ASV of *Streptococcus mitis/oralis* to be positively correlated with the highest lung damage. While, *Leptotrichia* and unclassified *Lachnospiraceae* genera, and ASVs belonging to *Prevotella* genus, were associated with lower lung damage levels (Babenko et al., 2021). The other paper reported that ten circulating cytokines were significantly different between critical and noncritical COVID-19 patients. Conversely, thirteen nasopharyngeal cytokines were differentially regulated between critical and noncritical COVID-19 patients. In their study, the nasopharynx microbiota of patients with critical disease is significantly different from that of health controls, as well as COVID-19 with different severities. The study also found positive correlations of *Staphylococcus* with inflammatory cytokines (IL-6 and TNF) and negative correlations of microbial diversity and *Corynebacterium* with CCL2, which support their potential role in COVID-19 disease severity (Smith et al., 2021). The other paper reported that in COVID-19 patients strong correlations were found between species biomarkers, metabolic pathways, and associated with better clinical outcomes, especially *Moraxella lincolnii* and pathways of vitamin K2 biosynthesis that was associated to better respiratory status and lower inflammation, which could contributing to clinical outcomes in COVID-19 patients (Bai et al., 2022).

4. Discussion

This systematic review aimed to report the current evidence throughout the COVID-19 pandemic period from research articles that investigated the association between SARS-CoV-2 infection and the composition of the nasopharyngeal microbiota. Therefore, 31 articles were examined, and 29 reports concluded that there is an association between nasopharyngeal microbiota composition changes and COVID-19 infection, when compared to recovered or non-COVID-19 patients (Bai et al., 2022; Hursitoglu et al., 2022; Rahaman et al., 2022; Rattanaburi et al., 2022; Ventero et al., 2022; Babenko et al., 2021; Engen et al., 2021; Giugliano et al., 2021; Gupta et al., 2021; Hernández-Terán et al., 2021; Hoque et al., 2021 a; Hoque et al., 2021 b; Hurst et al., 2021; Kolhe et al., 2021; Liu et al., 2021; Llórens-Rico et al., 2021; Mahapatra et al., 2021; Merenstein et al., 2021; Miao et al., 2021; Nardelli et al., 2021; Rhoades et al., 2021; Rosas-Salazar et al., 2021; Rueca et al., 2021; Shilts et al., 2021; Smith et al., 2021; Ventero et al., 2021; Wang et al., 2021; Zhang et al., 2021; Mostafa et al., 2020). Otherwise, two studies did not find associations between the nasopharynx microbiota and SARS-CoV-2 infection (Braun et al., 2021; De Maio et al., 2020). However, it remains to be answered whether changes in the nasopharyngeal microbiota are the cause or a consequence of SARS-CoV-2 infection.

The nasopharyngeal microbiota of adults in healthy conditions normally is composed of the following genera *Staphylococcus*, *Haemophilus*, *Streptococcus*, and also could include

Sphingobacterium, *Prevotella*, *Bifidobacterium, Rothia,* and *Propionibacterium*, showing different microorganisms as dominant members of this microbiota (Flynn & Dooley, 2021; Cleary & Clarke, 2017). Most of the studies showed that the nasopharyngeal microbiota of COVID-19 patients it's different from that of control patients (negative for SARS-CoV-2), being characterized by lower diversity and higher colonization for opportunistic pathogens, such as *Rothia*, *Acinetobacter*, and *Pseudomonas* (Rhoades et al., 2021), what characterizes a microbiota in a possible dysbiosis state. There were also reports of co-infections with pathogens of clinical importance such as *Haemophilus influenzae*, *Moraxella catarrhalis*, human metapneumovirus (hMPV), and human alphaherpesvirus (Mostafa et al., 2020). These results are widely discussed in the literature, where bacterial coinfections including *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Haemophilus influenzae* are commonly related in patients hospitalized with COVID-19 (with moderate or critical symptoms), demonstrating a possible influence of the SARS-CoV-2 virus on the upper respiratory tract microbiota, what may impact on the severity and the clinical outcome of the patients with COVID-19 (Cohen et al., 2022; Moreno-Garcia et al., 2022).

The dysbiosis of the nasopharyngeal microbiota might be the reason for the higher susceptibility and severity of COVID-19 infection (Wang et al., 2020). Ten studies (Bai et al., 2022; Ventero et al., 2022; Babenko et al., 2021; Hernández-Terán et al., 2021; Kolhe et al., 2021; Lloréns-Rico et al., 2021; Merenstein et al., 2021; Shilts et al., 2021; Smith et al., 2021; Ventero et al., 2021) found associations between the nasopharynx microbiota and COVID-19 severity. The main correlations identified in SARS-CoV-2 patients were related to the changes in the microbiota composition, kind of immune response, functional pathways, genes families, metabolite production, progression of inflammation and production of cytokines (Bai et al., 2022; Ventero et al., 2022; Babenko et al., 2021; Hernández-Terán et al., 2021; Kolhe et al., 2021; Lloréns-Rico et al., 2021; Merenstein et al., 2021; Shilts et al., 2021; Smith et al., 2021;Ventero et al., 2021). One study found correlations between increasing in inflammatory cytokines levels and the co-infection with the genera *Staphylococcus* and/or *Corynebacterium* influencing the COVID-19 disease severity. High levels of interleukin-6 (IL-6) were reported in COVID-19 patients admitted to ICU, and the ones with bad outcome presented significantly higher values of this interleukin (Gorham et al., 2021). Bacterial pathogens as *Staphylococcus aureus, Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis, Dolosigranulum pigrum* and *Corynebacterium propinquum/pseudodiphtheriticum* are enriched in patients with viral infection (Edouard et al., 2018). These alterations at the microbiota found in COVID-19 patients may have a role at the inflammation progression and disease development, influencing the case severity (De et al., 2022).

The nasopharyngeal microbiota in COVID-19-recovered patients (negative for SARS-CoV-2 after the period of infection) was investigated in three studies. Differences in

nasopharyngeal microbiota composition were found when compared to COVID-19 and healthy patients (Hoque et al., 2021; Hursitoglu et al., 2021; Nardelli et al., 2021). Previous articles had already reported that the gut microbiota of recovered patients, compared to healthy controls, differed significantly in diversity and composition (Tian et al., 2021). The modifications found in microbiota diversity in COVID-19 patients, could be a consequence of immune responses triggered by the viral infection (Mizutani et al., 2022). In accordance, studies show that after the infection period recovered COVID-19 patients microbiota is reestablished to the normal state in asymptomatic and mild cases, what did not occur in severe patients (Kim et al., 2021). By other hand, a pro-inflammatory response also registered in severe cases of the disease, could be responsible for maintaining the unbalanced state of the microbiota for a longer period of time (Mizutani et al., 2022).

5. Conclusion

Up to now, the studies show that patients with COVID-19 show differences at the bacterial nasopharyngeal microbiota at diversity and composition levels when compared to controls, and that certain personal particularities such as presence of specific bacteria genus, immune response and other clinical aspects also contribute to the widely different clinical conditions and outcomes presented by different patients for the same disease.

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Declaration of competing interest

The authors declare no conflict of interest.

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Supplemental File 1 (S1 File)
The results of database search (2019 / Dec.—2022 / Apr.)

Pubmed (https://pubmed.ncbi.nlm.nih.gov/): Advanced Search Terms: (((((((COVID-19[Text Word]) OR (SARS-CoV-2[Text Word])) OR (severe acute respiratory syndrome coronavirus 2[Text Word])) OR (coronavirus disease 2019[Text Word])) OR (COVID19[Text Word])) AND (((((microbiota[Text Word]) OR (microbiome[Text Word])) OR (mycobiome[Text Word])) OR (bacterial[Text Word])) OR (microbial[Text Word]))) AND ((((((((((((airway microbiota[Text Word]) OR (airway microbiome[Text Word])) OR (nasopharyngeal microbiota[Text Word])) OR (nasopharyngeal microbiome[Text Word])) OR (respiratory microbiota[Text Word])) OR (respiratory microbiome[Text Word])) OR (oral microbiota[Text Word])) OR (oral microbiome[Text Word])) OR (oropharyngeal microbiota[Text Word])) OR (oropharyngeal microbiome[Text Word])) OR (lung microbiota[Text Word])) OR (lung microbiome[Text Word])) OR (throat microbiota[Text Word])) OR (throat microbiome[Text Word]))) AND ((((((((16S rRNA[Text Word]) OR (16S rDNA[Text Word])) OR (amplicon[Text Word])) OR (whole genome[Text Word])) OR (metagenomic[Text Word])) OR (Next-generation[Text Word])) OR (shotgun metagenomic[Text Word])) OR (multi-omics[Text Word]))

Results: 32 articles

Embase (https://www.embase.com/landing?status=yellow): Search: (COVID-19 OR SARS-CoV-2 OR "severe acute respiratory syndrome coronavirus 2" OR "coronavirus disease 2019" OR COVID19) AND (microbiota OR microbiome OR mycobiome OR bacterial OR microbial) AND ("airway microbiota" OR "airway microbiome" OR "nasopharyngeal microbiota" OR "nasopharyngeal microbiome" OR "respiratory microbiota" OR "respiratory microbiome" OR "oral microbiota" OR "oral microbiome" OR "oropharyngeal microbiota" OR "oropharyngeal microbiome" OR "lung microbiota" OR "lung microbiome" OR "throat microbiota" OR "throat microbiome") AND ("16S rRNA" OR "16S rDNA" OR amplicon OR "whole genome" OR metagenomic OR "Next-generation" OR "shotgun metagenomic" OR "multi-omics")

Results: 48 articles

Web of Science (https://www-webofscience.ez41.periodicos.capes.gov.br/wos/woscc/basic-search): Web of science/Advanced Search, Topics, Search Terms: (((TS=(COVID-19 OR SARS-CoV-2 OR severe acute respiratory syndrome coronavirus 2 OR coronavirus disease 2019 OR COVID19)) AND TS=(microbiota OR microbiome OR mycobiome OR bacterial OR microbial)) AND TS=(airway microbiota OR airway microbiome OR nasopharyngeal microbiota OR nasopharyngeal microbiome OR respiratory microbiota OR respiratory microbiome OR oral microbiota OR oral microbiome OR oropharyngeal microbiota OR oropharyngeal microbiome OR lung microbiota OR lung microbiome OR throat microbiota OR throat microbiome)) AND TS=(16S rRNA OR 16S rDNA OR amplicon OR whole genome OR metagenomic OR Next-generation OR shotgun metagenomic OR multi-omics)

Results: 64 articles

Scopus (https://www-scopus.ez41.periodicos.capes.gov.br/): advanced search, Title Abstract Keyword search, search terms: TITLE-ABS-KEY ("COVID-19" OR "SARS-CoV-2" OR "severe acute respiratory

syndrome coronavirus 2" OR "coronavirus disease 2019" OR "COVID19") AND ("microbiota" OR "microbiome" OR "mycobiome" OR "bacterial" OR "microbial") AND ("airway microbiota" OR "airway microbiome" OR "nasopharyngeal microbiota" OR "nasopharyngeal microbiome" OR "respiratory microbiota" OR "respiratory microbiome") AND (EXCLUDE (DOCTYPE , "re")) AND (EXCLUDE (DOCTYPE , "ch")) AND (EXCLUDE (DOCTYPE , "ed"))

Results: 115 articles

ScienceDirect (https://www-sciencedirect.ez41.periodicos.capes.gov.br/): Advanced Search, Title, abstract or author-specified keywords, Terms:(2019-2022) (COVID-19 OR SARS-CoV-2 OR "severe acute respiratory syndrome coronavirus 2" OR "coronavirus disease 2019" OR COVID19) AND ("airway microbiota" OR "airway microbiome" OR "respiratory microbiota" OR "respiratory microbiome")

Results: 79 articles

Supplemental File 2 (S2 File)

Nasopharyngeal bacteria	Taxa	Increased abundance	Decreased abundance	Common in both	Total reports	References
Actinobacteria	Phylum	6	0	9	15	Hernández-Terán et al., (2021), Engen et al., (2021), Giugliano et al., (2022), Mahapatra et al., (2021), Hoque et al., (2021), Hoque et al., (2021b), De Maio et al., 2020, Engen et al., (2021), Ventero et al., (2021), Gupta et al., 2021, Braun et al., (2021), Nardelli et al., 2021, Rhoades et al., (2021), Rahaman et al., (2021), Giugliano et al., (2022)
Aeromonas		0	0	1	1	Kolhe et al., (2021)
Amylobacter		0	0	1	1	Kolhe et al., (2021)
Bacteroidetes		5	2	9	16	Rattanaburi et al., (2021), Ventero et al., (2021), Gupta et al., 2021, Hoque et al., (2021), Hoque et al., (2021b), Hernández-Terán et al., (2021), Giugliano et al., (2022), De Maio et al., 2020, Kolhe et al., (2021), Hernández-Terán et al., (2021), Ventero et al., (2021), Gupta et al., 2021, Braun et al., (2021), Liu et al., (2021), Nardelli et al., 2021, Rahaman et al., (2021)
Balneola		0	0	1	1	Kolhe et al., (2021)
Basidiomycota		0	0	1	1	Rahaman et al., (2021)
Candidatus Saccharibacteria		1	1	0	2	Rueca et al. (2021), Giugliano et al., (2022)
Cyanobacteria		3	0	0	3	Kolhe et al., (2021), Hoque et al., (2021), Hoque et al., (2021b)
Firmicutes		7	0	11	18	Rattanaburi et al., (2021), Hernández-Terán et al., (2021), Ventero et al., (2021), Gupta et al., 2021, Giugliano et al., (2022), Mahapatra et al., (2021), Hoque et al., (2021b), De Maio et al., 2020, Hernández-Terán et al., (2021)Engen et al., (2021), Ventero et al., (2021), Gupta et al., 2021, Braun et al., (2021), Liu et al., (2021), Nardelli et al., 2021, Rhoades et al., (2021), Rahaman et al., (2021), Hoque et al., (2021)
Fusobacteria		1	3	4	8	Hoque et al., (2021), Nardelli et al., 2021, Giugliano et al., (2022), Hursitoglu et al., (2022), De Maio et al., 2020, Gupta et al., 2021, Braun et al., (2021), Nardelli et al., 2021
Litoricola		0	0	1	1	Kolhe et al., (2021)
Proteobacteria		5	3	9	17	Engen et al., (2021), Gupta et al., 2021, Giugliano et al., (2022), Hoque et al., (2021), Hoque et al., (2021b), Hernández-Terán et al., (2021), Merenstein et al., (2021), Nardelli et al., 2021, De Maio et al., 2020, Hernández-Terán et al., (2021), Engen et al., (2021), Ventero et al., (2021), Gupta et al., 2021, Braun et al., (2021), Nardelli et al., 2021, Rhoades et al., (2021), Rahaman et al., (2021)

Saccharibacteria		0	1	0	1	Hursitoglu et al., (2022)
Spirochaetes		0	2	0	2	Giugliano et al., (2022), Hursitoglu et al., (2022)
Tenericutes		2	0	0	2	Hoque et al., (2021b), Rueca et al. (2021)
TM7		1	0	0	1	Hernández-Terán et al., (2021)

Supplemental File 3 (S3 File)

Type of bacteria (phylum)	Representatives (genera or species)	Increased abundance	Decreased abundance	Common in both	Total studies	References
Actinobacteria	*Actinomyces*	3	4	0	7	Hernández-Terán et al., (2021), Liu et al., (2021), Hoque et al., (2021b), Hernández-Terán et al., (2021),Gupta et al., (2021), Merenstein et al., (2021), Miao et al.,(2021)
	Bifidobacterium	1	2	0	3	Mahapatra et al., (2021), Rueca et al. (2021), Giugliano et al., (2022)
	Corynebacterium	8	2	10	20	Rattanaburi et al., (2021), Hernández-Terán et al., (2021), Rosas-Salazar et al., (2021), Gupta et al., (2021), Hurst et al., (2021), Hoque et al., (2021b), Wang et al., (2020), Bai et al., (2022), Gupta et al., (2021), Smith et al., (2021), Hernández-Terán et al., (2021), Engen et al., (2021), Lloréns-Rico et al., (2021), Rhoades et al., (2021), Hurst et al., (2021), Shilts et al. (2022), Ventero et al., (2022), Babenko et al., (2021), Wang et al., (2020), Bai et al., (2022)
	Corynebacterium accolens	0	1	1	2	Mostafa et al., (2020), Bai et al., (2022)
	Corynebacterium pseudodiphtheriticum	0	0	1	1	Bai et al., (2022)
	Cutibacterium	1	0	1	2	Kolhe et al., (2021), Bai et al., (2022)
	Cutibacterium acnes	0	0	1	1	Bai et al., (2022)
	Lawsonella	2	0	0	2	Rosas-Salazar et al., (2021), Hurst et al., (2021)
	Micrococcus	1	0	0	1	Hoque et al., (2021b)
	Mycobacterium	2	0	0	2	Mahapatra et al., (2021), Hoque et al., (2021b)
	Mycobacterium avium	1	0	0	1	Mahapatra et al., (2021)
	Mycobacterium tuberculosis	1	0	0	1	Mahapatra et al., (2021)
	Mycobacteroides	1	0	0	1	Mahapatra et al., (2021)
	Mycolicibacterium	1	0	0	1	Mahapatra et al., (2021)
	Propionibacterium	1	0	0	1	Hoque et al., (2021b)
	Rhodococcus	1	0	0	1	Hoque et al., (2021b)
	Rothia	1	1	0	2	Mahapatra et al., (2021), Gupta et al., (2021)
	Scardovia	1	0	0	1	Rueca et al., (2021)
	Streptomyces	2	0	0	2	Mahapatra et al., (2021), Hoque et al., (2021b)
Bacteroidetes	*Bacteroides*	1	0	0	1	Rosas-Salazar et al., (2021)
	Elizabethkingia	1	0	0	1	Rahaman et al., (2021)

	anophelis					
	Flavobacterium	1	0	0	1	Mahapatra et al., (2021)
	Flectobacillus	0	1	0	1	Kolhe et al., (2021)
	Porphyromonas	0	1	0	1	Gupta et al., (2021)
	Prevotella	4	1	3	8	Ventero et al., (2021), Liu et al., (2021), Smith et al., (2021), Hoque et al., (2021), Miao et al.,(2021), Ventero et al., (2021), Babenko et al., (2021), Wang et al., (2021)
	Prevotella 6	0	1	0	1	Gupta et al., (2021)
	Prevotella 7	0	1	0	1	Gupta et al., (2021)
	Prevotella histicola	1	0	0	1	Liu et al., (2021)
	Prevotella melaninogenica	1	0	0	1	Hoque et al., (2021
Firmicutes	*Alloiococcus*	0	1	0	1	Hernández-Terán et al., (2021)
	Anaerococcus	1	0	0	1	Rosas-Salazar et al., (2021)
	Bulleidia	1	0	0	1	Rueca et al. (2021
	Clostridium	1	1	0	2	
	Coprobacillus	1	0	0	1	Hoque et al., (2021)
	Dolosigranulum	2	2	4	8	Rosas-Salazar et al., (2021), Hurst et al., (2021), Giugliano et al., (2022), Smith et al., (2021), Rhoades et al., (2021),, Hurst et al., (2021), Shilts et al. (2022), Wang et al., (2021)
	Filifactor	0	0	0	0	(ventero et al., 2021 or 2022)
	Fusobacterium	1	1	0	2	Hoque et al., (2021), Nardelli et al., (2021),
	Fusobacterium periodonticum	0	1	0	1	Nardelli et al., (2021)
	Gemella	1	0	0	1	Gupta et al., (2021)
	Granulicatellata	0	1	0	1	Gupta et al., (2021)
	Halanaerobium	1	0	0	1	Rueca et al. (2021
	Lacticaseibacillus	0	1	0	1	Giugliano et al., (2022)
	Megasphaera	0	0	1	1	Wang et al., (2020)
	Megasphaera micronuciformis	1	0	0	1	Liu et al., (2021)
	Pediococcus	0	0	1	1	Wang et al., (2020)
	Peptoniphilus	2	0	0	2	Rosas-Salazar et al., (2021), Hurst et al., (2021)
	Peptostreptococcus	1	0	0	1	Smith et al., (2021)
	Rhodobacter	1	0	0	1	Hoque et al., (2021b)
	Selenomonas	1	0	1	2	Hernández-Terán et al., (2021), Hernández-Terán et al., (2021)
	Staphylococcus	8	0	10	18	Rattanaburi et al., (2021), Hernández-Terán et al., (2021), Rosas-Salazar et al., (2021), Hurst et al., (2021), Giugliano et al., (2022), Smith et al., (2021), Hoque et al., (2021), Hoque et al., (2021b), Hernández-Terán et al., (2021), Engen et al., (2021), Lloréns-Rico et al., (2021), Rhoades et al., (2021), Hurst et al., (2021), Shilts et al. (2022), Ventero et al., (2022), Babenko et al., (2021), Wang et al., (2021), Bai et al., (2022)

	Staphylococcus aureus	1	0	1	2	Giugliano et al., (2022), Bai et al., (2022)
	Streptococcus	4	3	7	14	Hernández-Terán et al., (2021), Ventero et al., (2021), Giugliano et al., (2022), Hoque et al., (2021), Gupta et al., (2021), Giugliano et al., (2022), Miao et al.,(2021), Rattanaburi et al., (2021), Ventero et al., (2021), Rhoades et al., (2021), Hurst et al., (2021), Ventero et al., (2022), Babenko et al., (2021), Wang et al., (2021)
	Streptococcus. parasanguinis	1	0	0	1	Hoque et al., (2021)
	Streptococcus sanguinis	1	0	0	1	Liu et al., (2021)
	Streptococcus sp. LPB0220,	1	0	0	1	Hoque et al., (2021)
	Veillonella	4	3	3	10	Hernández-Terán et al., (2021),Ventero et al., (2021), Liu et al., (2021), Hoque et al., (2021), Gupta et al., (2021), Giugliano et al., (2022),Miao et al.,(2021), Hernández-Terán et al., (2021),Ventero et al., (2021), Babenko et al., (2021)
	Veillonella atypica	1	0	0	1	Hoque et al., (2021)
	Veillonella dispar	2	0	0	2	Liu et al., (2021), Hoque et al., (2021)
	Veillonella parvula	1	0	0	1	Hoque et al., (2021)
Proteobacteria	*Acinetobacter*	3	0	0	3	Gupta et al., (2021), Hoque et al., (2021b), Miao et al.,(2021)
	Acinetobacter baumannii	0	1	0	1	Giugliano et al., (2022)
	Acinetobacter junii	1	0	0	1	Hoque et al., (2021)
	Actinobacillus	1	1	1	3	Hernández-Terán et al., (2021), Gupta et al., (2021), Hernández-Terán et al., (2021)
	Bradyrhizobium	1	0	0	1	Hoque et al., (2021b)
	Brevundimonas	0	1	0	1	Giugliano et al., (2022)
	Burkholderia	1	0	0	1	Hoque et al., (2021b)
	Comamonas	0	1	0	1	Kolhe et al., (2021)
	Escherichia	1	0	0	1	Hoque et al., (2021)
	Escherichia-Shigella,	0	0	1	1	Engen et al., (2021)
	Haemophilus	2	5	2	9	Hernández-Terán et al., (2021), Gupta et al., (2021), Hernández-Terán et al., (2021), Gupta et al., (2021), Merenstein et al., (2021), Nardelli et al., (2021), Miao et al.,(2021), Ventero et al., (2021), Wang et al., (2020)
	Halothiobacillus	1	0	0	1	Mahapatra et al., (2021)
	Jannaschia	0	1	0	1	Kolhe et al., (2021)
	Klebsiella	1	0	0	1	Miao et al.,(2021)
	Klebsiella pneumoniae	2	0	0	2	Rahaman et al., (2021), Giugliano et al., (2022)
	Lautropia	1	0	0	1	Rattanaburi et al., (2021)
	Lautropia mirabilis	1	0	0	1	Liu et al., (2021)
	Methylobacterium	1	0	0	1	Hoque et al., (2021b)

	Moraxella	2	0	4	6	Gupta et al., (2021), Hurst et al., (2021), Engen et al., (2021),Ventero et al., (2021), Hurst et al., (2021), Wang et al., (2021)
	Morganella	0	0	1	1	Engen et al., (2021)
	Neisseria	2	3	3	8	Hernández-Terán et al., (2021), Liu et al., (2021), Gupta et al., (2021), Merenstein et al., (2021), Miao et al.,(2021), Hernández-Terán et al., (2021), Rhoades et al., (2021), Wang et al., (2021)
	Neisseria flavescens	1	0	0	1	Hoque et al., (2021)
	Neisseria gonorrhoeae	1	0	0	1	Giugliano et al., (2022)
	Neisseria meningitidis	1	0	0	1	Rahaman et al., (2021)
	Neisseria subflava	1	0	0	1	Hoque et al., (2021)
	Pelomonas	1	0	0	1	Miao et al.,(2021)
	Proteus	0	1	1	2	Engen et al., (2021), Giugliano et al., (2022)
	Pseudomonas	3	0	1	4	Rattanaburi et al., (2021), Gupta et al., (2021), Hoque et al., (2021b), Babenko et al., (2021)
	Pseudomonas aeruginosa	0	1	0	1	Giugliano et al., (2022)
	Pseudomonas plecoglossicida	1	0	0	1	Rahaman et al., (2021)
	Ralstonia	2	0	0	2	Gupta et al., (2021), Miao et al.,(2021)
	Rhodopseudomonas	1	0	0	1	Hoque et al., (2021b)
	Salmonella	1	0	0	1	Rueca et al. (2021
	Serratia	1	0	0	1	Rueca et al. (2021)
	Sphingomonas	1	0	0	1	Miao et al.,(2021)
	Stenotrophomonas	1	1	1	3	Gupta et al., (2021), Gupta et al., (2021), Babenko et al., (2021)
	Stenotrophomonas maltophilia	1	0	0	1	Rahaman et al., (2021)
	Streptococcus agalactiae	1	0	0	1	Rahaman et al., (2021)
	Streptococcus mitis	1	0	0	1	Hoque et al., (2021)
	Streptococcus pneumoniae	1	0	0	1	Giugliano et al., (2022)
	Streptococcus salivarius K12	1	0	0	1	Hoque et al., (2021)
	unkn_Epsilonproteobacteria	1	0	0	1	Rueca et al. (2021)
Fusobacteria	*Leptotrichia*	2	2	0	4	Ventero et al., (2021), Hoque et al., (2021), Gupta et al., (2021), Nardelli et al., 2021,
	Streptobacillus	1	0	0	1	Rueca et al. (2021)
Cyanobacteria	*Anabaena*	1	0	0	1	Hoque et al., (2021b)
	Cyanothece	1	0	0	1	Hoque et al., (2021b)

	Nodularia	1	0	0	1	Hoque et al., (2021b)
	Nostoc	1	0	0	1	Hoque et al., (2021b)
Spirochaetes	*Treponema*	0	1	0	1	Giugliano et al., (2022)
Tenericutes	*Mycoplasma*	1	0	1	1	Mahapatra et al., (2021)
	Mycoplasma pneumonia	1	0	0	1	Mahapatra et al., (2021)
Verrucomicrobia	*Lentimonas*	1	0	0	1	Kolhe et al., (2021)

4. CAPÍTULO II — Artigo Científico Experimental

O segundo capítulo desta dissertação apresenta os resultados científicos experimentais na forma de um artigo completo intitulado "**Differences in the nasopharynx microbial community of individuals with respiratory infection: comparisons between outpatients and hospitalized patients, with and without COVID-19**".

Esse artigo será submetido à revista *Microbes and Infection,* com fator de impacto de (2021 – 2022): **9.57**

- https://www.sciencedirect.com/journal/microbes-and-infection
- https://www.pasteur.fr/fr/ceris/publications-scientifiques/microbes-infection

Differences in the nasopharynx microbial community of individuals with respiratory infection: comparisons between outpatients and hospitalized patients, with and without COVID-19

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Running title: Nasopharyngeal microbiota in COVID-19 patients

Abstract

The upper respiratory tract is considered the main route for initial SARS-CoV-2 infection; however, the relation of the nasopharynx microbiota with the asymptomatic or severe COVID-19 has not yet been well established. We characterized the nasopharyngeal bacterial microbiota by 16S rRNA NGS gene sequencing of 96 individuals with symptoms of acute respiratory infection (ARI) or severe acute respiratory infection (SARI) positive and negative for SARS-CoV-2, divided into two age categories 20 – 40 years and over 60 years. Individuals were categorized as SARS-CoV-2 positive (24 ARI+, and 25 SARI+), or SARS-CoV-2 negative (23 ARI-, and 24 SARI-). The microbial community profiles differed significantly in SARS-CoV-2 groups (ARI compared to SARI) at both genus and species levels, across all age categories. Without age influence, SARI+ group showed a significantly lower diversity with decrease of *Bacillus* and enrichment of potential pathogenic genus *Streptococcus* spp., *Veillonella* spp., and *Staphylococcus* spp. In contrast, *Bacillus* genus was increased in ARI+ and SARI- groups. Based on data generated, we conclude that there are significant differences in the microbiota profiles of patients with acute versus severe acute respiratory infections with and without COVID-19, suggesting that the microbiota composition could be an indicator of susceptibility to SARS-CoV-2 infection and disease severity.

Keywords: Nasopharynx; Microbiota; SARS-CoV-2; Acute respiratory infection; Severe acute respiratory infection; 16S rRNA

1. Introduction

At the end of 2019, the SARS-CoV-2 virus emerged in the city of Wuhan, China [1]. As of December 23, 2022, the number of COVID-19 cases surpasses 651 millions, accounting for more than 6.6 millions deaths worldwide [2]. SARS-CoV-2 is a positive-sense single-stranded RNA virus with a genome of approximately 30 kb belonging to the *Betacoronavirus* genus of the Coronaviridae family [3, 4]. Individuals infected with SARS-CoV-2 can be asymptomatic or have clinical manifestations of COVID-19, which range from mild symptoms such as fever, cough, sore throat among others, to more severe symptoms, including pneumonia, dyspnea, acute respiratory distress syndrome and even multiple organ failure and death [5, 6]. The main route of infection and replication of the virus is the upper airways, which is a site of high expression of the angiotensin-converting enzyme 2 (ACE2) [7, 8], to which the SARS-CoV-2 surface glycoprotein spike (S) binds to enter the cell [9].

Several factors are hypothesized as associated with SARS-CoV-2 susceptibility and COVID-19 disease severity . Evidence suggests an association between the composition of the microbiota and regulation of ACE2 expression in the respiratory and gastrointestinal tracts, indicating a possible impact of the microbiota on the clinical outcome of COVID-19 [10]. The healthy microbiota of the nasopharynx is diverse and dynamic, functioning as an interface between the airways and the external environment [11]. The upper respiratory tract microbiota is rich in components such as specialized communities of bacteria, fungi, and viruses that are fundamental to the healthy composition of the respiratory system [12]. In adults, the nasopharyngeal microbiota is more diversified and generally reduced in abundance, predominantly dominated by commensal members of the genera *Corynebacterium*, *Dolosigranulum*, *Lactobacillus* and potentially opportunistic pathogens

such as *Staphylococcus*, *Haemophilus* and *Streptococcus* [11, 13]. The microbial community may also include pathobionts genera with species related to human diseases, including *Sphingobacterium, Prevotella, Rothia, Propionibacterium,* and commensal like *Bifidobacterium,* with changes occurring in inter-individual bacterial community profile as a result of individual's age and lifestyle [12, 13]. Elderly individuals (>65 years) present bacterial communities in the nasal cavity dominated by *Streptococcus*, *Prevotella*, *Veillonella* and *Staphylococcus* [12].

Viral infections of the upper respiratory tract can cause alterations in the composition of commensal microbiota, favoring the increase of pathogenic species [14]. Disruption of the microbial community is also related to immune responses, and might decrease the defenses against infections of viral origin [15]. The microbiota can act in modulating systemic immunity through the production of metabolite mediators; these mediators influence microbiota-host interactions impacting the regulation of the innate immune response and the production of antimicrobial substances that could act against pathogens [16-18]. In the respiratory tract, the first defense of the immune system is the mucus layer containing immunoglobulin A (IgA) which traps particles and microbial pathogens; a second defense are epithelial cell layers produced antimicrobial substances [19]. For example, in COVID-19 it has been proposed that the microbiota in the airways may help to prevent SARS-CoV-2 infection through a colonization resistance mechanism. In this sense, the microbiota could prevent the establishment of a virus infection and the invasion to other mucosal surfaces [18]. In this context, commensal bacteria play an essential role in the host's immune response against a pathogen infection [12].

Recent studies have investigated the associations between SARS-CoV-2 infection and the microbiota in different human body systems in COVID-19 patients [20, 21]. When

comparing the microbiota profile of individuals infected with SARS-CoV-2 with that of non-infected individuals, differences in diversity and composition were found mainly in the microbiota of the respiratory and gastrointestinal tracts [20, 21]. These studies have emphasized a possible link between the microbiota and disease severity in SARS-CoV-2 infected patients, in whom the commensal bacterial composition is altered, favoring the enrichment of opportunistic pathogens, which can be indicative of dysbiosis in patients with COVID-19 [20, 21]. As recently reported, commensal *Staphylococcus* species may be associated with the reduction of entry factors of SARS-CoV-2 virus in the host's nasal epithelium, possibly functioning as a defense mechanism and promoting host protection against SARS-CoV-2 [22]. Patients with COVID-19 may also be co-infected with microorganisms of clinical relevance, such as *Haemophilus influenzae*, *Moraxella catarrhalis*, human metapneumovirus (HMPV) and human alphaherpesvirus (HSV) [23]. Moreover, higher abundance of potentially opportunistic pathogens, including *Veillonella*, *Staphylococcus*, *Corynebacterium*, *Neisseria*, *Actinobacillus*, and *Selenomonas* has been found in COVID-19 patients with different disease severity when compared to healthy controls [24]; and *Prevotella* sp. have been associated with disease severity [25].

To help establish whether there are associations between the microbiota and severe outcomes in cases of COVID-19, we analyzed the microbiota of the nasopharynx of outpatients with acute respiratory infection (ARI) and hospitalized patients with severe acute respiratory infection (SARI), with and without SARS-CoV-2.

2. Materials and Methods

2.1. Experimental design and patients

A cross-sectional study was conducted with suspected COVID-19 cases admitted to a hospital in Rio Grande do Sul, Brazil, during the mortality peak of the COVID-19 outbreak (January–March 2021). Nasopharyngeal samples were collected from each patient for diagnosis of SARS-CoV-2 by RT-qPCR at the Central Laboratory of the Health Department of the State of Rio Grande do Sul (LACEN/CEVS/SES-RS). All samples were collected in a sterile tube containing viral transport medium using a nasal swab stored at -80 ºC until DNA extraction. The samples were obtained on onset of symptoms, before any treatment was administered to the patients. Each sample was accompanied by an investigation file containing sociodemographic and clinical data of the patients. The study was approved by the Research Ethics Committee of UFCSPA (CAAE: 30714520.0.0000.5345 and CAAE 75118217.9.0000.5345). All samples and clinical data of patients were managed anonymously.

2.2. Selection of Samples

A selection of 96 samples was based on a stratified sampling provided by LACEN/CEVS/SES-RS. The stratifying variables were (i) syndrome (hospitalized patients with Severe Acute Respiratory Infection – SARI, and non-hospitalized patients with Acute Respiratory Infection – ARI); (ii) SARS-CoV-2 (positive and negative); and (iii) age (adults 20–40 years old, and elderly >60 years old). A random sampling was performed in each strata corresponding to a specific combination of stratifying variables. Because some groups did not contain enough samples, we complemented the final experiment with samples from different strata. Based on these criteria, samples were divided into the following groups: patients infected with SARS-COV-2 and ARI symptoms (ARI+: 20–40

years, n=12; and >60 years, n=12), or SARI symptoms (SARI+: 20–40 years, n=13; and >60 years, n=12); and patients negative for SARS-CoV-2 with ARI symptoms (ARI–: 20–40 years, n=14; and >60 years, n=9); or SARI symptoms (SARI–: 20–40 years; n=11; and >60 years, n=13) (Table 1).

2.3. DNA extraction and Sequencing

DNA from nasopharyngeal swab samples was isolated using the DNeasy PowerSoil Kit (Qiagen), according to the manufacturer's instructions. Before extraction, samples were centrifuged (3,000 rpm for 10 min) and the pellet resuspended in 50 µL volume of viral transport medium. The resulting DNA samples were quantified using a Qubit fluorometer (Thermo Fisher Scientific, USA). Polymerase chain reaction (PCR) was performed with Taq Platinum High Fidelity DNA polymerase (Thermo Fisher Scientific), following the thermal profile established by Illumina. Bacterial composition was characterized by PCR amplification of the V4 variable region of the *16S rRNA* gene using previously described primers [26] containing the Illumina adaptor sequences. Amplicons were indexed and sequenced on the Illumina Miseq system using Reagent Kit v2, 500 cycles (Illumina®, USA).

2.4. Analysis of 16S rRNA gene amplicon sequences

Paired end sequence data were imported for 16S rRNA taxonomic analysis using QIIME2 v2021.4 [27]. Sequence data were first denoised using QIIME DADA2 and a taxonomic classifier was downloaded and trained using the function Qiime feature-classifier classify-sklearn and the classifier gg-13-8-99-515-806-nb-classifier (https://github.com/qiime2/q2-feature-classifier). Outputs from QIIME2 were exported as

feature tables with counts at the genus level for downstream 16S rRNA analyses, due to not having enough counts at the species level for further analysis.

2.5. Statistical analysis

Taxonomic genera counts were first normalized by size factors and variance stabilization using DESeq2 v. 1.34.0 [28]. Alpha-diversity analyses were conducted using the normalized counts with the diversity function of vegan v2.5-7 [https://CRAN.R-project.org/package=vegan], with statistical significance between different groups being conducted using the Wilcoxon test (p-value <0.05). For Bray-Curtis beta-diversity analyses, Bray-Curtis distances between samples were first calculated using the function vegdist of vegan. Bray-Curtis distances were plotted after undergoing classic multidimensional scaling with significance being determined using a sample PERMANOVA with the adonis function of vegan. Differential enrichment analyses were conducted using edgeR v. 3.36.0 [29], with counts normalized using TMM and dispersion estimates. Differentially expressed genera were identified using glmQLFTest (FDR <0.05).

3. Results

3.1. Cohort description

Nasopharyngeal samples from 96 individuals were included in the analyses of the microbiota over the course of SARS-CoV-2 infection; samples were grouped according to syndrome (ARI or SARI) and age (20 – 40 years or > 60 years). Patient characteristics as sex, ethnicity, symptoms, and outcome are described in Table 1. Patients with ARI were not hospitalized, and symptoms included fever, cough, sore throat, coryza, headache, anosmia, among others. All patients with ARI had clinical outcomes of cure, while individuals with symptoms of SARI required hospitalization. In addition to the

above-mentioned symptoms, these individuals had dyspnea, respiratory distress and hypoxemia; furthermore, in the SARI groups high fatality rates were observed among COVID-19 patients SARI+ > 60 years (Table 1).

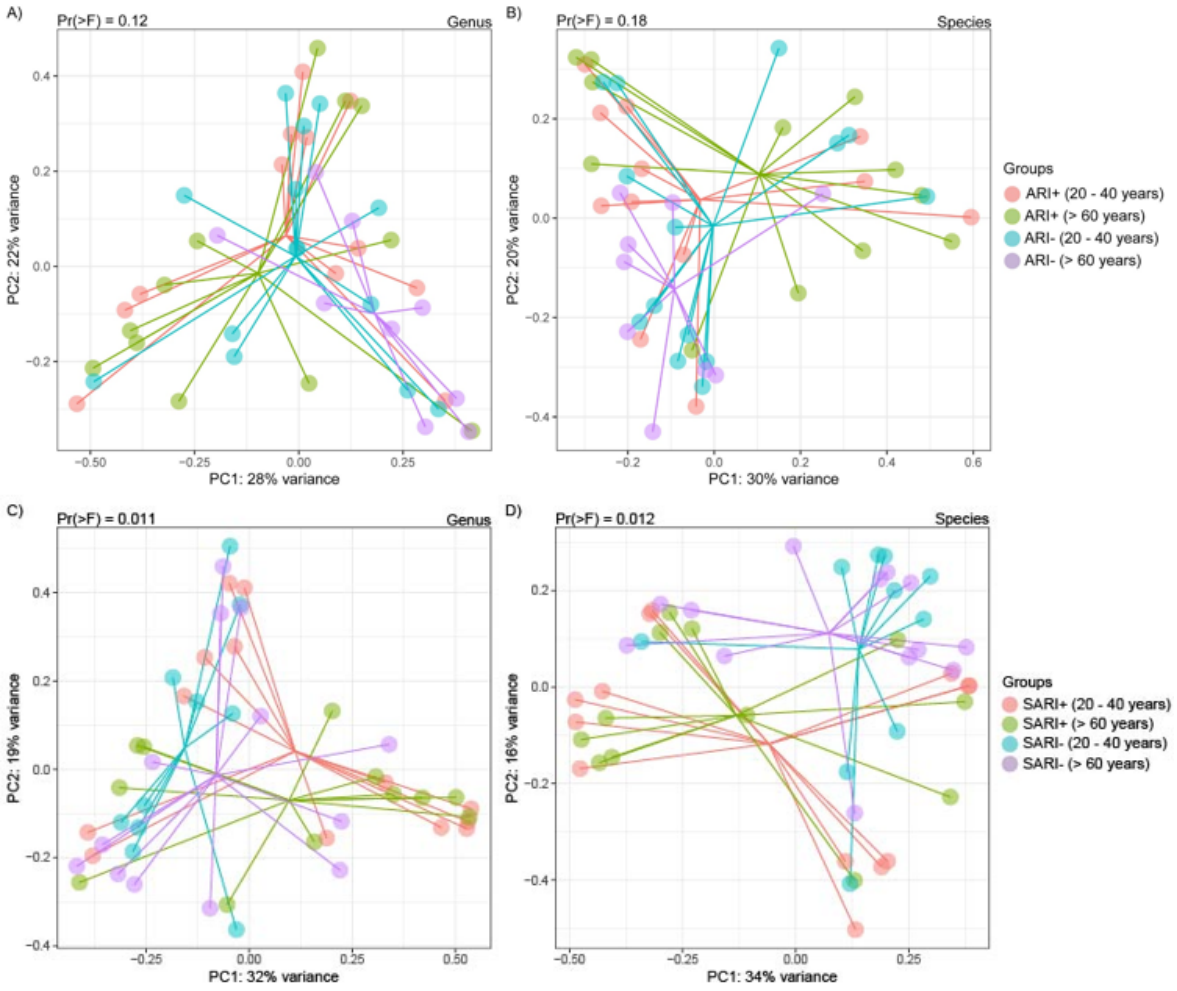
Table 1: Demographic and clinical characteristics of patients

Characteristics	Value for group (%)							
	ARI+ (20–40 years) n=12	**ARI+ (>60 years)** n=12	**ARI- (20–40 years)** n=14	**ARI- (>60 years)** n=9	**SARI+ (20–40 years)** n=13	**SARI+ (>60 years)** n=12	**SARI- (20–40 years)** n=11	**SARI- (>60 years)** n=13
Sex [N (%)]								
Female	6 (50)	6 (50)	8 (57)	4 (44)	7 (54)	7 (58)	9 (82)	8 (62)
Male	6 (50)	6 (50)	6 (43)	5 (56)	6 (46)	5 (42)	2 (18)	5 (38)
Race/ethnicity [N(%)]								
White	8 (67)	4 (33)	9 (64)	6 (67)	11 (85)	8 (67)	5 (45)	3 (23)
Black	0	0	0	0	0	0	0	0
Other/unknown[a]	4 (33)	8 (67)	5 (36)	3 (33)	2 (15)	4 (33)	6 (55)	10 (77)
Symptoms [N (%)]								
Fever	7 (58)	4 (33)	4 (28)	1 (11)	6 (46)	4 (33)	2 (18)	0 (0)
Cough	8 (67)	4 (33)	8 (57)	5 (56)	6 (46)	3 (25)	1 (9)	1 (8)
Sore throat	4 (33)	1 (8)	7 (50)	4 (44)	0 (0)	0 (0)	0 (0)	0 (0)
Dyspnea	2 (17)	2 (17)	4 (28)	2 (22)	10 (77)	9 (75)	3 (27)	1 (8)
Respiratory distress	0 (0)	0 (0)	0 (0)	0 (0)	10 (77)	10 (83)	3 (27)	2 (15)
O_2<95%	0 (0)	0 (0)	0 (0)	0 (0)	10 (77)	9 (75)	3 (27)	1 (8)
Diarrhea	0 (0)	0 (0)	0 (0)	0 (0)	1 (8)	1 (8)	0 (0)	0 (0)
Vomiting	0 (0)	0 (0)	0 (0)	0 (0)	1 (8)	1 (8)	0 (0)	0 (0)
Hyposmia	0 (0)	1 (8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ageusia	4 (33)	2 (17)	0 (0)	1 (11)	0 (0)	0 (0)	0 (0)	0 (0)
Headache	10 (83)	2 (17)	8 (57)	1 (11)	0 (0)	0 (0)	0 (0)	0 (0)
Coryza	6 (50)	0 (0)	7 (50)	3 (33)	0 (0)	0 (0)	0 (0)	0 (0)
Asymptomatic	0 (0)	3 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Other[b]	7 (58)	7 (58)	6 (43)	1 (11)	0 (0)	0 (0)	0 (0)	0 (0)
Outcome [N (%)]								
Cure	12 (100)	12 (100)	14 (100)	9 (100)	10 (77)	1 (8)	10 (91)	12 (92)
Fatality	0 (0)	0 (0)	0 (0)	0 (0)	3 (23)	11 (92)	1 (9)	1 (8)

Data expressed as N%, number (percentage). a: Other ethnicities or not mentioned in the patient's file. b: Other symptoms or not mentioned in the patient's file. O_2<95%: oxygen saturation below 95 percent.

3.2. Nasopharyngeal microbiota composition comparisons between adult and elderly patients

The *16S rRNA* variable region 4 (V4) was amplified and sequenced from nasopharyngeal DNA samples from all patients in our cohort. The reads obtained allowed the classification of bacterial taxa at the genus and species levels. The total read counts obtained from each group are shown in Supplementary Figure 1**.** We first compared taxonomic diversity at the genus and species levels across samples using beta-diversity between age groups for each condition. Data were visualized using Bray-Curtis principal-coordinate analysis (PCoA) (Fig. 1A-1H). The only significant difference was observed when comparing SARI patients positive with or negative for SARS-CoV-2 (SARI+ and SARI-) (Fig. 1C, 1D). It was possible to notice a significant association at genus ($p=0.011$) and species levels ($p=0.012$), grouping the samples according to the syndrome presented and the diagnosis for SARS-CoV-2, and not by age group (Fig. 1C, 1D). In contrast, no significant differences were found when comparing the groups, regardless of SARS-CoV-2 infection ($p> 0.05$) or disease severity (Fig. 1A and 1B; Fig. 1E and 1F; and Fig. 1G and 1H).


A)
Pr(>F) = 0.12
Genus
PC2: 22% variance
PC1: 28% variance
B)
Pr(>F) = 0.18
Species
PC2: 20% variance
PC1: 30% variance
Groups
ARI+ (20 - 40 years)
ARI+ (> 60 years)
ARI- (20 - 40 years)
ARI- (> 60 years)
C)
Pr(>F) = 0.011
Genus
PC2: 19% variance
PC1: 32% variance
D)
Pr(>F) = 0.012
Species
PC2: 16% variance
PC1: 34% variance
Groups
SARI+ (20 - 40 years)
SARI+ (> 60 years)
SARI- (20 - 40 years)
SARI- (> 60 years)

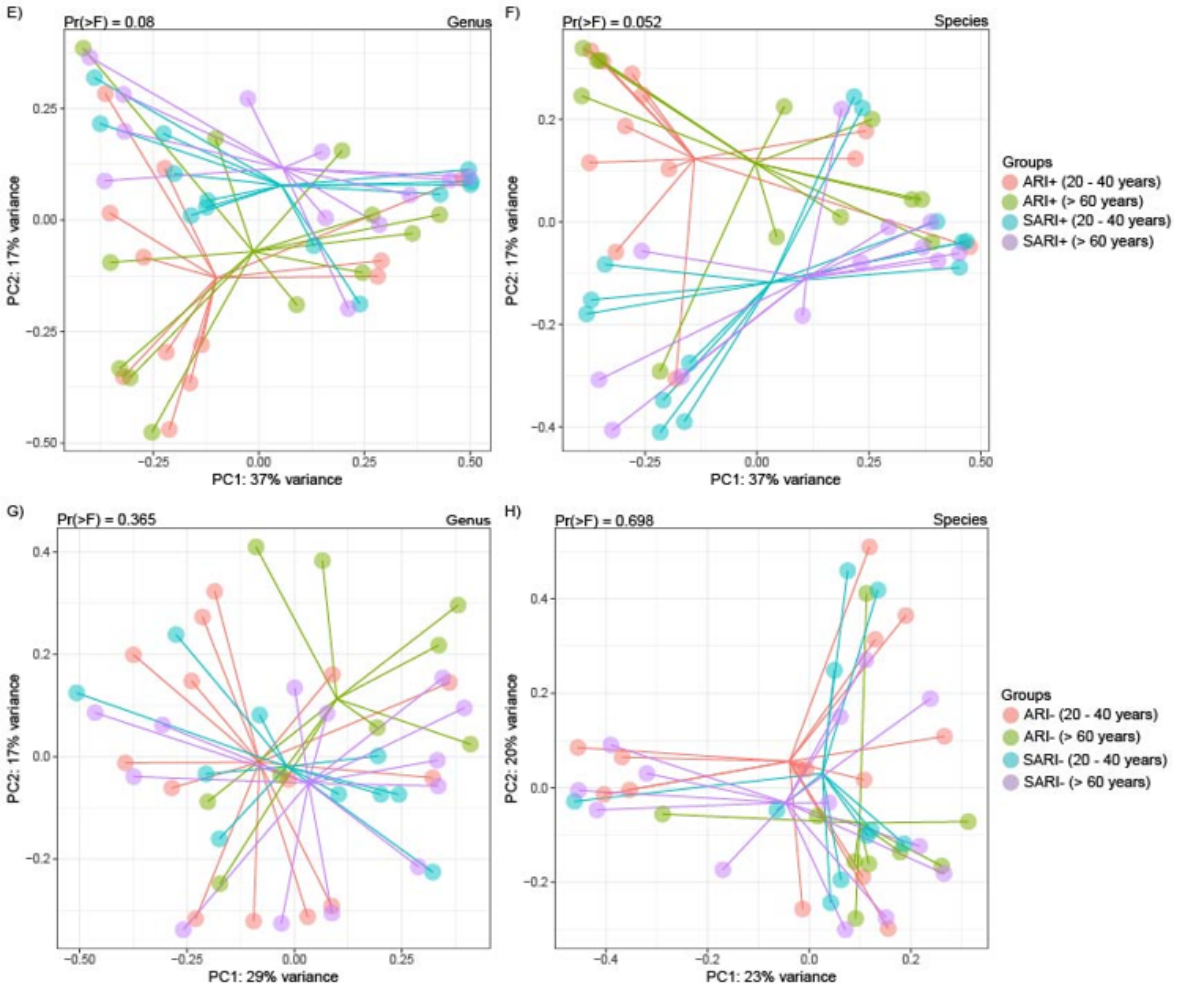


Figure 1: Nasopharyngeal microbiota composition diversity by age group comparisons. Beta-diversity analyses based on the Bray-Curtis dissimilarity index were tested at the genus (**A**, **C**, **E** and **G**) and species (**B**, **D**, **F**, **H**) levels. Analysis of bacterial community composition diversity between groups (**A**, **B**): ARI+ (20–40 years and >60 years) vs. ARI- (20–40 years and >60 years); (**C**, **D**): SARI+ (20–40 years and >60 years) vs. SARI- (20–40 years and >60 years); (**E**, **F**): ARI+ (20–40 years and >60 years) vs. SARI+ (20–40 years and >60 years); (**G**,**H**): ARI- (20–40 years and >60 years) vs. SARI- (20–40 years and >60 years). Points represent individual samples (tested by PERMANOVA analysis with $p<0.05$).

3.3. Nasopharyngeal microbiota composition comparison between individuals with SARI and ARI symptoms with or without SARS-CoV-2 infection

3.3.1. Alpha-diversity

As there were no significant differences in microbiota composition based on age, we combined groups to increase statistical power. Comparisons in these analyses were

done across four groups: ARI+ vs. ARI- and SARI+ vs. SARI-. To determine richness and distribution of genera across the samples tested, we used alpha-diversity measured by the Shannon diversity index (Fig. 2). Significant differences were found among SARS-CoV-2 infected individuals presenting ARI or SARI symptoms, with the ARI+ group as the most diverse and SARI+ group the least diverse (Wilcoxon test, p=0.027) (Fig. 2A). The bacterial richness was not significantly different between the other groups (Wilcoxon test, p>0.05) (Fig. 2B, 2C and 2D).

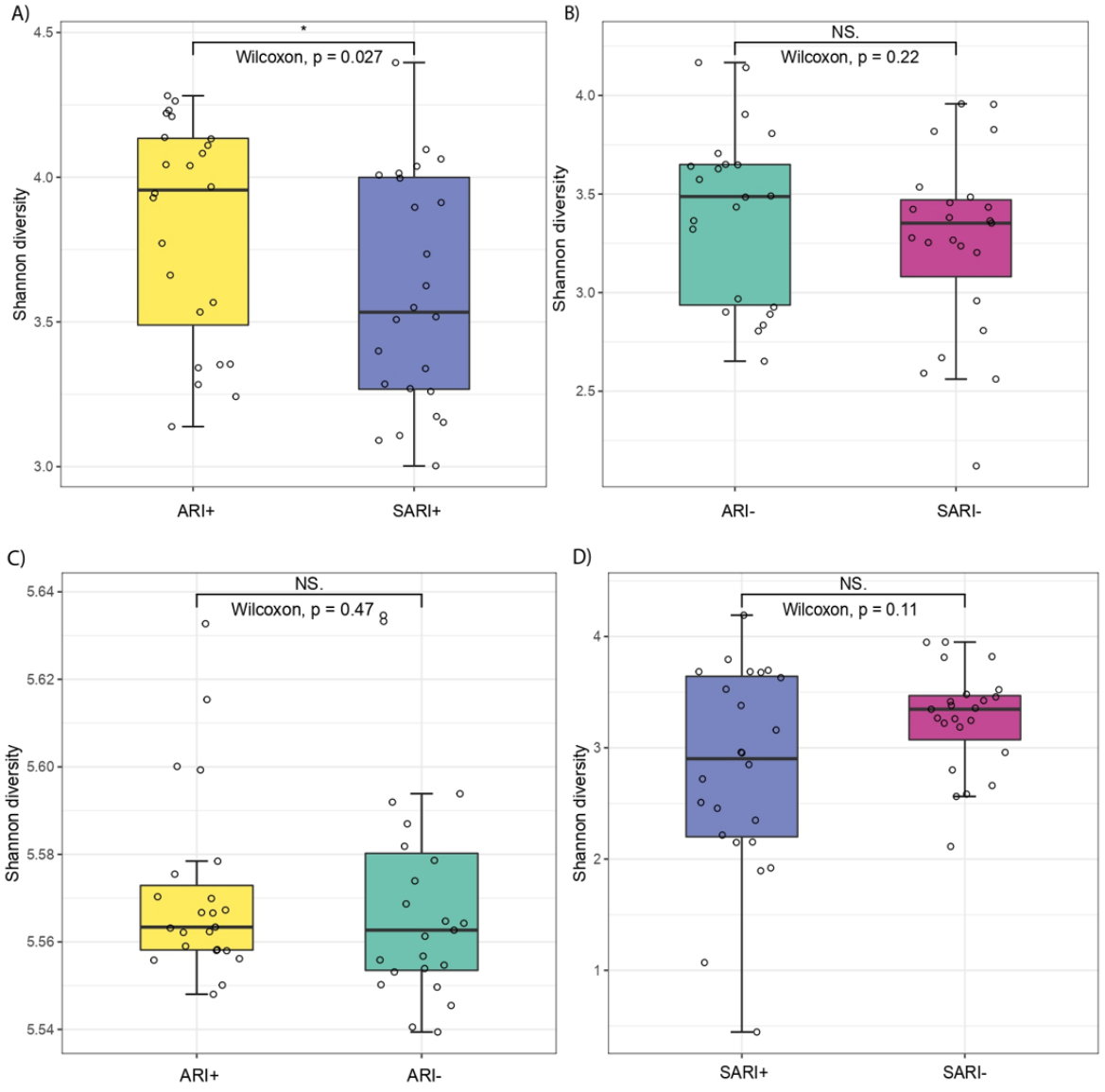


Figure 2: Alpha-diversity of the nasopharyngeal microbiota between patients with ARI or SARI and with and without SARS-CoV-2. The boxplots indicate the average Shannon diversity index among groups (**2A – 2D**). **A:** ARI+ (yellow) vs. SARI+ (blue); **B:** ARI- (green) vs. ARI- (purple); **C:** ARI+ (yellow) vs. ARI- (green); **D:** SARI+ (blue) vs. SARI- (purple), (Wilcoxon test, $p< 0.05$).

3.3.2. Beta-diversity

The beta-diversity analysis showed differences in the nasopharyngeal microbiota composition between patients with different acute respiratory symptoms (ARI or SARI) and SARS-CoV-2 diagnostic (positive or negative), at genus (Fig. 3) and species levels (Fig. 4). As measured by Bray-Curtis distance matrix, the principal-coordinate analysis (PCoA) showed differences between ARI+ and SARI+ groups (PERMANOVA analysis: $p=0.013$; Fig. 3A). Significant differences were also observed comparing SARI+ and SARI- individuals (PERMANOVA analysis: $p=0.001$; Fig. 3D). Other comparisons between groups did not show significant differences (PERMANOVA analysis: $p> 0.05$), with samples clustering closely, indicating that the bacterial communities were similar in these analyses (Fig. 3B, 3C). Furthermore, when analyzing the beta-diversity at the species level, results revealed differences in composition between ARI+ and SARI+ patients (PERMANOVA analysis: $p=0.005$; Fig. 4A). Likewise, differences were found when comparing SARI+ and SARI- groups (PERMANOVA analysis: $p=0.003$; Fig. 4D). In contrast, no differences were observed between ARI- and SARI- (Fig. 4B), neither between ARI+ and ARI- groups (Fig. 4C) (PERMANOVA analysis: $p>0.05$).

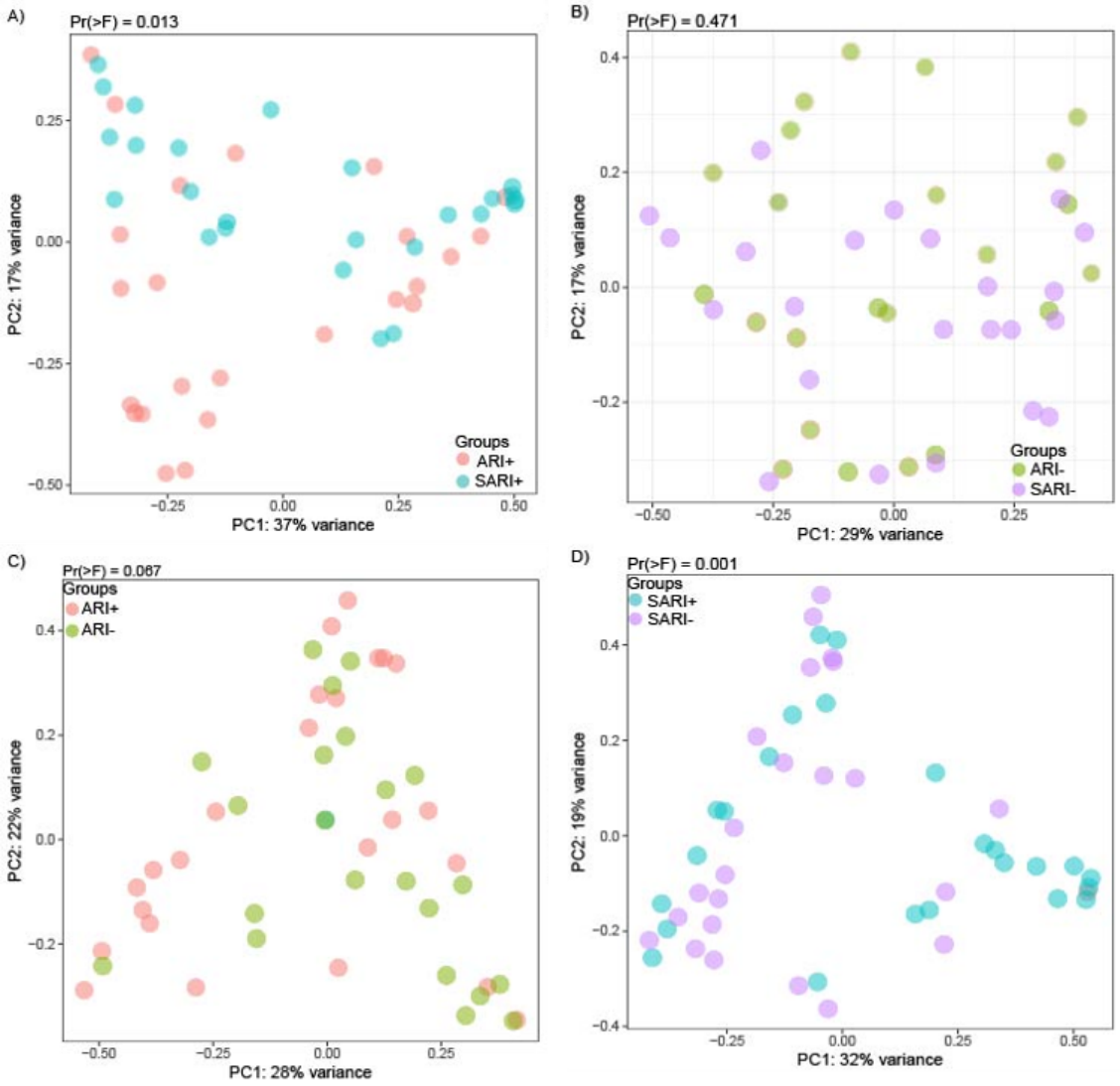


Figure 3: Beta-diversity analysis of the nasopharyngeal microbiota of patients with ARI or SARI with positive or negative diagnosis for SARS-CoV-2 infection. Principal coordinate analysis (PCoA) plots performed by the Bray-Curtis dissimilarity were colored by sample groups and each dot represents an individual. **A**: ARI+(red) vs. SARI+ (blue); **B**: ARI- (green) vs. SARI- (purple); **C**: ARI+ (red) vs. ARI- (green); **D**: SARI+ (blue) vs. SARI- (purple) (tested by PERMANOVA analysis; statistically significant when $p < 0.05$).

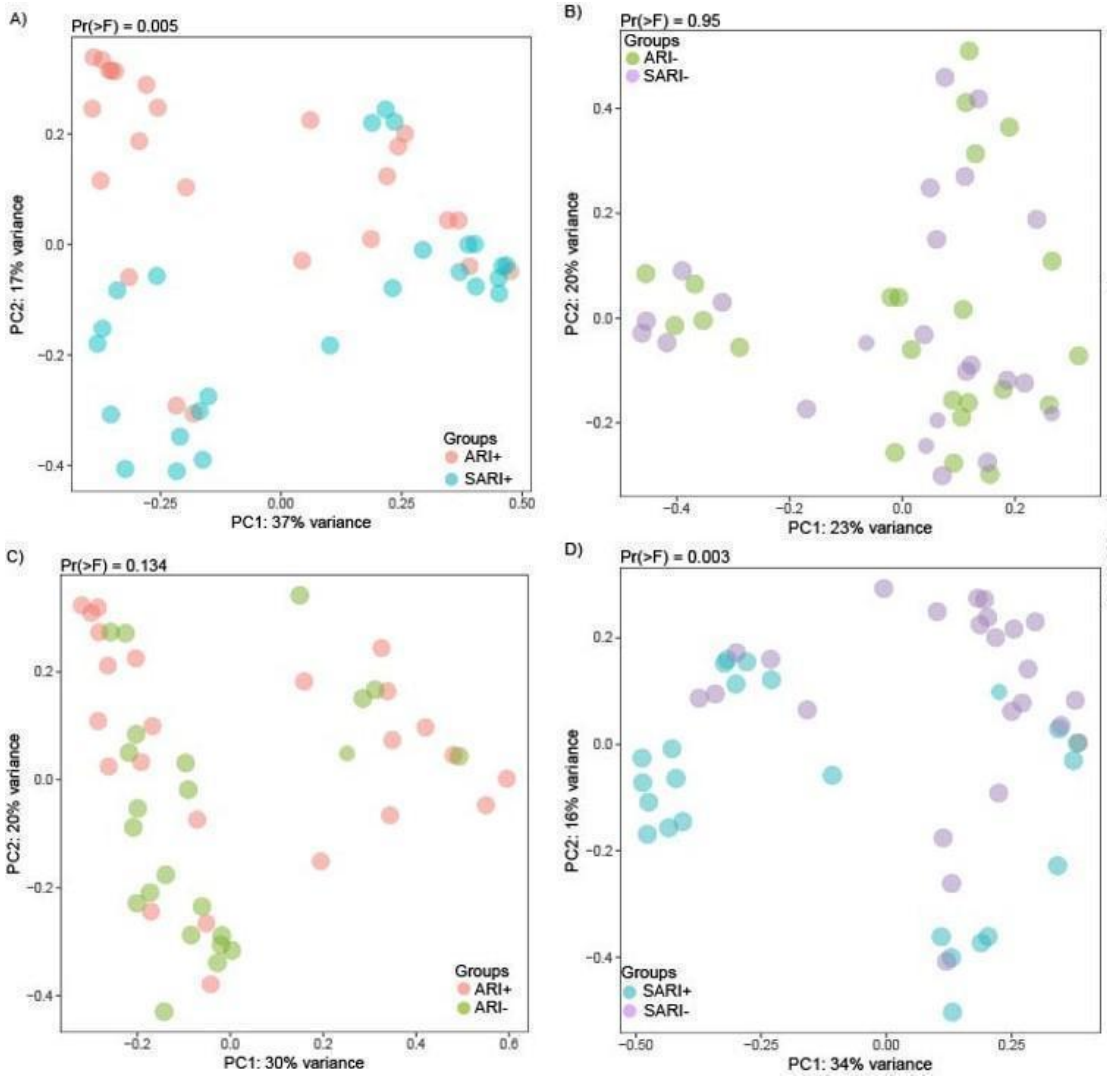


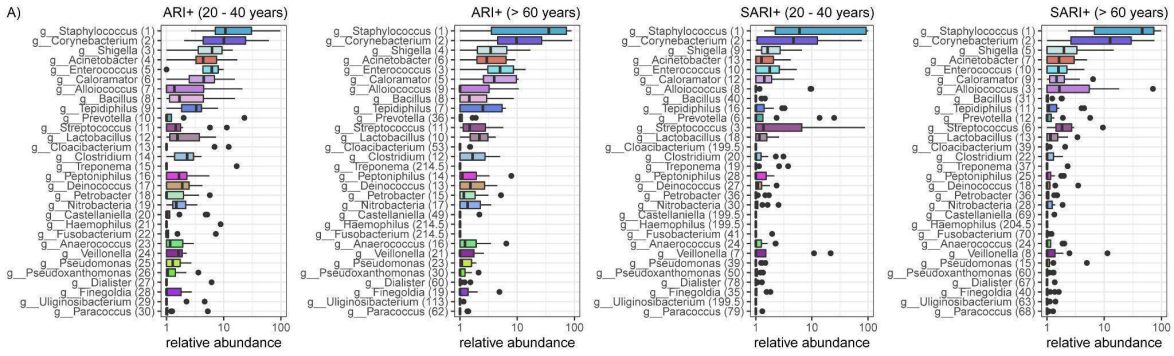
Figure 4: Beta-diversity principal-coordinate analysis (PCoA) of nasopharyngeal microbiota composition in patients positive or negative for SARS-CoV-2 with or without ARI or SARI at the species level. Each dot represents an individual in the colored sample. **A:** ARI+ (red) vs. SARI+ (blue), **B:** ARI-(green) vs. SARI- (purple), **C:** ARI+ (red) vs. ARI- (green), **D:** SARI+ (blue) vs. SARI- (purple).

3.4. Nasopharyngeal microbiota taxonomic profile of SARI and ARI individuals

3.4.1. Relative abundance between genera and species

The relative abundance of genera was compared across the different groups of individuals by analyzing the top 30 most abundant taxa; results showed variations of taxa among groups (Fig. 5). Comparison of the bacterial composition relative abundance at genus level showed that *Staphylococcus* spp., *Corynebacterium* spp., *Shigella* spp.,

Acinetobacter spp., *Enterococcus* spp., and *Caloramator* spp. were the most abundant genera in both ARI+ and SARI+ groups (Fig. 5A). Notably, differences in bacterial composition between ARI+ and SARI+ were also observed. For example,the *Bacillus* genus was more abundant in the ARI+ groups and decreased in the SARI+ groups; *Streptococcus* spp. was increased in SARI+ (adults) samples; and *Alloiococcus* spp. was the most common genus in ARI+ groups and SARI+ (elderly) patients (Fig. 5A). When comparing SARI+ and SARI-, the most common genera in both groups were *Staphylococcus* spp. and *Corynebacterium* spp. (Fig. 5B). Overall, *Anaerococcus* spp., *Deinococcus* spp., *Peptoniphilus* spp., and *Nitrobacteria* spp. were found abundant in SARI- groups and reduced in SARI+. In addition, there were differences related to age in the abundance of some genera in SARI groups (SARI+ and SARI-). For example, the *Alloiococcus* genus was increased in elderly patients (>60 years) in both SARI+ and SARI- groups;whereas *Streptococcus* spp. was found highly abundant in adults (20–40 years) in the SARI+ group (Fig. 5B).



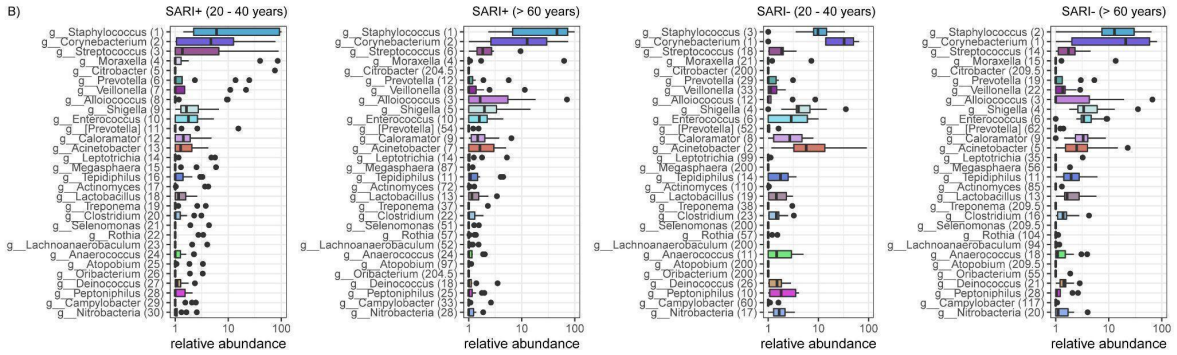


Figure 5: Bacterial composition of nasopharyngeal microbiota at genus level between ARI and SARI patients with or without SARS-CoV-2. The boxplots illustrate the relative abundance of genera between: **A)** ARI+ compared to SARI+ , and **B)** SARI+ compared to SARI-.

Additionally, we also characterized the relative abundance at the species level (top 50 taxa) (Supplementary Figure 2). Generally, the most common species among groups include *Staphylococcus lugdunensis*, *Enterococcus cecorum*, *Tepidiphilus margaritifer*, *Bacillus thermoamylovorans*, *Lactobacillus delbrueckii*, and *Deinococcus geothermalis*. However, some groups present species with different abundances depending on the type of symptom (ARI or SARI) and diagnosis (positive or negative for SARS-CoV-2). For example, *Paenibacillus barengoltzii* and *Veillonella dispar* were present on ARI+ adults, whereas *Streptococcus infantis* and *Sphingomonas echinoides* were found on ARI- adults. By contrast, *Streptococcus infantis* was abundant in SARI+ adults, and *Brevundimonas vesicularis* in SARI+ elderly group; *Bacillus thermoamylovorans,* in turn, was reduced in both groups. Lastly, *Sphingomonas echinoides* and *Gulbenkiania mobilis* were abundant in SARI- adults, while *Prevotella melaninogenica* was abundant in SARI- elderly patients (Supplementary Figure 2).

3.4.2. Differently represented genera

We also investigated whether there were bacteria differentially abundant between the study groups at the genus level (Fig. 6). Analysis of differential enrichment showed

that the genus *Bacillus* was significantly enriched among ARI+ individuals when compared to SARI+ patients (Fig. 6A). Additionally, *Streptococcus*, *Veillonella*, and *Staphylococcus* genera were enriched in SARI+ compared to SARI- patients; the latter, on the other hand, presented the *Bacillus* genus as the most enriched (Fig. 6D). When comparing ARI- and SARI-, as well as ARI+ and ARI- groups, no differential abundant genera were found (FDR >0.05) (Fig. 6B, 6C).

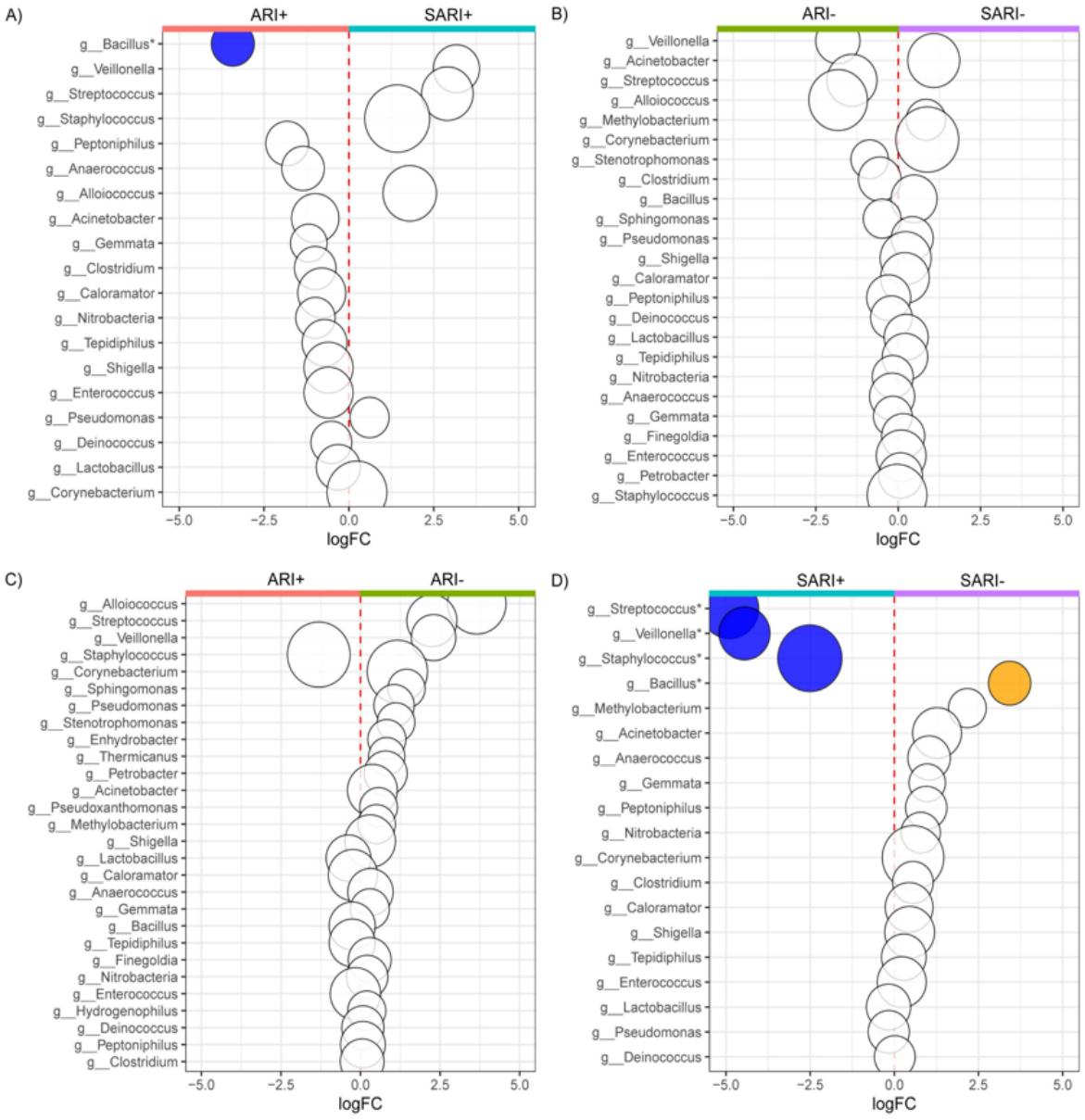


Figure 6: Differential abundant genera in the nasopharyngeal microbiota for each group obtained through glmQLFTest, at the genus level. Plots colored are significantly abundant

in the left group (blue) or the right group (orange). **A:** ARI+ (red) vs. SARI+ (blue), **B:** ARI- (green) vs. SARI- (purple), **C:** ARI+ (red) vs. ARI- (green), and **D:** SARI+ (blue) vs. SARI- (purple), (FDR <0.05).

4. Discussion

Our study characterized the nasopharyngeal microbiota composition of adults and elderly patients with symptoms of ARI and SARI, with or without SARS-CoV-2. We observed differences in bacterial diversity and composition (measured by alpha- and beta-diversity) at the genus and species levels. Our data show that the microbiota of SARI+ patients is different from that of ARI+ or even from SARI- patients, indicating a potential association between the microbiota of patients and COVID-19 disease severity.

Results are in accordance with previous studies investigating the upper respiratory tract microbiota composition, in which microbiota communities differ across COVID-19 and non-COVID-19 patients (controls) [20, 21]. Here, patients infected with SARS-CoV-2 and presenting severe acute respiratory symptoms (SARI+) have an upper airway microbiota that differ in composition and diversity, when compared to individuals with acute respiratory infection (ARI+), or even patients with severe acute respiratory infection and SARS-CoV-2 negative (SARI+ and SARI-). A previous study has shown that low initial diversity of the nasopharynx microbiota in hospitalized COVID-19 patients is associated with high fatality outcomes; this points to the nasopharyngeal microbiota of COVID-19 patients as a prognostic biomarker [30]. In addition, other studies suggest that changes in the composition and abundance of bacteria in the upper respiratory tract may remain altered after SARS-CoV-2 clearance [31, 32].

In agreement with previous studies [33, 34], no differences in the composition of the upper respiratory tract microbiota in ARI+ compared to ARI- patients were found. This

suggests that in these groups the microbiota is not impacted and continues to serve as a barrier against bacterial invasion caused by the virus, thus preventing aggravation of the disease, which could lead to hospitalization [33, 34].

Another analysis was conducted regarding comparisons between the patient's age, respiratory infection (ARI or SARI), and SARS-CoV-2 diagnosis; in this sense, differences were observed only when comparing SARI+ and SARI- groups, and such differences were associated with severe acute respiratory infection (SARI) and SARS-CoV-2 status. In a study by Hurst and colleagues (2021) in a cohort of 285 individuals, divided between children, adolescents and young adults (<21 years of age), an association between the age of the subjects and the diversity and composition of the nasopharyngeal microbiota was observed, as well as an association between age and the presence of respiratory symptoms arising from SARS-CoV-2 infection [35].

The groups ARI+ and SARI+ showed a relative abundance of the bacteria genera *Staphylococcus, Corynebacterium, Shigella, Acinetobacter, Enterococcus,* and *Caloramator*. Accordingly, the normal microbiota of the human nasopharynx is composed of the genera *Streptococcus, Corynebacterium, Staphylococcus, Moraxella, Haemophilus, Enhydrobacter, Bacillus, Lactobacillus,* and *Alloiococcus/Dolosigranulum*, with some variation from person to person [11]. Considering variations in the composition of the nasopharyngeal microbiota communities, the microbiota of healthy individuals may be dominated by different 'community types' that could include the genera *Moraxella*, *Fusobacterium*, or *Streptococcus* as dominants. Another profile could be a more diversified bacterial community composed of a mix of dominant genera including *Corynebacterium*, *Staphylococcus*, and/or *Dolosigranulum*; or even a glimpse of genera such as

Haemophilus, *Alloprevotella*, and *Neisseria* in smaller proportions [36]. This suggests that even under conditions of viral infections the natural microbiota remains dominant.

It is worth mentioning that a reduction in diversity and distribution of bacterial genera in the SARI+ group was clearly observed, with a highly relative abundance of *Staphylococcus*, and *Corynebacterium* genera. This is in accordance with the literature, as studies have shown that in hospitalized COVID-19 patients the *Staphylococcus* genus was significantly increased in severe cases [37], and associated with worse clinical outcomes in mechanically ventilated subjects [38]. In this sense, it was also seen that in adults with Influenza-like illness the nasopharyngeal microbiota was shown to be dominated by the genus *Corynebacterium* [39]. We also found that *Bacillus* at the genus level and *Bacillus thermoamylovorans* at the species level were significantly reduced in the nasopharyngeal microbiota of SARI+ patients. *Bacillus* spp. are considered opportunistic pathogens, and several members of the genus are related to a variety of clinical infections, causing human diseases[40, 41]. On the other hand, ongoing clinical studies report the potential use of *Bacillus* spp. as probiotics due to their advantage over other genera in forming endospores [42]. It has been seen that *Pseudomonas*, *Ralstonia,* and *Bacillus* genera comprise the oropharyngeal microbiota of patients with pneumonia due to influenza A virus infection [43]. Also, the *Bacillus* genus was enriched in samples from lower respiratory tract microbiota of infectious and inflammatory respiratory disease patients [44].

A study conducted with children with multisystem inflammatory syndrome (MIS-C), COVID-19 and healthy controls in France found that *Bacteroides uniformis, Bacillus thermoamylovorans*, and *Eubacterium dolichum* were dominant in the MIS-C group [45]. In this sense, our study also found that the nasopharyngeal microbiota of patients of the groups ARI+, ARI-, and SARI- group presented the genus *Bacillus* and the

species *Bacillus thermoamylovorans,* which could indicate less severity. In COVID-19 patients, several pathogens were found enriched in the nasopharyngeal microbiota, including *Afipia birgiae*, *Anaerobacillus alkalidiazotrophicus*, *Bacillus massiliamazoniensis*, *Corynebacterium accolens*, *Corynebacterium propinquum/pseudodiphtericum*, *Klebsiella pneumoniae*, *Moraxella catarrhalis*, *Staphylococcus capitis* subsp. *capitis*, and *Streptococcus pneumoniae.* These species were found to be enriched mainly in deceased patients when compared to asymptomatic COVID-19 patients, which shows a specific signature of the microbiota in COVID-19 infection at different stages of the disease [46]. So, considering the severe symptoms present by SARI+ patients, further studies are needed on the genus and species levels of *Bacillus* on these groups to understand the correlation between this genus and species, COVID-19 infection and disease severity.

In SARI+ patients the most dominant genera were *Streptococcus*, *Veillonella*, and *Staphylococcus*. These genera are considered members of the human commensal microbiota. In a previous study, the data revealed *Streptococcus*, *Prevotella*, *Veillonella,* and *Fusobacterium* as significantly dominant genera in patients without influenza viruses and SARS-CoV-2 infections [47], different from the present study, in which differential abundance of *Streptococcus*, *Veillonella*, and *Staphylococcus*, was found in SARI+ group. An explanation for this may be the fact that these genera present species of clinical significance, with high pathogenic potential of being associated with different types of infections in the respiratory tract [48, 49], and may be associated with dysbiosis in the respiratory tract microbiota of these patients. Besides, in the face of a respiratory viral infection, the virus may induce a disruption on the nasopharyngeal microbiota. The effect generated could affect biofilm colonies of the upper respiratory tract, and contribute to

migration of the virus to the lower respiratory airways, resulting in worse outcomes [50]. As seen in COVID-19, the virus infects respiratory epithelial cells, and after replication heads to the lungs resulting in more severe disease outcomes [6, 8]. Furthermore, the impact caused by the virus could interfere in the nutrition profile of the upper airway, providing a competition for nutrients among bacterial species, which in turn leads to modification of bacterial growth and microbiota profile [50].

5. Conclusion

The upper respiratory tract is considered the main route for SARS-CoV-2 virus fixation and dissemination; however, the role of nasopharynx microbiota in COVID-19 has not yet been established. Here, we characterized the nasopharyngeal bacterial microbiota profiles of 96 individuals with an acute respiratory infection (ARI) or severe acute respiratory infection (SARI), with (+) or without (-) SARS-CoV-2 using high-throughput sequencing. We found that SARI+ patients have a specific bacterial microbiota composition profile abundantly increased in *Streptococcus,* and decreased in *Bacillus*, and *Alloiococcus* genera, which was shown to be different from that of ARI+ patients, as well as SARI- individuals. The age variable does not seem to be a significant factor influencing the microbiota profile of most groups in this study. The composition of nasopharyngeal microbiota in SARI+ patients is enriched by potential opportunistic pathogens (*Streptococcus, Veillonella,* and *Staphylococcus*). These results contribute to the understanding of microbiota's role as an indicator of susceptibility to SARS-CoV-2 infection and disease severity. However, it is not possible to conclude if the changes found in the nasopharynx microbiota of these patients were a cause or an effect of SARS-CoV-2 infection.

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Declaration of competing interest

The authors declare no conflict of interest.

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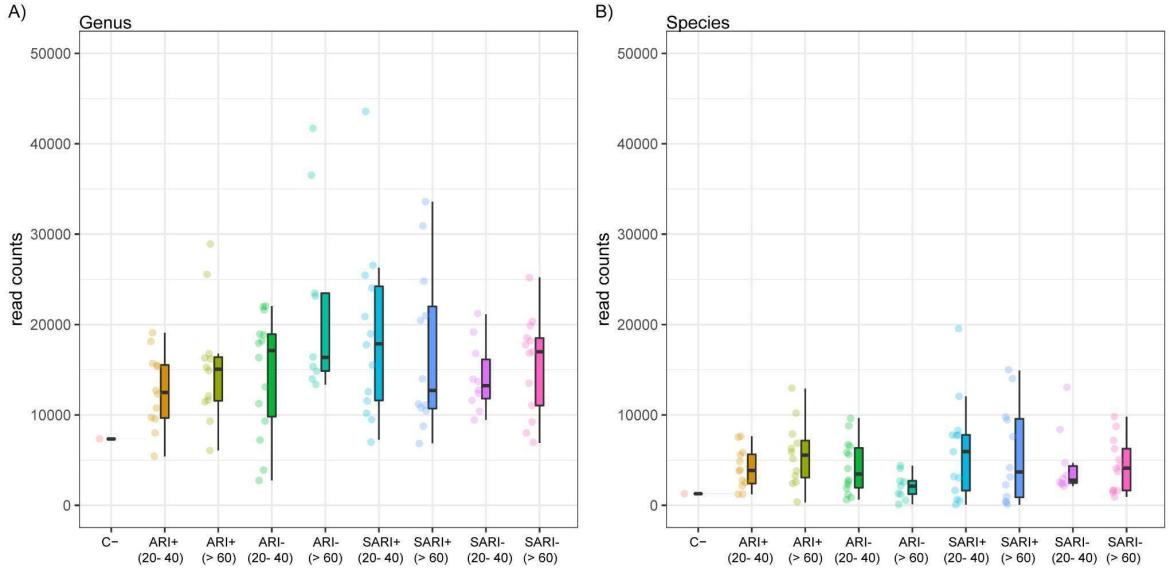
Supplementary Material



Supplementary Figure 1: Representation at the genus and species level in each study group, read counts statistics.

Supplementary Figure 2: Nasopharyngeal microbiota composition in ARI and SARI patients with or without SARS-CoV-2. The boxplots show the relative abundance at the species level.

5. DISCUSSÃO

A microbiota da nasofaringe é formada de comunidades microbianas que incluem bactérias, fungos, vírus e arqueas. Essas comunidades microbianas estabelecem relações ecológicas complexas entre si e o hospedeiro ao qual estão associados, fundamentais para a sobrevivência de ambos (FLYNN; DOOLEY, 2021). Entretanto, infecções virais causam danos diretos ao tecido celular ao qual infectam, gerando como resultado a exposição do tecido a patógenos oportunistas (DE STEENHUIJSEN et al., 2015). Neste contexto, os vírus também atuam desregulando uma gama de componentes do sistema imune do hospedeiro, e como consequências é vista uma redução na resistência à colonização por patobiontes e uma predisposição do hospedeiro a co-infecções por bactérias (DE STEENHUIJSEN et al., 2015).

A COVID-19 é uma doença respiratória sistêmica que resultou em uma pandemia de alta morbidade e mortalidade (ZHOU et al., 2020 a; ZHU et al., 2020; WU et al., 2020). Até 23 de dezembro de 2022, 651.918.402 milhões de casos foram confirmados em 228 países e territórios, incluindo 6.656.601 milhões de mortes atribuídas à doença, tornando-se uma das mais mortais da história (WHO, 2022). A pandemia instaurou uma crise econômica e nos sistemas de saúde mundiais (PAK et al., 2020; KAYE et al., 2021). Indivíduos infectados com SARS-CoV-2 podem ser assintomáticos ou desenvolvem sintomas variando em leves a graves, podendo progredir ao desfecho fatal (SALZBERGER et al., 2020). Os pacientes com COVID-19 podem apresentar sintomas de SRAG, que seria a forma mais grave da pneumonia causada por SARS-CoV-2 (SALZBERGER et al., 2020; BATTAGLINI et al., 2021).

Este trabalho visou ampliar o conhecimento sobre a microbiota do trato respiratório superior humano e investigar uma possível relação entre a microbiota e a infecção por SARS-CoV-2. Para tanto, neste estudo foi caracterizada a microbiota da nasofaringe de pacientes infectados e não infectados pelo SARS-CoV-2 e avaliada a existência de relação entre esses achados e os diferentes graus de severidade da doença. Somado a isso, uma revisão sistemática foi conduzida sobre a temática microbiota da nasofaringe e COVID-19, para agregar o conhecimento produzido por diversos grupos de pesquisa focados neste tema durante parte do período de pandemia.

Nos resultados do estudo experimental (Capítulo II), diferenças significativas ao nível de gênero e espécie tanto em diversidade como composição (alfa e beta diversidades)

foram encontradas entre os grupos SRAG+ e SG+; e SRAG+ e SRAG-. Esses resultados sugerem que a microbiota da nasofaringe dos pacientes SRAG+ apresente um perfil distinto de comunidades microbianas, caracterizado por um conjunto específico de microrganismos que se agrupam segundo a síndrome apresentada pelo paciente (gravidade do quadro) e o diagnóstico para SARS-CoV-2. As diferenças encontradas nos perfis de microbiota dos grupos analisados podem estar associadas com a infecção causada pelo SARS-CoV-2 e a severidade da COVID-19, determinante para o desfecho do caso, culminando ou não em hospitalização do paciente. Além disso, o grupo SRAG+ apresentou uma diversidade em gêneros bacterianos reduzida comparado ao grupo SG+. Como já visto na literatura, grande parte dos estudos com pacientes com COVID-19 em diferentes graus de severidade da doença apresentam uma microbiota alterada em diversidade e composição de táxons, caracterizada por um aumento significativo no número de patógenos oportunistas quando comparada a grupos controles (YAMAMOTO et al., 2020; LIU et al., 2021 b).

Em nosso estudo, os gêneros *Staphylococcus*, *Corynebacterium, Shigella*, *Acinetobacter*, *Enterococcus* e *Caloramator* se destacaram entre os mais abundantes em ambos os grupos analisados, positivos para COVID-19, SG+ e SRAG+. Esses gêneros bacterianos constituem a microbiota da nasofaringe em condições normais (CLEARY; CLARKE, 2017; FLYNN; DOOLEY, 2021) o que demonstra que, apesar da infecção causada pelo vírus, a microbiota tenta se manter resiliente frente a doença indicando a importância da presença desses gêneros para proteção do hospedeiro. Já comparando os pacientes SRAG com e sem COVID-19, os gêneros *Staphylococcus* e *Corynebacterium* foram os mais abundantes. Foi descrito que pacientes hospitalizados com COVID-19 apresentavam um aumento significativo do gênero *Staphylococcus* nos casos mais graves da doença (ZHONG et al., 2021). A presença de gêneros específicos pode significar que esses táxons estejam associados com a suscetibilidade e agravamento da COVID-19, funcionando como biomarcadores de severidade da doença.

É importante ressaltar a presença de gêneros diferencialmente abundantes nas comparações entre grupos realizadas em nosso estudo. O gênero *Bacillus* se mostrou mais abundante em pacientes SG+ quando comparados aos SRAG+, os quais interessantemente não apresentaram esse gênero. Por sua vez, o grupo SRAG+ apresentou como gêneros mais abundantes *Streptococcus, Veillonella* e *Staphylococcus,* diferente do grupo SRAG-,

que, em contrapartida, apresentou o gênero *Bacillus*. Os gêneros abundantes nos pacientes em estado mais grave (SRAG+) são conhecidos por englobarem espécies de importância clínica, podendo estar associadas com a evolução desses casos mais severos da COVID-19. Já o gênero *Bacillus*, mais abundante em pacientes negativos para COVID-19 ou com sintomatologia leve, apesar de possuir espécies causadoras de doenças em humanos, possui diversas espécies conhecidas pelos benefícios à saúde, seja na forma de probióticos ou ainda com aplicação na indústria farmacêutica, alimentícia e agrícola (TURNBULL; KRAMER; MELLING, 1996; SCHULTZ; BURTON; CHANYI, 2017). A presença do gênero *Bacillus* em pacientes SRAG- e SG+ pode estar relacionada a uma possível proteção contra o vírus SARS-CoV-2 ou ainda diminuição de suscetibilidade ao agravamento da COVID-19. Já a ausência do gênero no grupo SRAG+ pode estar associada a uma microbiota debilitada para o hospedeiro enfrentar a infecção viral, resultando em agravamento da COVID-19.

A grande maioria dos estudos analisados na revisão sistemática encontraram diferenças significativas na composição da microbiota da nasofaringe de pacientes com COVID-19 quando comparados aos grupos controles (29 estudos no total). Dois estudos não encontraram diferenças significativas entre os grupos COVID-19 e controles, o que é justificado por se tratarem de pacientes com COVID-19 com sintomas leves, nesses casos, a microbiota pode não ter sofrido alterações significativas e se mantido estável frente a infecção viral por SARS-CoV-2 (DE MAIO et al., 2020; BRAUN et al., 2021).

A revisão possibilitou elencar um conjunto de fatores considerados determinantes para a severidade da COVID-19: 1) a presença dos gêneros bacterianos *Cutibacterium* e *Lentimonas* (KOLHE et al., 2021), *Prevotella* spp. (VENTERO et al., 2021), entre outros; 2) variáveis clínicas e demográficas (HERNÁNDEZ-TERÁN et al., 2021); 3) composição de células imunes (MERENSTEIN et al., 2021); 4) conteúdo de citocinas circulantes (SMITH et al., 2021); e 5) condição das vias metabólicas de biossíntese de vitaminas (BAI et al., 2022). Foi vista uma correlação entre a composição da microbiota nasal, mais especificamente relacionada a presença de certos gêneros bacterianos, e a severidade da COVID-19 (KOLHE et al., 2021; VENTERO et al., 2021). Assim como, a presença de bactérias específicas foi correlacionada com variáveis clínicas associadas ao aumento do risco de mortalidade (HERNÁNDEZ-TERÁN et al., 2021). Além desses fatores, a composição da microbiota também foi associada a parâmetros imunológicos sistêmicos

(MERENSTEIN et al., 2021). E a carga viral da nasofaringe correlacionou-se positivamente com as respostas humorais verificadas contra o SARS-CoV-2, exceto com as respostas mediadas por interferon que foram associadas a comunidades microbianas protetoras (SMITH et al., 2021). Por fim, foram encontradas correlações entre espécies bacterianas e vias metabólicas associadas com uma melhor evolução clínica, como a via de biossíntese de vitamina K_2 (BAI et al., 2022). Esses diferentes fatores, cada um de seu modo, influenciam a composição da microbiota da nasofaringe, podendo ser este outro grande determinante da severidade e desfecho clínico da COVID-19.

Apenas seis estudos relataram o uso de antibióticos ou outros medicamentos pelos pacientes anteriormente a coleta das amostras (HERNÁNDEZ-TERÁN et al., 2021; LLORENS-RICO et al., 2021; MIAO et al., 2021; BAI et al., 2022; SHILTS et al., 2022; VENTERO et al., 2022). É sabido que o uso de antibióticos pode impactar de maneira negativa a composição da microbiota, reduzindo a diversidade de táxons e deixando o hospedeiro mais suscetível a infecções secundárias e até mesmo selecionando cepas resistentes a antibióticos (PATANGIA et al., 2021). Sabendo-se disso, alterações na microbiota encontradas nesses estudos podem ter sido influenciadas pelo uso de antibióticos por esses pacientes, e estendendo a relação podemos sugerir consequências na severidade da doença. Esse é um fator que precisa ser considerado em estudos de avaliação de microbiota, uma vez que o uso de antibióticos pode interferir nos resultados obtidos. Apesar de não constar na ficha preenchida pelos pacientes incluídos no nosso estudo, os profissionais do centro de saúde informaram que estes pacientes ainda não haviam usado antibióticos em função do quadro clínico em questão.

Ao compilar os dados dos estudos comparativos da microbiota da nasofaringe, alguns desafios metodológicos foram encontrados: 1) os diferentes tipos de técnica de amostragem empregados (amostras de suabes, lavagens nasais, teste de papel); 2) as diferenças entre os sítios corporais de amostragem (nasal, nasofaringe, orofaringe, lavado broncoalveolar, oral); 3) as diferentes técnicas de sequenciamento empregadas (metagenômico, *16S rRNA*, metatranscriptômica), o que dificultou estabelecer parâmetros comparativos entre os estudos analisados. Entretanto, um protocolo metodológico ainda não foi estabelecido para trabalhar com amostras de microbiota do trato respiratório superior em pacientes com COVID-19 (MANCABELLI et al., 2022). Com a produção crescente de estudos abordando essa temática, as diferentes análises realizadas poderão ser

utilizadas para se chegar a uma proposta sobre qual seria o método mais adequado para a avaliação e comparação da microbiota da nasofaringe.

Por fim, grande parte dos estudos apresentaram algumas limitações: 1) contaram com amostras pequenas (número limitado de participantes); 2) não consideraram a severidade da doença (somente pacientes com sintomas leves foram incluídos ou os sintomas apresentados pelos pacientes no momento da coleta não foram relatados); 3) não determinaram a carga viral de SARS-CoV-2; 4) e nem mesmo a variante de SARS-CoV-2 circulante no período da coleta das amostras, esses fatores podem ter influenciado os resultados obtidos nesses estudos. Porém, é sabido que nem sempre todos os parâmetros metodológicos são possíveis de serem considerados em um estudo, seja pela dificuldade de obtenção de dados dos pacientes amostrados, por conta do orçamento disponível ou falta de materiais adequados, além de outras particularidades que fogem do controle do pesquisador.

6. CONCLUSÕES

Conforme os resultados obtidos neste estudo, conclui-se que pacientes SRAG+ apresentam uma microbiota da nasofaringe menos abundante em composição e diversidade quando comparada a microbiota da nasofaringe de indivíduos SG+, e até mesmo diferente do grupo SRAG-. Além disso, a microbiota do grupo SRAG+ foi caracterizada por um aumento em espécies bacterianas que podem ser consideradas patógenos oportunistas.

No entanto, ficou evidente o quanto a análise da composição e abundância dos microrganismos que compõem a microbiota da nasofaringe pode contribuir para o entendimento da patogênese da COVID-19. E que fatores clínicos dos pacientes em associação com a composição da microbiota da nasofaringe e a maneira que o vírus interage com as comunidades microbianas pode influenciar a severidade da doença. Por fim, evidências são fornecidas sobre a relação da severidade da doença e possíveis tratamentos alternativos para a COVID-19, como uso de probióticos ou novas moléculas com efeito antimicrobiano. Contudo, ainda não é possível estabelecer se a infecção pelo vírus SARS-CoV-2 é causa ou efeito das alterações encontradas na microbiota dos pacientes com COVID-19, uma vez que dificilmente teremos um estudo com dados comparativos entre a microbiota antes de depois da infecção por SARS-CoV-2 para os mesmos indivíduos.

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ANEXO I

Ficha de investigação pacientes

MINISTÉRIO DA SAÚDE
SECRETARIA DE VIGILÂNCIA EM SAÚDE

Nº

e-SUS Notifica – MODELO 25/08/2020

FICHA DE INVESTIGAÇÃO DE SG SUSPEITO DE DOENÇA PELO CORONAVÍRUS 2019 – COVID-19 (B34.2)

Definição de caso: Indivíduo com quadro respiratório agudo, caracterizado por pelo menos dois (2) dos seguintes sinais e sintomas: febre (mesmo que referida), calafrios, dor de garganta, dor de cabeça, tosse, coriza, distúrbios olfativos ou distúrbios gustativos.
Em crianças: além dos itens anteriores considera-se também obstrução nasal, na ausência de outro diagnóstico específico.
Em idosos: deve-se considerar também critérios específicos de agravamento como síncope, confusão mental, sonolência excessiva, irritabilidade e inapetência.
Observação: Na suspeita de COVID-19, a febre pode estar ausente e sintomas gastrointestinais (diarreia) podem estar presentes.

UF de notificação: |__|__| **Município de Notificação:** _____

IDENTIFICAÇÃO

Tem CPF? (Marcar X) |__|Sim |__|Não	**Estrangeiro:** (Marcar X) |__| Sim |__|Não	**Profissional de saúde** (Marcar X) |__| Sim |__|Não	**Profissional de segurança** (Marcar X) |__| Sim |__|Não
CBO:		**CPF:** __|__|__|__|__|__|__|__|__|__|__	
CNS: __|__|__|__|__|__|__|__|__|__|__|__|__|__|__			
Nome Completo:			
Nome Completo da Mãe:			
Data de nascimento: ____|____|_____		**País de origem:**	
Sexo: (Marcar X) |__|Masculino |__|Feminino	**Raça/COR:** (Marcar X) |__|Branca |__|Preta |__|Amarela |__|Parda |__|Indígena - Etnia:_____ |__|Ignorado		**Passaporte:** __|__|__|__|__|__|__|__|__
CEP: __|__|__|__|__ - __|__|__			
Estado de residência: |__|__|	**Município de Residência:** _____		
Logradouro:		**Número:**	**Bairro:**
Complemento:			
Telefone Celular:		**Telefone de contato:**	

DADOS CLÍNICOS EPIDEMIOLÓGICOS

Data da Notificação: ___|___|____		**Data do início dos sintomas:** ___|___|____	
Sintomas: (Marcar X) |__|Assintomático |__| Febre |__|Dor de Garganta |__|Dispneia |__| Tosse |__| Coriza |__| Dor de Cabeça |__| Distúrbios gustatórios |__| Distúrbios olfativos |__|Outros_____			
Condições: (Marcar X) |__| Doenças respiratórias crônicas descompensadas |__| Doenças renais crônicas em estágio avançado (graus 3, 4 e 5) |__| Portador de doenças cromossômicas ou estado de fragilidade imunológica |__| Doenças cardíacas crônicas |__| Diabetes |__| Obesidade |__| Imunossupressão |__| Gestante |__| Puérpera (até 45 dias do parto)			
Estado do Teste: (Marcar X) |__|Solicitado |__|Coletado |__|Concluído |__|Exame Não Solicitado	**Data da Coleta do Teste:** ____|____|____	**Tipo de Teste:** (Marcar X) |__|RT – PCR |__|Teste rápido – anticorpo |__|Teste rápido – antígeno |__|Enzimaimunoensaio-ELISA |__|Eletroquimioluminescência- ECLIA |__|Quimioluminescência- CLIA	**Resultado do teste:** (Marcar X) |__|Negativo |__|Positivo |__|Inconclusivo ou Indeterminado

ENCERRAMENTO

Classificação final: (Marcar X) |__|Descartado |__|Confirmado Clínico Imagem |__|Confirmado Clínico-Epidemiológico |__|Confirmado Por Critério Clínico |__|Confirmado Laboratorial |__|Síndrome Gripal Não Especificada	**Evolução do caso:** (Marcar X) |__|Cancelado |__|Internado |__|Ignorado |__|Óbito |__|Em tratamento domiciliar |__|Cura |__|Internado em UTI
Data de encerramento: ____|____|_____	

Informações complementares e observações

e-SUS Notifica – MODELO DE FICHA DE NOTIFICAÇÃO DE COVID-19 25/08/2020

ANEXO II

UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Epidemiologia Molecular do Coronavírus SARS-CoV-2 no Rio Grande do Sul
Pesquisador: Ana Beatriz Gorini da Veiga
Área Temática:
Versão: 3
CAAE: 30714520.0.0000.5345
Instituição Proponente: Universidade Federal de Ciências da Saúde de Porto Alegre
Patrocinador Principal: Universidade Federal de Ciências da Saúde de Porto Alegre

DADOS DO PARECER

Número do Parecer: 3.978.647

Apresentação do Projeto:
Trata-se de um projeto que visa a compreensão da epidemiologia do SARS-CoV-2 no Rio Grande do Sul, através de análise dos casos, baseados em dados clínicos e moleculares registrados pelo Laboratório Central do Estado (LACEN) e pelo Centro Estadual de Vigilância em Saúde (CEVS. As amostras serão analisadas por imunoensaio e técnicas moleculares (PCR e sequenciamento). As sequências virais serão analisadas em relação às mutações genéticas, a fim de inferir relações filogenômicas entre as cepas virais circulantes no RS e aquelas de outras regiões. Paralelamente, será realizada análise da microbiota dos pacientes positivos para SARS-CoV-2 para relacionar a infecção viral com a comunidade bacteriana presente nesses indivíduos. Sendo possível então a compreensão das relações de co-infecções virais e bacterianas associadas à sintomatologia clínica (de assintomáticos a graves) e ao desfecho da doença. Tal abordagem, envolvendo diferentes aspectos da infecção pelo SARS-CoV-2 e busca o aprimoramento de conhecimentos sobre os coronavírus a nível nacional e internacional.

Objetivo da Pesquisa:
Objetivo Primário:
O objetivo geral deste projeto é estudar casos de infecção causadas pelo novo coronavírus, SARS-CoV-2, no Rio Grande do Sul, por meio de análises moleculares e de dados clínicos, contribuindo, assim para o conhecimento sobre a epidemiologia desse vírus e para o manejo e controle da doença por ele causada.

Endereço: Rua Sarmento Leite ,245
Bairro: Sarmento **CEP:** 90.050-170
UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3303-8804 **E-mail:** cep@ufcspa.edu.br

Objetivo Secundário:
Analisar dados de SG e SRAG do Rio Grande do Sul disponíveis no CEVS e identificar os casos de infecção por SARS-CoV-2.
Realizar análises dos dados demográficos e clínicos relacionados à infecção por SARS-CoV-2.
Sequenciar o genoma do SARS-CoV-2 a partir de amostras de nasofaringe dos pacientes infectados.
Construir árvores filogenéticas com base nas sequências de SARSCoV-2 obtidas e comparar com sequências disponíveis no GenBank de outras regiões geográficas.
Analisar as mutações que ocorreram no genoma das cepas de SARS-CoV-2 circulantes no RS.- Realizar análise da microbiota de amostras de pacientes com SRAG infectados e não infectados pelo SARS-CoV-2, a fim de identificar possível associação entre comunidades bacterianas e a infecção pelo SARS-CoV-2.
Analisar a presença de outros vírus respiratórios nas amostras

Avaliação dos Riscos e Benefícios:
Riscos:
O projeto tem risco mínimo. Apesar de ser baseado na análise de vírus respiratórios, todas as análises serão realizadas em laboratórios adequadamente equipados e os pesquisadores envolvidos possuem experiência na realização das técnicas a serem empregadas. Além disso, as amostras serão submetidas à inativação viral para manipulação no laboratório.
Benefícios:
Fornecer informações sobre o novo coronavírus SARS-CoV-2. contribuindo para o crescimento científico, capacitação e formação de recursos humanos e geração de publicação em nível internacional, abrindo perspectivas para outros estudos na área, integrando a clínica com a Biologia Molecular, a Microbiologia, a Epidemiologia, a Saúde Pública e outras áreas relacionadas. O surgimento do SARS-CoV-2 no final de 2019 aumentou o alerta da população e de agências de saúde do mundo todo para melhores medidas de prevenção e combate às infecções respiratórias causadas por vírus. O diagnóstico preciso é fundamental para estudos epidemiológicos e para a prevenção da disseminação da COVID-19, enquanto a caracterização molecular através do sequenciamento e análise filogenética do coronavírus contribuem para o conhecimento sobre a

circulação do vírus e para a tomada de medidas adequadas de prevenção à disseminação da COVID-19. As informações sobre o genoma desse vírus provêm de dados moleculares obtidos principalmente de amostras de outras regiões geográficas, não tendo sido sequenciado nenhum genoma de SARS-CoV-2 de amostras do Rio Grande do Sul. Devido à escassez de dados no nosso meio acerca desse vírus, o presente projeto visa sequenciar o genoma de SARS-CoV-2 de amostras do RS, relacionar os dados moleculares com dados epidemiológicos, e comparar os genomas com aquele de outras regiões geográficas, a fim de estudar afilogenia de cepas de SARS-CoV-2 circulantes no RS. A abordagem do problema sugerida neste projeto, inédita na nossa região, é fundamental para que condutas adequadas sejam tomadas em relação aos pacientes que apresentam sintomas severos COVID-19, evitando o óbito desses pacientes e contribuindo para o controle e o tratamento desse problema de saúde pública. Os modelos experimentais que serão desenvolvidos neste projeto poderão servir de base para estudos com outros vírus patogênicos humanos, com o desenvolvimento de bancos de dados e de programas computacionais que poderão ser utilizados por outros grupos de pesquisa, bem como poragências de controle e vigilância em saúde. O projeto contribuirá para a formação de profissionais capacitados a:- Elaborar e desenvolver projetos de pesquisa na área de Biologia Molecular, Microbiologia, Epidemiologia e Bioinformática.- Buscar soluções para problemas relacionados a essas área de pesquisa.- Empregar técnicas de laboratório adequadas para diferentes problemas e assuntos abordados nas pesquisas em Microbiologia, Bioinformática e Biologia Molecular, sejam elas técnicas clássicas ou que empreguem equipamentos de última geração. - Buscar bibliografia adequada.- Organizar os resultados obtidos para compartilhar e divulgar o conhecimento gerado através da elaboração de trabalhos a serem apresentados em eventos acadêmico-científicos nacionais e internacionais.- Organizar os resultados obtidos para e elaborar trabalhos para publicação de artigos científicos em revistas internacionais de impacto.- Colaborar em atividades acadêmico-científicas junto à Instituição.A colaboração de pesquisadores de diferentes instituições (UFCSPA, Secretaria de Vigilância Epidemiológica, LACEN-RS) para o desenvolvimento das atividades propostas será um dos pontos fortes do projeto, resultando no fortalecimento das linhas de pesquisa em Virologia e Biologia Molecular, o que promoverá também a abertura para parcerias em outros projetos futuros. Além disso, tal colaboração permitirá odesenvolvimento do projeto em rede, de forma a otimizar o uso dos equipamentos presentes nos diferentes laboratórios e acelerar a obtenção dos resultados. Fornecer informações sobre o novo coronavírus SARS-CoV-2. Contribuir para o crescimento científico, capacitação e formação de recursos humanos e geração de publicação em nível internacional, abrindo perspectivas para outros

estudos na área, integrando a clínica com a Biologia Molecular, a Microbiologia, a Epidemiologia, a Saúde Pública e outras áreas relacionadas. Contribuir para melhores medidas de prevenção e combate às infecções respiratórias causadas por vírus. Contribuir para a precisão de um diagnóstico, visando a prevenção da disseminação da COVID-19. A caracterização molecular através do sequenciamento e análise filogenética do coronavírus poderão contribuir para o conhecimento sobre a circulação do vírus e para a tomada de medidas adequadas de prevenção à disseminação da COVID-19. Obtenção de sequenciamento genômico de amostras do Rio Grande do Sul, relacionando os dados moleculares com dados epidemiológicos, e comparar os genomas com aquele de outras regiões geográficas, a fim de estudar afilogenia de cepas de SARS-CoV-2 circulantes no RS. Contribuir para condutas adequadas sejam tomadas em relação aos pacientes que apresentam sintomas severos COVID-19, evitando o óbito desses pacientes e contribuindo para o controle e o tratamento desse problema de saúde pública. Os modelos experimentais que serão desenvolvidos neste projeto poderão servir de base para estudos com outros vírus patogênicos humanos, com o desenvolvimento de bancos de dados e de programas computacionais que poderão ser utilizados por outros grupos de pesquisa, bem como por agências de controle e vigilância em saúde. O projeto contribuirá para a formação de profissionais capacitados nas áreas de Biologia Molecular, Microbiologia, Epidemiologia e Bioinformática.

Comentários e Considerações sobre a Pesquisa:

Trata-se de um projeto em caráter de urgência. A caracterização molecular através do sequenciamento e análise filogenética do coronavírus poderá contribuir para o conhecimento sobre a circulação do vírus e para prevenção à disseminação da COVID-19. Na atual conjuntura, a busca de métodos diagnósticos, estudos epidemiológicos e preventivos, são de extrema importância para impedir a disseminação do vírus.

Considerações sobre os Termos de apresentação obrigatória:

Todos os termos apresentados.

Recomendações:

Caso novos pesquisadores sejam incluídos no estudo através de emenda, os mesmos devem assinar o TCUD.

UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

Conclusões ou Pendências e Lista de Inadequações:

Solicitações atendidas.

Projeto aprovado, com duração prevista até dezembro de 2022.

Considerações Finais a critério do CEP:

De acordo com o parecer do Relator

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1537954.pdf	17/04/2020 17:32:36		Aceito
Projeto Detalhado / Brochura Investigador	Projeto_Coronavirus_2020_Final_CEP.pdf	17/04/2020 17:32:04	Ana Beatriz Gorini da Veiga	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	Declaracao_LACEN_UFCSPA.pdf	17/04/2020 17:31:16	Ana Beatriz Gorini da Veiga	Aceito
Declaração de Pesquisadores	ProjetoCorona_CEP_TermoDados_TSGregianini.pdf	17/04/2020 13:39:08	Ana Beatriz Gorini da Veiga	Aceito
Declaração de Pesquisadores	ProjetoCorona_CEP_TermoDados_ASeixas.pdf	17/04/2020 13:38:04	Ana Beatriz Gorini da Veiga	Aceito
Declaração de Pesquisadores	ProjetoCorona_CEP_TermoDados_Fabiana_Quoos_Mayer_ass.pdf	17/04/2020 13:36:01	Ana Beatriz Gorini da Veiga	Aceito
Declaração de Pesquisadores	ProjetoCorona_CEP_TermoDados_LeticiaGarayMartins.pdf	17/04/2020 13:35:51	Ana Beatriz Gorini da Veiga	Aceito
Declaração de Pesquisadores	ProjetoCorona_CEP_TermoDados_AnaMuterle.pdf	17/04/2020 13:35:38	Ana Beatriz Gorini da Veiga	Aceito
Declaração de Pesquisadores	ProjetoCorona_CEP_TermoDados_JPrichula.pdf	17/04/2020 13:35:24	Ana Beatriz Gorini da Veiga	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	ProjetoCoronavirus_Justificativa_TCLE.pdf	09/04/2020 19:24:15	Ana Beatriz Gorini da Veiga	Aceito
Outros	ProjetoCorona_CEP_TermoDados.pdf	09/04/2020 19:12:30	Ana Beatriz Gorini da Veiga	Aceito
Declaração de concordância	ProjetoCoronavirus_TermoAnuencia_Local.pdf	09/04/2020 19:04:36	Ana Beatriz Gorini da Veiga	Aceito
Outros	Projeto_Coronavirus_EntregaRelatorio.pdf	09/04/2020 19:02:48	Ana Beatriz Gorini da Veiga	Aceito
Folha de Rosto	ProjetoCorona_CEP_FolhaRosto.pdf	09/04/2020 18:52:49	Ana Beatriz Gorini da Veiga	Aceito

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Telefone: (51)3303-8804 **E-mail:** cep@ufcspa.edu.br

UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE

Continuação do Parecer: 3.978.647

Situação do Parecer:
Aprovado

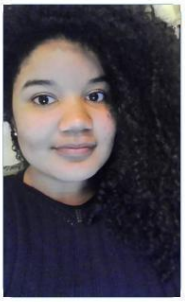
Necessita Apreciação da CONEP:
Não

PORTO ALEGRE, 17 de Abril de 2020

Assinado por:
Fernanda Bordignon Nunes
(Coordenador(a))

Endereço: Rua Sarmento Leite ,245
Bairro: Sarmento **CEP:** 90.050-170
UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3303-8804 **E-mail:** cep@ufcspa.edu.br

CURRÍCULO LATTES



Jordana Ariane Nunes da Rosa

Endereço para acessar este CV: http://lattes.cnpq.br/1653457458326436
ID Lattes: **1653457458326436**
Última atualização do currículo em 20/12/2022

Possui graduação em Ciências Biológicas (ênfase em genética) pela Universidade Luterana do Brasil (2015-2020). Atualmente é mestranda no Programa de Pós-Graduação em Biociências da Universidade de Ciências da Saúde de Porto Alegre (UFCSPA). Foi bolsista de iniciação cientifica CNPq e FAPERGS no Laboratório de Toxicidade Genética (TOXIGEN), da Universidade Luterana do Brasil no período de 2016-2019, tendo como experiência Cultura celular, teste SMART (Teste de Mutação e Recombinação Somática), Ensaio Cometa, Ensaio de micronúcleos, ensaios microbiológicos, extração de proteínas, entre outros. Tem interesse na área de Toxicologia, Biologia celular e molecular, Microbiologia, Biotecnologia. **(Texto informado pelo autor)**

Identificação

Nome	Jordana Ariane Nunes da Rosa
Nome em citações bibliográficas	ROSA, J. A. N.;Jordana A. N. da Rosa;DA ROSA, JORDANA A. N.
Lattes iD	http://lattes.cnpq.br/1653457458326436
Orcid iD	? https://orcid.org/0000-0002-6007-4509

Endereço

Formação acadêmica/titulação

2020
Mestrado em andamento em BIOCIÊNCIAS (Conceito CAPES 4).
Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil.
Título: MICROBIOTA DO TRATO RESPIRATÓRIO SUPERIOR DE PACIENTES ACOMETIDOS POR INFECÇÕES RESPIRATÓRIAS AGUDAS E SUA ASSOCIAÇÃO COM SARS-CoV-2.
Orientador: Adriana Seixas.
Coorientador: Ana Beatriz Gorini da Veiga.
Bolsista do(a): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES, Brasil.
Palavras-chave: microbiota bacteriana; nasofaringe; SARS-CoV-2; síndrome gripal; síndrome respiratória aguda grave.
Grande área: Ciências Biológicas
Grande Área: Ciências da Saúde / Área: Saúde Coletiva / Subárea: Epidemiologia.
Grande Área: Ciências Biológicas / Área: Microbiologia.

2015 - 2019
Graduação em Ciências Biológicas.
Universidade Luterana do Brasil, ULBRA, Brasil.
Título: EFEITO DAS PROTEÍNAS DE SEMENTES DE Morinda citrifolia L. (Noni) EM FUNGOS..
Orientador: Arlete Beatriz Becker Ritt.
Bolsista do(a): PROGRAMA UNIVERSIDADE PARA TODOS, PROUNI, Brasil.

Formação Complementar

2022 - 2022
Revisão Sistemática e Meta-análise. (Carga horária: 40h).
Universidade Estadual de Campinas, UNICAMP, Brasil.

2022 - 2022
Curso de Bioinformática Módulo Bioinformática e R. (Carga horária: 5h).
Simbiosis Empresa Júnior de Ciências Biológicas, SIMBIOSIS EMPJR., Brasil.

2021 - 2022
Metagenômica. (Carga horária: 60h).
NÚCLEO DE APRIMORAMENTO CIENTÍFICO, NACIENTIFICO, Brasil.

2021 - 2022 Epidemiologia. (Carga horária: 40h).
Instituto Federal de Educação, Ciência e Tecnologia do Rio Grande do Sul, IFRS, Brasil.

2020 - 2020 BIOSSEGURANÇA EM TEMPOS DA COVID-19. (Carga horária: 2h).
Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil.

2020 - 2020 Doenças ocasionadas por vírus respiratórios emergentes,incluindo o COVID-19. (Carga horária: 4h).
Fundação Oswaldo Cruz, FIOCRUZ, Brasil.

2020 - 2020 Gerenciamento de Resíduos. (Carga horária: 60h).
Instituto Federal de Educação, Ciência e Tecnologia do Rio Grande do Sul, IFRS, Brasil.

2020 - 2020 CULTIVO CELULAR: UMA FERRAMENTA PARA A PESQUISA E PARA ASSISTÊNCIA EM SAÚDE. (Carga horária: 3h).
Universidade Luterana do Brasil, ULBRA, Brasil.

2020 - 2020 How to write a Review paper. (Carga horária: 2h).
Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil.

2020 - 2020 R para não programadores: analisando dados do TCGA. (Carga horária: 2h).
Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil.

2020 - 2020 Transforme sua pesquisa com illumina Next Generation Sequencing (NGS). (Carga horária: 2h).
Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil.

2020 - 2020 Preparatory Course (TOEFL & TOEIC Bridge) nível B1/B2. (Carga horária: 12h).
Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil.

2019 - 2019 Inglês. (Carga horária: 30h).
Instituto Federal de Educação, Ciência e Tecnologia do Rio Grande do Sul, IFRS, Brasil.

2019 - 2019 Juventudes, Participação e Cuidado com a Água. (Carga horária: 30h).
Ministério do Meio Ambiente, MMA, Brasil.

Atuação Profissional

Universidade Luterana do Brasil, ULBRA, Brasil.

Vínculo institucional

2018 - 2019 Vínculo: Bolsista, Enquadramento Funcional: Bolsista de Iniciação Cientifica, Carga horária: 20, Regime: Dedicação exclusiva.

Outras informações Bolsista de Iniciação Científica (CNPq), laboratório de Toxicidade Genética (Toxigen).

Vínculo institucional

2016 - 2018 Vínculo: Bolsista, Enquadramento Funcional: Bolsista de Iniciação Cientifica, Carga horária: 20, Regime: Dedicação exclusiva.

Outras informações Bolsista de Iniciação Científica (FAPERGS), laboratório de Toxicidade Genética (Toxigen).

Atividades

08/2018 - 08/2019 Pesquisa e desenvolvimento, Unidade universitária Canoas.
Linhas de pesquisa
Caracterização do perfil genotóxico de produtos do metabolismo de cianobactérias in vitro e in vivo

08/2016 - 08/2018 Pesquisa e desenvolvimento, Unidade universitária Canoas.
Linhas de pesquisa
Estudo da atividade citotóxica, genotóxica e antigenotóxica, in vitro, do Artepelin C.

Fundação Universidade Federal de Ciências da Saúde de Porto Alegre, UFCSPA, Brasil.

Vínculo institucional

2020 - Atual Vínculo: Bolsista, Enquadramento Funcional: Bolsista de Mestrado, Carga horária: 40, Regime: Dedicação exclusiva.

Atividades

08/2020 - Atual Pesquisa e desenvolvimento, Departamento de Farmacologia e Toxicologia.
Linhas de pesquisa
Estudos funcionais e estruturais de moléculas com potencial biotecnológico

Linhas de pesquisa

1. Estudo da atividade citotóxica, genotóxica e antigenotóxica, in vitro, do Artepelin C.

2. Caracterização do perfil genotóxico de produtos do metabolismo de cianobactérias in vitro e in vivo

3. Estudos funcionais e estruturais de moléculas com potencial biotecnológico
Objetivo: Esta linha tem como objetivo o estudo de substâncias de diversas origens, incluindo compostos isolados de extratos de plantas medicinais, extratos e secreções animais, alimentos ou fármacos, buscando seus potenciais biotecnológicos, utilizando diversos modelos experimentais, e a caracterização de suas estruturas e mecanismos de ação (imunológico/ farmacológico), assim como a avaliação da segurança de seu uso através de estudos de genotoxicidade..
Grande área: Ciências Biológicas

Grande Área: Ciências Biológicas / Área: Farmacologia.
Grande Área: Ciências Biológicas / Área: Microbiologia.
Palavras-chave: Microbiologia; Biotecnologia.

Áreas de atuação

1.	Grande área: Ciências Biológicas / Área: Genética.
2.	Grande área: Ciências Biológicas / Área: Farmacologia / Subárea: Toxicologia.
3.	Grande área: Ciências Biológicas / Área: Genética / Subárea: Mutagenese.
4.	Grande área: Ciências Biológicas / Área: Biotecnologia.
5.	Grande área: Ciências Biológicas / Área: Bioquímica / Subárea: Biologia Molecular.

Idiomas

Inglês	Compreende Bem, Fala Pouco, Lê Bem, Escreve Pouco.
Espanhol	Compreende Razoavelmente, Fala Pouco, Lê Pouco, Escreve Pouco.
Português	Compreende Bem, Fala Bem, Lê Bem, Escreve Bem.

Prêmios e títulos

2020	EXAME DE PROFICIÊNCIA EM LÍNGUA ESTRANGEIRA - INGLÊS, Centro Universitário Assis Gurgacz.

Produções

Produção bibliográfica

Artigos completos publicados em periódicos

Ordenar por

Ordem Cronológica

1. CARDOZO, TATIANE R. ; DE CARLI, RAÍNE F. ; SEEBER, ALLAN ; FLORES, WLADIMIR H. ; **DA ROSA, JORDANA A. N.** ; KOTZAL, QUEILA S. G. ; LEHMANN, MAURICIO ; DA SILVA, FERNANDA R. ; DIHL, RAFAEL R. . Genotoxicity of zinc oxide nanoparticles: an in vivo and in silico study. Toxicology Research JCR, v. 8, p. 277-286, 2019.

Citações: WEB OF SCIENCE ™ 8

Resumos expandidos publicados em anais de congressos

1. **ROSA, J. A. N.**; SOUZA, A. P. ; LEHMANN, M. ; DIHL, R. R. . ESTUDO DA ATIVIDADE ANTIMUTAGÊNICA DO ARTEPELIN C NO TESTE DE MICRONÚCLEOS COM BLOQUEIO DA CITOCINESE (CBMN). In: 4º ENCONTRO ULBRA DE BOLSISTAS CNPq E FAPERGS, 2018, CANOAS. 4º ENCONTRO ULBRA DE BOLSISTAS CNPq E FAPERGS, 2018.

2. **ROSA, J. A. N.**; PAZ, F. A. N. ; SOUZA, A. P. ; LEHMANN, M. ; DIHL, R. R. . AVALIAÇÃO DA ATIVIDADE MUTAGÊNICA DO ARTEPELIN C NO TESTE DE MICRONÚCLEOS IN VITRO. In: 3º ENCONTRO ULBRA DE BOLSISTA CNPq E FAPERGS, 2017, CANOAS. 3º ENCONTRO ULBRA DE BOLSISTAS CNPq E FAPERGS, 2017.

Resumos publicados em anais de congressos

1. SOUZA, A. P. ; **ROSA, J. A. N.** ; SCHARDOSIM, R. F. C. ; MIRI, J. M. ; GRIVICICH, I. ; DIHL, R. R. . INVESTIGATION OF CITOTOXICITY, MUTAGENICITY AND ANTIMUTAGENICITY OF ARTEPILIN C IN HUMAN GLIOMA CELLS. In: XIV CONGRESSO DA ASSOCIAÇÃO BRASILEIRA DE MUTAGÊNESE E GENÔMICA AMBIENTAL, 2019, BENTO GONÇALVES/RS. XIV CONGRESSO DA ASSOCIAÇÃO BRASILEIRA DE MUTAGÊNESE E GENÔMICA AMBIENTAL, 2019.

2. PORTA, C. ; **ROSA, J. A. N.** ; SOUZA, A. P. ; LEHMANN, M. ; DIHL, R. R. . CARACTERIZAÇÃO DO PERFIL GENOTÓXICO DE PRODUTOS DO METABOLISMO DE CIANOBACTÉRIAS. In: XIX FÓRUM DE PESQUISA CIENTÍFICA E TECNOLÓGICA, 2019, CANOAS. XIX FÓRUM DE PESQUISA CIENTÍFICA E TECNOLÓGICA, 2019.

3. SOUZA, A. P. ; HONATEL, K. F. ; **ROSA, J. A. N.** ; LEHMANN, M. ; DIHL, R. R. . INVESTIGATION OF THE CYTOTOXICITY AND GENOTOXICITY OF ArtC IN HUMAN CELL LINES. In: II LATIN AMERICAN CONGRESS OF CLINICAL AND LABORATORIAL TOXICOLOGY, 2018, PORTO ALEGRE. II LATIN AMERICAN CONGRESS OF CLINICAL AND LABORATORIAL TOXICOLOGY, 2018.

4. **ROSA, J. A. N.**; SOUZA, A. P. ; LEHMANN, M. ; DIHL, R. R. . ANTIMUTAGENIC EFFECT OF ARTEPILLIN C IN THE CYTOKINESES-BLOCK MICRONUCLEUS (CBMN) ASSAY. In: II LATIN AMERICAN CONGRESS OF CLINICAL AND LABORATORIAL TOXICOLOGY, 2018, PORTO ALEGRE. II LATIN AMERICAN CONGRESS OF CLINICAL AND LABORATORIAL TOXICOLOGY, 2018.

5. **ROSA, J. A. N.**. AVALIAÇÃO DA ANTIMUTAGENICIDADE DO ARTEPELIN C, IN VITRO, NO TESTE DE MICRONÚCLEOS COM BLOQUEIO DA CITOCINESE (CBMN). In: XXIV SALÃO DE INICIAÇÃO CIENTÍFICA E TECNOLÓGICA, 2018, CANOAS. XXIV SALÃO DE INICIAÇÃO CIENTÍFICA E TECNOLÓGICA, 2018.

6. **ROSA, J. A. N.**; SOUZA, A. P. ; LEHMANN, M. ; DIHL, R. R. . ESTUDO DA ATIVIDADE ANTIMUTAGÊNICA DO ARTEPELIN C. In: III ENCONTRO DO PPG BIOCIÊNCIAS & ENCONTRO DE PESQUISA EM BIOLOGIA CELULAR, 2018, PORTO ALEGRE. III ENCONTRO DO PPG BIOCIÊNCIAS & ENCONTRO DE PESQUISA EM BIOLOGIA CELULAR, 2018.
7. SOUZA, A. P. ; PAZ, F. A. N. ; **ROSA, J. A. N.** ; FREITAS, D. S. S. ; LEHMANN, M. ; DIHL, R. R. . ASSESSMENT OF THE MUTAGENIC POTENTIAL OF ARTEPILIN C ON HUMAN CELLS. In: XII CONGRESSO DA MUTAGEN-BRASIL, 2017, RIBEIRÃO PRETO-SP. XII CONGRESSO DA MUTAGEN-BRASIL, 2017.
8. **ROSA, J. A. N.**; PAZ, F. A. N. ; SOUZA, A. P. ; LEHMANN, M. ; DIHL, R. R. . INVESTIGAÇÃO DA GENOTOXICIDADE DO ARTEPELIN C EM CÉLULAS HEPG2. In: XXIV MOSTRA UNISINOS DE INICIAÇÃO CIENTÍFICA E TECNOLÓGICA, 2017, SÃO LEOPOLDO. XXIV MOSTRA UNISINOS DE INICIAÇÃO CIENTÍFICA E TECNOLÓGICA, 2017.
9. **ROSA, J. A. N.**. AVALIAÇÃO DA MUTAGENICIDADE DO ARTEPELIN C NO TESTE DE MICRONÚCLEOS COM BLOQUEIO DA CITOCINESE. In: XXII SALÃO DE INICIAÇÃO CIENTÍFICA E TECNOLÓGICA, 2017, CANOAS. XXII SALÃO DE INICIAÇÃO CIENTÍFICA E TECNOLÓGICA, 2017.
10. **ROSA, J. A. N.**. ESTUDO DA ATIVIDADE CITOTÓXICA, GENOTÓXICA E ANTIGENOTÓXICA, IN VITRO, DO ARTEPELIN C. In: XVI FÓRUM DE PESQUISA CIENTÍFICA E TECNOLÓGICA, 2016, CANOAS. XVI FÓRUM DE PESQUISA CIENTÍFICA E TECNOLÓGICA, 2016.

Eventos

Participação em eventos, congressos, exposições e feiras

1. Encontro Anual do Grupo Arthromint.MICROBIOTA DO TRATO RESPIRATÓRIO SUPERIOR DE PACIENTES ACOMETIDOS POR INFECÇÕES RESPIRATÓRIAS AGUDAS E SUA ASSOCIAÇÃO COM SARS-CoV-2. 2022. (Encontro).
2. I simpósio de Imunoterapia para Câncer. 2022. (Simpósio).
3. Simpósio de Pós-Graduação em Ciências da Saúde. 2022. (Simpósio).
4. Ciclo de Palestras sobre Meio Ambiente e Saúde - SEMANA DO MEIO AMBIENTE DA UFCSPA 2021,. 2021. (Encontro).
5. Curso de Comunicação e Escrita Científica. 2021. (Simpósio).
6. II Brazilian South Symposium on Neuroscience. 2021. (Simpósio).
7. II Congresso UFCSPA: conectando experiências em saúde global. 2021. (Congresso).
8. III Seminário de Internacionalização da UFCSPA - "Como podemos melhorar o mundo juntos?". 2021. (Seminário).
9. INTRODUÇÃO À BIOINFORMÁTICA E BANCOS DE DADOS BIOLÓGICOS". 2021. (Encontro).
10. Minicurso de Bioinformática-Alinhamento de amostras. 2021. (Outra).
11. UFCSPA - Descubra um rico conteúdo de textos completos e enriqueça sua pesquisa com ScienceDirect. 2021. (Seminário).
12. Universidades, Inovação e Combate à Pandemia da COVID-19",. 2021. (Outra).
13. VI Encontro do PPG Biociências. 2021. (Encontro).
14. VI Encontro do PPG Biociências.MICROBIOTA DO TRATO RESPIRATÓRIO SUPERIOR DE PACIENTES ACOMETIDOS POR INFECÇÕES RESPIRATÓRIAS AGUDAS E SUA ASSOCIAÇÃO COM SARS-CoV-2. 2021. (Encontro).
15. WORKSHOP DIVULGAMICRO DE COMUNICAÇÃO e DIVULGAÇÃO CIENTÍFICA. 2021. (Encontro).
16. BioIn4Girls - Ciclo de Palestras em Bioinformática. 2020. (Encontro).
17. I Workshop Online de Bioinformática (WOB20). 2020. (Outra).
18. JORNADA VIRTUAL: Riesgo en Salud con el marco de la Resiliencia al Cambio Climático en Latinoamérica y El Caribe. 2020. (Encontro).
19. MINICURSO ENGENHARIA DE TECIDOS E CULTIVO CELULAR. 2020. (Seminário).
20. Simpósio de Doenças de Base Genética e Medicina Canabinoide. 2020. (Simpósio).
21. VACINAÇÃO E COVID-19- O ESTADO DA ARTE. 2020. (Seminário).
22. V Biosciences Meeting ? Crossing Borders.Microbioma & COVID-19. 2020. (Encontro).
23. PALESTRA PERÍCIA CRIMINAL E LOCAL DO CRIME. 2019. (Outra).
24. XIV CONGRESSO DA ASSOCIAÇÃO BRASILEIRA DE MUTAGÊNESE E GENÔMICA AMBIENTAL. INVESTIGATION OF CITOTOXICITY, MUTAGENICITY AND ANTIMUTAGENICITY OF ARTEPILIN C IN HUMAN GLIOMA CELLS. 2019. (Congresso).
25. XIX FÓRUM DE PESQUISA CIENTÍFICA E TECNOLÓGICA EXPOULBRA. CARACTERIZAÇÃO DO PERFIL GENOTÓXICO DE PRODUTOS DO METABOLISMO DE CIANOBACTÉRIAS. 2019. (Exposição).
26. 4º ENCONTRO ULBRA DE BOLSISTA CNPq E FAPERGS.ESTUDO DA ATIVIDADE ANTIMUTAGÊNICA DO ARTEPELIN C NO TESTE DE MICRONÚCLEOS COM BLOQUEIO DA CITOCINESE (CBMN). 2018. (Encontro).
27. 50º DEFESA DE DOUTORADO: AVALIAÇÃO DA ATIVIDADE ANTIMUTAGÊNICA DO RESVERATROL ATRAVÉS DO TESTE SMART EM DROSOPHILA MELANOGASTER. 2018. (Seminário).
28. 58º DEFESA DE TESE DE DOUTORADO: AVALIAÇÃO DA ATIVIDADE ANTIMUTAGÊNICA DOS ÁCIDOS CLOROGÊNICOS 3-ACQ E 5-ACQ E DOS ÁCIDOS CAFEICO E QUÍNICO ATRAVÉS DO TESTE SMART EM DROSOPHILA MELANOGASTER. 2018. (Seminário).
29. 96º SEMINÁRIO FORMAL DE APRESENTAÇÃO DE MESTRADO: AVALIAÇÃO DO POTENCIAL MODULADOR DAS PRÓPOLIS VERDE E MARROM SOBRE OS DANOS GENÉTICOS INDUZIDOS PELO ETIL METANOSULFONATO ATRAVÉS DO TESTE SMART EM DROSOPHILA MELANOGASTER. 2018. (Seminário).
30. APRESENTAÇÃO DE TCC DO CURSO DE CIÊNCIAS BIOLÓGICAS: ANÁLISE DA PARTICIPAÇÃO PÚBLICA NA FISCALIZAÇÃO E CONTROLE AMBIENTAL DA CIDADE DE MONTENEGRO/RS DA. 2018. (Outra).
31. APRESENTAÇÃO DE TCC DO CURSO DE CIÊNCIAS BIOLÓGICAS: AVALIAÇÃO DA GENOTOXICIDADE EM GASTRÓPODES EXPOSTOS EM ÁREA DE MINERAÇÃO DE CARVÃO A CÉU ABERTO (CANDIOTA). 2018. (Outra).
32. APRESENTAÇÃO DE TCC DO CURSO DE CIÊNCIAS BIOLÓGICAS: AVALIAÇÃO DA GENOTOXICIDADE E MUTAGENICIDADE EM MORCEGOS QUE VIVEM NA ÁREA DE MINERAÇÃO E QUEIMA DE CARVÃO (CANDIOTA-RS). 2018. (Outra).
33. APRESENTAÇÃO DE TCC DO CURSO DE CIÊNCIAS BIOLÓGICAS:ESTUDO ANATÔMICO DAS ESPÉCIES DO GÊNERO AKODON (RODENTIA, SIGMODONTINAE) COM OCORRÊNCIA NO RIO GRANDE DO SUL, BRASIL: VARIAÇÃO MORFOLÓGICA CRANIANA E DISTRIBUIÇÃO GEOGRÁFICA. 2018. (Outra).
34.