

Research Article

A pilot study of the impact of bacterial and fungal coinfections on mildly ill COVID-19 patients

Anastasia Vaganova¹^a, Danial Djulanov², Marina Uvarova³, Diana Zaitseva⁴, Diana Safarova⁵, Andrei Ivanov^{6,7}

¹ Institut of Translational Biomedicine, St Petersburg University, ² Infection Department, Luga Intredistrict Hospital, ³ Clinic Laboratory, North-West Centre for Evidence-Based Medicine, ⁴ Faculty of Pediatrics, St Petersburg State Pediatric Medical University, ⁵ Faculty of Biology, St Petersburg University, ⁶ Hospital, St Petersburg University, ⁷ Department of Histology, St Petersburg State Pediatric Medical University

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Abstract

Background

Coinfections and superinfections significantly impair prognosis in severely ill COVID-19 patients who may develop ventilator-associated pneumonia. However, the role of bacterial and fungal infections and/or lung colonization in patients with moderate COVID-19 who are not on mechanical ventilation remains controversial. Additionally, there is limited data on the impact of coinfections on pneumonia development in vaccinated subjects. To clarify this question, we summarize the data for patients treated in the single infectious department for a moderate form of COVID-19-associated pneumonia.

Methods

We evaluated the association of the medical condition on hospital admission and disease duration with anti-*Chlamydomphila pneumoniae* and anti-*Mycoplasma pneumoniae* quantitative IgM and sputum culture results in COVID-19 in patients (n=271).

Results

Non-pneumococcal *Streptococci* were the most frequent bacteria isolated from sputum (70% of the population; only one case of *St. pneumoniae*), followed by *Candida albicans* (15.6% of the population) and *Neisseria* spp. (13% of the population). Airway colonization with *C. albicans* and anti-*M. pneumoniae* IgM seropositivity was significantly associated with a higher CT score, especially in vaccinated patients; meanwhile, fungal pathogen *C. albicans* colonization was associated with prolonged hospital duration. Airway colonization with *C. albicans* was associated with slightly longer disease duration.

Conclusion

The results demonstrate that respiratory pathogens, at least *M. pneumoniae*, can contribute to the risk of COVID-19 onset and/or severity in the vaccinated population. Meanwhile, neither bacterial agents of atypical pneumonia nor lung colonization with opportunistic pathogens are essential for recovery in patients with moderate COVID-19 infection when appropriate treatment is provided.

INTRODUCTION

Respiratory viral infections are commonly associated with secondary bacterial pneumonia. At the beginning of 2020, in Russia, secondary infections were identified in 59.55% of influenza A cases, 23.63% of influenza B cases, 21.26%

of respiratory syncytial virus cases, and 42.42% of metapneumovirus cases.¹ The frequency of bacterial infection in adult COVID-19 patients varies from 5 to 9% in reports from Europe to 45.5% in Thailand and 55.4% in the USA.² The difficulty in differentiating a true infection from colonization may lead to overdiagnosis of bacterial infections.

a Corresponding author at: a.n.vaganova@spbu.ru

Taking into account this source of bias, up to 91.8% of COVID-19 patients may be considered to have bacterial co- or secondary infections, and up to 23.3% may be secondary infected by fungal pathogens,^{3,4} especially *Candida* spp.^{2,5}

A series of preventive and control measures for SARS-CoV-2 during the COVID-19 pandemic contained the spread of SARS-CoV-2 and decreased the infection of other pathogens, including *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*,⁶ *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis*.⁶⁻⁸ Therefore, in earlier reports, its frequency is low, with prevalence near 21% for bacterial and 12% for fungal co- or superinfections.⁹ Meanwhile, even at the beginning of the COVID-19 pandemic, in China, up to 80% of SARS-CoV-2-infected subjects had IgM positivity against at least one other respiratory agent.¹⁰ Following the reduction of pandemic restrictions, a resurgence of respiratory pathogen-spread cases appeared.^{7,8,11}

Bacterial pneumonia is a common coinfection in COVID-19 patients, most frequently caused by *H. influenzae*, *St. pneumoniae* or *Staphylococcus aureus*. Other opportunistic pathogens, such as *Klebsiella* spp., *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Escherichia coli*, were also identified as predominant co- or super-infection causative agents in several studies.^{2,4,8,12-16} All these microorganisms may be community-acquired, persistent causative agents of an underlying chronic infection, or nosocomial.^{1,12} Such pathogens as *St. pneumoniae* and *H. influenzae* were found in the hospital aerosols.¹⁷ While *St. pneumoniae* remained the main causative agent of secondary pneumonia acquired out of hospital, *S. aureus* and *H. influenzae* often cause hospital-acquired pneumonia.¹

Because bacterial coinfections and superinfections are widespread, empirical use of antibacterials specifically at the onset of the COVID-19 infection is justified.⁹ Coinfections or secondary infections in COVID-19 patients are associated with a poor prognosis.¹⁸ These patients also have a higher risk of admission to the ICU, septic shock, increased maximum CRP and ferritin values, higher proportions with fever, purulent sputum, peripheral leukocytosis, and consolidative opacity, and a longer duration of therapy.^{1-4,12,16,19,20}

Meanwhile, it is suggested that bacterial infections may play a less relevant role in non-severe COVID-19.²¹ In the present study, we estimate the diversity of bacterial and fungal respiratory pathogens in patients with moderate forms of COVID-19 who were hospitalized but did not need ICU (except four patients) or mechanical ventilation. The purpose of this research was to evaluate the impact of coinfection on the severity of COVID-19, particularly in vaccinated individuals who are expected to be protected against the coronavirus infection.

MATERIALS AND METHODS

DATA COLLECTION

We retrospectively analyzed the clinical, immunological, and microbiological findings in patients with COVID-19 (n

= 271), including COVID-19 and *Ch. pneumoniae* coinfection (n = 81), and COVID-19 and *M. pneumoniae* (n = 72) coinfection treated in the State Budgetary Institution of Healthcare in the Leningrad Region “Luga Interdistrict Hospital” in 2021–2022. In three subgroups, i.e., only COVID-19, COVID-19 and *Ch. pneumoniae* coinfection, and COVID-19 and *M. pneumoniae* coinfection, patients were selected randomly to reach the numbers 100, 100, and 100 subjects per group, respectively; however, part of the population was excluded from the analysis because of the lack of microbiological data.

All patients were examined at admission, and the severity of the lung involvement was estimated on the computed tomography (CT). The CT score was assessed as 0 for < 5% involvement, 1 for 5%–25% involvement, 2 for 26%–49% involvement, 3 for 50%–75% involvement, and 4 for > 75% involvement. The National Early Warning Score (NEWS) was estimated for all patients at admission according to NEWS2 standards.²² Data for CT score, NEWS, hospitalization duration, ICU, and previous anti-SARS-CoV-2 vaccination were collected from medical charts. None of the patients had *Mycobacterium tuberculosis* DNA in sputum.

Data for age, sex, sputum culture, anti-*Ch. pneumoniae* IgM, and anti-*M. pneumoniae* IgM were collected in the Laboratory Information System (LIS) of the North-West Centre for Evidence-Based Medicine laboratory as part of the routine diagnostic workflow and then re-obtained for the present study. All data were anonymized for the analysis.

All procedures performed in the study were in accordance with the ethical standards of the institutional research committee and national standards. For this type of retrospective study, formal consent is not required.

MICROBIAL ISOLATION AND IDENTIFICATION

The matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS) method was used for the identification of microorganisms in sputum. Clinical samples were cultivated in Columbia blood agar for 24 hours and Sabouraud agar, followed by colony transferring to the sample plate, matrix adding, and drying. MALDI Microflex Biotyper (Bruker Daltonics, Bremen, Germany) was used with α -cyano-4-hydroxycinnamic acid matrix according to the manufacturer’s guidelines.²³

Following national guidelines for laboratory diagnosis of community-acquired pneumonia, MUK 4.2.3115-13,²⁴ samples with 10⁵ CFU/mL or higher are considered positive.

AUTOMATED ELISA FOR ANTI-MYCOPLASMA PNEUMONIAE AND ANTI-CHLAMYDOPHILA PNEUMONIAE IGM EVALUATION

At admission, blood samples were collected to estimate anti-*M. pneumoniae* and anti-*Ch. pneumoniae* IgM levels. Blood samples were studied in the North-West Centre for Evidence-Based Medicine. ELISA test kits “CHORUS MYCOPLASMA PNEUMONIAE IgM” (DIESSE Diagnostica Senese, Italy) and “CHORUS CHLAMYDOPHILA PNEUMONIAE IgM” (DIESSE Diagnostica Senese, Italy) were applied to automatic testing with Chorus Trio (DIESSE Diagnostica

Senese, Italy) according to the manufacturer's guidelines. The samples with INDEX (ratio between the OD value of the sample and that of the cut-off) above 1.1 were considered positive.

STATISTICAL ANALYSIS

To estimate the impact of identified pathogens on CT score, NEWS value, and hospitalization duration, we evaluated the normality of values distribution using the Anderson–Darling test with the nortest R package (version 1.0-4). The test rejects the hypothesis of normality with a p -value less than 0.05. Since the hypothesis of normality was rejected, differences between groups were assessed for significance using the Mann–Whitney–Wilcoxon test. Mean value and standard deviation (SD) were also estimated. Data were visualized with the ggplot2 R package.²⁵

The microbial co-occurrence was analyzed and visualized using cooccur v.1.3. R package.²⁶ Only genera (or species) identified in at least five samples per group were analyzed.

RESULTS

STUDY POPULATION

The study population included 271 COVID-19 patients, who were treated in the infectious department of the Luga Interdistrict Hospital. Although most patients were from the Luga district of the Leningrad region of Russia, some patients were from the nearby parts of the Leningrad region, especially the Gatchina district and Slantsy district, and Saint Petersburg. The details for CT score, NEWS value, duration of hospitalization, age, and sex distribution in the population are summarized in [Table 1](#).

The CT score and NEWS values were favourable, and the duration of hospitalization was shorter in the vaccinated subjects ($p < 0.05$). Considering the potential differences in the disease progression in vaccinated and non-vaccinated patients, we further studied these groups separately. No differences in pathogen distribution were identified between vaccinated and unvaccinated groups ([Figure 1a](#)). In both groups, non-pneumococcal *Streptococci* were the predominant airway-colonizing bacteria (70% of the entire population; only one case of *St. pneumoniae*), followed by *C. albicans* (15.6% of the entire population) and *Neisseria* spp. (13% of the entire population).

When we evaluated the co-occurrence of different microorganisms in the population, we revealed the association between respiratory tract colonization by *Neisseria* spp. and *Streptococcus* spp., *Neisseria* spp. and *Rothia* spp. (including *R. mucilaginosa*, $n = 36$, and *R. dentocariosa*, $n = 5$), and *Neisseria* spp. and *Haemophilus* spp. (including *H. influenzae*, $n = 1$; *H. parainfluenzae*, $n = 10$; *H. haemolyticus*, $n = 6$; *H. parahaemolyticus*, $n = 1$), which was significant only in unvaccinated patients ([Figure 1bc](#)). Furthermore, *M. pneumoniae* coinfection was negatively associated with airway colonization by *Rothia* spp. The identified coexclusion between *Ch. pneumoniae* and *M. pneumoniae* may be

associated with selection bias and should be interpreted with caution. *Ch. pneumoniae* and *M. pneumoniae* IgM-positive patients were revealed in both groups (nine subjects were unvaccinated and three were vaccinated).

ASSOCIATION BETWEEN COINFECTIONS OR AIRWAY COLONIZATION BY OPPORTUNISTIC PATHOGENS AND DISEASE SEVERITY ON ADMISSION

Pathogens that were identified in less than 10% of the population were excluded from the analysis to avoid bias related to high group inequality. The association between *C. albicans*, *Ch. pneumoniae*, *M. pneumoniae*, and *Streptococcus* spp. infection or airway colonization with disease severity expressed in numerical form as CT score and NEWS was estimated. Because of the slightly higher frequency of *Rothia* spp. in unvaccinated patients (12%), we also evaluated its effect in this group.

In the entire population, lower respiratory tract colonization with *C. albicans* and anti-*M. pneumoniae* IgM positivity was significantly associated with higher CT scores, i.e., more pronounced lung damage ($p < 0.05$, [Figure 2a](#)). The association between anti-*M. pneumoniae* IgM positivity and lung damage is reproduced in vaccinated patients ($p < 0.05$, [Figure 2c](#)), but does not occur in the unvaccinated group ([Figure 2b](#)). For *C. albicans*, the association with more pronounced lung damage lost the statistical significance when both subgroups were analyzed separately. Also, in unvaccinated patients, we identified a negative association between colonization by *Rothia* spp. and a higher CT score ($p < 0.05$).

No associations with worse NEWS values were identified for any pathogen included in the analysis, both in vaccinated and unvaccinated patients.

ASSOCIATION BETWEEN COINFECTIONS OR AIRWAY COLONIZATION BY OPPORTUNISTIC PATHOGENS AND THE DURATION OF HOSPITAL STAY

When we estimated the impact of *M. pneumoniae*, *Ch. pneumoniae*, and the most frequent ($>10\%$ of cases) airway-colonizing bacteria on the hospitalization duration in our study group, neither atypical pneumonia causative agents nor colonization with opportunistic pathogenic bacteria were associated with prolonged hospital duration in the studied patient group. Meanwhile, the patients in whom *C. albicans* was isolated from sputum stayed in hospital a bit longer than others (11.8 ± 5.66 days vs. 13.6 ± 6.07 days, $p < 0.05$, [Figure 3a](#)). However, this tendency did not reach statistical significance when the groups of vaccinated and non-vaccinated patients were analyzed separately ([Figure 3bc](#)).

DISCUSSION

Commonly, the lung microbiome exhibits dynamic fluxes of microbial immigration and clearance. Thus, it demonstrates a fluid nature rather than a fixed one.²⁸ SARS-CoV-2 damages airway epithelium, promotes inflammation, and deregulates immunity and interferon counteraction towards bacterial colonization and proliferation.² Antimicro-

Table 1. Patient characteristics.

Group	Entire population	Unvaccinated*	Vaccinated
<i>n</i>	271	133	51
Age			
Mean±SD	61.7±16.55	62.7±16.7	61.7±15.91
Range	9-90	19-87	9-90
Gender			
Male	95	81	29
Female	136	52	23
Clinical and laboratory data			
CT score (mean±SD)	1.33±1.055	1.48±0.994	0.88±0.993**
NEWS (mean±SD)	3.42±2.135	3.46±2.144	2.84±2.23**
Duration of hospitalization (mean±SD)	12.01±5.75	12.3±5.44	10.7±5.78**
Anti- <i>Ch. pneumoniae</i> IgM seroprevalence***	81 (35%)	46 (34.6%)	19 (37.3%)
Anti- <i>M. pneumoniae</i> IgM seroprevalence***	72 (31.2%)	45 (33.8%)	16 (31.4%)
Sputum positivity for opportunistic pathogens	199 (86.1%)	116 (87.2%)	47 (92.2%)

* Data about vaccination was available only for a part of the population

**The difference between unvaccinated and vaccinated subjects is significant, i.e., $P < 0.05$

***These proportions are significantly biased by the preselection as described in the main text for the purpose of the present study and do not mirror the frequency of IgM in Luga Hospital inpatients, which was approximately 5.21% for *M. pneumoniae* and 6.67% for *Ch. pneumoniae*, as described elsewhere.²⁷

bial resistance of pathogens, aggressive use of immunomodulatory therapies, and overuse of antibiotics could contribute to the emergence of lung colonization by pathogens and secondary infections.^{13,21}

In our study, we identified a high frequency (86.1%) of lung colonization by bacteria or fungi; however, it was mostly opportunistic pathogens or commensal bacteria, like *Neisseria sp.* Also, despite the dynamic nature of lung microbiota, we identified several trends in microbial co-occurrence and co-exclusion in unvaccinated patients, and some of them may have clinical significance, as discussed below.

It is suggested that, despite the high risks related to superinfections in severely ill COVID-19 patients, these infections play a less relevant role in the early, non-severe stages of COVID-19.²⁹ In our data, we also did not find any correlations between patients' condition (i.e., CT score and NEWS) and the majority of opportunistic pathogens isolated from sputum in follow-up. However, in our population, we revealed the association between suggested *M. pneumoniae* coinfection (i.e., anti-*M. pneumoniae* IgM positivity at hospital admission) in COVID-19 patients and the grade of lung damage on CT. This relation is significantly more evident in vaccinated patients. The symptoms of COVID-19 and *M. pneumoniae*-associated pneumonia are similar, with fever, cough, and shortness of breath, and these diseases demonstrate similar radiologic findings.³⁰⁻³² Despite the importance and informativeness of CT in COVID-19 diagnosis, the sensitivity and specificity of pure ground-glass opacities are not very high, because many diseases similarly exhibit ground-glass opacities on chest imaging, including acute lupus pneumonia, diffuse interstitial pneumonia, pulmonary collagen vascular disease, acute

eosinophilic pneumonia, and atypical pathogen infection in the lungs.^{33,34}

M. pneumoniae infections are commonly comorbid with respiratory viral diseases.³⁵ We suggest that in *M. pneumoniae*-infected anti-SARS-CoV-2-vaccinated COVID-19 patients, this atypical pathogen may have a significant impact on disease severity in a notable proportion of patients who are protected from COVID-19 by immunization. Under previously published data, coinfection with *M. pneumoniae* is associated with higher mortality, length of hospital stay, and the need for mechanical ventilation in COVID-19 patients.³⁶⁻³⁸ Meanwhile, in our population, we did not reveal any associations between *M. pneumoniae* coinfection and the duration of hospitalization, apparently because of successful empirical fluoroquinolone treatment and mild illness severity.

As was previously identified, approximately 10% of hospitalized COVID-19 patients have positive respiratory fungal cultures across multiple studies, and the *Candida* spp. is the most frequently isolated fungal pathogen.²⁰ In patients from the Luga hospital, we identified a slight but significant impact of *C. albicans* on the duration of the disease. All patients received antibiotics from the first day of hospitalization; however, no antifungal treatment was administered prior to microbiological testing, and this is the most probable reason for the identified association. The positive association between an unfavourable CT score at hospital admission and a high *C. albicans* CFU number in sputum indicates that in the studied population, this pathogen may have originated from the community or patients' commensal microflora rather than from a nosocomial environment.

Suddenly, we revealed the association between more favourable CT and *Rothia* spp. isolation from sputum in the follow-up. Previously, data demonstrated the controversial

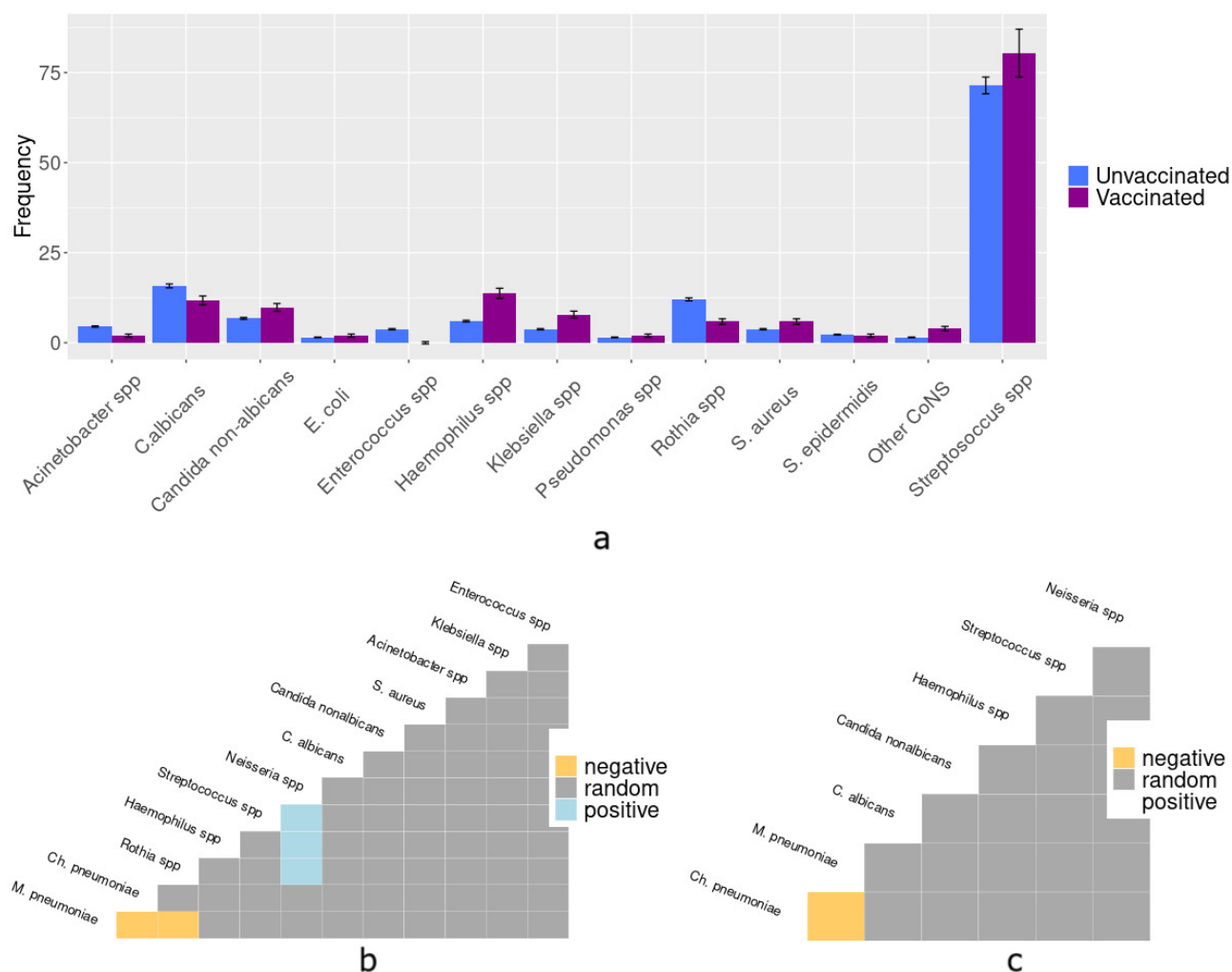


Figure 1. Frequency (a) (whiskers represent the confidence interval) and co-occurrence of different microorganisms in the sputum of unvaccinated (b) and vaccinated (c) COVID-19 patients. Pairs of bacterial genera or species that are more frequently cooccurring than expected are marked by blue, and pairs that are less frequently cooccurring than expected are marked by yellow ($p < 0.05$). CoNS – coagulase-negative staphylococci (*S. epidermidis* is represented separately and not included in this group on this plot).

role of this opportunistic pathogen in COVID-19 patients. It was identified that great *Rothia* spp. abundance may be associated with higher lung damage or higher risk of hospitalization,⁵ but inversely associated with severe COVID-19.³⁹ Apparently, *Rothia* spp does not develop severe illness in immunocompetent moderately ill COVID-19 patients who receive appropriate antibiotic therapy.⁴⁰ Furthermore, in our study, we revealed coexclusive relationships between anti-*M. pneumoniae* IgM and isolation of *Rothia* spp. from sputum. As described above, anti-*M. pneumoniae* IgM-positive patients have more pronounced ground-glass opacities on CT. Therefore, it cannot be excluded that the negative association of *Rothia* spp. and lung damage may be prone to bias related to the selection mode.

LIMITATIONS

The present study's major limitation is its focus on a narrow, homogeneous group of patients with moderately severe disease, all treated in the same department. The group

of patients is relatively small and does not allow for estimating the impact of some pathogens on disease severity and hospitalization duration. Also, there is some disproportion in the patient selection. So, in our group, 31% of patients have anti-*M. pneumoniae* IgM and 35% of patients have anti-*Ch. pneumoniae* IgM, despite the actual proportion of such patients in Luga Hospital being 5.21% and 6.67%, respectively.²⁷ Furthermore, the sputum samples were acquired for a microbiological study on different hospitalization days, which does not allow us to differentiate nosocomial infections from community-acquired cases. Similarly, it is difficult to distinguish a true infection from colonization, which may lead to an overdiagnosis of bacterial infections.¹⁸ especially in such samples as sputum, which is inevitably contaminated by oral flora. In the present study, we apply 10^5 CFU/mL as the detection limit for a positive sputum culture according to national guidelines for laboratory diagnosis of community-acquired pneumonia, MUK 4.2.3115-13 to discriminate clinically significant microbial growth.²⁴

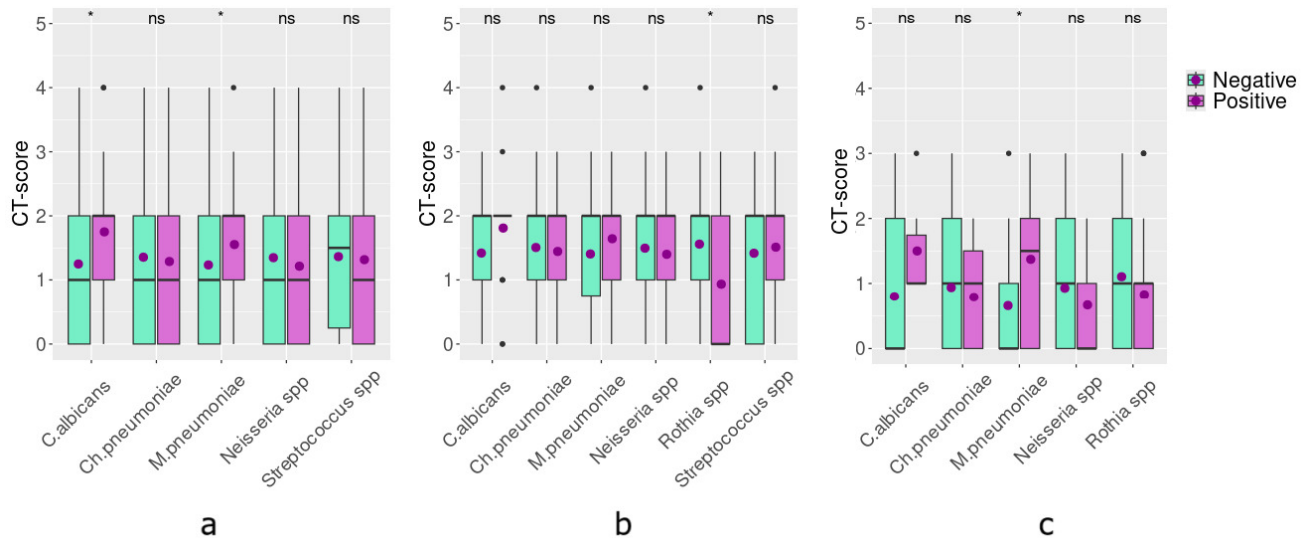


Figure 2. The impact of *Mycoplasma pneumoniae*, *Chlamydothyla pneumoniae*, and opportunistic pathogens on the lung damage grade identified by CT in the entire study group (n = 230, a), unvaccinated (n = 132, b), and vaccinated (n = 51, c) patients. Boxes represent the interquartile ranges (IQRs) between the first and third quartiles, and the line inside the box represents the median value. Whiskers represent the lowest or highest values within values 1.5 times the IQR from the first or third quartiles. Dots represent outliers.

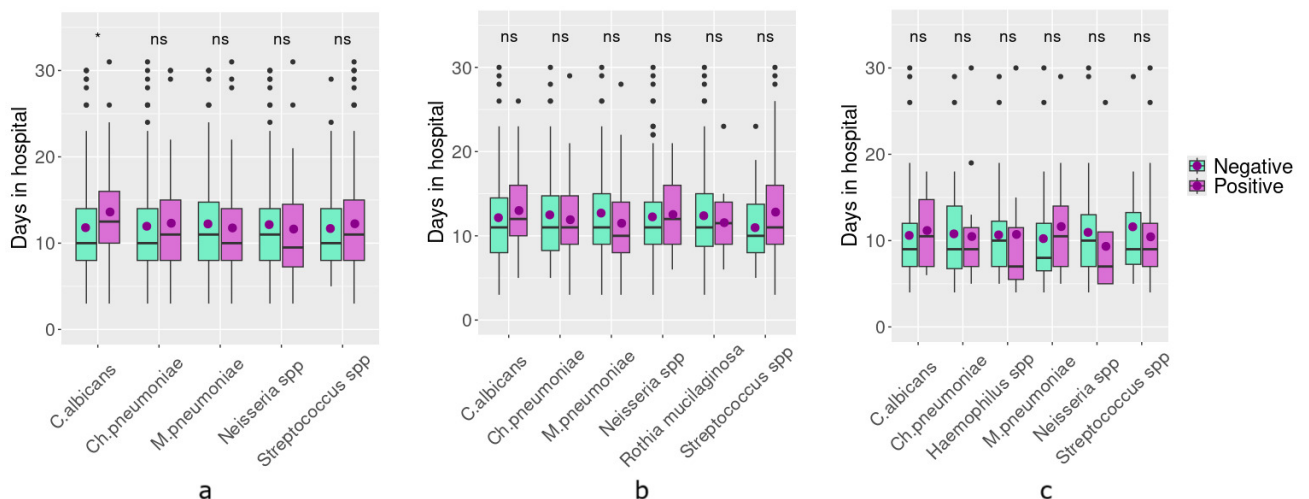


Figure 3. The impact of *Mycoplasma pneumoniae*, *Chlamydothyla pneumoniae* and opportunistic pathogens on the hospitalization duration in the entire study group (n = 230, a), unvaccinated (n = 132, b) and vaccinated (n = 51, c) patients. Boxes represent the interquartile ranges (IQRs) between the first and third quartiles, and the line inside the box represents the median value. Whiskers represent the lowest or highest values within values 1.5 times the IQR from the first or third quartiles. Dots represent outliers.

Despite these limitations, we confirm the level of pathogenic microorganism coinfection and superinfections is higher among COVID-19 patients, including subjects with moderate disease. Also, we revealed that coinfection, like *M. pneumoniae*, may significantly impact the disease severity in vaccinated patients. Meanwhile, the empirical use of antibacterial treatment offsets the negative impact of these pathogens in a moderate form of COVID-19 infection.

CONCLUSION

Considering the wide distribution of bacterial and fungal coinfections and superinfections in COVID-19 patients, we estimated the impact of the bacterial pathogens *Ch. pneumoniae* and *M. pneumoniae* and lung colonization with opportunistic pathogens from normal microbiota on the disease severity and duration in patients with moderate COVID-19 infection. We have revealed a negative effect of *M. pneumoniae* coinfection on lung appearance on CT; however, the general state didn't get worse in patients coin-

fects with SARS-CoV-2 and *M. pneumoniae*. The identified lung damage may mirror specific features of *M. pneumoniae*-related disease rather than a serious health risk. In view that this association is more pronounced in vaccinated patients, it may be speculated that the coinfection with *M. pneumoniae* rather than COVID-19 may be the reason for pneumonia onset and hospitalization, at least in vaccinated patients. Another important issue is a slight but significant association of *C. albicans* coinfection and longer hospitalization duration. The studied patient group was empirically treated with antibiotics from the first day of hospitalization, including third-generation cephalosporins and macrolides or fluoroquinolones. The delayed antifungal medication may be the reason for the identified *C. albicans* colonization effect. However, the increase in disease duration was not too high, and *C. albicans* coinfection was not too frequent to assume that empiric antifungal treatment would be particularly beneficial in this population.

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CONFLICT OF INTEREST

All authors have completed the ICMJE uniform disclosure form and declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

DATA ACCESSIBILITY STATEMENT

All data underlying the results are available as part of the article, and no additional source data are required.

ETHICAL APPROVAL

All procedures performed in the study were in accordance with the ethical standards of the institutional research committee and national standards. For this type of retrospective study, formal consent is not required.

AI STATEMENT

The authors confirm that no generative AI or AI-assisted technology was used to generate content.

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