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DOCTORAL THESIS

The Impact of Multiplex PCR in Diagnosing and Managing Bacterial and Fungal Infections in COVID-19 Patients

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STUDY 1: THE IMPACT OF MULTIPLEX PCR IN DIAGNOSING AND MANAGING BACTERIAL INFECTIONS IN COVID-19 PATIENTS SELF-MEDICATED WITH ANTIBIOTICS.

CONTEXT

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), emerged in late 2019 and swiftly became a global pandemic. Most COVID-19 cases are moderate respiratory illnesses, but complications can arise, including coinfections, acute respiratory distress syndrome, and sepsis. Coinfections with other respiratory diseases are common, and variations may depend on the SARS-CoV-2 variant, presenting significant challenges for diagnosis and treatment. Reports show a varying range of coinfection rates in patients, but exact data on community-acquired bacterial coinfections is limited. It's crucial to identify pathogens in coinfected COVID-19 patients due to their potential to increase disease severity and mortality.

Coinfections with SARS-CoV-2 usually occur simultaneously, while superinfections develop as the disease progresses. Despite this distinction, there's inconsistent reporting in literature, resulting in varied data on coinfection and superinfection rates. A noted study found that 3.5% of COVID-19 patients had a bacterial coinfection on admission, with 14.3% developing a superinfection during their hospital stay. Ventilator-associated pneumonia rates are notably higher in COVID-19 patients than non-COVID-19 patients. Common coinfecting pathogens include Streptococcus pneumoniae, Staphylococcus aureus, and various respiratory viruses. Coinfections with these viruses can worsen the clinical course, leading to heightened disease severity.

Using multiplex polymerase chain reaction (PCR) panels can quickly detect respiratory infections, aiding in treatment decisions. Sampling from the respiratory tract is vital for detecting organisms in patients developing complications. Modern PCR-based tests help diagnose bacterial infections concurrent with or following SARS-CoV-2 infection. The rise of antibiotic resistance, exacerbated by self-medication, poses global health risks. The ongoing study aims to assess if routine multiplex PCR screenings can help doctors identify secondary bacterial infections more accurately in self-medicating patients and predict the COVID-19 clinical trajectory.

SUMMARY OF FINDINGS

Data collected from 489 hospitalized COVID-19 patients who suffered a secondary bacterial infection revealed that 198 of them self-medicated with antibiotics before hospitalization. Notably, those who took antibiotics had a higher prevalence of pulmonary diseases like chronic bronchitis and COPD. The most commonly self-administered antibiotics were cephalosporins, macrolides, and penicillin, mainly sourced over-the-counter. Patients who self-medicated faced worse outcomes, with higher instances of ventilator-associated pneumonia, more severe bacterial superinfections, increased COVID-19 severity, and prolonged ICU stays.

Upon comparing the biological parameters of antibiotic users and non-users, the former showed signs of organ dysfunction with higher values in kidney and liver function tests. In terms of microbial identification, multiplex RT-PCR was superior to conventional culture techniques, especially in detecting bacterial infections among antibiotic takers. For example, PCR was significantly more accurate in identifying pathogens like Klebsiella spp., Staphylococcus aureus, and Pseudomonas aeruginosa.

The efficacy of multiplex RT-PCR also extended to identifying antibiotic resistance. Among antibiotic takers, the PCR test detected higher resistance rates to cephalosporins and macrolides compared to culture methods. Furthermore, PCR highlighted a greater prevalence of multi-drug resistance and infections with multiple pathogens. In evaluating patient outcomes

based on diagnostic method, those initially assessed with PCR had faster bacterial identification and treatment initiation, leading to shorter hospital stays.

Risk factor analysis emphasized the dangers of over-the-counter antibiotic use, as it was a significant independent risk factor for extended hospitalization. Other risk factors included smoking status, bacterial superinfection, reliance on only conventional bacterial cultures, and delays in bacterial sampling upon hospital admission.

Figure 1 – Frequency of self-medicated antibiotics among patients with COVID-19 and secondary bacterial infections.

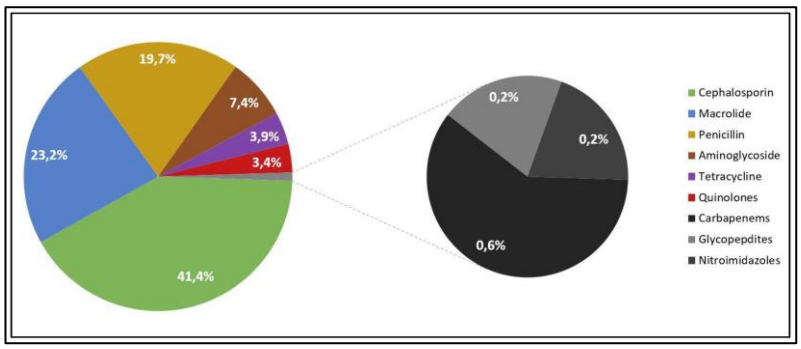
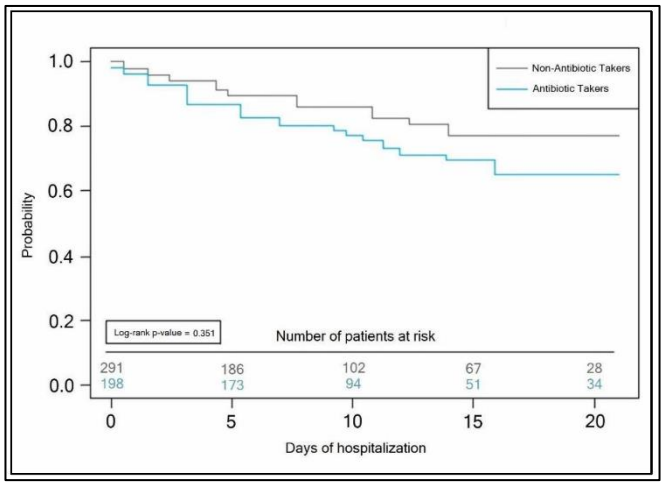


Figure 2 – Kaplan–Meyer probability curve of hospitalization duration by the antibiotic use status of COVID-19 patients.



CONCLUSIONS

The multiplex PCR technique is highly effective for detecting secondary bacterial infections in COVID-19 patients who have taken antibiotics on their own. Implementing this method for initial assessments in patients exhibiting sepsis symptoms or clinical decline can lead to reduced hospital stays and faster recovery. The importance of judicious antibiotic use, especially during the COVID era, is emphasized to prevent unnecessary antibiotic consumption and the rise of antimicrobial resistance. Furthermore, we recommend faster testing procedures for COVID-19 patients to minimize self-administered antibiotic use and stress the significance of integrating antimicrobial management strategies into the pandemic action plan.

STUDY 2: FUNGAL INFECTIONS IDENTIFIED WITH MULTIPLEX PCR IN SEVERE COVID-19 PATIENTS DURING SIX PANDEMIC WAVES.

CONTEXT

The onset of the COVID-19 pandemic, caused by SARS-CoV-2, led to a global health crisis. Beyond managing the primary virus, secondary complications like opportunistic fungal infections have significantly affected patient morbidity and mortality. Detection and understanding of these infections remain a challenge, with traditional methods such as culture and microscopy often yielding slow and imprecise results.

Advanced technologies like next-generation sequencing and MALDI-TOF MS have emerged, but they often require more resources and expertise than what's readily available. In contrast, multiplex PCR has proven to be a rapid, sensitive, and cost-effective tool for detecting fungal pathogens, even though its application for severe COVID-19 patients is still under-explored.

This study seeks to bridge the knowledge gap by analyzing fungal infections in severe COVID-19 patients during the first six pandemic waves. The aim is to determine the prevalence and types of fungal infections and examine potential links with patient characteristics and therapeutic strategies.

RESULTS

Out of the studied patients, 96 were diagnosed with fungal infections while 192 weren't. Those infected with fungal infections had a slightly higher average age of 64.6 years compared to 62.0 years for the uninfected, although this wasn't statistically significant. Men were more commonly diagnosed with fungal infections at 63.5%. A significant finding was that 35.4% of those with fungal infections were obese as opposed to 21.4% without infections. There were no significant variations in COVID-19 vaccination rates and smoking habits between the two groups. Furthermore, while the presence of pulmonary diseases was higher among those with fungal infections, only the Charlson comorbidity index showcased a significant difference.

Blood culture tests were more frequent in the fungal infections group, though without a significant difference. Differences in oxygen supplementation methods were noted; notably, ventilator usage was considerably higher in the fungal infections group (45.8% versus 18.8%). Clinically, those with fungal infections had a more challenging course: they were more frequently admitted to the ICU, had a longer ICU stay, faced a shorter time from symptom onset to death, and had a notably higher mortality rate (32.3% vs. 12.0%).

The most commonly identified fungal infection was Candida spp., with varying prevalence across the pandemic waves. Aspergillus spp. and Mucor spp. also had fluctuating detection rates across waves. Concerning clinical outcomes, ICU admissions and mortality differed significantly across the waves. With respect to drug resistance, Candida spp. and Aspergillus spp. infections showed significant resistance to multiple drugs, while Mucor spp. infections exhibited resistance mainly to one or two drugs. Notably, a large portion of Rhizopus spp. infections demonstrated resistance to three or more drugs.

The risk of death increased substantially with the degree of drug resistance. Specifically, patients resisting one drug showed nearly triple the mortality risk, and those resisting three or more drugs had an over six-fold higher risk. Mortality risk varied across pandemic waves, with the second, fourth, and fifth waves showing significantly higher risks. Aspergillus spp. and Mucor spp. infections correlated with a higher mortality risk, whereas Candida spp. infections did not significantly influence it.

Figure 3 – Kaplan–Meier analysis of survival between severe COVID-19 patients with and without fungal infections.

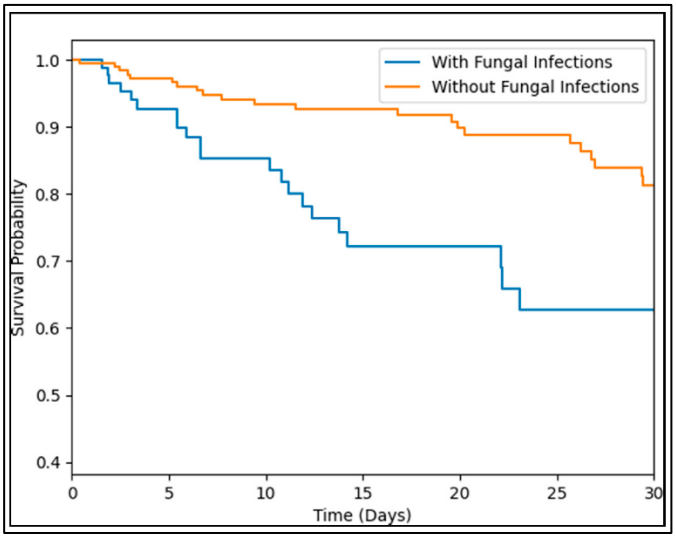
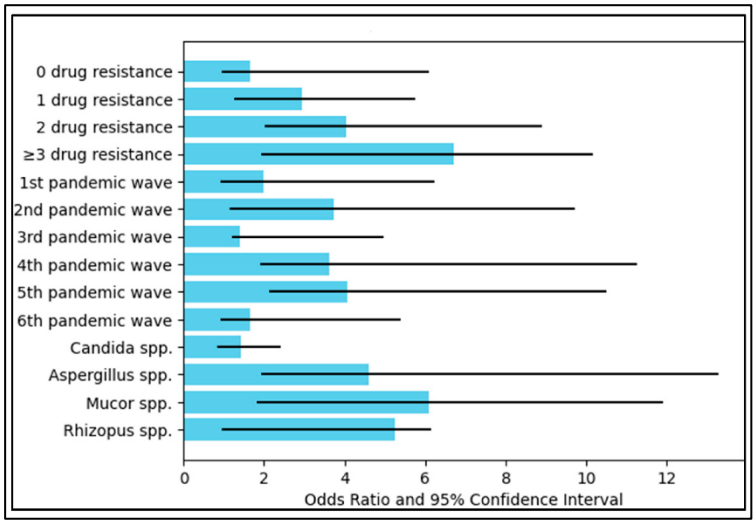


Figure 4 – Risk factor analysis for mortality.



CONCLUSIONS

In conclusion, this retrospective study reveals that fungal infections, identified through multiplex PCR, are an important factor in severe COVID-19 patients, impacting both hospitalization duration and mortality rates. The presence of fungal infections was associated with a higher rate of ICU admissions, longer ICU stays, and elevated mortality rates. Among the fungal infections, Candida spp. was most common across all pandemic waves, but Aspergillus spp. and Mucor spp. infections were associated with a significantly increased mortality risk. It was also observed that drug resistance, especially resistance to multiple drugs, contributed to a significant increase in mortality risk among severe COVID-19 patients with fungal infections. No significant disparities were found in the distribution of patients with fungal infections across different pandemic waves, suggesting that the dominant SARS-CoV-2 variant in circulation did not significantly influence the occurrence of fungal infections. These findings underscore the importance of early detection and management of fungal infections in severe COVID-19 patients to improve clinical outcomes.

STUDY 3: THE ASSESSMENT OF MULTIPLEX PCR IN IDENTIFYING BACTERIAL INFECTIONS IN PATIENTS HOSPITALIZED WITH SARS-COV-2 INFECTION: A SYSTEMATIC REVIEW.

CONTEXT

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the coronavirus disease (COVID-19). While most infected individuals experience mild to moderate symptoms, the presence of co-infections can lead to severe outcomes, with many fatalities linked to multiple concurrent infections. The most frequent bacterial co-infections are caused by pathogens such as Klebsiella pneumoniae, Streptococcus pneumoniae, and Staphylococcus aureus, among others. These can be community-acquired, signaling infection before or in conjunction with SARS-CoV-2, or nosocomial, indicating acquisition from a hospital post-COVID-19 diagnosis.

Clinical microbiology plays a pivotal role in detecting these pathogens, especially during the COVID-19 outbreak, demanding accurate and swift testing methods. Multiplex polymerase chain reaction (PCR) has emerged as an effective testing procedure, offering a quicker alternative to the traditional PCR by amplifying multiple target sequences simultaneously. Real-time PCR also expedites the process by eliminating post-amplification steps and detecting RNA-based genetic material. Though antibiotics are not a primary treatment for COVID-19, they are administered to counter potential bacterial co-infections. However, this has raised concerns about the increase in antimicrobial resistance, emphasizing a judicious approach to antibiotic use. Given the implications of bacterial co-presence in COVID-19 patients, this systematic review was undertaken to explore the clinical characteristics and outcomes of these patients and evaluate the efficiency of multiplex PCR in identifying bacterial infections..

RESULTS

Nine studies were evaluated, with the majority being retrospective in nature, and each conducted in a different country. Most of the participants in these studies were older male patients hospitalized due to COVID-19. ICU admission rates varied between 6.7% to 34.3%, with the median hospital stay ranging from 7 to 24 days. Mortality rates among the patients ranged from 5.5% to 38.7%, with some studies noting the need for mechanical ventilation in significant proportions of the patients. Several studies also highlighted a variety of clinical symptoms upon admission, including fever and cough, with prevalent comorbidities such as diabetes, cardiovascular disease, and chronic kidney disease.

All studies identified Staphylococcus aureus in the sample, and many found Klebsiella spp. Differentiation between community-acquired and nosocomial infections highlighted varying prevalences of bacterial pathogens such as Chlamydophila pneumonia, S. pneumoniae, Pseudomonas aeruginosa, and others. High rates of bacterial co-infection were found in critically ill patients, often involving pathogens like Pseudomonas aeruginosa, Klebsiella pneumoniae, and Staphylococcus aureus. Distinctions between Gram-negative and Gram-positive bacterial prevalence varied between studies.

Studies reported substantial rates of antibiotic use prior to hospitalization, with percentages as high as 82%. Commonly used antibiotics included cephalosporins, macrolides, ampicillin, and azithromycin. Antibiotic-resistance genes were found in a subset of patients, indicating the challenge of bacterial infections in some cases.

Multiplex PCR, particularly the BioFire® FilmArray® Pneumonia plus Panel, showed promise in rapidly detecting various pathogens and antibiotic-resistance genes. While many studies didn't report on the sensitivity and specificity of the multiplex PCR, those that did generally found high levels of both, suggesting that it could be an effective tool in bacterial detection.

Table. Demographic data and clinical outcomes

Study	Age (median)	Gender (% female - male)	ICU admissions (%)	Duration of hospitalization (median)	Mortality rate (%)
Alosaimi [35]	52.0	33.0% - 77.0%	29.0	ND	19.0
Bogdan [36]	>18.0	49.0% - 51.0%	6.7	12.4	5.5
Cohen [37]	67.0	30.0% - 70.0%	ND	24.0	38.7
Foschi [38]	>18.0	ND	100	ND	ND
Huang [39]	72.0	31.8% - 68.2%	9.5	7.0	8.5
Karolyi [40]	62.5	20.0% - 80.0%	100	7.0	36.7
Maataoui [41]	57.0	18.0% - 82.0%	100	19.0	56.0
Rothe [42]	58.5	36.5% - 63.5%	23.4	9.0	4.5
Soto [43]	61.7	29.0% - 71.0%	ND	ND	31.0

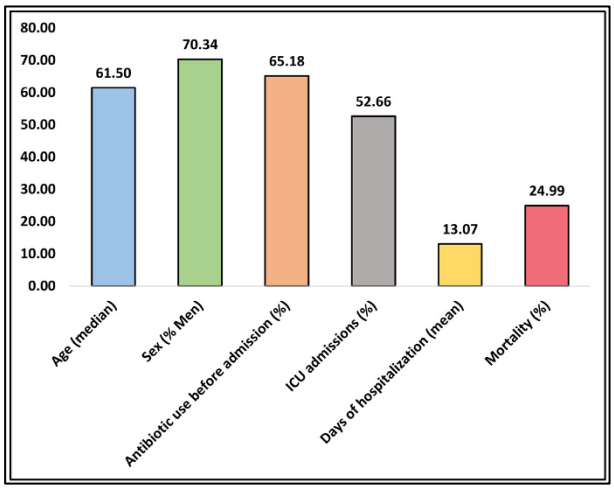


Figure 5 – Summary of findings.

CONCLUSIONS

Bacterial infections that are identified in COVID-19 patients can lead to more negative outcomes, particularly when it comes to patients with a severe infection who are hospitalized with COVID-19. Taking into account the high rates of co-infection and the high percentage of antibiotics used by individuals prior to hospitalization, effective methods of timely diagnosis and treatment need to be implemented in order to decrease the mortality rate and prevent the misdiagnosis of infections. This will allow for a reduction in the number of infections that are incorrectly diagnosed. Regarding the different PCR assays, both multiplex PCR and RT-PCR have high sensitivity and specificity, regardless of the panel used for bacterial detection, therefore making them ideal methods of detecting bacterial co-infections in COVID-19 patients.