Article

The Close Correlation Between COVID-19 and Bacterial Etiologies of Hospital-Acquired Infections (HAIs) As a Co-infection

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Abstract: This study was conducted on COVID-19 patients aged 20–60 years of both sexes, who were hospitalized at Mosul General Hospital and Tikrit Teaching Hospital during the period from 16 February to 13 July 2024. A total of 80 throat swab samples were collected to investigate bacterial co-infections accompanying COVID-19. Of these, 72 samples (90%) were positive for bacterial growth, while 8 samples (10%) were negative. Among the Gram-positive bacterial isolates, Staphylococcus aureus was the most prevalent (24 isolates; 33.33%), followed by Streptococcus pneumoniae (17 isolates; 23.61%) and Streptococcus pyogenes (14 isolates; 19.44%). S. aureus is a known pathogen responsible for a wide range of respiratory infections. S. pneumoniae, which colonizes the nasopharynx, may become pathogenic and is a leading cause of secondary respiratory tract infections. S. pyogenes possesses several virulence factors that facilitate tissue invasion and bacterial dissemination, contributing to severe co-infections (Ahmed et al., 2021). In addition to Gram-positive bacteria, Gram-negative pathogens were also identified. Pseudomonas aeruginosa was the most common among them (10 isolates; 13.88%), known for its persistence in moist environments and association with nosocomial infections. Escherichia coli (5 isolates; 6.94%) and Klebsiella pneumoniae (2 isolates; 2.77%) were also isolated, both of which are opportunistic pathogens in immunocompromised or hospitalized patients. The findings of this study revealed a concerning level of antimicrobial resistance. S. aureus showed 100% resistance to amoxicillin/clavulanic acid (Augmentin), while S. pneumoniae exhibited complete resistance to most antibiotics tested. S. pyogenes demonstrated 100% resistance to ceftriaxone, Augmentin, and ampicillin. Similarly, Gram-negative isolates showed high resistance levels to commonly used antibiotics, indicating a critical challenge in managing bacterial co-infections in COVID-19 patients.

Keywords: COVID-19, bacterial co-infections, antimicrobial resistance

1. Introduction

With the outbreak of the COVID-19 pandemic in late 2019, health systems around the world faced enormous challenges, not only in dealing with the novel coronavirus SARS-CoV-2 , but also in dealing with the complications resulting from concomitant infections, especially secondary bacterial infections. This infection has emerged as a critical problem, especially in hospitals, where it has worsened the clinical condition of patients and increased mortality rates, especially in intensive care units (ICU) [1]. Studies indicate that bacterial infections associated with COVID-19 occur in two main forms: co-infections that occur concurrently with viral infections, and secondary infections that arise during hospital stays, often as a result of medical interventions such as mechanical ventilation or intravenous catheters. Data have shown that the incidence of bacterial infections among

COVID-19 patients ranged between 7% and 15%, rising to more than 30% in critically ill patients [2].

The most prominent factors contributing to the occurrence of these infections include prolonged hospital stays, weakened immunity caused by the virus or immunotherapy, and excessive and misguided use of antibiotics. This indiscriminate use of antibiotics, especially in the early days of the pandemic, contributed to the rise of bacterial resistance to antibiotics (antimicrobial resistance - AMR), which has become a growing global threat [3].

Common pathogens in these infections include Staphylococcus aureus (including MRSA), Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii, and other resistant Gram-negative bacteria, and have been isolated in high proportions from patients hospitalized for long periods or on mechanical ventilation [4].

The COVID-19 pandemic has seen a significant increase in antibiotic resistance rates among bacterial isolates, particularly in hospitals that have received large numbers of patients infected with the virus, particularly in intensive care units. Several factors have contributed to this increase, most notably: the widespread and unrestricted use of antibiotics, weak infection control protocols in some facilities, and delays in accurate laboratory diagnosis of secondary bacterial infections . Multiple studies indicate that a significant proportion of patients with COVID-19 received antibiotics despite the absence of clear evidence of a bacterial infection. A systematic review found that more than 70% of patients received antibiotics, while the percentage of confirmed bacterial infections did not exceed 8%. This unjustified use has created a fertile environment for the emergence of resistant bacterial strains [5].

Multidrug resistance (MDR) has become a great global health challenge because there are no antibiotics (chemical) for treating such infections including co-infections . The vast majority of several human pathohens show antibiotic resistance like ; (MRSA) , multidrug-resistant streptococcus , klebsiella , and carbapenem-resistant Pseuodomonas aeruginosa (CRE) [6].

2. Materials and Methods

80 samples were collected from patients admitted to Tikrit Teaching Hospital and Mosul General Hospital, who were confirmed to be infected with the Coronavirus, for both sexes, aged 20-60. These samples were taken by swab and were urgently transferred to the laboratory, where they were cultured on MacConkey agar and EMB medium to grow Gram-negative bacteria, and on blood agar to grow hemolytic bacteria, as well as on mannitol agar and nutrient agar to grow Gram-positive bacteria. These samples were then incubated for 24-48 hours at 37°C to detect bacterial species causing secondary infection associated with Coronavirus, which is considered a hospital-acquired infection. It is diagnosed phenotypically and through biochemical tests such as oxidase, coagulase, and catalase tests, in addition to sensitivity tests to optochin and bacitracin. The sensitivity of the isolated bacterial species in this study to 10 antibiotics was examined, and resistance was determined by measuring the diameter of inhibition around the disc according to the standard method (Kirby and Bauer 1966), and then comparing the result with the global table of measurements of diameters of inhibition (CLSI, 2024).

3. Results and Discussion

coronavirus (COVID-19) admitted to Mosul General Hospital and Tikrit Teaching Hospital, who were receiving artificial respiration due to hypoxia resulting from COVID-19. This test was conducted to identify bacteria associated with this pandemic. Seventy-two samples showed positive growth (90%), while eight samples were negative growth (10%).
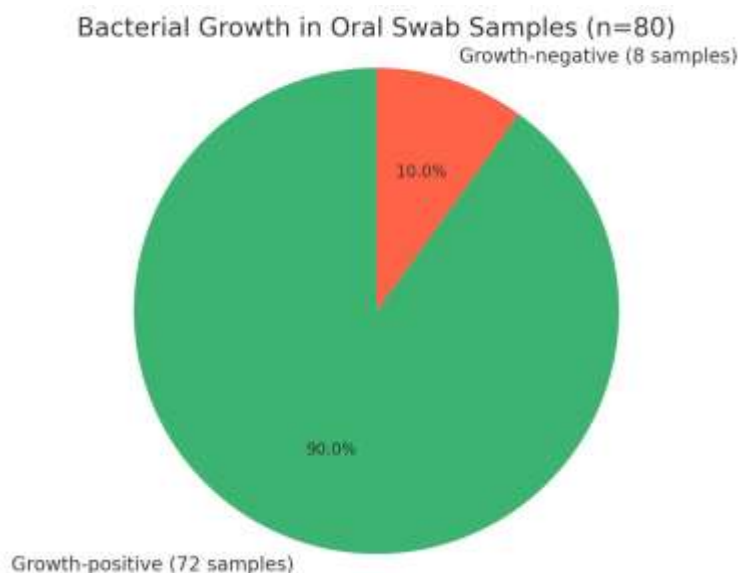
Figure 1. Distribution of bacterial growth in oral swab samples collected from hospitalized COVID-19 patients (n = 80).

The pure isolates obtained were distributed among 55 Gram-positive bacteria (76.38%), while 17 Gram-negative bacteria (23.61%). The results of the current study also showed that the highest isolation rate of Gram-positive bacteria was staphylococcus aureus bacteria, at 33.33%. This result is close to the findings of [7]. These bacteria are the dominant bacteria in hospital infections that affect patients with COVID-19, causing widespread respiratory infectionsCo-infection with S. aureus leads to complications such as secondary pneumonia and bacteremia , which increases the length of hospital stay and the need for mechanical ventilation. *Streptococcus pneumoniae* followed with an isolation rate of 23.61%. This bacteria is considered one of the main causes of secondary infections associated with COVID-19, as it normally colonizes the respiratory tract and enters the lungs through inhalation . This study is consistent with [8]. It inhabit where oxygen is stored in nose and pharynx , it transmitted through droplets (coughing and sneezing) or direct contact and it can enter the lungs by inhalation , It was discovered to be one of the most common causes of respiratory tract infection therefore it is a major of secodary infection Unlike influenza virus, which is strongly associated with secondary Streptococcus pneumoniae infection, the novel coronavirus appears less associated with this type of infection, but the reported cases have shown high clinical severity. *Streptococcus pyogenes* was isolated in 19.44% of cases and is considered secondary cause of COVID-19 infection. This finding is consistent with a study by Musher. This bacterium causes a wide range of diseases such as sore throat, scarlet fever, and skin infections. During the COVID-19 pandemic, increased rates of secondary infections associated with this bacterium were observed in some patients infected with SARS-CoV-2, especially in severe cases requiring intensive care. Co-infection between S. pyogenes and COVID-19 can lead to a worsening of the clinical condition due to an overstimulation of the inflammatory response, increasing the likelihood of sepsis or septic shock, which increases mortality rates [9]. Regarding the Gram-negative bacteria isolated from patients, *Pseudomonas aeruginosa* constituted the largest proportion among them, as 10 samples were isolated, representing 13.88% of the total percentage of all isolates. This study is consistent with [10]. It is one of the most common Gram-negative bacterial species wich causes nosocomial infection and associated with COVID-19 infection.

Five E. coli isolates were also isolated, representing 6.94% of the total causative agents. respectively , where these bacteria are naturally transient microflra of the body , but it converts to a an oppurtunistic in hospitalized persons [11]. This study is consistent

with [12]. E. coli coinfection among COVID-19 patients is not common, but it poses a therapeutic challenge due to multiple antibiotic resistance .

Two isolates of *Klebsiella pneumoniae* were obtained, the lowest percentage of isolates in the current study, with two isolates and a percentage of 2.77%. This study is consistent with [13]. Co-infection with Klebsiella pneumoniae and the coronavirus (COVID-19) is a serious complication that affects the course of the disease, especially in critical cases due to its high virulence and resistance to antibiotics. It causes ventilator-associated pneumonia and is considered an opportunistic pathogen that infects the body when immunity is affected and weakened.

The results of the current study showed that Staphylococcus aureus bacteria are resistant to most of the antibiotics used in the study, which include ampicillin, levofloxacin, and ceftriaxone, with a resistance rate of 87.5%, 79.16%, and 66.66%, respectively. Resistance to Azithromycin, Amoxicillin\Clavulanic acid, Gentamicin and Amikacin reached 100%, 91.66%, 62.5% and 75%, respectively.
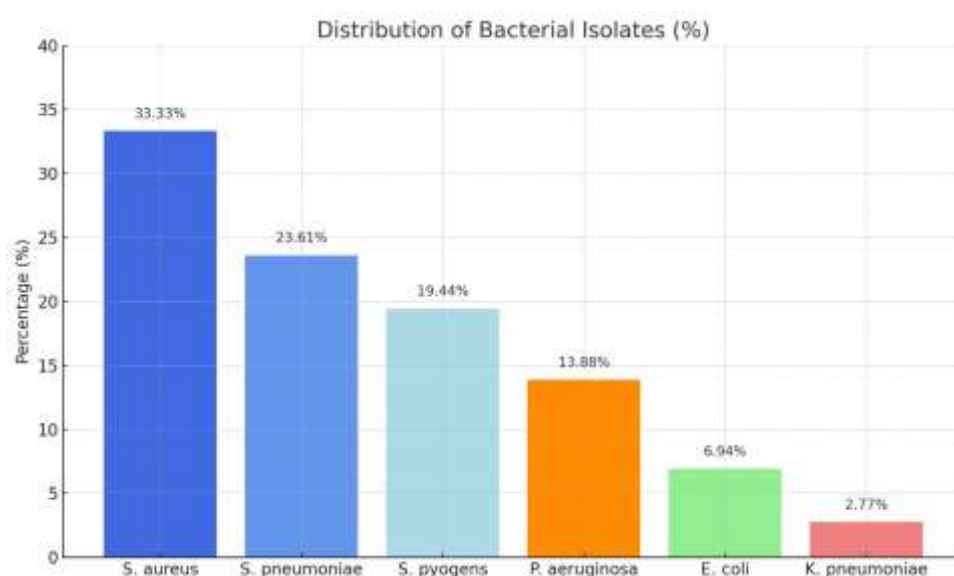

Figure 2. Distribution of Gram-positive and Gram-negative bacterial isolates among COVID-19 patients with hospital-acquired infections.

The increasing resistance of bacterial isolates to antibiotics has become a global problem that threatens human life due to the excessive use of antibiotics and the misuse of these antibiotics by members of society due to the lack of health awareness [14]. As for Staphylococcus pneumoniae bacteria, our current study showed that it is 100% resistant to the antibiotics amikacin, gentamicin, and ampicillin, and 88.23%, 64.70%, and 70.58% to the antibiotics azithromycin, levofloxacin, and amoxicillin/clavulanic acid, respectively . These results are consistent with [15] .

Regarding of Staphylococcus pyogenes, the results showed that the 14 isolates of this bacteria were resistant to the antibiotics Ampicillin, Gentamicin and Amikacin at a rate of 92.85%, 78.57% and 71.42%, respectively, and 100% resistant to the antibiotics Levofloxacin, Ceftriaxone and Amoxicillin/Calvolanic acid, and 57.14% to Azithromycin. This result is consistent with what the researcher [16] reached. These antibiotics possess the beta-lactamase enzyme that breaks down the beta-lactam ring in these antibiotics, which is attributed to the great effectiveness in resisting these antibiotics.

As for Gram-negative bacteria, Pseudomonas aeruginosa, facultative aerobe, it is known for its high ability to survive in moist environments, such as laundries and ventilators . It causes a variety of infections, including respiratory and urinary tract infections and sepsis, and targets immunocompromised patients or those who use invasive medical devices for extended periods [17] which is the most common type of Gram-negative bacteria associated with

hospital infections associated with COVID-19, its isolates varied in their resistance to antibiotics, as their resistance to ampicillin, ceftriaxone, levofloxacin, and azithromycin reached 80%, 70%, 50%, and 60%, respectively, while they were completely resistant to amoxicillin/clavulanic acid, amikacin, and gentamicin. The MexAB-OprM and MexcD-OprJ pumps are among the main mechanisms used by these bacteria to expel antibiotics outside the cell, which reduces their effectiveness [18].

The *E. coli* isolates under study were relatively more susceptible to antibiotics compared to isolates of other bacterial species, as 40% resistance was recorded to amoxicillin/clavulanic acid, amikacin, levofloxacin, and ceftriaxone, 60% to gentamicin, 20% to azithromycin, and 100% to ampicillin.This reseult is consistent with [19].

.As for the Klebsiella pneumoniae species, two isolates were obtained from patients infected with COVID-19, and they were 100% resistant to all antibiotics except for azithromycin and amoxicillin/clavulanic acid, as resistance to these two antibiotics was 50%. This study differs from the results of the study [20].

Table 1. Percentage of Bacterial isolates resistance to antibiotics.

Percentage of Bacterial isolates resistance to antibiotics					Table :		
Bacteria	**AMP**	**LEV**	**CRO**	**AMC**	**AZM**	**AMK**	**GEN**
Staphylococcus aur	87.5%	79.16%	66.66%	100%	91.66%	75%	62.5%
Streptococcus pneumoniae	100 %	64.70 %	88.23%	70.58%	88.23%	100%	100%
Streptococcus pyog	92.85%	100%	100%	100%	57.14%	71.42%	78.57%
Pseuodomonas aeruginosa	80%	50%	70%	100%	60%	100%	100%
Escherichia coli	100%	40%	40%	40%	20%	40%	60%
Klebsiella pneumon	100%	100%	100%	50%	50%	100%	100%

4. Conclusion

This study demonstrates a high prevalence of bacterial co-infections among hospitalized COVID-19 patients, with 90% of oral swab samples showing positive bacterial growth. The majority of isolates were Gram-positive bacteria, notably Staphylococcus aureus (33.33%), Streptococcus pneumoniae (23.61%), and Streptococcus pyogenes (19.44%), which are well-documented causes of respiratory tract infections and are known to complicate viral illnesses by inducing severe secondary infections. Among Gram-negative isolates, Pseudomonas aeruginosa (13.88%) was most prevalent, followed by Escherichia coli and Klebsiella pneumoniae, consistent with previous findings highlighting their role in nosocomial infections and ventilator-associated pneumoniae. Alarmingly, the antibiotic susceptibility profiles revealed high levels of multidrug resistance (MDR), with S. aureus, S. pneumoniae, and S. pyogenes displaying complete resistance to several commonly used antibiotics, including ampicillin, amoxicillin/clavulanic acid, and ceftriaxone. Similar resistance patterns were observed in P. aeruginosa and K. pneumoniae, reflecting a global trend of increasing antimicrobial resistance, but also highlight higher resistance rates compared to earlier reports where susceptibility was more varied. The overuse and empirical prescribing of antibiotics during the COVID-19 pandemic likely contributed to these resistance patterns, emphasizing the urgent need for robust antimicrobial stewardship programs and precise diagnostic strategies to mitigate the emergence and spread of resistant pathogens in healthcare settings.

The findings of this study emphasize the alarming levels of antibiotic resistance among bacterial isolates from hospitalized COVID-19 patients, particularly Gram-positive organisms. Staphylococcus aureus demonstrated high resistance rates to key antibiotics, notably azithromycin and amoxicillin/clavulanic acid, which supports the observations of

Urmi et al. concerning the global misuse and overprescription of antibiotics [14]. Likewise, Streptococcus pneumoniae and Streptococcus pyogenes showed significant resistance, likely associated with the production of beta-lactamase enzymes. Among Gram-negative pathogens, Pseudomonas aeruginosa exhibited broad resistance, particularly driven by active efflux mechanisms such as MexAB-OprM, . In contrast, E. coli isolates showed comparatively lower resistance levels, whereas Klebsiella pneumoniae isolates were almost entirely resistant to all tested antibiotics except two. These results underscore the urgent need for robust antimicrobial stewardship programs and infection control strategies in hospital settings during pandemics like COVID-19.

REFERENCES

[1] X. Chen and others, "Bacterial co-infection raises in-hospital mortality of COVID-19 patients," *Front. Microbiol.*, vol. 14, p. Art. no. 1206476, 2023.

[2] B. J. Langford and others, "Bacterial co-infection and secondary infection in patients with COVID-19: A living rapid review and meta-analysis," *Clinical Microbiology and Infection*, vol. 26, no. 12, pp. 1622–1629, 2020.

[3] T. M. Rawson and others, "Bacterial and fungal co-infection in individuals with coronavirus: A rapid review to support COVID-19 antimicrobial prescribing," *Clinical Infectious Diseases*, vol. 71, no. 9, pp. 2459–2468, 2020.

[4] J. S. Musuuza and others, "Prevalence and outcomes of co-infection and superinfection with SARS-CoV-2 and other pathogens: A systematic review and meta-analysis," *PLoS One*, vol. 16, no. 5, p. Art. no. e0251170, 2021.

[5] D. Rawat and others, "High prevalence of multidrug-resistant bacteria in COVID-19 patients admitted to ICU: Experience from a tertiary care hospital in India," *Infection Prevention in Practice*, vol. 3, no. 2, p. Art. no. 100113, 2021.

[6] A. Kumar and N. Chorida, "Bacterial resistance against antibiotics," in *Drug Resistance in Bacteria, Fungi, Malaria, and Cancer*, G. Arora and others, Eds., Cham, Switzerland: Springer, 2017, pp. 171–192.

[7] A. Elabadi and others, "Bacterial co-infection in critically ill COVID-19 patients with severe pneumonia," 2021.

[8] A. B. Brueggemann and others, "Changes in the incidence of invasive disease due to Streptococcus pneumoniae, Haemophilus influenzae, and Neisseria meningitidis during the COVID-19 pandemic in 26 countries and territories in the Invasive Respiratory Infection Surveillance Initiative: A prospective analysis of surveillance data," *Lancet Digit. Health*, vol. 3, no. 6, pp. e360–e370, 2021.

[9] L. Lansbury and others, "Co-infections in people with COVID-19: A systematic review and meta-analysis," *Clinical Infectious Diseases*, vol. 71, no. 10, pp. 2459–2468, 2020.

[10] A. Rhoades-Pinski and others, "Acute SARS-CoV-2 infection is associated with an expansion of bacterial pathogens in the nose, including Pseudomonas aeruginosa," *bioRxiv*, 2021.

[11] E. Sharifipour and others, "Evaluation of bacterial co-infections in COVID-19 patients admitted to ICU," *Arch. Clin. Infect. Dis.*, vol. 15, 2020.

[12] R. Shrestha and others, "Co-infection of uropathogenic Escherichia coli among COVID-19 patients," *Journal of the Nepal Medical Association*, vol. 60, no. 245, pp. 1–5, 2022.

[13] S. Ahmed and M. Hussein, *Financial Data Analysis Using Artificial Intelligence*. Dar Al-Nahda, 2021.

[14] M. R. Urmi and others, "Antibiotic resistance patterns of Staphylococcus spp. isolated from fast foods sold in different restaurants of Mymensingh, Bangladesh," *J. Adv. Vet. Anim. Res.*, vol. 8, no. 2, 2021.

[15] T. Dessie and others, "Multiresistant bacterial pathogens causing bacterial pneumonia and analyses of potential risk factors from Northeast Ethiopia," *Int. J. Microbiol.*, 2021.

[16] D. Kebede and others, "Prevalence and antibiotic susceptibility profiles of Streptococcus pyogenes among pediatric patients with acute pharyngitis at Felege Hiwot Comprehensive Specialized Hospital, Northwest Ethiopia," *BMC Microbiol.*, vol. 21, no. 1, pp. 1–10, 2021.

[17] H. Mahmoudi and others, "Prevalence of Pseudomonas aeruginosa in COVID-19 patients: A systematic review and meta-analysis," *Front. Microbiol.*, vol. 13, p. Art. no. 859099, 2022.

[18] Z. J. Resko and others, "Evidence for intracellular Pseudomonas aeruginosa," *J. Bacteriol.*, vol. 206, p. Art. no. e00109-24, 2024.

[19] F. Poursina and others, "Biofilm formation in non-multidrug-resistant Escherichia coli isolated from patients with urinary tract infection in Isfahan, Iran," *Adv. Biomed. Res.*, vol. 7, p. Art. no. 40, 2018.

[20] J. Xie and others, "Clinical features and antimicrobial resistance profiles of important Enterobacteriaceae pathogens in Guangzhou representative of Southern China, 2001–2015," *Microb. Pathog.*, vol. 107, pp. 206–211, 2022.