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Identification and antibiotics Sensitivity of Secondary Bacterial Infection in COVID-19 (SARS-CoV-2) Pneumonia patients in Kirkuk/Iraq

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ABSTRACT

The unusual virus, now known as Coronavirus 2 (SARS-CoV-2), spread fast throughout China and other regions of the world due to its remarkable ability for human-to-human transmission. Patients with COVID-19 commonly experience significant morbidity and mortality from subsequent bacterial infections.
Aim: To determine the incidence of typical bacterial infections in COVID-19 pneumonia, including Klebsiella pneumoniae, Pseudomonas aerogenusa, Escherichia coli, and Staphylococcus aureus. Hundred sputum samples were taken from individuals who had been identified as having the developing Coronavirus and were dwelling in intensive care units in Hospitals (Al Shifa hospital) in Kirkuk city/Iraq. The BDph Phoenix m50 procedure was used to test the antibiotic sensitivity of samples collected on various culture mediums, then transferred to pure cultures and diagnosed. The total of 100 patients, 60% of them had a secondary bacterial infection, whereas 40% of them did not. The most common isolated species was Staphylococcus aureus identification at (40%) followed by K. pneumoniae (33%), Pseud. aeruginosa (22%), and E. coli (5%). The majority of the identified strains were resistant to the study's antibiotics. This study demonstrates that SARS-CoV-2 infected critically sick individuals had a very high frequency of subsequent bacterial infection. The pathogenic bacteria that were identified for this investigation were resistant to the majority of the antibiotics that were utilized.

Introduction

A pneumonia outbreak was brought on by a brand-new Coronavirus strain in Wuhan, Hubei Province, China in December 2019 [1]. Due to its exceptional capacity for human-to-human transmission, the rare virus, which is now known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has spread quickly throughout China and other parts of the world [2]. With 227 million cases and 4,664,000 fatalities worldwide from COVID-19, it is the biggest pandemic of our generation [WHO, 2022]. According to WHO recommendations for the clinical care of COVID-19 [3], physicians should obtain upper respiratory and blood samples for bacterial cultures and only initiate therapy with experimental antibiotics in the most severe cases. As demonstrated in the 1918 influenza pandemic, where the majority of fatalities were linked to concomitant bacterial infection [4], the infection is thought to expose people to respiratory viral infection, which can increase illness severity and mortality. The worst outcomes for those with COVID-19 infection are more frequently linked to bacterial co-infection than to the direct result of viral infection [5]. According to another study [6], bacterial infections affect between 3 and 30% of COVID-19 cases. In the current COVID-19 pandemic, Zhou *et al.* observed that secondary bacterial infections were the cause of death in 50% of patients, whereas bacterial and fungal infections were discovered in another analysis [7]. It is expected that immunological damage brought on by the virus, as well as dysregulation of both innate and adaptive immune responses, would lead to the destruction of the airway epithelium and its barrier function, facilitating bacterial colonization [8]. According to several studies carried out globally [9], gram-negative bacteria were more prevalent than gram-positive bacteria in COVID-19 patients. The treatment of COVID-19 patients, as well as guaranteeing the reliable administration of antibiotics and avoiding side effects of antibiotic usage, depends on knowing the age of patients with acute respiratory illness who also have bacterial infections [10]. Furthermore, this information may improve COVID-19 patient antimicrobial therapy recommendations [11].

Aim: To determine the incidence of typical bacterial infections in COVID-19 pneumonia, including *Klebsiella pneumoniae, Pseudomonas aerogenusa, Escherichia coli, and Staphylococcus aureus.*

Material and method

Study population

A total of 100 individuals of all ages and sexes took part in this study from December 2021 to April 2022. All patients (100) had positive PCR results for nasal and pharyngeal swabs, and all were diagnosed using the Iraqi national criteria for the diagnosis and treatment of COVID-19 [12], in addition to the WHO interim guidelines. This group consisted of those with severe infections who needed to be hospitalized (n = 100). The developing coronavirus (Covid-19)-infected patients were sent to Kirkuk's Al-Shifa Hospital.

Cultivation of samples

All (100) patients had sputum samples collected. The streak plate method, as reported by Cheesbrough (1985) [13], is used to plate sputum samples onto blood agar, McConkey agar, and nutrition agar plates for 18 to 24 hours at 37 °C. The characteristics of colony morphology, which included form, size, surface roughness, edge, height, color, and opacity, were assessed visually in pure colonies. The properties of the morphology of the various media are displayed in Table 1.

Table 1. Table title. Table captions should always be positioned *above* the tables.

Bacteria	Nutrient agar	Blood agar	MacConky agar
Klebsiella pneumoniae	Circular, Greyish white, Dome-shaped	Circular, Greyish white, Dome-shaped ,γ-Hemolysis	Circular, Pink – Red, Convex,
Pseudomonas aerogenusa	large opaque and flat colonies with irregular margins, green-colored	mucoid-type colonies with a typical metallic sheen, β-hemolysis	round, flat, and colorless colonies
Escherichia coli	Large greyish white colonies	Non hemolytic or Beta (β) hemolytic	Bright pink colonies
Staphylococcus aureus	Large Yellow or white colonies	light to golden yellow colonies surrounded by a clear zone of hemolysis	No growth

The bacteria were diagnosed through phenotypic and microscopic examination and through biochemical tests that included (oxidase, catalase, indole, coagulase, motility test, citrate utilization test and urea hydrolysis test) as illustrated in table 2.

Table 2. Table title. Table captions should always be positioned *above* the tables.

Pathogenic bacteria	catalase	coagulase	Oxidase	Motility test	Urea test	Citrate Utilization test	Indole test
Staphylococcus Aureus	+	+	-	Non motile	+	+	-
Klebsiella pneumoniae	+	-	-	Non motile	+	+	-
Pseudomonas Aerogenusa	+	-	+	motile	-	+	-
Escherichia coli	+	-	-	motile	-	-	+
Staphylococcus Aureus	+	+	-	Non motile	+	+	-

Then, the results were confirmed by The BD Phoenix™ Automated Microbiology System which is intended for the in vitro rapid identification (ID) and quantitative determination of antimicrobial susceptibility by minimal inhibitory concentration (MIC) of gram positive and gram negative bacteria.

Result and discussion

SARS-CoV-2 patients have a higher risk of developing severe illness that requires admission to an intensive care unit [14]. Critically sick COVID-19 patients may be more susceptible to secondary infection because of the interaction between drug-and virus-induced immunosuppression [15]. The severity of the sickness and the likelihood of mortality can both be worsened by this secondary infection [16]. In the city of Kirkuk, 100 sputum samples were taken from individuals who had been identified as having the developing coronavirus and were dwelling in intensive care units in Al-shifa hospitals. Of the total of 100 patients, 60% of them (60 patients) had a secondary bacterial infection, whereas 40% of them (40 patients) did not. When Khan and Shaib et al. (2021) analyzed 70 lower respiratory tract samples from COVID-19 patients, 51 (72.9 %) of them demonstrated microbiological evidence of bacterial infection by culture [17], but in another study, the incidence rate was 8.7 % [18]. The number of patients, their age, sex, and whether they experienced a subsequent bacterial infection are all displayed in Table 3.

Table 3. Number and percentage of COVID-19 pneumonia patients

		Positive secondary bacterial Infection(n=60)	Patient without secondary bacterial infection(n=40)
Gender:	Female	22(36.7%)	23(57.5%)
	male	38(63.3%)	17(42.5%)
Age groups:	15-30	2(3.3%)	18(45%)
	31-45	23(38.3%)	12(30%)
	More than 46 years	35(58.3%)	10(20%)

In this study, men (63.3%) were more likely than women to get bacterial infections (36.7%). De Bruyn (2022) [19] also demonstrates that the male sex is potentially a risk factor since it is more prone than the female sex to secondary bacterial infection.

In comparison to other age groups, older people over the age of 46 had a greater risk for bacterial infection. Similar findings were found in other studies that discussed conditions, such as the presence of comorbidities (hypertension, diabetes, heart disease, etc.), that led this group to develop severe COVID-19 illness needing mechanical ventilation [20].

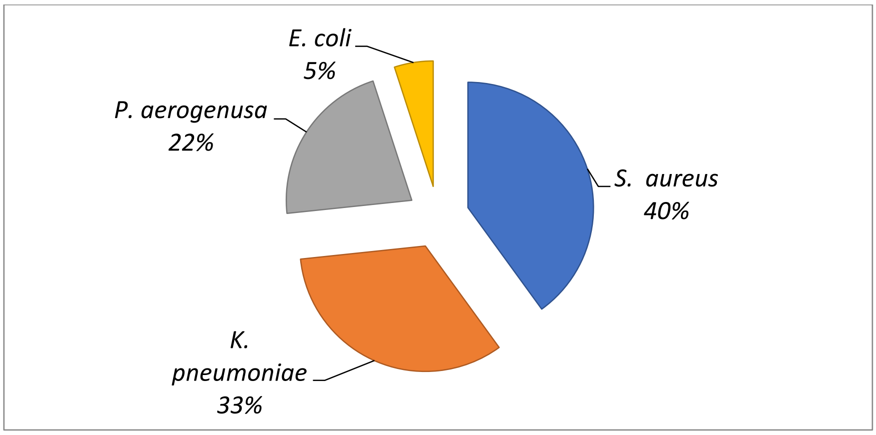

Figure 1. Percentage of Secondary Bacterial Infection Species in COVID-19 pneumonia Patients under study

As it's illustrated in (figure 1) Staphylococcus aureus was the most common isolate (40%) followed by K. pneumonia (33%), P. aerogenousa (22%) and E. coli (5%). This results were consistent with what is found by (Elabadi et al..,2021), which found that among 101 patients Staphylococcus aureus was the main microorganism identified then in a second degree Klebsiella spp., E. coli, P. aeruginosa [21]. Besides COVID-19 infection these bacteria were found to cause secondary infection in other viruses that cause upper respiratory tract infection such as a study conducted to Kirkuk city that show among 150 children admitted to hospital for upper respiratory tract infection (para influenza virus infection) 119 of them had secondary infection S. pyogenes (27.10%), Staph. Aureus (15.88%), K. pneumonia (33.3%), with E. coli and P. aeruginosa (8.3%) [22].

Table 3. Number and percentage of secondary bacterial infection against antibiotic under study depend on phoenix M50

Antibiotics	*S. aureus* (N=24)		*K. pneumonia* (N=20)		*P. erogenous* (N=13)		*E. coli* (N=3)	
	R	**S**	**R**	**S**	**R**	**S**	**R**	**S**
Amikacin	-	-	18 90%	2 10%	3 23%	10 77%	0	3 100%
Ampicillin	19 79.2%	5 20.8%	18 90%	2 10%	-	-	3 100%	0
Cefipem	-	-	15 75%	5 25%	4 30.8%	9 69.2%	1 33.3%	2 66.7%
Ceftazidime	10 41.7%	14 58.3%	14 70%	6 30%	3 23%	10 77%	2 66.7%	1 33.3%
Tetracycline	19 79.2%	5 20.8%	18 90%	2 10%	10 77%	3 23%	3 100%	0
Ceftriaxone	6 25%	18 75%	15 75%	5 25%	9 69.2%	4 30.8%	1 33.3%	2 66.7%
Ciprofloxacin	16 66.7%	8 33.3%	12 60%	8 40%	8 61.5%	5 38.5%	1 33.3%	2 66.7%
Levofloxacin	-	-	10 50%	10 50%	5 38.5%	8 61.5%	1 33.3%	2 66.7%
Erythromycin	15 62.5%	9 37.5%	-	-	8 61.5%	5 38.5%	1 33.3%	2 66.7%
Gentamycin	14 58.3%	10 41.7%	7 35%	13 65%	3 23%	10 77%	0	3 100%
Imipenem	-	-	8 40%	12 60%	0	13 100%	1 33.3%	2 66.7%
Meropenem	-	-	2 10%	18 90%	0	13 100%	0	3 100%
Piperacillin/ tazobactam	15 62.5%	9 37.5%	17 85%	3 15%	-	-	3 100%	0
Tmp/ smx	16 66.7%	8 33.3%	12 60%	8 40%	-	-	1 33.3%	2 66.7%
Azithromycin	15 62.5%	9 37.5%	10 50%	10 50%	2 15.4%	11 84.6%	2 66.7%	1 33.3%
Vancomycin	4 16.7%	20 83.3%	-	-	-	-	-	-

The results revealed that 40% of isolates was staphylococcus aureus isolates shown in figure (1), which represents 24 gram-positive bacteria isolates. This finding is in line with previous research (Al-Abadi et al., 2021) [21].

Staphylococcus aureus is a known pathogen associated with secondary pneumonia during influenza infection. Its dissemination to the lungs is attributed to a combination of environmental changes and immune responses that create suitable conditions for *Staphylococcus aureus* infection [23]. Staphylococci isolates was Methicillin-resistant *Staphylococcus aureus* (MRSA) which was resistance to all other resident agents, including ampicillin, azithromycin, erythromycin, gentamicin, and ciprofloxacin. Vancomycin resistance was not found. This could be a result of both the extensive use of antibiotics and the general lack of health knowledge among the population. Azithromycin resistance in staphylococci may result from ribosomal changes brought on by the 23s rRNA methylase enzyme produced by the ermA, ermB, and ermC genes [24]. The second pathogen isolated is *Klebssiela pneumonia. Klebsiella* is a part of the normal flora in humans that colonizes the nasal and digestive tract, the colonization can turn into an infection when the host immunity fails to control the pathogen growth (immunocompromised patient) [25]. Meropenem and imipenem are very sensitive to *K. pneumonia,* although the bacteria are resistant to the other antibiotics. These results are in line with what was discovered (AL-Khikani *et al*., 2020) [26]. Due to the synthesis of beta-lactam enzymes, extended-spectrum beta-lactams, and biofilm formation, as well as a variety of internal and external reasons, klebssiella become more resistant to antimicrobials [27]. This finding was similar to that of Mahmoudi, H. (2020) [28]. The rate of pseudomonas infection was 22% and that it was the third pathogen recovered from COVID-19 patients. However, in another investigation, *P. aerogenous* was scored at 78% following S. aureus infection, which was at 85% [29]. Except for amikacin, cefepim, meropenem, and imipenem, *P. aeruginosa* strains were resistant to most antibiotics. In addition to being a typical opportunistic pathogen of the respiratory system, Pseudomonas aeruginosa is also recognized as the most typical gram-negative bacterial species linked to serious hospital-acquired infections in various hospitals [30]. The rate of isolation of *E. coli* was the lowest (5%)and this result was consistent with that show rate of isolation of *E. coli* in respiratory tract in COVID-19 patients was 1.3% while *E. coli* isolation from UTI infection in COVID-19 patients was higher (11.9%) above the other pathogenic bacteria that causes UTI in COVID-19patients [31]. Probably this is due to *E. coli* is the most prevalent bacterium that cause UTI [32]. Amikacin, meropenem, and imipenem were completely effective against *E. coli* isolates. Tetracycline resistance was seen in every bacterial isolate, and this finding is consistent with research from 2015 [33]. The high antimicrobial resistance among COVID-19 patients testing positive for bacterial co-infections is a cause of great concern as it adds to the overall morbidity and mortality.

Conclusion

This investigation into the presence of superinfections in individuals with COVID-19's lower respiratory tracts is similar to several other investigations carried out in other countries. The latest research emphasizes how crucial it is to concern about bacterial infections in COVID-19 patients. Specifically, in patients with bacterial infections that may develop as a result of the first infection occurring during or after COVID-19. The pathogenic bacteria that were recovered in this investigation demonstrate resistance to drugs commonly used to treat life-threatening bacterial infections. It is essential to take the appropriate precautions to prevent secondary injuries and restrict the spread of antibiotic-resistant strains by refraining from the indiscriminate use of antibiotics since the combination of viral and bacterial illnesses may raise the risk of mortality. COVID-19-related secondary bacterial infections must be treated seriously in future research and are regarded as one of the significant factors that might cause mortality.

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