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Bacterial Coinfections and Antibiogram Profiles Among ICU Coronavirus Disease 2019 Patients

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Abstract

Help fight infections and limit the spread of antibiotic resistance. This study was designed to investigate the frequency and etiology of bacterial infections in patients with coronavirus disease 2019 admitted to the ICU and exploring their antibiogram sensitivity. The fact that patients with a viral respiratory infection are more likely to develop a bacterial infection and the recently reported prevalence of bacterial coinfection among coronavirus disease 2019 patients in the ICU and its association with serious disease complications. However, the role of cobacterial infection still needs a lot of study and research. Nine COVID-19 patients admitted to the ICU were registered in this study. Bacterial sampling was performed using the blind mini-bronchoalveolar lavage (BAL) technique, VITEK 2 compact system was used for bacterial identification. Antibiotic susceptibility testing was performed by two methods; the first according to Clinical and Laboratory Standards Institute (CLSI) guidelines using disc diffusion assay with 19 antibiotics and the second method by VITEK 2-AST. Of the nine COVID-19 patients in the ICU, four patients (44.5 %) developed bacterial coinfection. Klebsiella pneumoniae was the most frequently reported pathogen, followed by Proteus mirabilis and Acinetobacter baumannii, respectively. The results proved the extensive multidrug resistant of cobacterial pathogens to tested betalactams, carbapenems, aminoglycosides, quinolones, tetracycline, rifamycin, and sulfonamide, however, P. mirabilis showed considerable susceptibility to amikacin, while colistin was highly active against A. baumannii, in addition to sensitivity of K. pneumoniae for aztreonam and colistin. This study is a useful guide to prescribe appropriate treatment and strict supervision of antibiotic stewardship programs and infection control to stop the spread of antibiotic resistance within hospitals.

Keywords: Antibiotic sensitivity, Bacterial coinfections, Coronavirus disease 2019, ICU

1. Introduction

It has been realized that patients with viral infections of the respiratory system are more susceptible to bacterial infection and the serious complications associated with it, worse than those resulting from each infection alone [1]. Previous viral epidemics and outbreaks have seen an escalation in morbidity and mortality due to the presence of bacterial coinfections [2]. In this respect, through the influenza pandemic (1918–1919), most deaths were due to secondary bacterial infections associated with the effects of the hyper virulent virus that causes fatal pneumonia [3]. In such a situation, bacterial coinfection was associated with the pandemic coronaviruses (SARS-CoV-1) and (MERS-CoV) at rates of 20, 30 %, respectively. Increased mortality and morbidity from viral infections of the respiratory tract have been reported when combined with bacterial coinfections [4]. In coronavirus disease 2019 (COVID-19) patients, if a second pathogen is detected at the time of diagnosis it is described as a coinfection, while the determination of the discovery of the second pathogen during hospitalization is considered a superinfection [5]. The acute respiratory syndrome caused by the coronavirus (SARS-CoV-2) led to the emergence of the COVID-19 pandemic in December 2019,
which caused 761 071 826 infections including 6 879 677 deaths globally, of which 515 852 cases and 24 819 deaths in Egypt, according to WHO reports, in March 2023 [6]. It has been shown that many factors affect the severity of COVID-19, including the immune status of the infected people and existing comorbidities [7]. Health care professionals have been alarmed by the presence of bacterial coinfection with COVID-19 and the consequent overlapping of clinical symptoms with bacterial pneumonia leading to an increased risk of mortality and morbidity associated with bacterial coinfection [8]. Moreover, invasive forms of COVID-19 infection submit patients to serious complications such as acute kidney injury, septic shock, and sepsis, which necessitate admission to the ICU [9]. However, hospitalization increases the risk of infection associated with health care, which increases the severity of the disease and makes treatment difficult, in addition to the occurrence of life-threatening complications, as well as the excessive consumption of antibiotics [10,11]. *Klebsiella pneumonia*, *Staphylococcus* species, *Acinetobacter* species, *Escherichia coli*, *Pseudomonas* species, *Enterococcus* species, and *Enterobacter* species are associated with hospital-acquired infections [12]. Previous protocols for the treatment of respiratory viral infections assisted the use of antibiotics, so at the beginning of the pandemic, preliminary guidelines for the treatment of COVID-19 recommended the use of antibiotics [13].

The overuse of antibiotics, especially during a pandemic, and bacteria associated with nosocomial infections are highly resistant to antibiotics, which all contradict the principles and objectives of the antimicrobial stewardship programs and is associated with high rates of morbidity and death, as well as cost [14]. In 2019, about 700 000 intensive care patients died around the world, and multidrug resistant bacteria are likely to be involved [5]. The judicious use of antibiotics to treat patients, especially in the ICU, requires a continuous update of frequent antibiogram [15].

The current study aims to determine the frequency and types of bacterial coinfections and antibiotic resistance profiles in COVID-19 patients admitted to ICU using the automated VITEK 2 system for the wise and rational use of antibiotics.

2. Patients and methods

2.1. Study design

This prospective observational cohort study was conducted in 2022 (1–15 January), at Kasr Elshefaa Hospital, Alexandria, Egypt. COVID-19 patients diagnosed with RT-PCR were admitted to the ICU. Mini-BAL samples were collected by board-certified physicians as a routine clinical procedure in the ICU, and we used the remaining samples in this study. There was no interaction with the patients and thus consent to the participation form was not required. Data confidentiality and patient privacy were maintained in accordance with the Declaration of Helsinki.

2.2. Population

All patients had been in the ICU for more than 48 h and were older than 18 years; the microbiological investigation and treatment decisions were not standardized and were made by the attending physicians. All patients had received a third-generation Cephalosporin as per protocol for severe COVID-19 presentation at the time [16].

Patients’ data were collected from electronic medical records accessed from the laboratory department of Kasr Elshefaa Hospital; the data collected included demographics (patient’s age, sex).

2.3. Sampling for investigation of bacterial coinfection

Nine patients were given mechanical ventilation for more than 48 h (to exclude potential community-acquired infection).

Bacterial sampling was performed using the blind mini-bronchoalveolar lavage (BAL) technique; a single-sheathed, plugged, sterile 50 cm telescoping catheter was inserted into the endotracheal tube, a second sterile catheter was then passed through the first one. Twenty milliliters sterilized physiological saline was injected through the catheter and reaspirated with the same syringe [17].

2.4. Bacterial identification

Recovered bacterial isolates were checked for purity; after Gram staining, freshly pure bacterial isolates were subcultured on blood agar plates and suspensions of each isolate was prepared in 0.45 % saline and adjusted to 0.5 0.5 McFarland turbidity. Ultimately, the simple standardized suspension was applied to GN-ID card and the GN-ID cassette was loaded to the VITEK 2 compact system (BioMérieux Inc., Marly-l’Etoile-france, France) chamber [18].

All biochemical data were collected from tests performed at the time of suspicion of cobacterial infections. Fungal or viral infections were not considered in this study.
2.5. **Antibiogram testing**

The antibiotic sensitivity of the identified cobacterial pathogens was determined by the standard disk-diffusion method, according to Clinical and Laboratory Standards Institute (CLSI) guidelines [19,20]. The following 19 antimicrobial agents were tested: Ticarcillin, Clavulanic Acid, Piperacillin, Tazobactam, Ceftazidime, Cefepime, Aztreonam, Imipenem, Meropenem, Amikacin, Gentamicin, Tobramycin, Ciprofloxacin, Pefloxacin, Minocycline, Colistin, Rifampicin, Trimethoprim, and Sulfamethoxazole. The range of applied concentrations was 5–100 μg/ml.

Antibiotic susceptibility has been further examined and the MIC values were defined by VITEK 2 compact system using ASTN093 card according to the manufacturer’s instructions [21].

3. **Results**

3.1. **Characteristics of coronavirus disease 2019 patients**

A total of nine patients with COVID-19, viral infection was confirmed by RT-PCR were consecutively admitted to the ICU. The group of patients consisted of six (66.6 %) males and three (33.3 %) females. The mean age of the patients was 52 (48–70 years). Microbiological examination showed that three (50 % of males) males and one (33 % of females) female with mean age 55 (52–70 years) have developed cobacterial bacterial infection (Table 1).

3.2. **Bacterial isolates**

Of the nine COVID-19 patients in the ICU, four patients developed bacterial coinfection and we used the mini-BAL technique to isolate the bacterial pathogens from these patients. Three bacterial isolates were cultivable; all of them (100 %) were Gram negative. After running for about 4 h, the VITEK 2 system correctly identified the isolates as; *Klebsiella pneumoniae*, *Proteus mirabilis*, *Acinetobacter baumannii*. We found that *K. pneumoniae* was the most frequently reported (75 %) organism among the other bacterial isolates, followed by *P. mirabilis* and *A. baumannii* isolates, at 50 and 25 %, respectively (Fig. 1).

The identification information of VITEK 2 system; bionumber generated from the GN-ID card, probability percentage and analysis time for the three identified isolates are listed in Table 2.

3.3. **Antibiogram testing**

According to CLSI recommendations, we used disk diffusion assay with 19 antibiotics to determine the sensibility of the cobacterial pathogens obtained from hospitalized COVID-19 patients, we used the same antibiotics that are in the VITEK 2 cards. Our investigations prove that *A. baumannii* were resistant to 93 % of the tested antibiotics, while it was only sensitive to Colistin (10 μg/ml), indicated phenotypically by the inhibition zone of 13 mm. Similarly, *K. pneumoniae* was resistant to 88 % of all the tested antibiotics, while it was sensitive to Aztreonam (30 μg/ml; ZI = 14 mm) and Colistin (10 μg/ml; ZI = 12 mm). In like manner, *P. mirabilis* showed the maximum resistance (94 %), it was only susceptible to Amikacin (30 μg/ml; ZI = 15 mm) (Table 3, Fig. 2).

The VITEK 2 system susceptibility testing results for *K. pneumoniae*, *P. mirabilis*, and *A. baumannii* are listed in Table 4. *P. mirabilis* showed the highest rate of resistance (93.3 %), while recording intermediate susceptibility for Amikacin only. In addition, *A. baumannii* were sensitive to Colistin only, while they were resistant to 13 other types of tested antibiotics, recording a resistance rate of 92.8 %. Although *K. pneumoniae* was susceptible for two types of antibiotics (Aztreonam and Colistin), it recorded a resistance rate of 86.6 %, as it showed resistance to 13 types of tested antibiotics. Some organisms did not have a result of some of the listed antimicrobials (symbolized NR in Table 4). This may be because the test is not suitable for that particular organism/group of antimicrobials (indicated in the CLSI guidelines).

4. **Discussion**

Patients with a viral infection of the respiratory tract are prone to bacterial infection. Moreover, this cobacterial infection leads to complications worse than any infection alone and increases the
consumption of antibiotics as well as life-threatening outcomes comes as the disease becomes more aggressive and difficult to treat. The exact role of superinfection/coinfection in COVID-19 patients remains unclear [4,22]. Admission of patients to the ICU especially for long periods makes them more susceptible to secondary bacterial infections which increase the risk of death [23].

Therefore, the current study aimed to investigate the prevalence of bacterial coinfection among COVID-19 patients in ICU. The exact role of superinfection/coinfection in COVID-19 patients remains unclear [4,22]. Admission of patients to the ICU especially for long periods makes them more susceptible to secondary bacterial infections which increase the risk of death [23].

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Table 4. Antibiotic susceptibility testing of cobacterial pathogens by VITEK 2 system.

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I, intermediately susceptible; NR, test is not relevant for that particular organism/antimicrobial combination; R, resistant; S, sensitive.
COVID-19 patients admitted to ICU and examine the antibiotic resistance profile for the isolated cobacterial pathogens. Herein, the mini-BAL revealed that 44.4 % of the ICU COVID-19 patients have been infected with cobacterial pathogens, this high percentage may be due to excessive exposure of ICU patients to catheters, including the urinary, endotracheal, and arteriovenous tubes [24]. As well, the coinfection could be used as an indicator of disease virulence since more than 50 % of the hospitalized COVID-19 mortality were related to the presence of a fungal, bacterial, and/or viral coinfection [25]. Likewise, sputum samples of Chinese COVID-19 patients in ICU indicated that more than half of them experienced bacterial infection [26].

In our investigations we performed a mini-BAL technique to isolate potential bacterial pathogens associated with COVID-19 patients. Mini-BAL is a useful technique for identifying the etiology of pneumonia by sampling the respiratory tract (including the lower respiratory tract) through bronchoscopy [27]. Our mini-BAL sampling revealed six culturable bacterial isolates obtained from four COVID-19 patients, and we recorded 33 % of women with COVID-19 admitted to the ICU presenting cobacterial infection, while the percentage rose to 50 % for men's. Males appear to be more prone to coinfection. In the same context, Mutua et al. [28] revealed that males are three times more likely to contract coinfection than women. In addition to the fact that the male sex is more susceptible to infection, the length of stay in the hospital, especially admission to intensive care, are considered risk factors that could lead to the presence of coinfection [29].

Here, we used the VITEK 2 system to identify and test the antibiotic sensitivity of the bacterial isolates. The VITEK 2 is an automated microbiological system for identifying bacterial isolates at the species level and determining their sensitivity to prescribed antibacterial [30]. Most of the clinical isolates are identified accurately and quickly based on the biochemical reactions used by VITEK for identification, in addition to conducting antibiotic sensitivity tests of these isolates (using an algorithm of growth kinetics monitored by VITEK) [31].

According to VITEK 2 system identification, K. pneumoniae was the most frequent pathogen in our samples followed by P. mirabilis and A. baumannii in order, have been given the bionumbers (66011 34753565010, 0013000240442210 and 02410101035 00312 respectively), bionumbers may have epidemiological value [32].

Recently, Pourajam et al. [33] reported that, K. pneumoniae and A. baumannii was the most prevalent secondary bacterial infection among COVID-19 patients admitted to ICU in Iran through the first wave of the pandemic and patients with bacterial infection showed comprehensive antibiotics resistant. Moreover, a comparable study conducted in the USA to determine the prevalence of cobacterial pathogens in COVID-19 patients, the respiratory pathogens panel revealed that Staphylococcus aureus and P. mirabilis are the prevalent pathogens [34]. Similarly, the microbiological examination of the respiratory tract of hospitalized COVID-19 patients in Kazakhstan showed that K. pneumoniae, E. coli and A. baumannii are the prominent microbiota in percentages of 23, 12, and 11 %, respectively [35].

Antimicrobial resistance (AMR) poses a major global public health threat, rapid and accurate detection of AMR, together with judicious supervision of the use of appropriate antimicrobials in treatment, is essential to control the emergence and spread of AMR [36]. Public health and clinical microbiology laboratories are an important resource for monitoring emerging microbial threats and monitoring the development and spread of AMR. Results obtained from these laboratories can be used to monitor microbial evolution, emerging strains, and mutations for early detection of outbreaks, which is critical to containing these epidemics [37]. In the current study, the disk diffusion assay revealed that, the isolated cobacterial pathogens; K. pneumonia, A. baumannii, and P. mirabilis were highly resistant to the 19 tested antibiotics, the resistance rates were recorded as 88, 93, and 94 %, respectively. The disc diffusion test is the most widely used routine AST test in clinical microbiology laboratories since its development in the 1940's, it has been standardized for use in antibiotic susceptibility testing for the most common clinical human bacterial pathogens [38].

In the current study, cobacterial pathogens isolated from COVID-19 patients showed extensive antibiotic resistant. The isolated pathogens displayed more than 88 % resistance to the selected antibiotics including; beta-lactam, carbapenem, aminoglycoside, quinolone, tetracycline, rifamycin, and sulfonamide antibiotics. However, P. mirabilis was resistant to aminoglycoside (Amikacin), K. pneumonia was resistant to monobactam (Azteronam), while the polymyxin (Colistin) was efficient for controlling K. pneumonia and A. baumannii. Recently, Colistin was used clinically for treatment of multidrug resistant bacterial infections [39].

Hence, all of our isolates showed resistant to more than three classes of the tested antibiotics, it could be categorized as multidrug resistant pathogens [21]. In line with our results, Pseudomonas aeruginosa, K. pneumonia, E. coli and A. baumannii were the most
commonly pathogens isolated from sputum samples of COVID-19 patients in ICU in western Romania, more than 80 % of isolated cobacterial pathogens proved to be multidrug resistant [40]. In a comparative study exploring bacterial coinfection in ICU COVID-19 patients, 73 % of patients showed invasive infection with carbapenem-resistant A. baumannii, 60.7 % recorded ventilator-associated pneumonia and 26.6 % suffering blood stream Infections [41]. Recently, hospitalized SARS-CoV-2 patients in Libya were examined for identifying hospital associated infections, sequencing revealed that K. pneumoniae, Citrobacter freundii, E. coli, and A. baumannii were the predominant isolates, moreover, AST were performed by disk diffusion assay using Mueller–Hinton agar to characterize the carbapenem and colistin resistance isolates [42].

Although the diagnostic routine relies on traditional methods such as disk diffusion method to determine the sensitivity of bacteria to antibiotics based on the bacterial response to antimicrobials by examining the phenotypes, the results take at least 18–24 h for most clinically important bacteria, including the preceding isolation and identification, some organisms may require more time such as anaerobes and some slow-growing fastidious bacteria such as group of the HACEK (Haemophilus, Aggregatibacter, Kingella, Eikenella corrodens, and Cardiobacterium hominis species) Brucella species, etc. [43]. Currently, automated devices such as VITEK 2 system (bioMerieux), are used to identify bacteria, determine their antibiotic susceptibility and have been used efficiently and effectively in most clinical microbiology laboratories to reduce time (6–12 h) and improve cost-effectiveness. They measure slight changes in growth and susceptibility to antimicrobials using optical systems [36]. Accordingly, we employed the VITEK 2 system for AST, and its results were 100 % identical to those results that we obtained from the disk diffusion assay; the average time for results was 4–9 h (including the antibiogram card installation). In the same regard, VITEK 2 AST testing of Gram-negative bacterial pathogens manifested 97.4 % agreement with disk diffusion method [44].

Antibiogram testing declared the multidrug resistant potential of our isolates to selected sulfonamide, rifamycin, tetracycline, quinolones, and carbapenems. Although K. pneumoniae was sensitive to Azteronam, it was resistant to the rest of the tested beta-lactams, while A. baumannii and P. mirabilis were resistant to all the tested beta-lactams. Besides, the sensitivity of P. mirabilis to Amikacin, K. pneumoniae and A. baumannii showed extended resistant to all the tested aminoglycosides. Likewise, carbapenem-resistant K. pneumoniae, A. baumannii have been colonizing hospitalized SARS-CoV-2 patients [42]. In line with our results, bacterial screening of endotracheal aspirate from COVID-19 patients confirmed the prevalence of Klebsiella spp., E. coli, P. aeruginosa, methicillin-sensitive S. aureus, Streptococcus pneumoniae, methicillin-resistant S. aureus and Enterobacter spp, the antibiotic profile showed the high resistance of these pathogens to selected sulfonamide and beta-lactams, however all of them were sensitive to amikacin [45]. In the current examination A. baumannii and K. pneumoniae were susceptible to colistin. As well, Sahu et al. [46] reported that, E. coli, P. aeruginosa, and K. spp were the most common Gram-negative copathogens isolated from COVID-19 patients and the antibiotic susceptibility testing revealed that colistin was the most efficient drug.

Based on the above, we can recommend the use of the VTech system for accurate bacterial identification and low-time antibiotic susceptibility testing, which increases workflow in clinical microbiology laboratories.

5. Conclusion

The current study indicates that COVID-19 patients in ICUs are more prone to bacterial coinfection, which may increase disease virulence and risk of death.

We used the blind mini-BAL technique to sample pulmonary bacteria from COVID-19 patients admitted to the ICU, and used an automated VITEK 2 system to identify bacterial isolates and find out their antibiotic sensitivity. K. pneumoniae, P. mirabilis, and A. baumannii are the most frequently reported bacterial pathogens with widespread multidrug resistance. This study is a useful guide to prescribe appropriate treatment and strict supervision of antibiotic stewardship programs and infection control to stop the spread of antibiotic resistance within hospitals.

Conflicts of interest

There are no conflicts of interest.

References


