Al-Azhar Bulletin of Science

Volume 34 | Issue 3 Article 4

2023
Section: Botany and Microbiology

Bacterial Coinfections and Antibiogram profiles among ICU COVID-19 patients

Mohamed M. Elsharkawy
Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Cairo, Egypt

Ahmed M. Eid
Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Cairo, Egypt, aeidmicrobiology@azhar.edu.eg

Nancy M. ATTIA
Department of Microbiology, Medical Research Institute, Alexandria University, Alexandria, Egypt

Amr Fouda
Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Cairo, Egypt

How to Cite This Article

Elsharkawy, Mohamed M.; Eid, Ahmed M.; ATTIA, Nancy M.; and Fouda, Amr (2023) "Bacterial Coinfections and Antibiogram profiles among ICU COVID-19 patients," *Al-Azhar Bulletin of Science*: Vol. 34: Iss. 3, Article 4.
DOI: https://doi.org/10.58675/2636-3305.1656

ORIGINAL ARTICLE

Bacterial Coinfections and Antibiogram Profiles Among ICU Coronavirus Disease 2019 Patients

Mohamed Mohamed Elsharkawy [a], Ahmed Mohamed Eid [a,*], Nancy Mohamed Attia [b], Amr Fouda [a]

[a] Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Cairo, Egypt
[b] Department of Microbiology, Medical Research Institute, Alexandria University, Alexandria, Egypt

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 beta-lactams, 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,

Received 13 July 2023; revised 16 August 2023; accepted 24 September 2023.
Available online 27 November 2023

* Corresponding author at: Al-Azhar University, El-Nasr Road, Nasr City, Cairo 11884, Egypt.
E-mail address: aeidmicrobiology@azhar.edu.eg (A.M. Eid).

https://doi.org/10.58675/2636-3305.1656

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 co-infection 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 contradicts 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., Marky-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

Table 1. Characteristics of the study patients.

Characteristics	All patients ($N = 9$)	SARS-CoV-2 ($N = 9$) (100 %)	SARS-CoV-2/bacterial coinfection ($N = 4$) (44.4 %)
Age (years), mean	52	52	55
Sex [*n* (%)]			
Male	6 (66.66)	6 (66.66)	3 (50)
Female	3 (33.33)	3 (33.33)	1 (33)

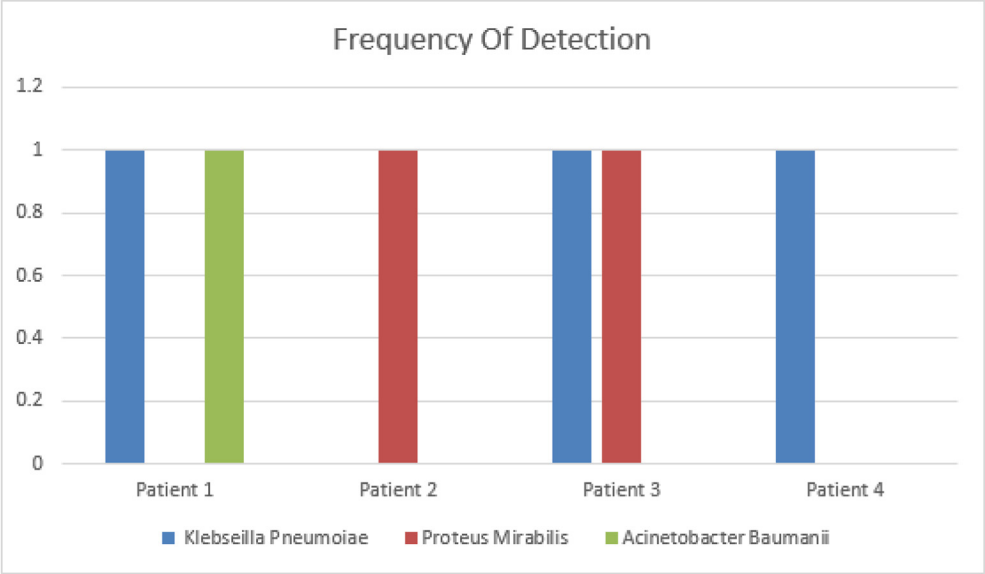

Fig. 1. Frequency of Gram-negative cobacterial pathogens among COVID-19 patients in ICU. COVID-19, coronavirus disease 2019.

Table 2. Identification outcomes of the VITEK 2 system for co bacterial pathogens isolated from coronavirus disease 2019 patients.

Bacterial species	Probability (%)	Bionumber	Analysis time (h)	Isolate source
Klebsiella pneumoniae	99	6601134753565010	4.78	Bronchoalveolar lavage
Proteus mirabilis	99	0013000240042210	3.93	Bronchoalveolar lavage
Acinetobacter baumannii	99	02410101035003 12	5.57	Bronchoalveolar lavage

Table 3. Susceptibility of cobacterial pathogens for selected antibiotics (disc-diffusion method).

Antibiotic	Concentration (μg/ml)	Susceptibility		
		Klebsiella pneumoniae	*Proteusmirabilis*	*Acinetobacter baumannii*
Ticarcillin	100	R	R	R
Clavulanic Acid	100	R	R	R
Piperacillin	100	R	R	R
Tazobactam	10	R	R	R
Ceftazidime	30	R	R	R
Cefepime	30	R	R	NR
Aztreonam	30	S	R	NR
Imipenem	10	R	R	R
Meropenem	15	R	R	R
Amikacin	30	R	S	R
Gentamicin	15	R	R	R
Tobramycin	15	R	R	R
Ciprofloxacin	5	R	R	R
Pefloxacin	10	NR	NR	NR
Minocycline	30	NR	NR	R
Colistin	10	S	R	S
Rifampicin	5	NR	NR	NR
Trimethoprim	20	R	R	R
Sulfamethoxazole	20	R	R	R

NR, test is not relevant for that particular organism/antimicrobial combination; R, resistant; S, sensitive.

consumption of antibiotics as well as life-threatening out 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

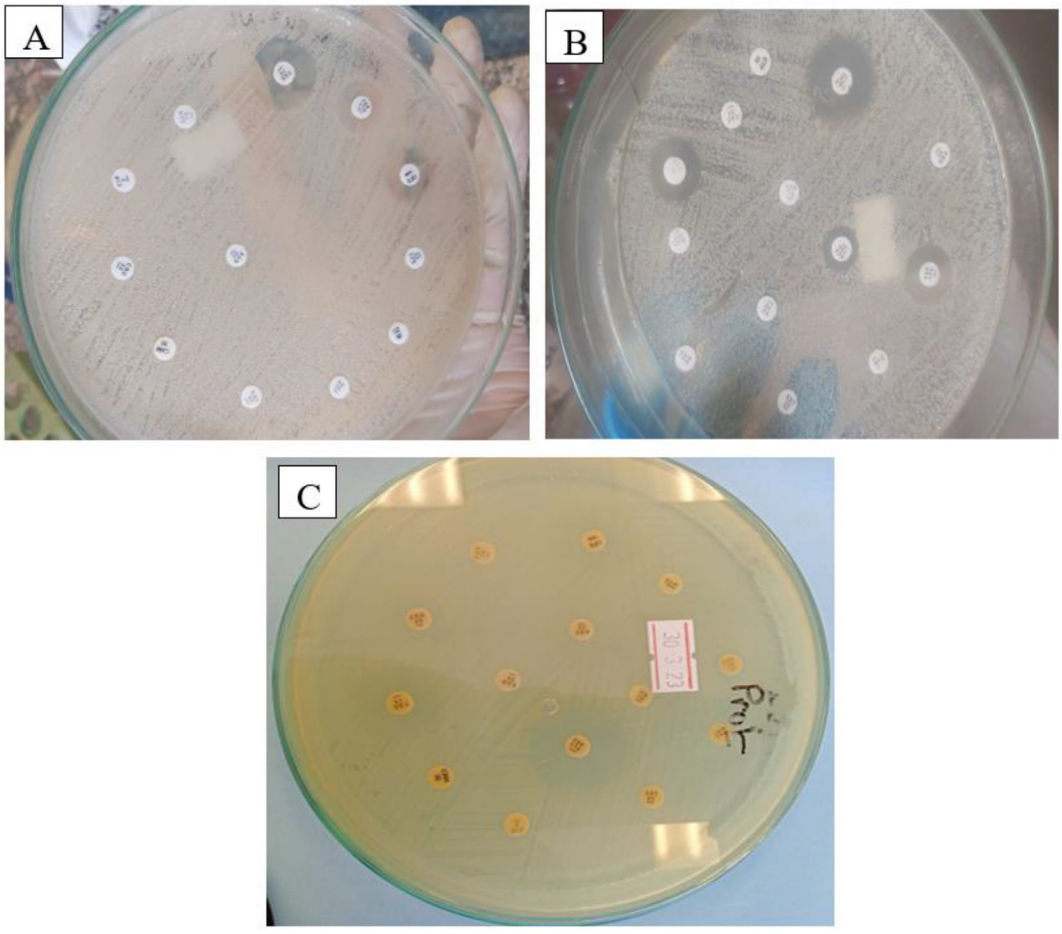

Fig. 2. Susceptibility of cobacterial pathogens for selected antibiotics. (a) K. pneumonia, (b) A. baumannii and (c) P. mirabilis.

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

Antimicrobial	*Klebsiella pneumoniae*		*Proteus mirabilis*		*Acinetobacter baumannii*	
	MIC	Interpretation	MIC	Interpretation	MIC	Interpretation
Ticarcillin	≥128	R	≥128	R	≥128	R
Ticarcillin/Clavulanic Acid	≥128	R	≥128	R	≥128	R
Piperacillin	≥128	R	≥128	R	≥128	R
Pipracillin/Tazobactam	≥128	R	64	R	≥128	R
Ceftazidime	≥64	R	≥64	R	≥64	R
Cefepime	≥64	R	≥64	R		NR
Aztreonam	≤1	S	16	R		NR
Imipenem	≥16	R	≥16	R	≥16	R
Meropenem	≥16	R	≥16	R	≥16	R
Amikacin	≥64	R	16	I	≥64	R
Gentamicin	≥16	R	≥16	R	≥16	R
Tobramycin	≥16	R	≥16	R	≥16	R
Ciprofloxacin	≥4	R	≥4	R	≥4	R
Pefloxacin		NR		NR		NR
Minocycline		NR		NR	≥16	R
Colistin	≤0.5	S	≥16	R	≤0.5	S
Rifampicin		NR		NR		NR
Trimethoprim/Sulfamethoxazole	≥320	R	≥320	R	≥320	R

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, 0013000240042210 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 (bioMérieux), 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

[1] Arnold FW, Fuqua JL. Viral respiratory infections: a cause of community acquired pneumonia or a predisposing factor? Curr Opin Pulm Med 2020;26:208−14.

[2] Rice TW, Rubinson L, Uyeki TM, Vaughn FL, John BB, MillerIII RR, et al. Critical illness from 2009 pandemic influenza A (H1N1) virus and bacterial co-infection in the United States. Crit Care Med 2012;40:1487.

[3] Morris DE, Cleary DW, Clarke SC. Secondary bacterial infections associated with influenza pandemics. Front Microbiol 2017;8:1041.
[4] Santos AP, Gonçalves LC, Oliveira ACC, Queiroz PHP, Ito CRM, Santos MO, et al. Bacterial co-infection in patients with COVID-19 hospitalized (ICU and not ICU): review and meta-analysis. Antibiotics 2022;11:894.
[5] Garcia-Vidal C, Sanjuan G, Moreno-García E, Puerta-Alcalde P, Garcia-Pouton N, Chumbita M, et al. Incidence of co-infections and superinfections in hospitalized patients with COVID-19: a retrospective cohort study. Clin Microbiol Infect 2020;27:83−8.
[6] WHO coronavirus (COVID-19) dashboard. https://covid19.who.int/. [Accessed 25 March 2023].
[7] Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): a review. JAMA 2020;324:782−93.
[8] Lansbury L, Lim B, Baskaran V, Lim WS. Co-infections in people with COVID-19: a systematic review and meta-analysis. J Infect 2020;81:266−75.
[9] Phua J, Weng L, Ling L, Egi M, Lim CM, Divatia JV, et al. Intensive care management of coronavirus disease 2019 (COVID-19): challenges and recommendations. Lancet Respir Med 2020;8:506−17.
[10] Langford BJ, So M, Raybardhan S, Leung V, Westwood D, MacFadden DR, et al. Bacterial coinfection and secondary infection in patients with COVID-19: a living rapid review and meta-analysis. Clin Microbiol Infect 2020;26:1622−9.
[11] Cavalcante GA. BACTERIAL coinfection in patients with COVID-19. Rev Multidiscip Em Saúde 2021;2:14.
[12] Díaz JML, Mendoza-Olazarán S, Camacho-Ortiz A, Flores-Treviño S, Garza-González E. One-year surveillance of ESKAPE pathogens in an intensive care unit of monterrey, Mexico. Chemotherapy 2012;58:475−81.
[13] WHO. Clinical management of COVID-19: interim guidance, 27 May 2020. World Health Organization; Geneva: 2020. https://iris.who.int/handle/10665/332196?locale-attribute=pt&show=full.
[14] Alshaikh FS, Godman B, Sindi ON, Seaton RA. Kurdi A Prevalence of bacterial coinfection and patterns of antibiotics prescribing in patients with COVID-19: a systematic review and meta-analysis. PLoS One 2022;17:e0272375.
[15] Dalton KR, Rock C, Carroll KC, Davis MF. One health in hospitals: how understanding the dynamics of people, animals, and the hospital built-environment can be used to better inform interventions for antimicrobial-resistant gram-positive infections. Antimicrob Resist Infect Control 2020;9:1−17.
[16] Alhazzani W, Møller MH, Arabi YM, Loeb M, Gong MN, Fan E, et al. Surviving sepsis campaign: guidelines on the management of critically ill adults with coronavirus disease 2019 (COVID-19). Intensive Care Med 2020;46:854−87.
[17] Erden V, Basaranoglu G, Beycan I, Delatioglu H, Hamzaoglu NS. Reproducibility of mini-BAL culture results using 10 ml or 20 ml instilled fluid. Intensive Care Med 2003;29:1856.
[18] Ligozzi M, Bernini C, Bonora MG, De Fatima M, Zuliani J, Fontana R. Evaluation of the VITEK 2 system for identification and antimicrobial susceptibility testing of medically relevant gram-positive cocci. J Clin Microbiol 2002;40:1681−6.
[19] Ibrahim AG, Fouda A, Elgammal WE, Eid AM, Elsenety MM, Mohamed AE, et al. New thiadiazole modified chitosan derivative to control the growth of human pathogenic microbes and cancer cell lines. Sci Rep 2022;12:21423.
[20] Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 23rd informational supplement. CLSI document M100-S23. Wayne, PA: Clinical Laboratory Standards Institute; 2013.
[21] Al-Muhanna AS, Al-Muhanna S, Alzuhairi MA. Molecular investigation of extended-spectrum beta-lactamase genes and potential drug resistance in clinical isolates of Morganella morganii. Ann Saudi Med 2016;36:223−8.
[22] Feldman C, Anderson R. The role of co-infections and secondary infections in patients with COVID-19. Pneumonia 2021;13:5.
[23] Elabbadi A, Turpin M, Gerotziafas GT, Teulier M, Voiriot G, Fartoukh M. Bacterial coinfection in critically ill COVID-19 patients with severe pneumonia. Infection 2021;49:559−62.
[24] Zhu X, Ge Y, Wu T, Zhao K, Chen Y, Wu B, et al. Co-infection with respiratory pathogens among COVID-2019 cases. Virus Res 2020;285:198005.
[25] Bazaid AS, Barnawi H, Qanash H, Alsaif G, Aldarhami A, Gattan H, et al. Bacterial coinfection and antibiotic resistance profiles among hospitalised COVID-19 patients. Microorganisms 2022;10:495.
[26] Yang S, Hua M, Liu X, Du C, Pu L, Xiang P, et al. Bacterial and fungal co-infections among COVID-19 patients in intensive care unit. Microb Infect 2021;23:104806.
[27] Morandi L, Torsani F, Forini G, Tamburrini M, Carnevale A, Pecorelli A, et al. The additional value of lower respiratory tract sampling in the diagnosis of COVID-19: a real-life observational study. Diagnostics 2022;12:2372.
[28] Mutua JM, Njeru JM, Musyoki AM. Multidrug resistant bacterial infections in severely ill COVID-19 patients admitted in a national referral and teaching hospital, Kenya. BMC Infect Dis 2022;22:877.
[29] Fehér Á, Szarvas Z, Lehoczki A, Fekete M, Fazekas-Pongor V. Co-infections in COVID-19 patients and correlation with mortality rate. Minirev Physiol Int 2022;109:1−8.
[30] Stefaniuk E, Mrówka A, Hryniewicz W. Susceptibility testing and resistance phenotypes detection in bacterial pathogens using the VITEK 2 System. Pol J Microbiol 2005;54:311−6.
[31] Garcia-Garrote F, Cercenado E, Bouza E. Evaluation of a new system, VITEK 2, for identification and antimicrobial susceptibility testing of enterococci. J Clin Microbiol 2000;38:2108−11.
[32] Bannerman TL, Kleeman KT, Kloos WE. Evaluation of the Vitek Systems Gram-positive identification card for species identification of coagulase-negative staphylococci. J Clin Microbiol 1993;31:1322−5.
[33] Pourajam S, Kalantari E, Talebzadeh H, Mellali H, Sami R, Soltaninejad F, et al. Secondary bacterial infection and clinical characteristics in patients with COVID-19 admitted to two intensive care units of an academic hospital in Iran during the first wave of the pandemic. Front Cell Infect Microbiol 2022;12:784130.
[34] Lehmann CJ, Pho MT, Pitrak D, Ridgway JP, Pettit NN. Community acquired co-infection in COVID-19: a retrospective observational experience. Clin Infect Dis 2020;72:1450.
[35] Lavrinenko A, Kolesnichenko S, Kadyrova I, Turmukhambetova A, Akhmaltdinova L, Klyuyev D. Bacterial co-infections and antimicrobial resistance in patients hospitalized with suspected or confirmed COVID-19 pneumonia in Kazakhstan. Pathogens 2023;12:370.
[36] Gajic I, Kabic J, Kekic D, Jovicevic M, Milenkovic M, Mitic Culafic D, et al. Antimicrobial susceptibility testing: a comprehensive review of currently used methods. Antibiotics 2022;11:427.
[37] Galar A, Kulldorff M, Rudnick W, O'Brien TF, Stelling J. Biochemical phenotypes to discriminate microbial subpopulations and improve outbreak detection. PLoS One 2013;8:e84313.
[38] Clinical and Laboratory Standards Institute. M02: performance standards for antimicrobial disk susceptibility tests. 13th ed. Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2018.
[39] Okdah L, Leangapichart T, Hadjadj L, Olaitan AO, Al-Bayssari C, Rizk R, et al. First report of colistin-resistant Klebsiella pneumoniae clinical isolates in Lebanon. J Glob Antimicrob Resist 2017;9:15−6.
[40] Cut TG, Mavrea A, Cumpanas AA, Novacescu D, Oancea CI, Bratosin F, et al. A retrospective assessment of sputum samples and antimicrobial resistance in COVID-19 patients. Pathogens 2023;12:620.

[41] Montrucchio G, Corcione S, Lupia T, Shbaklo N, Olivieri C, Poggioli M, et al. The burden of carbapenem-resistant Acinetobacter baumannii in ICU COVID-19 patients: a regional experience. J Clin Med 2022;11:5208.
[42] Slimene K, Ali AA, Mohamed EA, El Salabi A, Suliman FS, Elbadri AA, et al. Isolation of carbapenem and colistin resistant Gram-negative bacteria colonizing immunocompromised SARS-CoV-2 patients admitted to some Libyan hospitals. Microbiol Spectr 2023;11:e0297222.
[43] Khan ZA, Siddiqui MF, Park S. Current and emerging methods of antibiotic susceptibility testing. Diagnostics 2019; 9:49.
[44] Wen H, Xie S, Liang Y, Liu Y, Wei H, Sun Q, et al. Direct identification, antimicrobial susceptibility testing, and extended-spectrum β-lactamase and carbapenemase detection in Gram-negative bacteria isolated from blood cultures. Infect Drug Resist 2022;15:1587−99.
[45] Mahmoudi H. Bacterial co-infections and antibiotic resistance in patients with COVID-19. GMS Hyg Infect Control 2020;15:Doc35.
[46] Sahu C, Singh S, Pathak A, Singh S, Patel SS, Ghoshal U, et al. Bacterial coinfections in COVID: prevalence, antibiotic sensitivity patterns and clinical outcomes from a tertiary institute of Northern India. J Fam Med Prim Care 2022;11:4473−8.