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Detecting Pathogenic Agents in Mechanically-Ventilated, Critically-Ill COVID-19 Patients with Ventilator-Associated *Pneumonia*

Alireza Abdollahi [1], Mohammadreza Salehi [2], Ali Ahmadi [3], Sadegh Khodavaisy [3], Seyed Ali Dehghan Manshadi [2], Mehdi Norouzi [4, 5], Pegah Afarinesh Khaki [6], Mahsa Norouzi Shadehi [2], Maryam Shadkam [6], Mahsa Abdorahimi [7], Reza Keikhaei [8], Ehsan Shiralipour [3] and Ronak Bakhtiari [5, 9, *]

[1]Department of Pathology, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran
[2]Department of Infectious Diseases and Tropical Medicine, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran
[3]Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[4]Department of Virology, School Public Health, Tehran University of Medical Sciences, Tehran, Iran
[5]Research Center for Clinical Virology, Tehran University of Medical Sciences, Tehran, Iran
[6]Central Laboratory, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran
[7]Department of Microbiology, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran
[8]School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[9]Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

[*]*Corresponding author*: Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran. Tel: +98-9121782075, Email: rounakbakhtiari@yahoo.com

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Abstract

Background: The coronavirus disease 2019 (COVID-19) pandemic has been a global shock since its initial spread in 2019. Medically, patients with coronavirus disease, especially those with pneumonitis, face serious life-threatening risks and often require mechanical ventilation and intensive care. Ventilator-associated pneumonia (VAP) remains a significant concern for critical care providers. Recent reports have highlighted the susceptibility of patients with confirmed COVID-19 receiving mechanical ventilation to nosocomial pneumonia (NP).

Methods: This study was a cross-sectional study conducted in the intensive care unit (ICU) at Imam Khomeini Hospital Complex (IKHC) in Tehran, Iran, within April 2020 to April 2021. The study focused on critically ill COVID-19 patients who required mechanical ventilation and met the criteria for VAP. Standard biochemical assays were used to identify pure colonies in patients' sample cultures, and antimicrobial susceptibility tests were conducted to assess antimicrobial resistance profiles. The findings were analyzed statistically using SPSS software (version 23.0).

Results: Out of 93 endotracheal aspirate samples, 64 samples tested positive for bacteria. Among the 64 eligible patients with positive cultures, 42 (65.6%) and 22 (34.4%) patients were male and female, respectively, with a mean age of 60.56 ± 13.58 years. A total of 52 patients (81.25%) had underlying conditions, such as hypertension, diabetes, and kidney or heart diseases. According to the study results, the most common pathogens were extensively drug-resistant (XDR) *Klebsiella pneumoniae* (7%) and *Acinetobacter baumannii* (23%). Additionally, 80% of *Klebsiella pneumoniae* and 90% of *Acinetobacter baumannii* were observed to be multi-drug resistant ($P < 0.05$).

Conclusions: The COVID-19 pandemic has posed significant risks to critically ill patients, often necessitating mechanical ventilation and intensive care. Furthermore, VAP remains a serious challenge in this context, with high rates of XDR *K. pneumoniae* and *A. baumannii*. Effective infection control measures and surveillance are critical to mitigating the risk of NP in these vulnerable patients.

Keywords: COVID-19, Ventilator-Associated Pneumonia, Antimicrobial Resistance, Iran

1. Background

The recent coronavirus disease (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been a global challenge since December 2019. Typically, 5 - 15% of patients require hospitalization, and in severe cases, intensive care with mechanical ventilation support becomes necessary due to the development of severe respiratory disease (1, 2). Consequently, intensive care units (ICUs) worldwide have

been under significant strain.

Despite three years of dealing with the pandemic, ventilator-associated pneumonia (VAP), which occurs after 48 hours or longer of intubation (3), remains a critical technical issue for healthcare providers. Nearly 42.7% of VAP-related deaths are projected to occur in COVID-19 patients (4). Although COVID-19-related fatalities have predominantly affected the elderly with underlying health conditions, nosocomial pneumonia (NP) in ICUs, especially when patients are intubated, remains a significant risk factor. Furthermore, elderly individuals with underlying conditions, such as hypertension, diabetes, dyslipidemia, and cardiovascular disease (CVD), face a higher risk of mortality from COVID-19 (5). The situation can worsen in cases of lower respiratory tract infections (RTIs) (6, 7).

Studies have also indicated that patients with COVID-19 are more likely to develop community-acquired *pneumonia* (CAP); however, VAP-related mortality rates are nearly three times higher than those linked to CAP (8-10). *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii*, and *Klebsiella pneumoniae* are among the most significant microbial agents responsible for VAP, leading to high mortality rates worldwide (11-16). Numerous COVID-19 patients develop secondary bacterial infections (SBIs), which exacerbate disease severity and can result in death, particularly among those requiring invasive mechanical ventilation. In general, COVID-19 patients have low rates of bacterial coinfections and secondary infections; nevertheless, critically ill ICU patients exhibit higher rates (17).

Secondary bacterial infections are most common among critically ill patients (34.5%), followed by moderate and mild cases (3.9% and 8.3%, respectively) (18). Fast and accurate identification of bacteria as either resident or pathogenic microorganisms in COVID-19 patients should be a crucial step in managing these cases (19-21). However, the data on the rate of SBIs in hospitalized COVID-19 patients, especially those in critical condition, are limited (22).

2. Objectives

This study aimed to assess the frequencies and characteristics of SBIs and antimicrobial resistance (AMR) profiles in critically ill COVID-19 patients with VAP in ICUs.

3. Methods

This cross-sectional study aimed to identify microbial pathogens causing VAP in critically ill COVID-19 patients. The study included patients with confirmed COVID-19 who were admitted to the ICUs at Imam Khomeini Hospital Complex (IKHC) in Tehran, Iran, within April 2020 to April 2021.

3.1. Patients

The inclusion criteria consisted of patients with confirmed COVID-19, verified through SARS-CoV-2 reverse transcription-polymerase chain reaction (RT-PCR) testing on respiratory secretions, who required mechanical ventilation (for more than 48 hours) and exhibited VAP criteria, which included new or persistent infiltrations in chest radiographs, fever exceeding 38°C or hypothermia below 36°C, a white blood cell count exceeding 10 000 or dropping below 5000 cells/mL, and a decrease in the ratio of arterial oxygen partial pressure/fractional inspired oxygen (PaO_2/FiO_2) or the presence of purulent tracheal secretions (23).

3.2. Sample Collection

Endotracheal aspirate (ETA) samples were collected using sterile tubes following standard clinical protocols (24). These samples were promptly transported to a microbiology laboratory and processed using conventional methods. Initially, the samples were cultured on blood, eosin methylene blue (EMB), and chocolate agars (Merck, Germany) and then incubated at 37°C for 24 - 72 hours in an environment with 5% CO_2 under standard conditions. Plates with colony counts $\geq$ 104 colony-forming units (CFU/mL) were selected for further analysis. Pure colonies were identified through conventional biochemical assays.

3.3. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing (AST) was conducted for various antibiotics, including cefoxitin (FOX, 30 μg), imipenem (IMP, 10 μg), ceftazidime (CAZ, 30 μg), amikacin (AK, 30 μg), azithromycin (AZM, 15 μg), gentamicin (GM, 10 μg), ceftriaxone (CRO, 30 μg), ciprofloxacin (CP, 5 μg), trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 μg), levofloxacin (LEV, 5 μg), erythromycin (E, 15 μg), piperacillin-tazobactam (PIT, 100/10 μg), rifampicin (RIF, 5 μg), and ampicillin-sulbactam (AMS, 10/10 μg) (Rosco, Taastrup, Denmark). The testing was performed on Muller-Hinton media (Merck, Germany) using the Kirby-Bauer disk diffusion method, following the Clinical and Laboratory Standards Institute (CLSI) guidelines. Additionally, E-tests (Liofilchem, Italy) were employed to determine bacterial susceptibilities to vancomycin and colistin, adhering to the manufacturer's instructions. The plates were then incubated at 37°C for

18 hours. The interpretation of AST results was based on established protocols to identify multidrug-resistant (MDR) (25) and extensively drug-resistant (XDR) (26) bacteria. According to the criteria provided by the Centers for Disease Control and Prevention (CDC), bacterial isolates that exhibited resistance to three or more antimicrobials were classified as MDR isolates; nevertheless, XDR isolates were those susceptible to just one or a maximum of two categories of antimicrobials. Control strains of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used for quality control purposes.

3.4. Statistical Analysis

For the statistical analysis, the data were imported into Microsoft Excel Plus 2019 Software and subsequently analyzed using SPSS software (version 23.0; IBM, USA). Continuous variables were presented as means ± standard deviation (SD), and comparisons between two groups were performed using Student's *t*-test. Categorical variables were reported as counts (frequencies) and proportions (percentages), with multiple comparisons conducted using the chi-square test. Statistical significance was indicated when $P < 0.05$.

4. Results

Out of 241 COVID-19-verified patients, 93 met the criteria for diagnosing VAP. Among the 64 patients with positive cultures of ETA, 42 (65.6%) and 22 (34.4%) subjects were male and female, respectively, with a mean age of 60.56 ± 13.58 years. Furthermore, 52 patients (81.25%) had underlying diseases, including hypertension, diabetes, kidney, and heart diseases. Eventually, 55 patients (86%) died, with an average age of 62 ± 2 years, among whom 78.2% (43 patients) had a history of underlying diseases. Moreover, the length of hospitalization in *K. pneumoniae*-positive patients was longer than that in *A. baumannii*-positive patients. Demographic data of the patients, including age, gender, underlying disease, and length of hospitalization in ICUs, are reported in Table 1. No significant associations were observed between patients' demographic data and VAP ($P > 0.05$).

The three most prevalent bacteria included *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa*, isolated from 28 (43.8%), 26 (40.6%), and 3 (4.7%) samples, respectively. The most prevalent isolated pathogens are listed in Tables 2 and 3. Significant associations were reported between infections with *K. pneumoniae* and *A. baumannii* in male and female subjects over 50 years ($P < 0.001$ and $P < 0.01$, respectively). However, these associations were not significant in male and female patients under 50 years ($P < 0.1$).

In this study, 75.4% of *K. pneumoniae* isolates were resistant to colistin; however, 88.46% of *A. baumannii* isolates were resistant to this antimicrobial. This high-level resistance to a last-resort antimicrobial could lead to increases in mortality rates in patients with MDR pathogens. As seen in Table 1, 92.3% (n = 24) and 75% (n = 21) of the patients with *A. baumannii* and *K. pneumoniae*-positive cultures died. The highest carbapenem-resistant (100%) rate belonged to *A. baumannii*, as bacteria showed resistance to meropenem. All strains of *A. baumannii* were also resistant to amikacin (100%). Table 4 shows the frequency of drug-resistance patterns in commonly isolated bacterial species. In general, resistance patterns of *K. pneumoniae* and A. baumannii isolates were not different between the non-survived and survived patients. The assessments of AST revealed that 80% of *K. pneumonia* and 90% of *A. baumannii* were MDR; nevertheless, 7% of *K. pneumonia* and 23% of *A. baumannii* were XDR with resistance to almost all the antimicrobials ($P = 0.0012$).

5. Discussion

Coronavirus disease 2019, a viral pneumonia with a rapidly unique outbreak, is considered a novel public health hazard, posing a global threat to nations. Recent studies suggest that SARS-CoV-2 originated in animals and evolved into different variations, crossing species barriers to infect humans (27, 28). In previous epidemics of severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), patients receiving invasive mechanical ventilation were susceptible to SBIs, leading to higher mortality rates (29, 30).

Traditionally, *P. aeruginosa*, *Acinetobacter* spp., and MRSA were identified as the most common causes of CAP and VAP. However, recent reports have highlighted *K. pneumoniae* and Acinetobacter spp. as the predominant pathogens in these patients (13, 15, 16). These findings align with a prior study by the current authors, which also identified *K. pneumoniae* and *A. baumannii* as the most prevalent pathogens. This study primarily focused on SBIs of the lower respiratory tract (RT) in critically ill COVID-19 patients intubated in ICUs. Surprisingly, microbiological examinations revealed that all collected specimens were contaminated by bacteria (64/64, 100%). These data suggested an association between VAP and increased patient mortality rates (55/64, 86%).

Similarly, a study in Egypt showed that specimens from critically ill COVID-19 patients under mechanical ventilation were positive for bacteria (197/197, 100%). Furthermore, significant relationships were observed

Table 1. Demographic and Clinical Characteristics of the Patients [a]

Characteristics	Details
Total number of patients	64 (100)
Male patients	42 (65.6)
Female patients	22 (34.4)
Patients' ICU length of stay (days)	22 ± 12.2
ICU length of stay for *Acinetobacter baumannii*-positive patients (days)	20 ± 13.7
ICU length of stay for *Klebsiella pneumonia*-positive patients (days)	25.2 ± 11
Mortality rates for *Acinetobacter baumannii*-positive patients	24 (92.3)
Mortality rates for *Klebsiella pneumonia*-positive patients	21 (75)
Patients with underlying diseases	52 (81.25)
Expired patients	55 (85.9)
Survived patients	9 (14.1)
Mean age of the expired patients (y)	62 ± 2

[a] Values are presented as No. (%) or mean ± SD.

Table 2. Type and Frequency of the Isolated Pathogens from Critically Ill Coronavirus Disease 2019 (COVID-19) Male Patients

Bacteria	No. (%) of Patients	Age < 50	Age > 50	P-Value
Klebsiella pneumonia	28 (43.8)	3	16	< 0.001
Acinetobacter baumannii	26 (40.6)	4	10	
Pseudomonas aeruginosa	3 (4.7)	-	2	
Klebsiella oxytoca	1 (1.6)	-	1	
Enterococcus faecalis	1 (1.6)	1	-	
Achromobacterdenitrificans	1 (1.6)	-	1	
Coagulase-negative *staphylococci*	1 (1.6)	-	1	
Stenotrophomonas maltophilia	1 (1.6)	-	1	
***Klebsiella* spp.**	1 (1.6)	-	-	
Mixed growth	1 (1.6)	-	1	

Table 3. Type and Frequency of the Isolated Pathogens from Critically Ill Coronavirus Disease 2019 (COVID-19) Female Patients

Bacteria	No. (%) of Patients	Age < 50	Age > 50	P-Value
Klebsiella pneumonia	9 (39.2)	2	7	
Acinetobacter baumannii	12 (52.1)	3	9	
Pseudomonas aeruginosa	1 (4.35)	-	1	0.002
***Klebsiella* spp.**	1 (4.35)	-	1	

between VAP and mortality rates (31). Nevertheless, studies in China and the UK reported that only 13.9% and 6.1% of COVID-19 ICU patients developed SBIs, respectively (32, 33). Differences in the results of these studies might be attributed to various variables affecting the quality of care and the frequency of ICU-acquired infections, including the type of ICUs, equipment quantity, admission/discharge criteria, and patient-to-nurse ratios.

In recent years, researchers have documented the occurrence of hypervirulent strains of both bacteria with resistance to various antimicrobial categories (34, 35). In a 2021 study in Egypt, the most frequently isolated bacteria from critically ill COVID-19 patients intubated in ICUs included *K. pneumoniae* and *A. baumannii* (33). Statistically, *K. pneumoniae* and Acinetobacter spp. exhibited the highest incidence rates in ICUs, as indicated by a 2019

Table 4. Frequency of Drug Resistance Patterns in Commonly Isolated Bacterial Species

Bacteria and Pattern [a]	No. (%)	P-Value
Klebsiella pneumonia		0.0012
XDR	2 (7)	
MDR	23 (80)	
MBL	8 (29)	
Acinetobacter baumannii		
XDR	6 (23)	
MDR	23 (90)	
MBL	21 (81)	

Abbreviations: MDR, multi-drug resistant; XDR, extensively drug resistant; MBL, metallo-β-lactamase.
[a] Drug resistance pattern.

study in Tehran, Iran (36). In a study conducted in India in 2018 (37), *A. baumannii* and *Klebsiella* spp. were the most prevalent bacteria isolated in Mysuru, India.

All bacterial isolates in the present study displayed high resistance to the highlighted antimicrobials. This resistance could be attributed to the scheduled administration of antimicrobials for COVID-19, which might have controlled other more susceptible pathogens, allowing resistant bacterial survivors to evade management protocols. Metallo-β-lactamase (MBL)-producing *K. pneumoniae* and *A. baumannii* were also identified as causative microorganisms of VAP in participants. Clinically, VAP was suspected after prolonged mechanical ventilation, similar to the scenario in the current study. This extended duration allowed for bacterial superinfections. The fatality rate of VAP in ICU patients typically varies from 20% to 50%, and it might be much higher when caused by antimicrobial-resistant bacteria (38).

The current study unexpectedly reported high rates of SBI VAP and significant ($P < 0.001$) mortality rates of 87.5% in ICUs. In some cases, mortality rates of patients in other studies exhibited significant differences between the two groups of *A. baumannii* and *K. pneumoniae*-positive patients. Overall, mortality rates were significantly higher in patients with *A. baumannii* infections (84.8%) than in those with *K. pneumoniae* infections (44.5%) ($P < 0.001$) (39, 40). In contrast to the current results, differences in the mortality rates of patients with *A. baumannii* and *K. pneumoniae* infections were insignificant in 37.5% and 32.8% of the patients, respectively.

Studies have generally shown that 75% of *K. pneumoniae* (41) and 86.3% of *A. baumannii* (42) strains were MDR. This trend was also observed in the current study, as AST revealed significant MDR rates of 80% for *K. pneumoniae* and 90% for *A. baumannii* isolates, respectively. Geographic distance, hygiene levels, specimen types, study dates, sample sizes, and antimicrobial use restrictions might account for these inconsistencies. In another study, 58% of *K. pneumoniae* (43) and 69.6% of *A. baumannii* (44) strains were reported as MDR.

Overall, the existing AMR situation is critical and must be addressed following CDC standards and recommendations. Adequate staffing is essential to improve infection control and reduce burnout among overworked healthcare workers. Additionally, medical equipment should be thoroughly disinfected before transferring between patients' rooms in COVID-19 ICUs, and handwashing and hygiene facilities are crucial, preferably equipped with touchless sensors (45). Furthermore, the predicted increase in AMR is a result of inappropriate and extensive antimicrobial use, particularly during the COVID-19 pandemic. To prevent potentially fatal overuse of antimicrobials, patients should receive empirical treatments with the most appropriate antimicrobials based on clinical findings and global standards. In light of the current microbiological findings, empirical therapy should be promptly adjusted.

5.1. Study Limitations

The current study has certain limitations. It only included infections confirmed by cultures, potentially omitting some cases. Moreover, the study was confined to a single institution with its unique local epidemiology of AMR, which might limit the generalizability of the findings.

5.2. Conclusions

In conclusion, the COVID-19 pandemic poses a severe health risk to individuals, particularly those with pneumonitis who require critical care and mechanical ventilation. The present study has

highlighted the significant problem of VAP in critically ill COVID-19 patients, particularly in the context of highly drug-resistant *K. pneumoniae* and *A. baumannii*. This finding underscores the urgent need for targeted antimicrobial strategies in such cases. These findings emphasize the crucial importance of stringent infection control protocols and surveillance programs to reduce the incidence of NP in vulnerable patients.

Overall, VAP remains a serious concern in critically ill COVID-19 patients, and as demonstrated in this study, there is an urgent need for action plans to enhance epidemic control efforts. Since the COVID-19 pandemic persists, exploring various solutions to address this critical issue is essential. One potential solution could involve the development of novel drugs targeting severe bacterial infections, particularly those caused by *K. pneumoniae* and *A. baumannii*. Such advancements can contribute to more effective treatment of emerging pandemics.

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Footnotes

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