Evaluation of Bacterial Coinfection and Antibiotic Resistance in Patients with COVID-19 Under Mechanical Ventilation

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Abstract

Patients with confirmed SARS-CoV-2 are principally at risk of emerging superinfections, particularly those caused by Gram-negative bacteria. Therefore, in this retrospective cohort study, we investigated the presence of bacteria in endotracheal aspirate samples in severe COVID-19 patients under mechanical ventilation between 20 February 2020 and 21 September 2020 in Mazandaran Heart Center Hospital, Iran. Outcomes were compared between ICU patients with confirmed SARS-CoV-2 (corona group) and those who suffer from other disease (non-corona group). Out of 38 subjects who met the diagnostic criteria for ventilator-associated pneumonia (VAP) in ICU, 22 and 16 patients in corona and non-corona groups, respectively, were enrolled in the study. Hospital length of stay in 27% of case in corona group was > 10 days. Also, SOFA score was > 10 in 64% and 25% of corona and non-corona groups, respectively ($P < 0.05$). Moreover, the number of death was significantly higher among corona patients (45%) than non-corona group (6%) in ICU ($P < 0.05$). *Acinetobacter* spp. were the most common bacteria in nine corona patients (41%) that were 100% resistant to amikacin, gentamycin, cefixime, and imipenem antibiotics. The prevalence of antibiotic resistance among pathogens isolated from patients with COVID-19 under mechanical ventilation in ICU highlighted the importance of preventing coinfections caused by this pathogen, suggesting an essential standardized approach to antibiotic stewardship in patients with COVID-19 for successful treatment.

Keywords SARS-CoV-2 · Ventilator-associated pneumonia · Bacterial coinfection · Antibiotic resistance

Introduction

SARS-CoV-2, first identified in Wuhan, China, is responsible for the illness named coronavirus disease 2019 (COVID-19), which has quickly become epidemic worldwide in the twenty-first century with high rate of mortality and morbidity [1–3]. This new pathogen is a beta coronavirus, which arrives into the lungs and heart, kidney, or gastrointestinal cells by fusion with human angiotensin-converting enzyme 2 (ACE2) receptor, and the spike protein of SARS-CoV-2 with a functional polybasic cleavage site plays a vital role in this [4]. The symptoms of disease are associated with age, immune system status, and patient's underlying disease ranged from asymptomatic/mildly symptomatic emersions to acute respiratory failure with higher mortality [5–7]. In case of severe COVID-19 disease due to respiratory failure, patients requiring ventilation support by mechanical ventilation and endotracheal intubation and outstripping the intensive care unit (ICU) bed valence in the most affected

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countries that increased risk to acquire bacterial ICU-pneumonia in this condition [8].

The previous studies reported that secondary bacterial infections, particularly with *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Staphylococcus aureus* increased in pneumonitis epidemics and pandemics [9–11]. Based on a systematic review, bacterial coinfections were detected in 7% and 14% of COVID-19 hospitalized cases and ICU patients, respectively. In these patients, bacterial coinfection was reported as a predictor of mortality [12]. Also, secondary bacterial infections were reported in 15% of hospitalized adult patients from COVID-19 cases in Wuhan [13]. Furthermore, it is known that the mortality rate can be up to 60% when ventilator-associated pneumonia (VAP) is caused by MDR pathogens [14].

Given that COVID-19 disease is a growing problem with implications for worldwide health and economy, the diagnosis and antimicrobial resistance pattern of bacterial coinfection are essential to ensure a better clinical outcome in these cases [15]. Therefore, in this survey, we investigated the presence of bacteria in endotracheal aspirate samples in severe COVID-19 patients under mechanical ventilation in Mazandaran Heart Center Hospital, Iran.

Subjects and Methods

Study Population and Data Collection

This retrospective cohort study was approved by the joint Ethical Committees of Mazandaran University of Medical Sciences, Sari, Iran (Ethics No. IR.MAZUMS.REC.1399.8461). Patients with confirmed SARS-CoV-2 under mechanical ventilation hospitalized in ICU between 20 February 2020 and 21 September 2020 in Mazandaran Heart Center Hospital, Sari, Iran, were included in the current study as corona group. The patients under mechanical ventilation in ICU suffering from other disease in this period were considered non-corona group. According to the clinical guidelines (version 5) by Scientific Committee of the Iran's National Headquarters of COVID-19 Disease Management, SpO₂ < 93% or RR > 30, fever ≥ 37.8 °C, computed tomography (CT) scan with radiographic abnormalities in the lung, positive real-time PCR, lymphopenia, and positive results of C-reactive protein (CRP) were considered the diagnostic criteria of COVID-19 [16]. VAP is defined in these patients as severe pneumonia happening more than 48 h after the initiation of invasive mechanical ventilation [17].

Quantitative culture techniques for tracheal secretion aspirates are more reliable if appropriate cutoff criteria are applied. When patients experience pneumonia, pathogens are available in the lower respiratory tract secretions at concentrations of at least 10⁵ to 10⁶ CFU/ml. The

current diagnostic threshold proposed for tracheal aspirate is 10⁶ CFU/ml [18].

In this study, endotracheal aspirate specimens were obtained during routine clinical specimen collection evaluated immediately after assembly according to the clinical and laboratory standard institute (CLSI) instructions in the hospital's microbiology laboratory [19].

Statistical Analysis

All data were expressed as number (%) and compared by Fisher's exact test. Differences were considered statistically significant when $P < 0.05$. Demographic, clinical, and laboratory data at admission were used to calculate SOFA for the prediction of mortality risk in ICU patients [20].

Results

Out of 38 patients under mechanical ventilation hospitalized in ICU from 20 February 2020 to 21 September 2020, a total of 22 patients in corona group meet the diagnostic criteria for VAP, were included in the study and compared with a total of 16 patients in non-corona group in Mazandaran Heart Center Hospital, Sari, Iran. Sixty-eight percent of patients was female in corona groups. Hospital length of stay in 27% of case in corona group was > 10 days. Also, SOFA score was > 10 in 64% and 25% of corona and non-corona groups, respectively ($P < 0.05$). Moreover, the number of death was significantly higher among corona patients (45%) than non-corona group (6%) in ICU ($P < 0.05$) (Table 1).

In this study, most of the isolated bacteria were *Acinetobacter* spp. (41%) in corona group. The other organisms in this group were *Pseudomonas aeruginosa* (9.1%), *Klebsiella* spp., *E. coli*, *Staphylococcus aureus*, *Staphylococcus epidermis* (4.5%), *Candida* spp. (18.2%), and *diphtheroid* (13.6%) in corona patients (Table 2). In non-corona group, 8 patients were found negative for bacterial infections in endotracheal aspirate specimen culture. The organisms in this group were *Acinetobacter* spp. (4 patients), *Klebsiella* spp. (2 patients), and *Candida* spp. (2 patients). There was no significant difference between the corona and non-corona groups according to the microbiologic culture results ($P > 0.5$).

In our study, *Acinetobacter* spp. were 100% resistant to amikacin, gentamycin, cefixime, and imipenem. *Pseudomonas aeruginosa* was more resistant to co-trimazol and cefixime. Also, *Klebsiella* spp. were 100% resistant to co-trimazol, cefixime, amikacin, gentamycin, ciprofloxacin, and ceftazidime. Moreover, *E. coli* was 100% resistant to co-trimazol and cefixime in corona group (Table 2). Furthermore, *Acinetobacter* spp. and *Klebsiella* spp. were resistant to almost all antibiotics used with the exception of colistin in non-corona group.

Table 1 Comparison of demographic features, risk factors, and clinical characteristics in corona and non-corona groups (data are the number)

Variables	Corona group ^a (N = 22)	Non-corona group ^b (N = 16)	P-value
Gender			
Male	7 (32)	3 (19)	0.469
Female	15 (68)	13 (81)	
Age group (years)			
40–50	2 (9)	1 (6)	0.370
50–60	3 (14)	3 (19)	
60–70	6 (27)	4 (25)	
> 70	11 (50)	8 (50)	
Length of stay (days)			
< 10	16 (73)	8 (50)	0.187
> 10	6 (27)	8 (50)	
Positive PCR	9 (41)	-	-
SOFA			
< 10	8 (36)	12 (75)	0.040
10–20	12 (54)	4 (25)	
> 20	2 (10)		
Final outcome			
Recovery	12 (55)	15 (94)	0.012
Death	10 (45)	1 (6)	

^aAll patients with confirmed SARS-CoV-2 infection^bAll patients suffering from other disease

Discussion

Patients with confirmed SARS-CoV-2 are principally at risk of emerging superinfections, particularly those caused by Gram-negative bacteria with MDR infections [21]. However, the pattern of coinfections and the microbiological profile in these cases remain an essential information gap in the literature [22]. To reveal this aspect, we investigated microbiologic data from 22 severe COVID-19 patients under mechanical ventilation. Based on the result of this study, 59.1% of patients in corona group showed the presence of Gram-negative bacteria, where *Acinetobacter* spp. and *Pseudomonas aeruginosa* were the most common species, respectively.

Mazzariol et al., Zhang et al., and Chen et al. reported 58.1%, 29.8%, and 5.1% of bacterial coinfections/superinfection in COVID-19 patients [15, 23, 24]. Based on previous study from Nahavand Hospitals, Hamedan, Iran, of 340 patients with COVID-19, secondary bacterial infections were reported in 12.46% of patients. The most common bacteria isolated were *Klebsiella* species, *S. aureus*, and *E. coli*. [25]. Also, Sharifipour et al. reported that of 19 positive corona cases, admitted to ICUs in Qom, Iran, 18 (95%) of patients died. All patients were found positive for bacterial coinfections caused by *Acinetobacter baumannii* (90%) and *Staphylococcus aureus* (10%) [26]. Out of the 22 cases with severe COVID-19 in our survey, 45% died in ICU. These

Table 2 Resistance of bacteria to different classes of antibiotics associated to VAP in corona group patients

Antibiotic	Microorganism N (%)						
	<i>Acinetobacter</i> spp. 9 (41)	<i>Pseudomonas aeruginosa</i> 2 (9.1)	<i>Klebsiella</i> spp. 1 (4.5)	<i>E. coli</i> 1 (4.5)	<i>Staph. aureus</i> 1 (4.5)	<i>Staph. epidermidis</i> 1 (4.5)	Others* 7 (31.8)
Ampicillin	NT	NT	NT	NT	100	100	NT
Penicillin G	NT	NT	NT	NT	100	100	NT
Cephalithin	NT	NT	NT	NT	NT	100	NT
Clindamycin	NT	NT	NT	NT	0	100	NT
Oxacillin	NT	NT	NT	NT	0	100	NT
Vancomycin	NT	NT	NT	NT	0	25	NT
Ceftriaxone	50	75	NT	0	NT	NT	NT
Co-trimazole	50	100	100	100	NT	NT	NT
Cefixime	100	100	100	100	NT	NT	NT
Amikacin	100	25	100	0	NT	NT	NT
Gentamycin	100	25	100	0	NT	NT	NT
Nitrofurantoin	100	25	NT	0	NT	NT	NT
Ciprofloxacin	50	50	100	0	NT	NT	NT
Colistin	50	25	0	NT	NT	NT	NT
Ceftazidime	50	NT	100	0	NT	NT	NT
Imipenem	100	NT	NT	NT	NT	NT	NT

**Candida* spp. 4 (18.2), *diphtheroid* 3 (13.6); NT not tested

findings indicated a significant role of bacterial secondary infection in COVID-19 patients.

Evans et al. reported that overall mortality in ICU patients with COVID-19 under mechanical ventilation at the St George's Hospital, London, was 35%. Most of ICU patients did not have bronchoalveolar lavages undertaken, and therefore bacterial coinfections in this study were not evaluated [27]. Based on Yang et al. study, the mortality rate was 32 cases in 37 critically ill patients with COVID-19 documented mechanical ventilation. Among the dead patients, secondary infections were reported in 4 cases with highly drug-resistant organisms [28].

One recent study by Temperoni et al. indicated that the MDR microorganisms were detected 64.5% in 48 COVID-19 patients under mechanical ventilation in ICU of Pesaro Hospital. Also, similar to our results, Gram-negative were more than Gram-positive bacteria in MDR strains. They detected a high prevalence of *A. baumannii* Carbapenem-resistant colonization with an infection rate of 75% [29]. Based on our findings, *Acinetobacter* spp. was 100% resistant to amikacin, gentamycin, cefixime, and imipenem antibiotics. Emergence of MDR Gram-negative bacteria are commonly associated to protracted use of external devices in patients such as urinary catheters, mechanical ventilation, central venous lines, to prolonged length of stay [30]. Drug-resistant bacteria present a serious threat to human health [28]. Montrucchio et al. evaluated Carbapenem-resistant *Klebsiella pneumoniae* (CP-Kp) in ICU-admitted COVID-19 individuals at "Città della Salute e della Scienza" hospital in Turin (Italy) from 1 March to 20 May 2020. In this study, bacterial coinfection was reported in three patients with VAP caused by *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Also, the mortality related to CP-Kp septic shock was 28.6% in these patients [31].

Recently, Mazzariol et al. reported that the most frequently isolated bacterial species in bronchial aspirate samples from mechanically ventilated patients with severe COVID-19 were *Pseudomonas aeruginosa*. The majority of these isolates showed Carbapenem resistance [15]. In other study, Grasselli et al. reported that in patients undergoing extracorporeal membrane oxygenation, colonization by multidrug-resistant-Gram-negative bacteria is common and is associated with more than ten-fold odds for following infections that are related with an increased risk of death [32]. Among Gram-positive bacteria, *Staph. aureus* and *Staph. epidermidis* were the most common species in patients. However, in Temperoni et al. survey, *Staph. aureus*, *Staph. faecalis*, and *Staph. faecium* were the most common species. Also, *E. faecium* showed to be MDR in 71.4% of the patients [29]. Moreover, emergence of *Candida* spp. was observed in 18.2% of cases. Senok et al. in evaluation of Dubai Health Authority hospitals and Sheikh Khalifa General Hospital Umm Al Quwain patients stated that predominance of

Gram-negative pathogens, emergence of *Candida* species, and prevalence of isolates harboring drug-resistance genes are of concern [33]. Eventually, a widespread consumption of antimicrobials, the immune dysregulation, and less adherence to the infection control and prevention measures are the main reasons of the high incidence of bacterial and fungal infections in severe COVID-19 cases [34].

Conclusions

The prevalence of antibiotic resistance among pathogens isolated from patients with COVID-19 under mechanical ventilation in ICU highlighted the importance of preventing coinfections caused by this pathogen, suggesting an essential standardized approach to antibiotic stewardship in patients with COVID-19 for successful treatment.

Author contribution ANJ, MM, and MMi designed the study. MMi provided patients' information and collected data. MM drafted the manuscript. MMO and MM analyzed the data. All authors read and approved the final manuscript.

Availability of data and material It will be available upon request.

Code availability Not applicable.

Declarations

Ethics approval This study was approved by the joint Ethical Committees of Mazandaran University of Medical Sciences, Sari, Iran (Ethics No. IR.MAZUMS.REC.1399.8461).

Consent to participate Not applicable.

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Conflict of interest The authors declare no competing interests.

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