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The significant role of Carbapenems-resistant Acinetobacter Baumannii in mortality rate of patients with COVID-19

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\textbf{ABSTRACT}

\textbf{Background:} Infections caused by Acinetobacter baumannii, especially carbapenem-resistant (CR) strains, pose important challenges in patients with COVID-19 infection. Therefore, in the present study, we investigated co-infection and antimicrobial resistance patterns, as well as the role of A. baumannii in the outcome of patients with COVID-19.

\textbf{Materials and methods:} Between February 2019 and January 2021, 141 patients with A. baumannii infections were detected from seven different hospitals (A to G) in Arak, Iran, and the antibacterial susceptibility pattern of these isolates was evaluated using disk diffusion and E-test methods. Forty-seven of these patients were co-infected with COVID-19. During the study, the data about the clinical course, demographic data, and the role of A. baumannii infections in the mortality rate of COVID-19 patients were collected.

\textbf{Results:} Hospitals A and B reported the most patients, with 53 (38%) and 47 (33%), respectively. Additionally, most cases (105 cases, 75%) were reported from surgical and general ICUs. Mechanical ventilators were detected as predisposing factors in 95 (67%) patients, and infection was detected in 20% of patients on the 10th day after intubation. All of the A. baumannii isolates were resistant to different classes of antibiotics, such as carbapenems. Notably, 33% (47 patients) were also positive for COVID-19, and 68% (32 patients) died due to the infection. Statistical analysis showed a significant role of A. baumannii co-infection in the mortality rate of COVID-19 patients (p-value 0.05).

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Conclusion: co-infection with A. baumannii is one of the most important challenges in COVID-19 patients. Our results showed that all isolated bacteria were CR and significantly increased mortality rates in COVID-19 patients.

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El papel significativo de Acinetobacter Baumannii resistente a los carbapenémicos en la tasa de mortalidad de pacientes con COVID-19

Antecedentes: Las infecciones causadas por Acinetobacter baumannii, especialmente las cepas resistentes a carbapenem (CR), plantean desafíos importantes en pacientes con infección por COVID-19. Por lo tanto, en el presente estudio investigamos los patrones de coinfección y resistencia a los antimicrobianos, así como el papel de A. baumannii en el desenlace de los pacientes con COVID-19.

Materiales y métodos: entre febrero de 2019 y enero de 2021, se detectaron 141 pacientes con infecciones por A. baumannii de siete hospitales diferentes (A a G) en Arak, Irán, y se evaluó el patrón de susceptibilidad antibacteriana de estos aislamientos mediante difusión en disco y E- métodos de prueba. Cuarenta y siete de estos pacientes estaban coinfectados con COVID-19. Durante el estudio, se recopilaron datos sobre datos demográficos, curso clínico y el papel de las infecciones por A. baumannii en la tasa de mortalidad de los pacientes con COVID-19.

Resultados: Los hospitales A y B reportaron mayor número de pacientes con 53 (38%) y 47 (44%), respectivamente. Además, la mayoría de los casos (105 casos, 75%) se informaron desde la UCI quirúrgica y general. Los ventiladores mecánicos se detectaron como factores predisponentes en 95 (67%) pacientes y la infección se detectó en el 20% de los pacientes al décimo día después de la intubación. Todos los aislamientos de A. baumannii fueron resistentes a diferentes clases de antibióticos, como los carbapenémicos. El 33,6% (47 pacientes) también dieron positivo a COVID-19, y el 68% (32 pacientes) fallecieron a causa de la infección. El análisis estadístico mostró un papel significativo de la coinfección por A. baumannii en la tasa de mortalidad de los pacientes con COVID-19 (valor de p 0,05).

Conclusión: la coinfección con A. baumannii es uno de los desafíos más importantes en pacientes positivos para COVID-19. Nuestros resultados mostraron que todas las bacterias aisladas eran CR y aumentaron significativamente las tasas de mortalidad en pacientes con COVID-19.

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Introduction

Carbapenem-resistant (CR) Gram-negative bacteria, including Acinetobacter baumannii, are the most important bacteria in patients admitted to intensive care units (ICU). Unfortunately, CR-A. baumannii have been detected in different parts of the world; hence, World Health Organization (WHO) has put this bacterium on the critical priority list of antibiotic-resistant bacteria [1,2].

The carbapenems family, including imipenem, meropenem, ertapenem, and doripenem, are appropriate antibiotics for treating infections caused by multi-drug resistant (MDR) bacteria. In recent years, CR-isolates have been reported from different parts of the world that lead to the use of less effective and more toxic antibiotics such as colistin and tigecycline or the use of antibiotic combinations regimens with controversial results in terms of toxicity, efficacy, and collateral environmental damage [2,3]. Noteworthy, prior use of antibiotics, admission to hospital wards with intense healthcare exposure, and a high density of CR-A. baumannii, such as ICU, as well as long usage of mechanical ventilation and intravenous catheters, are the most important risk factors for infection with CR-A. baumannii [4–6].

Additionally, MDR A. baumannii infections were reported as one of the most prevalent bacterial co-infection in patients with COVID-19 [7,8]. The use of corticosteroids and anti-IL6-directed therapies in COVID-19-positive patients suppresses the immune system and causes a high incidence of microbial co-infections in these patients. Additionally, Coronavirus leads to cytokine storming, ciliary dysfunction, microvascular hypercoagulability, and, consequently, innate immune dys-regulation. Moreover, other risk factors such as mechanical ventilation, invasive procedures, industrial-grade oxygen
administration, and prolonged hospital stays are other risk factors that increase the risk of microbial co-infections in patients with COVID-19 [9].

Therefore, the data about local epidemiology, etiological distribution of causative agents and antibiotic resistance, and factors associated with poor patient outcomes are pivotal to guiding infection control and antimicrobial stewardship policies and assist clinicians in choosing the best therapeutic approach. In this regard, in the present study, we evaluated co-infection, antimicrobial resistance patterns, and the role of A. baumannii in the outcome of patients with COVID-19.

Methods and material

Study design

We performed the current multicenter retrospective descriptive study and evaluated the prevalence of A. baumannii among hospitalized patients in seven different hospitals in Arak (A, B, C, D, E, F, and G) between February 2019 and January 2021. The A. baumannii co-infection in COVID-19-positive patients was assessed, and the impact of this bacterium on the outcomes of these patients was compared with COVID-19 patients without A. baumannii co-infections. Informed consent forms were collected from all patients before the study, and their information was kept confidential. The approach to the disease was under the guidelines provided by Iran National Health and adapted from the WHO guidelines.

Inclusion and exclusion criteria

All patients infected with A. baumannii and positive COVID-19 patients with or without A. baumannii co-infection that their clinical data were available at the mentioned hospitals were included in the present study. In this regard, patients whose clinical data were not accessible were excluded from the final analysis.

Bacterial isolates

A total of 141 patients with A. baumannii infection were included in this study. These patients had been hospitalized in wards such as ICU, Neurosurgery, Infectious, Orthopedics, etc. The bacterial isolates were collected from various clinical samples such as wounds, abscesses, tracheal aspirate, sputum, ascites, bronco alveolar lavage (BAL), and blood. The isolates were identified using conventional biochemical tests, and amplification of 16S rRNA and blaOXA-51-like carbapenemase gene by polymerase chain reaction (PCR) using primers was reported in a recently published study [10,11].

Antibacterial susceptibility testing

The disk diffusion method (DDM) was performed for evaluation of isolated A. baumannii susceptibility to various antibiotics disks (Mast (Liverpool, UK) and BD (New Jersey, USA)) according to clinical and laboratory standard institute (CLSI) guidelines. In this regard, the susceptibility of isolated bacteria was evaluated against different antibiotic agents including ceftazidime (30 μg), amikacin (30 μg), tetracycline (10 μg), cefotaxime (30 μg), gentamicin (10 μg), piperacillin/tazobactam (100/10 μg), co-trimoxazole (2.5 μg), ciprofloxacin (5 μg), cefepime (30 μg), ampicillin (10 μg), meropenem (10 μg), and imipenem (10 μg). Pseudomonas aeruginosa ATCC 27853 and Escherichia coli ATCC 25922 was used as quality control strain [10,12].

The prevalence of MDR A. baumannii was also investigated. MDR was defined as acquired resistance to at least one agent in three or more antimicrobial categories. Additionally, extensively drug-resistant (XDR) was defined as bacterial isolates that remain susceptible to only one or two antimicrobial categories [3].

The minimum inhibitory

Minimum inhibitory concentrations (MICs) of CR-A. baumannii isolates to meropenem and imipenem were evaluated using E-test according to CLSI guidelines [13].

COVID-19 detection

Patients with different clinical manifestations such as sore throat, fever, cough, and rhinorrhea as well as probable respiratory distress were considered as COVID-19 suspected patients (especially if they had a travel history to a COVID-19-affected country or city or positive history of the close relationship with a confirmed or highly suspected COVID-19 patients). Data from different diagnóstico methods such as clinical signs, chest exams, laboratory findings, and reverse transcription (RT)-PCR tests using both nose and throat swab samples lead to the final diagnóstico of COVID-19 [14].

Statistical analysis

Excel software (Microsoft, Redmond, WA, USA) and SPSS v.20.0

Software (SPSS Inc. Chicago, IL, USA) was used to assess descriptive statistics. A P-value of <0.05 was considered statistically significant.

Results

The population included 141 patients with A. baumannii infection, and 66% (93 patients) were male. Hospitals A and B reported the most patients, with 53 (38%) and 47 (33%), respectively. Additionally, most cases (105 cases, 74%) were reported from surgical and general ICUs.

Mechanical ventilators were detected as predisposing factors in 95 (67%) patients, and infection was detected in 20% of patients on the 10th day after intubation. Accordingly, A. baumannii was isolated in 64 patients (45%) from secretions of the airways and lungs, such as Tracheal aspiration, BAL, sputum, and aspiration of lung secretion. Furthermore, the wound of 13 (9%) patients were infected with this bacterium.
All of the A. baumannii isolates were resistant to amikacin, gentamycin, co-trimoxazole, cefazidime, ciprofloxacin, piperacillin/tazobactam, cefepime, imipenem, and meropenem. Afterward, we used the E-test to evaluate bacterial susceptibility to imipenem and meropenem. E-test results confirmed DDM findings (Fig. 1), and all isolates were resistant to carbapenems; therefore, all the isolates were determined as XDR.

Notably, 33% (47 patients) of patients were infected by A. baumannii and were positive for COVID-19. Most of these patients were reported from hospitals A, C, and B (Fig. 2), and 68% (32 patients) died due to the infection. The total mortality rate in patients who were infected with CR-A. baumannii was 50% (71 patients). Therefore, A. baumannii infection alone was detected in 39 patients died. In this regard, hospitals A, B, and C reported the highest mortality rate at 48%, 20%, and 14.1%, respectively.

Additionally, the highest mortality rate was reported in September in the males (62%, 44/71) and in the patients aged 60 to 69. The underlying predisposing conditions such as cancer, pneumonia, hypertension, hyperlipidemia, and polytrauma were just reported in 31% (22/71) of the patients. Furthermore, 81% (58/71) of dead patients had mechanical ventilation.

On the other hand, we assessed 5078 COVID-19-positive patients without A. baumannii co-infection was reported from the mentioned hospitals and during the study time. The reported mortality rate for these patients was 22% (1103/5078). Therefore, statistical analysis showed a significant role of A. baumannii co-infection in the mortality rate of COVID-19 patients (p-value 0.05).

### Discussion

A. baumannii is one of the most important bacterial pathogens with high antibiotic resistance characteristics that could lead to a high mortality rate in patients, especially those admitted to the ICU. In this regard, in the present study, we analyzed A. baumannii epidemiology and antibacterial resistance profiles in hospitalized patients (seven different hospitals) in Arak between February 2019 and January 2021.

Furthermore, we evaluated the clinical properties and the outcome of patients with co-infection of A. baumannii and COVID-19. Our population in the present study included 141 patients infected with A. baumannii. Most of these patients were reported form the ICU and mechanically ventilated. Our analysis also showed that all of the isolated A. baumannii were CR, while none of the patients were exposed to carbapenems before the acquisition of CR- A. baumannii. Moreover, our patients had an unusually short length of stay before CR- A. baumannii acquisition (median ten days), which could be related to the severe pneumonitis in these patients, immunosuppression due to COVID-19 treatments such as corticosteroids and lymphopenia or a combination of these.

Previous studies also reported CR- A. baumannii as responsible for the most severe infections in ICUs worldwide [6,15]. The total mortality rate in the current study was more than 50%, which shows the very high importance of CR- A. baumannii infections. Besides, co-infection with CR- A. baumannii significantly increased the mortality rate in COVID-19-positive patients.

As mentioned, most of the dead patients had mechanical ventilation. Previous studies reported that 8% to 28% of mechanically ventilated patients develop Ventilator-associated pneumonia (VAP), which is 3 to 10 times more than patients who are not mechanically ventilated. Recent studies reported that several risk factors, such as trauma, duration of mechanical ventilation, sepsis, lung, and neurological disorders, acute respiratory distress syndrome, and previous use of antibiotics, increase the rate of VAP in patients [16,17]. Therefore, mechanical ventilation and its long duration should be avoided as much as possible, and patients with mechanical ventilation should continuously be screened for possible A. baumannii infection.

In line with our results, a recently published study also reported that all of their A. baumannii isolates collected from COVID-19 patients were CR [7]. Additionally, Sathyakamala et al. reported Acinetobacter species as a bacterial pathogen with maximum antimicrobial resistance among COVID-19 and non-COVID-19 patients admitted to the ICU [8]. Furthermore,
a study from Iran reported A. baumannii infections as the most bacterial co-infections of the respiratory tract in COVID-19 patients admitted to ICU [18]. Pourajam et al., in their recently published study from Isfahan, IRAN, reported a high prevalence of CR-Gram-negative bacilli in COVID-19 patients admitted to ICUs, with a high proportion of K. pneumoniae followed by A. baumannii [19]. We found that A. baumannii isolates were CR in both COVID-19 and non-COVID-19 patients, suggesting a nosocomial horizontal spread throughout hospital departments. To this end, the hospital A reported the most infected and dead patients. Therefore, CR-A. baumannii outbreaks in this hospital could be partly associated with shortages in personnel, personal protective equipment, and dedicated medical equipment, which had affected the conventional infection prevention and control practices [7]. To this end, the hands of healthcare workers can act as vehicles for transmission either from a colonized patient to other patients or contaminated surfaces to patients. To this end, environmental sampling in a CR- A. baumannii hospital endemic should be performed after terminal cleaning to ensure no reservoir has been left. Besides, COVID-19 infection probably leads to the spread of A. baumannii because of neglect of contact isolation measures similar to the SARS outbreak period rather than facilitating bacterial infections [20,21]. Therefore, CR-A. baumannii is one of the most important bacterial pathogens that could lead to higher mortality rates in COVID-positive patients; hence, promptly detecting and treating patients is essential. To this end, Casarotta et al. reported that the use of combination therapy of high-dose of tigecycline, intravenous colistin, and ampicillin/sulbactam could be effective for the treatment of MDR- A. baumannii in patients with COVID; however, more detailed studies are needed in this field [22].

**Limitations**

We should report some limitations in the present study. First of all, the study’s retrospective design does not allow to control for all confounding factors. Additionally, as mentioned, our attention was focused on A. baumannii infections (single infection); therefore, we did not collect data about other possible co-infections. Finally, we did not perform molecular typing for isolated bacteria. Therefore, molecular typing should be considered in future evaluation because molecular typing could show exactly the source of the infection.

**Conclusions**

CR-A. baumannii is one of the most important bacterial pathogens that could lead to high mortality rates in COVID-19 patients. Mechanical ventilation is one of the most important risk factors that could increase the rate of A. baumannii infections. Noteworthy, hospital transmission of this bacterium is linked to invasive procedures, environmental contamination, and patient vulnerabilities. To this end, environmental cleaning is particularly important as enforcing strict hand hygiene compliance policies among healthcare workers.

**Declarations**

**Abbreviations**

| CR | Carbapenem-resistant |
| ICU | intensive care units |
| XDR | extensively drug-resistant |
| MDR | multi-drug resistant |
| DDM | disk diffusion method |
| MICs | Minimum inhibitory concentrations |

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Not applicable.

**Competing interests**

The authors declare to have no competing interests.

**Consent for publication**

Not applicable.

**Availability of data and materials**

Data sharing does not apply to this article as no datasets were generated during the current study.

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**Author contribution**

EGHR and AM conceived and designed the study. NS, ZH and EA collected the data. AS wrote the paper. AS, AM and EGHR participated in manuscript editing. All authors have read and approved the manuscript.

**Ethics approval and consent to participate.**

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