Acinetobacter baumannii producing ESBLs and carbapenemases in the Intensive Care Units developing fosfomycin and colistin resistance

Sura A. Abdulateef
Department of Applied Sciences, University of Technology- Iraq
Mustafa S. Al-Salmani
Department of Molecular and Medical Biotechnology, College of Biotechnology, Al-Nahrain University, Baghdad, Iraq
Hasan A. Aal Owaif
Department of Plant Biotechnology, College of Biotechnology, Al-Nahrain University, Baghdad, Iraq

*Corresponding author. E-mail: hasan.abdulhadi@nahrainuniv.edu.iq

How to Cite

Abstract
Acinetobacter baumannii is responsible for causing difficult-to-treat healthcare-associated infections globally, owing to its resistance to antibiotics. The intensive care unit (ICU) settings mediate spread of multidrug resistance (MDR) strains. This research aimed to evaluate non-susceptible colistin and fosfomycin A. baumannii, harboring extended-spectrum beta-lactamases (ESBLs) and carbapenemases in ICU setting. During the period of 2019-2021, this study obtained 200 A. baumannii isolates out of 1410 burns samples from an ICU setting. The antibiotic sensitivity, ESBLs and carbapenemase production were determined using clinical and laboratory standards institute (CLSI) 2020. The colistin (mcr-1 and mcr-2) and fosfomycin (fosA3) resistance genes was amplified. The highest resistance was to ceftazidime (98%), cefepime (86%), tetracycline (84%), levofloxacin (78%) and piperacillin-tazobactam (76%), while the highest sensitivity was to meropenem (63%) and tigecycline (62%). ESBL production was determined in 94% and carbapenemases were observed in 54% of A. baumannii. Four isolates (2%) were found to carry the mcr-1 gene, and three isolates (1.5%) were found to carry the mcr-2 gene. Moreover, the fosA3 was not detected in the isolates. This study showed that MDR A. baumannii was high in ICU settings. The spread of antibiotics considered the last line of defense against infections is a concern that necessitates surveillance and control measures.

Keywords: Acinetobacter baumannii, Carbapenemase, Colistin, ESBL, Fosfomycin

INTRODUCTION
Acinetobacter baumannii nosocomial infections worldwide resist most antibiotics. The bacterium is responsible for more than 1% of nosocomial infections, often affecting patients with disabilities in hospital intensive care units (ICUs). Patients with clinical strains of A. baumannii incur an average additional cost of $ 61 more than the cost of their disease, and these patients are hospitalized 13 days longer (Tsioultis et al., 2016). Strains of A. baumannii have been identified with multidrug resistance (MDR) and pan drug resistance (PDR). Different definitions of A. baumannii have been proposed with multidrug resistance and resistance to all drugs. However, MDR strains are commonly characterized as those that are resistant to at least three different classes of antibiotics or as resistant to a key antibiotic in treatment and PDR is considered to be resistant to all groups of antibiotics available for the experimental treatment of infections of this bacterium (Lee et al., 2017). The clinical significance of this bacterium, especially during the last 15 years, with its ability to obtain resistance indices, has posed serious problems for antibiotic treatment. The reason for the increased isolation of this bacterium in hospitals is not well understood. Broad-spectrum antibiotics effectively overcome resistant bacteria (Kyriakidis et al., 2021). MDR A. baumannii was isolated for the first time after the quake. A. baumannii is resistant to the many antibiotics available because it is in close contact with other gram-negative bacteria in the hospital environment and is also subject to bombardment by the widespread use of antibiotics.

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Therefore, in addition to their inherent ability to acquire resistance, they can also obtain resistance mechanisms from other gram-negative genera through plasmids, integrons, and transposons (Wong et al., 2017). ESBL-producing isolates encoding ESBL enzymes have been documented worldwide. MDR isolates contain plasmids encoding ESBLs and genes encoding resistance to fluoroquinolones and aminoglycosides. In addition, carbapenemase enzymes, especially metallobeta-lactamases, are prevalent and have been identified in A. baumannii isolated from different regions (Hamidian and Nigro, 2019). On the other hand, strains with resistance to fosfomycin and colistin have also caused serious problems in the treatment process, and the study of the prevalence of these strains can provide a useful information for treatment. One of the mechanisms of resistance to fosfomycin occurs through the chromosomal fosA gene (Sharma et al., 2017). On the other hand, the colistin resistance occurs via both chromosomal and the presence of plasmid-encoded mcr genes (Petrillo et al., 2016). This study aimed to evaluate non-susceptible colistin and fosfomycin A. baumannii, harboring carbapenemases and ESBL in ICU setting.

MATERIALS AND METHODS

Ethical approval
Before collecting the sample, the patient's permission was obtained. Also this study was approved by the specialized committee at the College of Biotechnology/ Al- Nahrain University.

Collection of samples
A total of 1410 burns samples were obtained from ICU settings of different hospitals in Baghdad governorate and were inoculated onto the McConkey agar (Merk) and blood agar medium. The cultured plates were incubated at 37 °C for 24-48 hr. The initial identification was done using common biochemical tests. After that, the isolates were preserved in slants containing the trypticase soy broth with 20% glycerol in the freezer.

Sensitivity to antibiotics
The Kirby-Bauer standard diffusion agar disk method was used to determine sensitivity. Antibiotics used in this study were 10 different disks from MAST CO Company, based on the CLSI 2020. Antibiotics included: ceftazidime (30 µg), amikacin (30 µg), ceftazidime (30 µg), imipenem (10 µg), minocycline (30 µg), meropenem (10 µg), levofloxacin (5 µg), gentamicin (10 µg), piperacillin-tazobactam (10-100 µg), tigecycline (15 µg) and tetracycline (30 µg).

ESBLs and carbapenemases phenotypic test
Ceftazidime disks 30 µg (CAZ) + clavulanic acid 10 µg (CIA) and cefotaxime 30 µg disks (CTX) + clavulanic acid 10 µg with ceftazidime and cefotaxime alone were placed and incubated at 37 °C for 24 hours. ESBL production was indicated by a difference in size of 5 mm relative to ceftazidime plus clavulanic acid. Meropenem disk 10 µg (MER) was placed alone, MER was placed alone, and MER + phenylbronic acid 10 µg for incubation. Carbapenemase production was confirmed in the aforementioned two cases by a ≥5 mm increase in the diameter of the growth inhibition zone (Ibrahim et al., 2017).

DNA extraction
DNA extraction was done by boiling method by first taking a loop full of isolated bacteria into a cryotube containing 100 µl sterile distilled water. The cryotube containing bacterial isolate and distilled water was boiled at 100 °C for 10 min and after centrifugation for 5 min at 12000rpm, the supernatant, which contained the DNA was poured into another sterile tube. The desired DNA was stored at the -20 °C to be used for PCR in later steps (Ahmed and Dablool, 2017). The primers used to detect fosA3, mcr-1 and mcr-2 are listed in Table 1.

RESULTS AND DISCUSSION

Patients’ demographic data
Out of 1410 burns samples from an ICU setting, 200 A. baumannii isolates were identified. Significant differences were observed for age (11-79 years) and sex (112 males and 88 females). Moreover, age range of 41-60 years was significantly more prevalent (Table 2). Prior hospital residence was also a significant risk factor. The diabetic and cancer patients and patients with a history of taking antibiotics for a previous infection did not represent risk factor.

Table 1. Primers used in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence: 5’——3’</th>
<th>Product size (bp)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>fosA3</td>
<td>F: CCTGCCATTTATCAGCGATG&lt;br&gt;R: CGGTTATCTTCCATACCTCAG</td>
<td>271</td>
<td>(Loras et al., 2021)</td>
</tr>
<tr>
<td>mcr-1</td>
<td>F: CGGTACGTCGTTGTTC&lt;br&gt;R: TGCTTAATCAGTGAGGCACC</td>
<td>400</td>
<td>(Liu et al., 2016)</td>
</tr>
<tr>
<td>mcr-2</td>
<td>F: TGGTACAGCCCCCTTATT&lt;br&gt;R: GCTTGAGATGGTATG</td>
<td>297</td>
<td>(Xavier et al., 2016)</td>
</tr>
</tbody>
</table>
Antibiotic susceptibility
The highest resistance was to ceftazidime (98%), cefepime (86%), tetracycline (84%), levofloxacin (78%) and piperacillin-tazobactam (76%). Other resistance rates included imipenem (68%), minocycline (66%), meropenem (68%), gentamicin (58%), amikacin (56%) and tigecycline (44%). Thus, 76% of A. baumannii were MDR (Table 3).

Phenotypic and genotypic detection of ESBLs and carbapenemases
The ESBL production was determined in 94% and carbapenemases were observed in 54% of A. baumannii. The mcr-1 and mcr-2 genes were detected in four and three isolates respectively (Table 4, Fig.1 and Fig. 2). Moreover, the fosA3 was not detected in the isolates (Fig. 3).

In this study in ICU wards, resistance to carbapenemases included 68% and carbapenemase production was observed among nearly 80% of them. ICU settings can disseminate MDR strains (Puzniak et al., 2021). A study reported that carbapenem-resistant A. baumannii strains increased worldwide over the past decade (Potron et al., 2015). Another research showed that carbapenem-resistant A. baumannii increase was associated with the MDR (Lee et al., 2017). A study on A. baumannii isolates in Iran found that 49% were resistant to imipenem. Moreover, the bla oxa-23-like gene was detected in 21.7% of isolates, and bla oxa-24-like genes were detected in 17.3% of isolates (Kooti et al., 2015). A study conducted on the risk factors for A. baumannii with extensive resistance concluded that some factors, such as a history of antibiotic treatment, host innate defense conditions, invasive medical care such as tracheostomy, and hospitalization conditions are the factors involved in the acquisition of infections by MDR A. baumannii. Among the mentioned factors, prior antibiotic treatment, especially the history of treatment with third-generation cephalosporins and then mechanical ventilation had the more significant impact (Puzniak et al., 2021). In Egypt, a study was conducted on infections in the ICU. Although the predominant bacteria in this study were Klebsiella isolates, the highest resistance to carbapenems was found in A. baumannii isolates (Potron et al., 2015). Moreover, they reported the first A. baumannii with the colistin resistance gene (mcr-1) among 40 carbapenem resistant A. baumannii isolates (Abdulzahra et al., 2018). In a study conducted in Brazil, out of 20 patients admitted for infection with A. baumannii, seven patients had colistin-resistant strains (Leite et al., 2016). This study showed ESBL production in 94% and car-
bapenemases in 54% of A. baumannii. Additionally, the mcr-1 and mcr-2 genes were detected in four (2%) and three (1.5%) of isolates. Among these, five were males and two patients were females. All these isolates were MDR, but one was not ESBL and carbapenemase producers. Although colistin resistance mechanisms are diverse, the focus of this study was limited to plasmid-mediated resistance. Prior studies have detected mcr genes in A. baumannii, P. aeruginosa and Enterobacteriaceae from ICU settings worldwide (Janssen et al., 2020; Papathanakos et al., 2020). In a study in Greece, among A. baumannii from ICU, 49% of them were resistant to colistin (Papathanakos et al., 2020). In another study in South Africa, 77% of the isolates showed resistant to colistin (Snyman et al., 2020). In Iraq, colistin resistance emerged in A. baumannii in Baghdad City in 2020 (Al-Kadmy et al., 2020; Kareem, 2020). This study showed that tigecycline was the most effective antibiotic against MDR strains.

**Conclusion**

This study identified specific antibiotics to which A. baumannii isolates showed high resistance levels, including ceftazidime, cefepime, tetracycline, levofloxacin, and piperacillin-tazobactam. The study also revealed that 76% of A. baumannii isolates were classified as MDR. Furthermore, the presence of genes conferring resistance to colistin, including mcr-1 and mcr-2, was detected in some of the isolates. Tigecycline was found to be the most effective antibiotic against MDR strains, highlighting its potential usefulness in treating A. baumannii infections. Top of Form These findings can inform the development of strategies for preventing and managing A. baumannii infections in healthcare settings.

**Conflict of interest**

The authors declare that they have no conflict of interest.

### Table 4. The demographic data of patients infected with A. baumannii carrying mcr genes and strains’ characteristics

<table>
<thead>
<tr>
<th>Gene</th>
<th>Patient gender</th>
<th>Age (years)</th>
<th>ESBL</th>
<th>Carbapenemase</th>
<th>MDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>mcr-1</td>
<td>Male</td>
<td>55</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>mcr-1</td>
<td>Male</td>
<td>65</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>mcr-1</td>
<td>Female</td>
<td>71</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>mcr-1</td>
<td>Male</td>
<td>73</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>mcr-2</td>
<td>Male</td>
<td>54</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>mcr-2</td>
<td>Female</td>
<td>46</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>mcr-2</td>
<td>Male</td>
<td>74</td>
<td>Yes</td>
<td>No</td>
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REFERENCES


