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Research Article

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

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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. baumanni* 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 (Tsioutis *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.

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 metallo-beta-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 tryptic case 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: cefepime (30 µg), amikacin (30 µg), ceftazidime (30µg), 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 30µg (CAZ) + clavulanic acid 10 µg (ClA) and cefotaxime 30µ 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 10µg (MER) was placed alone, MER was placed alone, and MER + phenylbronic acid 10µg 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. baumanni* 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

Primer	Sequence: 5'—→3'	Product size (bp)	Ref.
fosA3	F: CCTGGCATTTTATCAGCAGT R: CGGTTATCTTTCCATACCTCAG	271	(Loras *et al*., 2021)
mcr-1	F: CGGTCAGTCCGTTTGTTC R: TGCTTAATCAGTGAGGCACC	400	(Liu *et al*., 2016)
mcr-2	F: TGGTACAGCCCCTTTATT R: GCTTGAGATTGGGTTATGA	297	(Xavier *et al*., 2016)

Table 2. Demographic data of patients

Data	No. (%)	*p value*
Male	112 (56%)	0.044
Female	88 (44%)	0.061
Age range (years)		
1-20	12 (6%)	>0.05
21-40	42 (21%)	>0.05
41-60	88 (44%)	0.041
61-80	58 (29%)	0.211
>80	0.00	-
Diabetic patients	84 (42%)	0.067
Cancer patients	21 (10.5%)	>0.05
Prior hospitalization (patients have a history of being hospitalized in the past).	142 (71%)	0.010
Prior antibiotics use (patients have a history of taking antibiotics for a previous infection).	82 (41%)	0.069

Table 3. Isolates resistance pattern (N = 200)

Disk/ resistance	Susceptibility %	Intermediate %	Resistance %
Ceftazidime	2	0.0	98
Cefepime	13	1	86
Tetracycline	14	2	84
Levofloxacin	14	8	78
Piperacillin-Tazobactam	18	6	76
Imipenem	22	10	68
Minocycline	30	4	66
Meropenem	63	0.0	68
Amikacin	40	4	56
Gentamicin	35	7	58
Tigecycline	62	4	44

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 carbapenems 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* $_{\text{oxa-23-like}}$ gene was detected in 21.7% of isolates, and *bla* $_{\text{oxa-24-like}}$ 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-

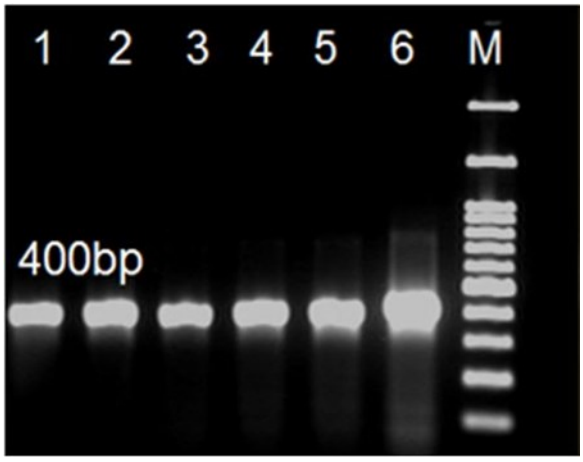

Fig. 1. *The PCR products of mcr-1 (400bp); M: 100bp DNA marker, agarose 1% at 70 volts, 60min.*

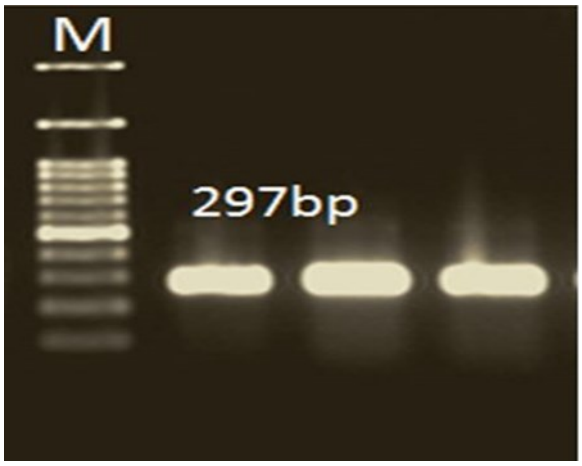

Fig. 2. *The PCR products of mcr-2 (297bp); M: 100bp DNA marker, agarose 1% at 70 volts, 60min.*

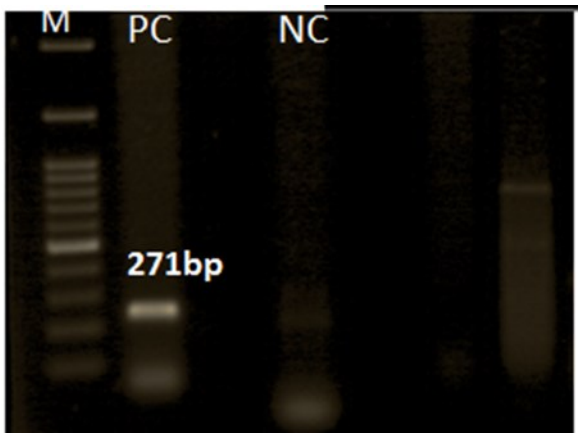

Fig. 3. *The results of gel electrophoresis of the fosA3 gene; PC: positive control, NC: negative control, M: 100bp DNA marker, agarose 1% at 70 volts, 60min.*

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. baumanni*, *P. aeruginosa* and *Enterobacteriaceae* from ICU settings worldwide (Janssen *et al*., 2020; Papathanakos *et al*., 2020). In a study in Greece, among *A. baumanni* 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

Gene	Patient gender	Age (years)	ESBL	Carbapenemase	MDR
mcr-1	Male	55	Yes	Yes	Yes
mcr-1	Male	65	Yes	Yes	Yes
mcr-1	Female	71	Yes	Yes	Yes
mcr-1	Male	73	Yes	Yes	Yes
mcr-2	Male	54	Yes	Yes	Yes
mcr-2	Female	46	No	Yes	Yes
mcr-2	Male	74	Yes	No	Yes

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