Iranian Journal of Microbiology
Volume 15 Number 3 (June 2023) 359-372

ORIGINAL ARTICLE

High burden of MDR, XDR, PDR, and MBL producing Gram negative bacteria causing infections in Kermanshah health centers during 2019-2020

Sepide Kadivarian[1], Mosayeb Rostamian[2], Shirin Dashtbin[3], Sara Kooti[4], Zahra Zangeneh[5], Ramin Abiri[6], Amirhooshang Alvandi[7*]

[1]Department of Microbiology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran
[2]Infectious Diseases Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran
[3]Department of Microbiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
[4]Department of Basic Sciences, Behbahan Faculty of Medical Sciences, Behbahan, Iran
[5]Department of Biology, Ilam Branch, Islamic Azad University, Ilam, Iran
[6]Fertility and Infertility Research Center, Research Institute for Health Technology, Kermanshah University of Medical Sciences, Kermanshah, Iran
[7]Department of Microbiology, School of Medicine, Medical Technology Research Center, Research Institute for Health Technology, Kermanshah University of Medical Sciences, Kermanshah, Iran

Received: January 2023**, Accepted:** May 2023

ABSTRACT

Background and Objectives: Microorganisms producing Metallo-Beta-Lactamase (MBL) are a threat and cause of concern as they have become one of the most feared resistance mechanisms. This study was designed to explore the prevalence of MBL production in clinical isolates of Gram negative bacteria using phenotypic MBL detection.
Materials and Methods: A total of 248 isolates were collected from various clinical samples and were evaluated for carbapenem resistance and MBL production. All strains were screened for MBL production using Double Disk Confirmatory Test (DDCT).
Results: The results of screening for MBL production using phenotypic disk diffusion method showed that in the 85 isolates were carbapenemase positive; including, 10 (16.1%) *Klebsiella pneumoniae*, 9 (14.5%) *Escherichia coli*, 58 (93.6%) *Acinetobacter baumannii*, and 8 (12.9%) *Pseudomonas aeruginosa* isolates. Also, 83 (97.6) Carbapenemase-producing isolates were resistant to at least four classes of antimicrobials (MDR).
Conclusion: *A. baumannii* was the most common carbapenem resistant bacterium in medical centers in Kermanshah. Significant multiple drug resistance (MDR) incidence was observed compared to different classes of antibiotics.

Keywords: Metallo-beta-lactamase; Antibiotic resistance; Multiple drug resistance; Gram negative bacteria

*Corresponding author: Amirhooshang Alvandi, PhD, Department of Microbiology, School of Medicine, Medical Technology Research Center, Research Institute for Health Technology, Kermanshah University of Medical Sciences, Kermanshah, Iran. Tel: +98-918-8398490 Fax: +8334276477 Email: ah_alvandi@kums.ac.ir

INTRODUCTION

The last line of drugs to treat infections caused by the most resistant bacteria such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacteriaceae* are carbapenem antibiotics. The World Health Organization (WHO) made a global priority to direct research and discovery of new antibiotics to a list of 12 published antibiotic-resistant bacteria, particularly the carbapenem-resistant bacteria (1, 2).

To face this global pandemic crisis, it is essential to understand resistance mechanisms and have a better categorization of pathogens. Distribution of carbapenemases among distinctive bacterial species and genera are chromosomally and plasmid-mediated and leads to a stronger expansion of resistance (3). The hydrolysis of carbapenems through production of carbapenem hydrolytic enzymes is the main resistance mechanism (4). Since 1993 and according to the Ambler classification, β-lactamase enzymes include A, B, C and D classes (5). Class A, B and D enzymes use carbapenemases to hydrolyze drugs but type C enzymes mainly hydrolyze cephalosporins. In addition, class A, C, and D enzymes have serine in their core catalytic region while type B enzymes have zinc instead and are considered metallo-β-lactamase (MBL) (6). In fact, carbapenem resistance in most cases results from fundamental mechanisms related to the manufacturing of carbapenemases (carbapenem hydrolases) and β-lactamase enzymes related to structural mutations (ESBL and AmpC cephalosporinase) (6, 7). Mutations are related to porin in the outer membrane or the presence of an efflux pump in specific gram-negative bacteria (8, 9). However, microorganisms producing carbapenemase are the most threatening because they have the main mechanism of resistance (9).

Accurate and timely diagnosis of this type of resistance contributes to infection control, although it may be challenging in clinical laboratories (10). Various phenotypic assays have been proposed to identify MBLs based on their metal chelating ability, such as EDTA inhibition of MBL activity (11). The phenotypic experiments that have been used for this purpose include Disk Diffusion Test (DDT) and Double Disk Confirmatory Test (DDCT). This research was carried out to identify MBLs generated isolates using phenotypic method.

MATERIALS AND METHODS

Reagents and materials. Sodium chloride (NaCl) was purchased from Sigma-Aldrich (St. Louis, MO, United States). Brain Heart Infusion (BHI) broth, Müller Hinton Agar (MHA), Blood Agar (BA), Eosin Methylene Blue Agar (EMB Agar), MacConkey Agar (MAC), Triple Sugar Iron (TSI), and TBE buffer were purchased from Merck Co., Germany). Antibiotic discs were purchased from Mast Company (MASTDISCS combi-D73C, Bootle, UK). Standard strains of *Pseudomonas aeruginosa* ATTC 27853, *E. coli* ATTC 25922 bacteria, and *K. pneumoniae* ATCC 700603 were purchased from Pasteur Institute (Tehran, Iran).

Study design. Clinical isolates collected from patients hospitalized in different hospitals from August 2019 to August 2020 were evaluated using standard and clinical samples.

Collection and identification of bacterial isolates. A total of 248 bacterial isolates (i.e. *Enterobacteriaceae* and non-fermenter) were collected from different medical centers. After Re-cultivation of the isolates obtained from different clinical samples including urine, sputum, blood, tissue and wound, trachea, and other secretions, the isolates were identified using standard microbiological techniques (Gram staining and biochemical tests). Demographic information of patients including age and sex was also collected.

Determination of antibiotic susceptibility pattern by Disk Diffusion Test (DDT). Antimicrobial susceptibility testing was performed using Kirby Bauer standard disk diffusion method on Müller-Hinton agar plates (Merck, Germany), and according to the instructions of the International Institute for Laboratory Standards (CLSI2018) as follows: Using the pure and fresh culture of each isolate, a standard half-McFarland microbial suspension (equivalent to 1.5×10^8) was prepared. After the bacteria were uniformly spread on the surface of Müller-Hinton agar medium, the plates were placed at room temperature for 15 minutes. Antibiotic disks were placed in the laboratory for a few minutes to reach room temperature for testing. The disks were then inoculated on the medium and incubated for 18-24 hours. *Pseudomonas aeruginosa* ATTC 27853, *E. coli* ATTC 25922

bacteria, *K. pneumoniae* ATCC 700603 and *Acinetobacter baumannii* ATCC 19606 were used for quality control. After incubation, the diameter of the growth inhibition zone around each disk was measured and compared with the values provided in the 2018 CLSI guidelines, and the results were reported as sensitive, intermediate, or resistant.

Phenotypic detection of MBLs by double disk confirmatory test (DDCT). Confirmation of MBL production was performed using DDCT test. First, a standard half-McFarland microbial suspension (equivalent to 1.5×10^8) was prepared and passed on Müller-Hinton agar medium. Two disks, namely imipenem and imipenem + EDTA, were placed on a passage of Müllerhinton agar medium. After disk placement, the plates were incubated for 16-18 hours at 37°C. After 16-18 hours, the diameter of the inhibition zone around each disk was measured. An increase in the diameter of inhibition zone ≥7 mm around the imipenem + EDTA disk relative to the imipenem disk alone was reported as a metallo-beta-lactamase-producing sample. In this test, the control strain of *E. coli* ATCC 25922 was used as the test control. Findings were reported according to the CLSI 2018 Table.

Design of primers. To detect the bla_{SPM} and bla_{VIM} genes, the complete sequence of bla_{SPM} and bla_{VIM} genes (FASTA format) as the target gene was retrieved from the NCBI database. Primer sequences were designed and analyzed using OligoCalc and oligoanalyzer programs. Primers for detection of MBL genes are shown in Table 1.

PCR assay. DNA extraction of bacteria was performed using AccuPrep Genomic DNA extraction kit according to the manufacturer's instructions. PCR was used to detect the presence of genes encoding MBL as follows: primary denaturation at 95°C for 3 minutes, secondary denaturation at 95°C for 1 minute, and annealing temperatures of 55°C and 48°C, for the bla_{SPM} and bla_{VIM} genes for 1 min, respectively. Initial extension was at 72°C for 1 min, and final extension was at 72°C for 5 minutes. Polymerase chain reaction (PCR) by Thermal Cycler (Bio Rad, Singapore) in 30 cycles and a final volume of 25 µl including 12.5 µl of Master Mix 1X (Amplicon, Denmark) 1 µl of each 0.6 µM primer pair (Bioneer, Korea), 2 µl of genomic DNA with a concentration of 50 ng, and the volume completed with 8.5 µl distilled water. Confirmation of PCR products was performed using electrophoresis (BIO RAD, Singapore) on 1.5% agarose gel and standard DNA ladder/1kb.

RESULTS

Demographic characteristics of patients and frequency of bacterial isolates. According to age groups, most isolates were related to patients (16.1%) aged 55-65 years, followed by those (15.7%) aged 55-45 years. The patients included 133 (53.6%) women, 94 (37.9%) men and 21 (8.4%) children. A collection of 248 Gram-negative bacilli containing 62 isolates of each of the bacteria were collected from medical centers: *Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Most isolates were obtained from ICU (33.1%) followed by outpatient (19.3%) and surgery (18.5%) departments. It is worth noting that most frequent clinical isolates were isolated from urine 111 (44.7%), followed by trachea 64 (25.8%), blood 27 (10.8%), sputum 23 (9.3%), tissue and wound 15 (6.5%) and other secretions 8 (3.2%). The isolates with the highest carbapenem resistance were related to *A. baumanii* (96.7%) followed by *E. coli* (22.5%). The highest and lowest rates of resistance to imipenem were detected in *A. baumanii* 58 (93.6%) and *K. pneumonia* 7 (11.3%), respectively. Of the 87 isolates resistant to imipenem, 85 were identified as carbapenemase-producing isolates, as shown in Table 2. These 85 carbapenemase-producing isolates showed high resistance to all classes of antibiotics. Most carbapenemase isolates were isolated from trachea (49.4%) and

Table 1. Sequence of primers

No.	Name	Sequence (5′→3′)	Product size	Reference
1	SPM F	GAG ATC GGA ATG AAC TCA CC	491 bp	This study
	SPM R	CTC AGA ATC CTT CGC TTC AG		
2	VIM F	GTTGCTTTTGATTGATACAG	155 bp	This study
	VIM R	GGAAGTCCAATTTGCTTC		

sputum (16.4%). The pattern of imipenem resistance and the frequency of MBL isolates of phenotype and positive genotype isolated from Gram-negative bacilli are shown in Table 3. It was found that the incidence of imipenem-resistant infections was higher in men 43 (49.5%) than in women 40 (46%). Details of the

Table 2. Characteristic and antibiogram of 85 carbapenemase-producing Gram negative bacilli isolates.

Bacteria	Total no. of isolates	Specimen no. and type	Penicillins	Aminoglycosides		Cephalosporins					Quinolones	Carbapenems			Sulfonamides	Monobactames
			Ampicillin	Gentamicin	Tobramycin	Ceftazidime	Cefopodoxime	Cefotaxime	Ceftriaxone	Cefazoline	Ciprofloxacin	Imipenem	Meropenem	Ertapenem	Trimethoprim/ Sulfamethoxazole	Aztreonam
K. pneumonia	10	Tracheal:3 Urine:4 Tissue and wound: 2 Other secretions: 1	10 (100)	7 (70)	7 (70)	8 (80)	8 (80)	8 (80)	7 (70)	7 (70)	7 (70)	6 (60)	8 (80)	7 (70)	6 (60)	7 (70)
E. coli	9	Tracheal:3 Urine:4 Blood: 2	9 (100)	7 (77.8)	9	7 (77.8)	7 (77.8)	9 (100)	9 (100)	9 (100)	9 (100)	9 (100)	8 (88.9)	7 (77.8)	8 (88.9)	9 (100)
A. baumannii	58	Tracheal:33 Urine:2 Blood: 9 Sputum: 14	ND	54 (93.1)	58 (100)	57 (98.3)	ND	58 (100)	58 (100)	ND	58 (100)	58 (100)	58 (100)	ND	58 (100)	ND
P. aeruginosa	8	Tracheal:3 Urine:2 Tissue and wound: 1 Blood: 1 Other secretions: 1	ND	5 (62.5)	5 (62.5)	6 (75)	ND	ND	ND	ND	7 (87.5)	5 (62.5)	7 (87.5)	ND	ND	6 (75)
Total no. (%)	85	Tracheal:42 Urine:12 Tissue and wound: 3 Blood: 12 Other secretions: 2 Sputum: 14	19 (100)	73 (85.9) 152 (89.4%)	79 (92.8)	78 (91.8)	15 (78.9)	75 (97.4) 258 (93.1)	74 (96.1)	16 (84.2)	81 (95.3) 81 (95.3)	78 (91.7)	81 (95.3) 173 (91.5)	14 (73.7)	72 (93.5) 72 (93.5)	22 (81.5) 22 (81.5)

Table 3. Prevalence of imipenem resistant Gram-negative bacilli isolates, Percent of positive MBL (phenotype and genotype) and isolation of carbapenem-resistant isolates from different types of clinical samples.

Bacteria	No. of isolates (%)	Imipenem resistant (%)	MBL phenotype positive (%)	MBL genotype positive (%)	Specimen no. and types					
					Urine	Sputum	Tracheal	Blood	Tissue and wound	Other secretions
K. pneumoniae	62 (25%)	7 (11.3%)	10 (16.1%)	1 (1.6%)	48	0	6	3	4	1
E. coli	62 (25%)	12 (19.4%)	9 (14.5%)	0	50	0	8	4	0	0
A. baumannii	62 (25%)	58 (93.6%)	58 (93.6%)	0	3	16	33	10	0	0
P. aeruginosa	62 (25%)	10 (16.1%)	8 (12.9%)	6 (9.7%)	10	7	17	10	11	7
Total	248 (100%)	87 (35.1%)	85 (34.3%)	7 (2.8%)	111	23	64	27	15	8

antibiotic resistance pattern between the samples and different hospital units (i.e., Pediatrics, Infectious diseases, Outpatient, Burns unit, Surgery, and ICU) are given in Tables 4 and 5.

Results of antimicrobial susceptibility profile and phenotypic screening. The pattern of antibiotic resistance is shown in Table 6. Of the 248 isolates examined, the highest percentage of antibiotic resistance was related to ampicillin (87.9%). Also, high antibiotic resistance to cefotaxime (77.9%), trimethoprim-sulfamethoxazole (73.1%), ceftriaxone (74.2%), ceftazidime (62.1%), ciprofloxacin (61.7%), tobramycin (57.7%) and gentamicin (48.4%) was observed. In general, resistance was high in different classes of antibiotics. The highest resistance was observed in 248 bacterial isolates in the class of penicillins (87.9%) followed by cephalosporins (68.2%). The results of the present study also showed that the resistance of isolates in Quinolone, Sulphonamides, Aminoglyco-

Table 4. Antibiotic resistance pattern by different hospital wards.

Antibiotics	ICU	Children	Inpatient	Outpatient	Surgery	Burn	Infectious	Total (%)	
AMP	21	10	12	37	20	3	6	109 (87.9)	109 (87.9)
CAZ	69	8	12	20	29	6	10	154 (62.1)	592 (68.2)
CPD	17	7	9	19	18	3	3	76 (61.29)	
CTX	58	8	11	20	32	5	11	145 (77.95)	
CRO	60	9	12	14	23	6	14	138 (74.2)	
CZ	21	2	11	17	21	3	4	79 (63.7)	
MEM	53	3	6	2	17	4	8	93 (37.5)	214 (28.76)
EMP	13	2	4	7	5	2	1	34 (27.41)	
IMP	48	5	5	5	13	4	7	87 (35.08)	
TN	61	7	9	12	32	6	15	143 (57.66)	263 (53.02)
GM	55	4	8	10	26	4	13	120 (48.38)	
AZ	15	11	12	8	31	3	7	87 (46.77)	87 (46.77)
CIP	56	12	20	14	30	6	15	153 (61.7)	153 (61.7)
STX	61	10	10	14	28	4	9	136 (73.11)	136 (73.11)

AMP: Ampicillin, CAZ: Ceftazidime, CPD: Cefopodoxime, CTX: Cefotaxime, CRO: Ceftriaxone, CZ: Cefazoline, MEM: Meropenem, EMP: Ertapenem, IMP: Imipenem, TN: Tobramycin, GM: Gentamicin, AZ: Aztreonam, CIP: Ciprofloxacin, STX: Trimethoprim/ Sulfamethoxazole.

Table 5. Antibiotic resistance pattern of according to various clinical samples.

Antibiotics	Urine	Sputum	Tracheal	Blood	Tissue and wound	Other secretions	Total (%)
AMP	85	0	13	7	4	0	109 (87.9)
CAZ	65	20	15	21	19	14	154 (62.1)
CPD	61	0	7	5	3	0	76 (61.29)
CRO	55	16	44	16	5	2	138 (74.2)
CZ	59	0	11	5	3	1	79 (63.7)
CTX	63	16	45	17	4	0	145 (77.95)
MEM	14	17	43	13	6	0	93 (37.5)
EMP	20	0	8	3	3	0	34 (27.41)
IMP	12	14	42	14	3	2	87 (35.08)
TN	30	22	51	25	1	5	143 (57.66)
GM	39	18	44	14	5	0	120 (48.38)
AZ	55	1	15	8	3	3	87 (46.77)
CIP	56	20	51	21	3	1	153 (61.7)
STX	60	14	45	12	4	1	136 (73.11)

Table 6. Antimicrobial resistance pattern of different species of gram negative (n= 248) by disc diffusion method.

Classes of antibiotics	Antibiotics	Gram Negative Bacteria (%)											
		K. pneumonia (62)			*E. coli* (62)			*A. baumannii* (62)			*P. aeruginosa* (62)		
		S	I	R	S	I	R	S	I	R	S	I	R
Penicillins	Ampicillin	2 (3.2%)	2 (3.2%)	58 (93.5%)	9 (14.5%)	2 (3.2%)	51 (82.2%)	ND	ND	ND	ND	ND	ND
Aminoglycosides	Gentamicin	46 (74.2%)	0	16 (25.8%)	31 (50%)	1 (1.6%)	30 (48.3%)	5 (8.1%)	0	57 (91.9%)	45 (72.6%)	0	17 (27.4%)
	Tobramycin	20 (48.3%)	12 (19.3%)	30 (32.2%)	15 (24.2%)	5 (8.06%)	42 (38.7%)	0	0	62 (100%)	36 (58.1%)	7 (11.3%)	19 (30.6%)
Cephalosporins	Ceftazidime	28 (45.1%)	6 (9.6%)	28 (45.1%)	13 (20.1%)	12 (19.3%)	37 (59.6%)	1 (1.6%)	0	61 (98.4%)	28 (45.1%)	0	34 (54.9%)
	Cefopodoxime	31 (50%)	1 (1.6%)	30 (48.3%)	16 (25.8%)	0	46 (74.2%)	ND	ND	ND	ND	ND	ND
	Cefotaxime	26 (41.9%)	2 (3.2%)	34 (54.8%)	11 (17.7%)	2 (3.2%)	49 (3.2%)	0	0	62 (100%)	ND	ND	ND
	Ceftriaxone	34 (54.8%)	1 (1.6%)	27 (45.1%)	13 (20.1%)	0	49 (79.03%)	0	0	62 (100%)	ND	ND	ND
	Cefazoline	29 (46.7%)	4 (6.4%)	29 (46.7%)	3 (4.8%)	9 (14.5%)	50 (80.6%)	ND	ND	ND	ND	ND	ND
Quinolones	Ciprofloxacin	36 (58.06%)	0	26 (41.9%)	11 (17.7%)	5 (8.06%)	46 (74.2%)	0	0	62 (100%)	34 (54.9%)	9 (14.5%)	19 (30.6%)
Carbapenems	Imipenem	53 (85.4%)	2 (3.2%)	7 (11.3%)	48 (77.4%)	2 (3.2%)	12 (19.3%)	4 (6.5	0	58	52 (83.9%)	0	10 (16.1%)
	Meropenem	48 (77.4%)	3 (4.8%)	11 (17.7%)	49 (79%)	0	13 (21%)	%) 0	0	(93.5%) 62	54 (87.1%)	1 (1.6%)	7 (11.3%)
	Ertapenem	41 (66.1%)	4 (6.4%)	17 (27.4%)	43	2 (3.2%)	17 (27.4%)	ND	ND	(100%) ND	ND	ND	ND
Sulfonamides	Trimethoprim/ Sulfamethoxazole	36 (58.1%)	0	26 (41.9%)	14 (22.6%)	0	48 (77.4%)	0	0	62	ND	ND	ND
Monobactames	Aztreonam	29 (46.7%)	5 (8.06%)	28 (45.1%)	12 (1.6%)	1 (19.3%)	49 (79.03%)	ND	ND	(100%) ND	51 (82.3%)	1 (1.6%)	10 (16.1%)

ND=Not determined; S=Sensitive; I= Intermediate; R=Resistant

sides, monobactam, and carbapenems was (61.7%), (73.1%), (53.02%), (46.7%), and (34.5%), respectively. In general, the resistance profiles were categorised into MDR, XDR and PDR according to Algammal (12). Carbapenemase-producing strains that were resistant to at least three classes of antimicrobials, were classified as multiple resistance (MDR), resistance to all but two or less classes of antimicrobials, as extensive drug resistance (XDR), and resistance to all classes of available antimicrobial agents were considered par-drug resistant (PDR) (12, 13). It is classified, and resistant to all classes of available antimicrobial agents is known as PDR. Also, 83 (97.6) Carbapenemase-producing isolates were resistant to at least four classes of antimicrobials (MDR). The findings of the present study showed that 82 (96.4) isolates were resistant to at least 5-7 antimicrobial classes and were identified as XDR isolates. Also, 65 (76.4) isolates resisted all antimicrobial classes (7 batches) and were known as PDR isolates. The most common patterns of phenotypic resistance to different classes of antimicrobials among different strains are shown in Table 7. Fig. 1 shows the pattern of antimicrobial resistance by gender, indicating that resistance to penicillin class (92.3%) in children was higher than that in men and women, with men having high resistance to ampicillins (88.6%) and cephalosporins (72.8%) and women having high resistance to ampicillins (86.6%) and sul-

Table 7. Distribution of MDR, XDR, and PDR patterns of bacterial agents among 85 carbapenemase-producing

Bacteria	Resistance profile	Antibiotics No.	Antibiotic resistant isolates No (%)	The most common phenotypic resistance patterns	Number of antimicrobial resistance classes
K.pneumoniae =10	8 MDR	14	4	Penicillins, Aminoglycosides, Cephalosporins, Quinolones, Carbapenems, Sulfonamides and Monobactames	4-7
		13	2		
		12	1	Penicillins, Aminoglycosides, Cephalosporins, Quinolones and Carbapenems	
		9	1	Penicillins, Cephalosporins, Quinolones and Monobactames	
	7 XDR	14	4	Penicillins, Aminoglycosides, Cephalosporins, Quinolones, Carbapenems, Sulfonamides and Monobactames	5-7
		13	2		
		12	1	Penicillins, Aminoglycosides, Cephalosporins, Quinolones and Carbapenems	
	4 PDR	14	4	Penicillins, Aminoglycosides, Cephalosporins, Quinolones, Carbapenems, Sulfonamides and Monobactames	7
E. coli =9	9 MDR	14	6	Penicillins, Aminoglycosides, Cephalosporins, Quinolones, Carbapenems, Sulfonamides and Monobactames	6-7
		13	1		
		12	1		
		10	1	Penicillins, Aminoglycosides, Cephalosporins, Quinolones, Carbapenems and Monobactames	
	9 XDR	14	6	Penicillins, Aminoglycosides, Cephalosporins, Quinolones, Carbapenems, Sulfonamides and Monobactames	6-7
		13	1		
		12	1		
		10	1	Penicillins, Aminoglycosides, Cephalosporins, Quinolones, Carbapenems and Monobactames Penicillins,	
	6 PDR	14	6	Aminoglycosides, Cephalosporins, Quinolones, Carbapenems, Sulfonamides and Monobactames	7
A.baumanii =58	58 MDR	8	58	Aminoglycosides, Cephalosporins, Quinolones, Carbapenems, and Sulfonamides	5
	58 XDR	8	58	Aminoglycosides, Cephalosporins, Quinolones, Carbapenems, and Sulfonamides	5
	53 PDR	8	53	Aminoglycosides, Cephalosporins, Quinolones, Carbapenems, and Sulfonamides	5
P.aeruginosa =8	8 MDR	8	2	Aminoglycosides, Cephalosporins, Quinolones, Carbapenems, and Monobactames	3-5
		6	1	Cephalosporins, Quinolones, Carbapenems, and Monobactames	
		5	1	Aminoglycosides, Cephalosporins, Quinolones, and Monobactames	
			1	Aminoglycosides, Cephalosporins, and Quinolones	
			1	Aminoglycosides, Cephalosporins, and Carbapenems, Quinolones,	
			1	Carbapenems, and Monobactames	
		4	1	Quinolones, Carbapenems, and Monobactames	
	8 XDR	8	2	Aminoglycosides, Cephalosporins, Quinolones, Carbapenems, and Monobactames	3-5
		6	1	Cephalosporins, Quinolones, Carbapenems, and Monobactames Aminoglycosides,	
		5	1	Cephalosporins, Quinolones, and Monobactames Aminoglycosides,	
			1	Cephalosporins, and Quinolones	
			1	Aminoglycosides, Cephalosporins, and Carbapenems,	
		4	1	Quinolones, Carbapenems, and Monobactames	
	2 PDR	8	1	Quinolones, Carbapenems, and Monobactames	5
Total no. (%)	83 MDR (97.6)		-	Aminoglycosides, Cephalosporins, Quinolones, Carbapenems, and Monobactames	
	82 XDR (96.4)				
	65 PDR (76.4)				

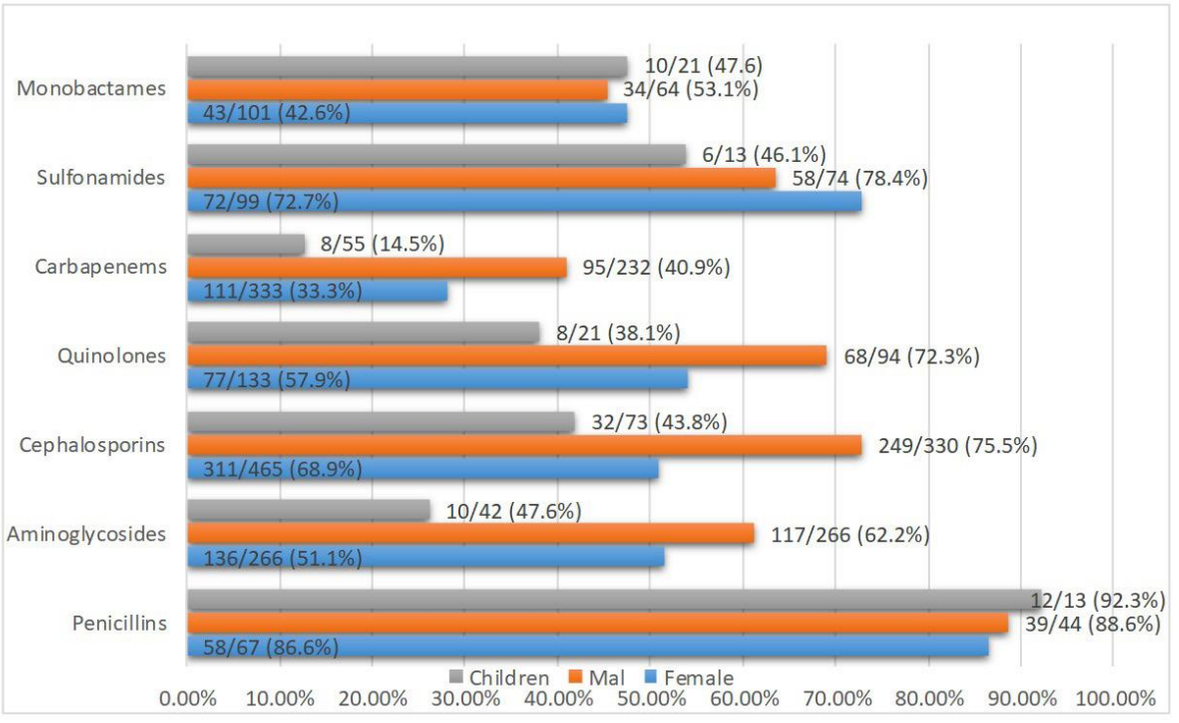

Fig 1. Distribution of antibiotic resistance of antibiotic classes by sex. Total no. R/ Total no. G (%)
R: Resistance, G: Gender

fonamides (63.5%). Also, the profile of antibiotic resistance of antibiotic classes by sex and total antibiotic resistance by gender to different antibiotic classes is shown in Tables 8 and 9, respectively.

Detection of carbapenemase activity phenotype. The results of diagnosis based on MBL phenotype using disk diffusion method showed that in the 85 carbapenemase isolates examined, there were 10 (16.1%) *K. pneumoniae* isolates, 9 (14.5%) *E. coli* isolates, 58 (93.6%) *A. baumannii* isolates, and 8 (12.9%) *P. aeruginosa* isolates. The positive result of MBL-producing isolates was detected as an increase in the diameters of the inhibition zone around the dual hybrid discs (imipenem and imipenem-EDTA) (difference in diameters of the inhibition zone ≥7) compared to the imipenem disc alone. Frequency of positive MBL Production of isolates according to different parts of the hospital is shown in Fig. 2.

Prevalence of carbapenemase genes. bla_{VIM} and bla_{SPM} genes were not isolated from any of the 85 phenotypically confirmed carbapenemase-producing isolates. The results of detection of bla_{VIM} and bla_{SPM} carbapenemase genes in carbapenemase-producing Gram-negative bacilli using PCR are shown in Fig. 3.

DISCUSSION

Increased antibiotic resistance seriously limits treatment choices, particularly given the significant carbapenem resistance among Gram-negative bacteria, which has been reported in many parts of the world. Therefore, the clinical impact of carbapenem resistance due to producing enzymes such as MBL has developed into a significant worldwide public health emergency (14).

In the present study an equal number of *K. pneumoniae*, *E. coli*, *A. baumannii* and *P. aeruginosa* (62 isolates each, 248 isolates totally) was collected from several medical centers in Kermanshah city, west of Iran. The highest number of isolates (111 isolates, 44.7%), was collected from urine samples. The results showed the prevalence of 35.1% resistance to carbapenems. That is, out of the 248 isolates studied, 87 isolates were imipenem-resistant, and out of these 87 isolates, 85 were carbapenemase-producing. This result is consistent with previous reports showing a frequency of 30.9% in Iraq (14), 36% in Eygept (15),

Table 8. The profile of antibiotic resistance of antibiotic classes by sex.

		Penicillins	Aminoglycosides		Cephalosporins					Quinolones	Carbapenems			Sulfonamides	Monobactames
Bacteria	Gender	Ampicillin	Gentamicin	Tobramycin	Ceftazidime	Cefopodoxime	Cefotaxime	Ceftriaxone	Cefazoline	Ciprofloxacin	Imipenem	Meropenem	Ertapenem	Trimethoprim/ Sulfamethoxazole	Aztreonam
K. pneumonia	F	R 34	8	12	18	18	19	15	15	14	4	7	10	16	14
		I 1	0	8	3	0	1	0	2	0	2	2	3	0	3
		S 3	30	18	17	20	18	23	21	24	32	29	25	22	21
	M	R 18	8	7	8	11	13	11	13	10	3	3	6	9	11
		I 1	0	3	2	0	1	0	1	0	0	1	1	0	2
		S 0	11	9	9	8	5	8	5	9	16	15	12	10	6
	Ch	R 5	0	1	2	1	2	1	1	2	0	1	1	1	3
		I 0	0	1	1	1	0	1	1	0	0	0	0	0	0
		S 0	5	3	2	3	3	3	3	3	5	4	4	4	2
E. coli	F	R 24	16	20	18	22	24	24	26	23	4	8	8	24	23
		I 0	0	2	5	0	1	0	1	3	2	0	2	0	0
		S 5	13	7	6	7	4	5	2	3	23	21	19	5	6
	M	R 21	11	19	16	20	20	19	19	18	6	4	7	19	20
		I 2	0	2	5	0	0	0	6	2	0	0	0	0	0
		S 2	14	6	4	5	5	6	0	5	19	21	18	6	5
	Ch	R 7	3	3	3	4	5	6	5	5	2	1	2	5	6
		I 0	1	1	2	0	1	0	2	0	0	0	0	0	1
		S 1	4	4	3	4	2	2	1	3	6	7	6	3	1
A. baumannii	F	R ND	29	32	32	ND	32	32	ND	32	28	32	ND	32	ND
		I ND	0	0	0	ND	0	0	ND	0	0	0	ND	0	ND
		S ND	3	0	0	ND	0	0	ND	0	4	0	ND	0	ND
	M	R ND	28	30	29	ND	30	30	ND	30	30	30	ND	30	ND
		I ND	0	0	1	ND	0	0	ND	0	0	0	ND	0	ND
		S ND	2	0	0	ND	0	0	ND	0	0	0	ND	0	ND
	Ch	R ND	-	-	-	ND	-	ND	ND	-	-	-	ND	-	ND
		I ND	-	-	-	ND	-	ND	ND	-	-	-	ND	-	ND
		S ND	-	-	-	ND	-	ND	ND	-	-	-	ND	-	ND
P. aeruginosa	F	R ND	9	10	16	ND	ND	ND	ND	8	6	4	ND	ND	6
		I ND	0	3	0	ND	ND	ND	ND	2	0	1	ND	ND	0
		S ND	25	21	18	ND	ND	ND	ND	24	284	29	ND	ND	28
	M	R ND	7	7	10	ND	ND	ND	ND	10	0	2	ND	ND	3
		I ND	0	2	0	ND	ND	ND	ND	4	16	0	ND	ND	1
		S ND	13	11	10	ND	ND	ND	ND	6	0	18	ND	ND	16
	Ch	R ND	1	2	2	ND	ND	ND	ND	1	0	1	ND	ND	1
		I ND	0	2	0	ND	ND	ND	ND	3	0	0	ND	ND	0
		S ND	7	4	6	ND	ND	ND	ND	4	8	7	ND	ND	7
Total no.	F	R 58	62	74	84	40	75	71	41	77	42	51	18	72	43
(%)		I 1	0	13	8	0	2	0	3	5	4	3	5	0	3
		S 8	71	46	41	27	22	28	23	51	87	79	44	27	55
	M	R 39	54	63	63	31	63	60	32	68	43	39	13	58	34
		I 3	0	7	7	0	1	0	7	6	0	1	1	0	3
		S 2	40	24	24	13	10	14	5	20	51	54	30	16	27
	Ch	R 12	4	6	7	5	7	7	6	8	2	3	3	6	10
		I 0	1	4	3	1	1	1	3	3	0	0	0	0	1
		S 1	16	11	11	7	5	5	4	10	19	18	10	7	10

Table 9. Total antibiotic resistance by gender to different antibiotic classes

Bacteria	Gender		Penicillins	Aminoglycosides	Cephalosporins				Quinolones	Carbapenems		Sulfonamides	Monobactames
			Ampicillin	Gentamicin Tobramycin	Ceftazidime	Cefopodoxime	Cefotaxime	Ceftriaxone Cefazoline	Ciprofloxacin	Imipenem	Meropenem Ertapenem	Trimethoprim/ Sulfamethoxazole	Aztreonam
Total no.	F	R	58/67	136/266	311/465				77/133	111/333		72/99	43/101
(%)			(86.6)	(51.1)	(68.9)				(57.9)	(33.3)		(72.7)	(42.6)
	M	R	39/44	117/266	249/330				68/94	95/232		58/74	34/64
			(88.6)	(62.2)	(75.5)				(72.3)	(40.9)		(78.4)	(53.1)
	Ch	R	12/13	10/42	32/73				8/21	8/55		6/13	10/21
			(92.3)	(47.6)	(43.8)				(38.1)	(14.5)		(46.1)	(47.6)

R: Resistance, I: Intermediate, S: Sensitivity, F: Female, M: Male, Ch: Children, G: Gender

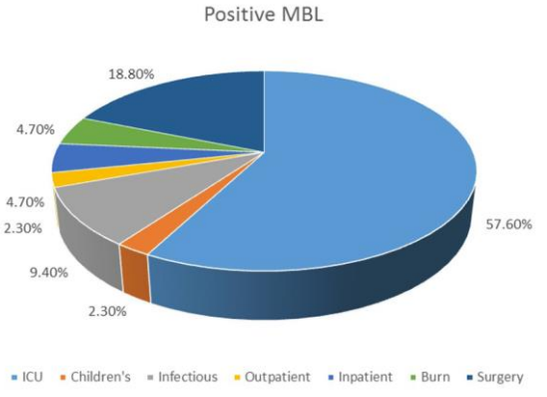

Fig. 2. Frequency of positive MBL Production of isolates according to different parts of the hospital

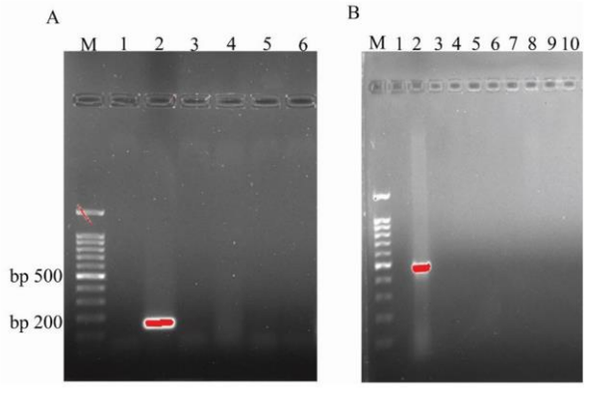

Fig 3. Agarose gel electrophoresis of PCR product of *VIM* and *SPM* genes with 155 bp, and 491 bp. A) M: marker 100bp, 1: negative control, 2: positive control, lanes 3 to 6: negative samples. B) M: marker 100bp, 1: negative control, 2: positive control, lanes 3 to 10: negative samples.

37.9% in Iran (13), 24.6% in China (16), 19% in Algeria (8), and 56% in Pakistan (17) for resistance to carbapenems. In some other studies, resistance to carbapenems was higher. A case in point is Tunisia with a resistance rate of 86.3% (18). In contrast, some studies observed lower carbapenems resistance rates such as 13.6% in Iran (19), 5.99% in Morocco (20), 2.9% in Ghana (21), and 2.82% in Turkey (22). These discrepancies could be attributed to several factors including different study times, regions, and populations.

To the best of our knowledge, there is no previous report about carbapenems resistance rate in *A. baumanii* and *E. coli* isolates in Kermanshah. However, previous studies done in Kermanshah reported carbapenems resistance prevalence of 36.02% (23) and 33.8% (24) for *P. aeruginosa*, and 6.7% for *K. pneumoniae* (25). In our study, we observed a prevalence of 16.1% and 11.3% for carbapenems-resistant *P. aeruginosa* and *K. pneumoniae* isolates, respectively, which is in agreement with previous studies conducted in Kermanshah. The most frequent carbapenem-resistant isolate in the present study was *A. baumanii*, which is in agreement with other studies from Algeria (8), Iraq (14), and Indonesia (26).

The mean prevalence of MBL-positive isolates was 34.3% in the present study. The most frequent MBL-positive isolates were *A. baumanii* (93.6%). This prevalence was low in other bacteria (16.1% of *K. pneumoniae*, 14.5% of *E. coli*, and 12.9% of *P. aeruginosa* isolates). Consistent with our results, Haji et al. in Iraq reported a prevalence of 27% (mean) for MBL-positive isolates, with the highest prevalence

being related to *A. baumanii* isolates (14). Also, in previous study in Kermanshah, Ranjbar et al. (27) in 2019 showed a high prevalence of MBL-positive *A. baumanii* with 91.4% prevalence. These results highlight the fact that the problem of antibiotic resistance of *A. baumanii* caused by the activity of MBL enzymes is very serious in this region. The prevalence of MBL-positive *P. aeruginosa* was reported to be 20% (24), 48.3% (28), and 17.7% (23) in the previous studies in Kermanshah (all in conducted in 2015), which are higher than the results obtained in our study (12.9%). The different results could be explained by the different study times or samples used.

In the present study, the highest percentage of antibiotic resistance was related to the class of penicillins (87.9%), followed by cephalosporins (68.2%), quinolone (61.7%), sulphonamides (73.1%), aminoglycosides (53.02%), monobactam (46.7%) and carbapenems (34.5%). The highest percentage of antibiotic resistance was related to ampicillin (87.9%). Similarly, in a previous study in Iraq, the prevalence of resistance to penicillins, cephalosporins, quinolones, sulphonamide, and aminoglycosides was high (14). The ICU, the surgery department, and the infectious diseases department were the departments where most resistant isolates were detected. This highlights the serious risk of microbial resistance for the patients' health and suggests that more attention should be paid to the control of resistant strains in these departments. Also, the resistance rate was higher in urine samples and among male subjects, introducing urine and male sex as possible risk factors. Consistent with our study, Haji et al. found more resistant strains in urine samples, but in contrast to our results, they observed more imipenem-resistant strains in strains isolated from female subjects (28).

As far as bacterial species are concerned, in our study 54.9% of *P. aeruginosa* isolates were resistant to ceftazidime. Similarly, a high resistance rate (43-97%) to cephalosporins of *P. aeruginosa* was reported in studies done in this region in 2015 (23, 24, 28). The frequency of carbapenems-resistant *P. aeruginosa* isolates was relatively low (16.1% for imipenem and 12.9 % for meropenem) in the present study. Abiri et al. (24) and Fazeli et al. (23) observed a higher resistance of *P. aeruginosa* to carbapenems in Kermanshah in 2015 (33.7% to imipenem, and 18.1% to meropenem in Abiri et al. and 36.0% to imipenem in Fazeli et al.).

Acinetobacter species are one of the main pathogens causing nosocomial infections and are most frequently associated with wound infections. They are the primary causes of 92% of infections associated to imipenem resistance (17). In our study, the highest resistance was observed in *A. baumanii* isolates in that they were highly resistant to aminoglycosides (gentamicin 91.9% and tobramycin 100%), cephalosporins (ceftazidime 98.4%, cefotaxime 100%, and ceftriaxone 100%), quinolones (ciprofloxacin 100%), carbapenems (imipenem 93.5% and meropenem 100%), and sulfonamides (trimethoprim/ sulfamethoxazole 100 %). This high resistance rate of *A. baumanii* to antibiotics was also previously reported in different parts of the world. For instance, the prevalence of carbapenems-resistant *A. baumanii* was very high and ranged between 77.5-100% in previous reports (13, 14, 29, 30).

Our results showed that in comparison to other bacteria studied here, *E. coli* and *K. pneumoniae* isolates had generally lower resistance to antibiotics, although they were also highly resistant to some antibiotics. *E. coli* isolates were highly resistant (about 75-85%) to penicillins, cephalosporins (except cefotaxime), quinolones, sulfonamides, and monobactames. *K. pneumoniae* isolates were highly resistant to penicillins (93.5%). Similar to our results, another study done in Kermanshah on 60 *K. pneumoniae* isolates, resistance to penicillins (ampicillin) was 100%, and the resistance to other antibiotics was lower (ranged from 6.67% to 66.67%) (25). We did not find any previous report on *E. coli* resistance in Kermanshah.

In the present study, of all carbapenemase-producing isolates studied, 97.6% were MDR, 96.4% were XDR, and 76.4% were PDR. The high prevalence of MDR, XDR and PDR profiles among carbapenemase-producing isolates has also been reported previously in this region (Kermanshah) (23), as well as in other countries (13, 14). The high percentages of MDR, XDR and PDR pattern amongst carbapenemase-producing Gram-negative bacterial strains in the present study may be due to increased selection pressure caused by self-medication, uncontrolled and excessive use of carbapenems and cephalosporins, and a lack of constant monitoring of these isolates in hospital environments, especially in ICU, and surgery, and infectious disease departments. For these reasons, in Kermanshah more monitoring activities are required to reduce the high prevalence of MDR, XDR and PDR isolates. The emergence of these isolates is a real threat since there is almost no antibiotic

that can be used to treat them, and thus, substantial rates of morbidity and mortality from infections with these strains are anticipated. According to some research, patients who are infected with *A. baumannii* have significant mortality rates (between 8% to 23% in hospitals and from 10% to 43% in ICUs) (31).

Two of the most important genes in carbapenemase resistance are bla_{VIM} and bla_{SPM} genes which were studied in the present study. However, none of them was isolated from our 85 phenotypically confirmed carbapenemase-producing isolates. Previous reports on bla_{VIM} gene in *K. pneumoniae* isolates in Kermanshah showed low prevalence (5%) of this gene (25). Low prevalence (2.2% or 8.3%) of bla_{VIM} gene was also reported among *P. aeruginosa* isolates of Kermanshah (25, 28). In contrast to these studies, a higher prevalence of bla_{VIM} gene (49%) was reported from carbapenem-resistant isolates in Iraq (14). The absence of bla_{VIM} and bla_{SPM} genes in our isolates showed there are other genes responsible for resistance to carbapenems that are recommended to be studied in future.

Carbapenemase-producing *Enterobacteriaceae* (CPE) are highly prevalent in some countries in Asia, particularly in India, Pakistan, and China (32). The prevalence of carbapenemase genes in Europe varies depending on the country and the healthcare setting. In some countries, such as Greece and Italy, the prevalence of carbapenemase-producing *Klebsiella pneumoniae* (CP-Kp) is high (33).

The prevalence of carbapenemase genes in North America is relatively low compared to other regions. However, some parts of the United States have reported a higher prevalence of carbapenemase-producing bacteria, particularly in healthcare settings (32, 34).

Carbapenemases in Enterobacterales from South America are being disseminated through clones, plasmids, and transposons, which are similar to those reported in other regions of the world (35).

In Iran, the prevalence of carbapenem resistance in *K. pneumoniae* and *E. coli* was primarily attributed to the presence of the bla_{OXA-48} gene (36). In a review article by Kopotsa et al. It was shown that the prevalence of both carbapenemases and the plasmid replicon groups associated with them has increased, with China, Canada, and the United States reporting a greater increase compared to other countries (37). Except for Angola and the Czech Republic, where OXA-181 and OXA-48-like carbapenemases were more prevalent, bla_{KPC} was the most commonly observed carbapenemase gene. Among the reported carbapenemases, $bla_{KPC-2/3}$ was responsible for 70% of the cases.

CONCLUSION

The results of the present study showed the clinical isolates that were recovered showed notable multidrug resistance to cephalosporins, penicillins, sulphonamides, aminoglycosides, and quinolones, which are taken as a serious risk to the public health. The resistance rate of MDR, XDR, and PDR to many antibiotic classes was high among our isolates. This warns us against infections brought on by these resistant germs and calls for sophisticated therapies. This study suggests ongoing antimicrobial susceptibility testing to identify isolates that are multidrug resistant, and the careful and limited use of antibiotics in sanitary procedures.

ACKNOWLEDGEMENTS

The authors of this article express their gratitude and appreciation to the Vice-Chancellor for Research and Technology of the Kermanshah University of Medical Science for accepting the costs of implementing this project. The present article has been registered with grand number: 97122 and ethical code: IR.KUMS.REC.1397.051.

REFERENCES

1. Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool MH, et al. Antibiotic resistance: a rundown of a global crisis. *Infect Drug Resist* 2018: 11: 1645-1658.
2. Giaccari LG, Pace MC, Passavanti MB, Gargano F, Aurilio C, Sansone P. Ceftolozane/Tazobactam for resistant drugs *Pseudomonas aeruginosa* respiratory infections: a systematic Literature review of the Real-World Evidence. *Life (Basel)* 2021; 11: 474.
3. Shaker OA, Gomaa HE, ElMasry SA, Halim RMA, Abdelrahman AH, Kamal JS. Evaluation of combined use of Temocillin disk and Mastdisks inhibitor combination set against polymerase chain reaction for detection of carbapenem-resistant *Enterobacteriaceae. Open Ac-*

cess Maced J Med Sci 2018; 6: 242-247.
4. Nordmann P, Poirel L. Strategies for identification of carbapenemase-producing *Enterobacteriaceae*. *J Antimicrob Chemother* 2013; 68: 487-489.
5. Deldar Abad Paskeh M, Mehdipour Moghaddam MJ, Salehi Z. Prevalence of plasmid-encoded carbapenemases in multi-drug resistant *Escherichia coli* from patients with urinary tract infection in northern Iran. *Iran J Basic Med Sci* 2020; 23: 586-593.
6. Bush K, Jacoby GA. Updated functional classification of beta-lactamases. *Antimicrob Agents Chemother* 2010; 54: 969-976.
7. Bush K, Fisher JF. Epidemiological expansion, structural studies, and clinical challenges of new β-lactamases from gram-negative bacteria. *Annu Rev Microbiol* 2011; 65: 455-478.
8. Bourafa N, Chaalal W, Bakour S, Lalaoui R, Boutefnouchet N, Diene SM, et al. Molecular characterization of carbapenem-resistant Gram-negative bacilli clinical isolates in Algeria. *Infect Drug Resist* 2018;11: 735-742.
9. Workneh M, Yee R, Simner PJ. Phenotypic methods for detection of carbapenemase production in carbapenem-resistant organisms: what method should your laboratory choose? *Clin Microbiol Newsl* 2019; 41: 11-22.
10. Hu W, Li M, Lu W, Guo S, Li J. Evaluation of MAST-DISCS combi Carba plus for the identification of metallo-β-lactamases, KPC and OXA-48 carbapenemase genes in *Enterobacteriaceae* clinical isolates. *Lett Appl Microbiol* 2020; 70: 42-47.
11. Owlia P, Saderi H, Karimi Z, Akhavi Rad SMB, Bahar MA. Phenotypic detection of Metallo-beta-Lactamase producing *Pseudomonas aeruginosa* strains isolated from burned patients. *Iran J Pathol* 2008; 3: 20-25.
12. Algammal AM, Hashem HR, Alfifi KJ, Hetta HF, Sheraba NS, Ramadan H, et al. atpD gene sequencing, multidrug resistance traits, virulence-determinants, and antimicrobial resistance genes of emerging XDR and MDR-Proteus mirabilis. *Sci Rep* 2021; 11: 9476.
13. Shokri D, Rabbani Khorasgani M, Fatemi SM, Soleimani-Delfan A. Resistotyping, phenotyping and genotyping of New Delhi metallo-β-lactamase (NDM) among Gram-negative bacilli from Iranian patients. *J Med Microbiol* 2017; 66: 402-411.
14. Haji SH, Aka STH, Ali FA. Prevalence and characterisation of carbapenemase encoding genes in multidrug-resistant Gram-negative bacilli. *PLoS One* 2021; 16(11): e0259005.
15. Makharita RR, El-Kholy I, Hetta HF, Abdelaziz MH, Hagagy FI, Ahmed AA, et al. Antibiogram and genetic characterization of carbapenem-resistant Gram-negative pathogens incriminated in healthcare-associated infections. *Infect Drug Resist* 2020; 13: 3991-4002.
16. Jin C, Zhou F, Cui Q, Qiang J, An C. Molecular characteristics of carbapenem-resistant *Enterobacter cloacae* in a tertiary Hospital in China. *Infect Drug Resist* 2020; 13: 1575-1581.
17. Ain NU, Iftikhar A, Bukhari SS, Abrar S, Hussain S, Haider MH, et al. High frequency and molecular epidemiology of metallo-β-lactamase-producing gram-negative bacilli in a tertiary care hospital in Lahore, Pakistan. *Antimicrob Resist Infect Control* 2018; 7: 128.
18. Kollenda H, Frickmann H, Ben Helal R, Wiemer DF, Naija H, El Asli MS, et al. Screening for carbapenemases in Ertapenem-resistant *Enterobacteriaceae* collected at a Tunisian Hospital between 2014 and 2018. *Eur J Microbiol Immunol (Bp)* 2019; 9: 9-13.
19. Jalalvand K, Shayanfar N, Shahcheraghi F, Amini E, Mohammadpour M, Babaheidarian P. Evaluation of phenotypic and genotypic characteristics of carbapnemases-producing *Enterobacteriaceae* and its prevalence in a referral Hospital in Tehran City. *Iran J Pathol* 2020; 15: 86-95.
20. Mahrach Y, Mourabit N, Arakrak A, Bakkali M, Laglaoui A. Phenotypic and molecular study of carbapenemase-producing *Enterobacteriaceae* in a regional hospital in northern Morocco. *J Clin Med Sci* 2019; 3: 113.
21. Codjoe FS (2016). Detection and characterisation of carbapenem-resistant gram-negative bacilli infections in Ghana: Sheffield Hallam University (United Kingdom).
22. Karabay O, Altindis M, Koroglu M, Karatuna O, Aydemir Ö A, Erdem AF. The carbapenem-resistant *Enterobacteriaceae* threat is growing: NDM-1 epidemic at a training hospital in Turkey. *Ann Clin Microbiol Antimicrob* 2016; 15: 6.
23. Fazeli H, Nazari F, Mirzaie M. The determination of metallo-beta-lactamase enzymes prevalence in *Pseudomonas aeruginosa* using etest and their antibiogram patterns in Kermanshah, Iran. *J Kerman Univ Med Sci* 2015; 22: 491-498.
24. Abiri R, Mohammadi P, Shavani N, Rezaei M. Detection and genetic characterization of metallo-β-lactamase IMP-1 and VIM-2 in *Pseudomonas aeruginosa* strains from different hospitals in Kermanshah, Iran. *Jundishapur J Microbiol* 2015; 8(9): e22582.
25. Zare A, Akya A, Nejat P. The frequency of bla_{VIM}, bla_{IMP}, bla_{KPC} and bla_{NDM} Carbapenemase genes in clinical isolates of *Klebsiella Pneumoniae* in Kermanshah medical centers. *JSSU* 2015; 23: 760-769.
26. Karuniawati A, Saharman YR, Lestari DC. Detection of carbapenemase encoding genes in *Enterobacteriace*, *Pseudomonas aeruginosa*, and *Acinetobacter baumanii* isolated from patients at Intensive Care Unit Cipto Mangunkusumo Hospital in 2011. *Acta Med Indones* 2013; 45: 101-106.
27. Ranjbar R, Farahani A. Study of genetic diversity, biofilm formation, and detection of Carbapenemase, MBL, ESBL, and tetracycline resistance genes in mul-

tidrug-resistant *Acinetobacter baumannii* isolated from burn wound infections in Iran. *Antimicrob Resist Infect Control* 2019; 8: 172.
28. Akya A, Salimi A, Nomanpour B, Ahmadi K. Prevalence and clonal dissemination of metallo-beta-lactamase-producing *Pseudomonas aeruginosa* in Kermanshah. *Jundishapur J Microbiol* 2015; 8(7): e20980.
29. Boral B, Unaldi Ö, Ergin A, Durmaz R, Eser ÖK ; Acinetobacter Study Group. A prospective multicenter study on the evaluation of antimicrobial resistance and molecular epidemiology of multidrug-resistant *Acinetobacter baumannii* infections in intensive care units with clinical and environmental features. *Ann Clin Microbiol Antimicrob* 2019; 18: 19.
30. Shamim S, Abbas M, Qazi MH. Prevalence of multidrug resistant *Acinetobacter baumannii* in hospitalized patients in Lahore, Pakistan. *Pakistan J Mol Med* 2015; 2: 23-28.
31. Nasiri MJ, Zamani S, Fardsanei F, Arshadi M, Bigverdi R, Hajikhani B, et al. Prevalence and mechanisms of carbapenem resistance in *Acinetobacter baumannii:* a comprehensive systematic review of cross-sectional studies from Iran. *Microb Drug Resist* 2020; 26: 270-283.
32. Gupta N, Limbago BM, Patel JB, Kallen AJ. Carbapenem-resistant *Enterobacteriaceae*: epidemiology and prevention. *Clin Infect Dis* 2011; 53: 60-67.
33. Giani T, Pini B, Arena F, Conte V, Bracco S, Migliavacca R, et al. Epidemic diffusion of KPC carbapenemase-producing *Klebsiella pneumoniae* in Italy: results of the first countrywide survey, 15 May to 30 June 2011. *Euro Surveill* 2013; 18: 20489.
34. CDC. Prevention. Antibiotic resistance threats in the United States, 2019: US Department of Health and Human Services, Centres for Disease Control and Prevention 2019.
35. Reyes JA, Melano R, Cárdenas PA, Trueba G. Mobile genetic elements associated with carbapenemase genes in South American Enterobacterales. *Braz J Infect Dis* 2020; 24: 231-238.
36. Nasiri MJ, Mirsaeidi M, Mousavi SMJ, Arshadi M, Fardsanei F, Deihim B, et al. Prevalence and mechanisms of carbapenem resistance in *Klebsiella pneumoniae* and *Escherichia coli*: a systematic review and meta-analysis of cross-sectional studies from Iran. *Microb Drug Resist* 2020; 26: 1491-1502.
37. Kopotsa K, Osei Sekyere J, Mbelle NM. Plasmid evolution in carbapenemase-producing *Enterobacteriaceae*: a review. *Ann N Y Acad Sci* 2019; 1457: 61-91.