Modified CIM-Test As a Useful Tool to Detect Carbapenemase Activity Among Extensively Drug- Resistant Klebsiella Pneumoniae, Escherichia Coli and Acinetobacter Baumannii

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Research Article

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Abstract

Background

Timely detection of carbapenemases is essential for developing strategies to control the spread of infections by carbapenem-resistant isolates. The purpose of our study was to determine the epidemiology of carbapenemase genes among carbapenem resistant isolates of *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Escherichia coli* and to compare efficacy of modified Hodge Test (MHT), Carba NP and modified carbapenem inactivation method (mCIM) tests.

Methods

A total of 122 carbapenem-resistant clinical isolates including 77 *K. pneumoniae*, 39 *A. baumannii*, and six *E. coli* were collected from hospitalized patients. Three phenotypic methods, including MHT, Carba NP test and mCIM were used for investigation of carbapenemase production. In addition, polymerase chain reaction (PCR) was performed to detect carbapenemase encoding genes.

Results

The sensitivity and specificity of the MHT were 75.0% and 100% respectively. In addition, CarbaNP displayed 80.8% sensitivity and 100% specificity, whereas the sensitivity and specificity were 90.4% and 100% for the mCIM test, respectively. Among carbapenem-resistant isolates, 70, 84 and 87 isolates exhibited positive results according to MHT, CarbaNP test and mCIM, respectively. PCR indicated the presence of one or more carbapenemase genes in 119 of carbapenem-resistant isolates, with bla_{KPC} and bla_{VIM} being the most commonly encountered. Co-production of 'KPC and VIM', 'KPC and IMP' and 'KPC and OXA-48' was detected in nine, seven and three isolates, respectively.

Conclusion

Our results confirm that the mCIM test is a useful tool for the reliable detection of carbapenemases-activity in enterobacterial isolates, especially in clinical microbiological laboratories with limited resources.

Introduction

The worldwide emergence and spread of carbapenemase producers represent a significant threat to public health, because infection with these bacteria is associated with high mortality rates and limited medical treatment options [1]. Particularly, this may pose a major problem as carbapenems are becoming more frequently needed to treat infections caused by Gram-negative bacteria that produce extended-spectrum beta-lactamases (ESBL) [2]. Carbapenemase enzymes are clustered in different classes that define their hydrolytic profiles; VIM, NDM, and IMP belong to the Ambler class B metallo-ß-lactamases (MBLs), *Klebsiella pneumoniae* carbapenemase (KPC) and GES belong to class A, and OXA-48 and OXA-48-like belong to class D [3]. These profiles are associated with resistance to carbapenems and to most ß-lactam antibiotics (except for aztreonam for class B and third-generation cephalosporins for class D).

Growing reports of carbapenemase-producing *Acinetobacter baumannii* strains and Enterobacteriaceae including *Klebsiella pneumoniae* and *Escherichia coli* are documented in the literature [4]. Mobile genetic elements (plasmids, transposons, etc.) that harbor carbapenemase genes have been documented to spread among Gram-negative bacterial species, making it important to rapidly and efficiently detect all Gram-negative carbapenemase producers to prevent their further spread [4].

Antimicrobial resistance surveillance is essential for providing the necessary information for formulating local, national and international antimicrobial guidelines. Timely detection of carbapenemases is essential for developing strategies to control the spread of infections by carbapenem- resistant isolates and related morbidity and mortality in particular regions. Phenotypic methods defined by the Clinical and Laboratory Standards Institute (CLSI) that clinical microbiology laboratories can apply to detect carbapenemase producers include the modified Hodge test (removed from the M100-S28 in 2018; CLSI), the Carba NP test, and most recently the modified carbapenem inactivation method (mCIM) [5, 6]. To date, only the Carba NP test has been endorsed for the detection of carbapenemase production among *A. baumannii* strains [7]. While the Carba NP test is relatively easy to implement in the clinical laboratory, the mCIM has been found to be sensitive and specific in several recent studies and easy to perform and implement, as it utilizes low-cost materials that are

readily available in clinical laboratories [6, 8]. The mCIM utilizes readily available reagents not requiring reagent preparation, and results are more objective in nature, as a zone diameter reading is used for interpretation of results [6]. Although molecular methods remain the gold standard, they are costly, limited by the targets used specifically in the test, and not accessible to all microbiology laboratories throughout the world [9]. These methods can only detect known carbapenemase encoding genes and the number of carbapenemase encoding genes and allelic variants thereof is expanding rapidly. In contrast, a phenotypic assay may detect carbapenemase activity irrespective of the carbapenemase encoding gene sequence.

The aim of this study was the characterization of carbapenemase activity in extensively drug-resistant (XDR) *Acinetobacter baumannii, Klebsiella pneumoniae* and *Escherichia coli* isolated from clinical specimens using phenotypic and molecular methods in a tertiary hospital in Tehran, Iran.

Methods

Bacterial isolates

A total of 122 non-duplicate carbapenem-resistant clinical isolates were analyzed. The isolates included 77 *K. pneumoniae*, 39 *A. baumannii*, and 6 *E. coli* had been collected at various wards between May 2017 and February 2019 in Milad hospital, Tehran, Iran. Bacterial identification was performed by standard biochemical and microbiological tests [10]. All isolates were stored at -70°C in trypticase soy broth with 15% glycerol and were subcultured twice on blood agar plates (Merck, Germany) prior to testing. As control, 18 carbapenem-resistant, KPC-2 type carbapenemase-producing *K. pneumoniae* clinical isolates were used in this study [11].

Antimicrobial susceptibility testing

The antimicrobial susceptibility of the all isolated bacteria was determined *in vitro*, utilizing the disc diffusion method in accordance with the CLSI criteria [7]. The tested antibiotics included ceftriaxone, cefotaxime, amoxicillin, cefepime, tazocin, ceftazidime, ciprofloxacin, gentamicin and colistin (MAST Diagnostics, Merseyside, UK). Susceptibility testing for meropenem and/or imipenem was performed according to the CLSI reference by using E-test method (Liofilchem, Italy). Carbapenem-resistant isolates were selected using the CLSI M100-S standard (27th edition) definition, i.e., not susceptible (intermediate or resistant, minimum inhibitory concentrations (MICs) of ≥2 μg/ml) to meropenem and/or imipenem, as defined by the current [12].

MHT

The MHT was carried out on all isolates to detect carbapenemase activity using ertapenem and meropenem as described by CLSI [7]. A well characterized strain of *Citrobacter freundii* that produced IMP-9-type metallo-beta-lactamase (MBL) and *E. coli* strain ATCC 25922 were used as positive and negative controls, respectively.

Carba NP and CarbAcineto NP

A selection of isolates has also been tested for carbapenemase activity with Carba NP and CarbAcineto NP as defined by CLSI [13]. The recommended QC strains were set up with each day of testing. Briefly, one inoculation loop (10 μl) of the isolate, recovered from Mueller-Hinton Agar (BD Ltd, USA), was re-suspended in 200 μl of 0.02% cetyl trimethyl ammonium bromide (CTAB) (Merck, Germany); then 100 μl of the bacterial suspension was added to 100 μl of diluted phenol red (Merck, Germany) solution containing 0.1 mM ZnSO4 (Merck, Germany) (pH = 7.5) supplemented with 6 mg/ml of imipenem (SciENcelab, Inc.). The phenol red solution, with no antibiotic, was used as a control tube for each isolate. Both tubes were vortexed and incubated at 37 °C for a maximum of two hours. The colour of the test tube changed to full yellow or orange, indicating carbapenemase-producing isolate while the control tube remained red.

The Modified Carbapenem Inactivation Method

The mCIM method was performed as described earlier by van der Zwaluw and colleagues [14], with some modifications. Briefly, a suspension was made with a 10 μL inoculation loop from a 1-day-old culture, taken from a Mueller-Hinton or blood agar plate in 500 μL of sterile tryptic soy broth (TSB; Merck, Germany) and vortexed to obtain a homogenous suspension. Subsequently, a 10 μg meropenem disk (BD Ltd, USA) was immersed in the suspension and incubated for 2–3 h at 37°C. After incubation, the disk was carefully picked from the suspension using a forceps, placed on a Mueller-Hinton Agar plate pre-inoculated with a susceptible *Escherichia coli* ATCC 25922 using a suspension equivalent to 0.5 McFarland and subsequently incubated overnight at 37°C in ambient air. A zone of ≤15 mm was considered positive for carbapenemase production, and a clear zone of ≥19 mm was considered negative. Zones of 16 to 18 mm or ≥19 mm with colonies present within the zone were considered indeterminate. Quality control was performed by testing a carbapenemase-

positive (*K. pneumoniae* ATCC BAA-1705) and a carbapenemase-negative (*K. pneumoniae* ATCC BAA-1706) control strain on each day of testing. For additional controls, meropenem disks were incubated in TSB or water alone (no organisms) for 2 h at 35°C. These and a dry meropenem disk were also applied to the *E. coli* lawn, and zones were evaluated at 18 to 20 h of incubation to ensure they fell within CLSI quality control (QC) ranges for meropenem and *E. coli* ATCC 25922 [15].

Detection of carbapenem resistance genes

Total DNA was extracted from all strains using the High Pure Template Kit (ROCHE) following the manufacturer's instructions. Five carbapenemase genes, including bla_{KPC}, bla_{NDM}, bla_{OXA-48}-like, bla_{IMP}, and bla_{VIM} genes, were amplified using previously described primers [16-19]. Amplification was performed in the thermal cycler (Eppendorf, Hamburg, Germany) and the amplified products were analyzed in 2% (w/v) agarose gel (100V for 45 min). Quality control included testing an isolate positive or negative for each target on each day of testing. Additionally, a 16S rRNA gene internal control was included in each PCR. Of note, multiplex polymerase chain reaction (PCR) was repeated for isolates that showed discrepant results in the form of negative PCR and positive Carba NP test or mCIM.

Statistical analysis

Data were analysed with SPSS 23.0 (SPSS Inc., Chicago, IL). Data are presented using the range, mean, standard deviation and median for quantitative variables and as frequency and percentage for categorical variables. Student's t-test was used to analyse numerical data. Statistical significance was set at $p < 0.05$.

Results

Bacterial isolates

During the study period, 122 nonduplicated isolates of *K. pneumoniae*, *A. baumannii*, and *E. coli*, which showed resistance to at least one of the tested carbapenems, were collected. The isolates were obtained from urine specimens (43, 35.2%), followed by tracheal aspirate/fluid (35, 28.7%), wound and soft tissue specimens (34, 27.9%), blood (6, 4.9%), sputum (3, 2.5%), bronchoalveolar lavage (1, 0.8%). The source of isolates was as follows: intensive care units (46, 37.7%), internal medicine (25, 20.5%), surgery (23, 18.9%), outpatient clinics (11, 9.0%), emergency (10, 8.2%), cardiac care unit (3, 2.5%), orthopedic (3, 2.5%), and pediatric intensive care unit (1, 0.8%). Patient's age ranged from 1 to 93 years old (61.4 ± 20.43), which 73 (59.8%) were from female patients, while 49 (40.1%) from males.

Antimicrobial susceptibility testing

The results of the antimicrobial susceptibility testing indicated that majority of *K. pneumoniae* and *A. baumannii* isolates were resistant to ceftriaxone, cefotaxime, cefepime, tazocin, amoxicillin clavulanic acid, ceftazidime and gentamicin, which indicates that these isolates were extensively drug-resistant (XDR). Antimicrobial susceptibility profiles of the isolates were shown in Table 1.

Phenotypic detection of carbapenemase production

To evaluate phenotypical tests for carbapenemase production, three methods the MHT, Carba NP test and mCIM were selected. All the positive controls were positive in all the three methods. There were no false positive results with all three methods. Discriminatory power of the methods in comparison to PCR was statistically significant ($p < 0.0001$). The positivity of MHT, Carba NP and mCIM tests according to the bacteria are shown in tables 2.

MHT test results

All 122 isolates of carbapenem-resistant bacteria were tested, and among them 70 (57.3%) isolates showed positive results by MHT. There were observed that 24 isolates had false negative results by MHT. All the 24 isolates of positive carbapenemase gene, which were negative by the MHT test, included 13, 10 and one isolates of *A. baumannii*, *K. pneumoniae* and *E. coli* respectively. The false negative results missed by MHT test were linked to bla_{VIM} (11/24), bla_{KPC} (6/24), bla_{IMP} (4/24) and bla_{OXA-48} (3/24) carbapenemases. The sensitivity of the MHT test was determined as 67.33%, and a specificity of 100% in the isolates studied (Table 3).

CarbaNP test results

Among the studied carbapenem-resistant bacteria, 84 (68.8%) isolates were positive by CarbaNP. The positivity of the CarbaNP test according to bacteria is given in Table 2. There were false negative results in 16 isolates in Carba NP test. The false negative results

missed by Carba NP test were linked to *bla*$_{VIM}$ (8/16), *bla*$_{IMP}$ (5/16) and *bla*$_{KPC}$ (3/16) carbapenemases. The test was found negative in all those isolates displaying no reduced sensitivity against any of the carbapenems. The sensitivity of the CarbaNP test was determined as 80.8%, and a specificity of 100% in the isolates studied (Table 3).

mCIM test results

All 122 isolates of carbapenem-resistant bacteria were tested, and among them 87 (71.3%) isolates showed positive results by mCIM (Table 2). It was observed that 12 isolates tested by mCIM were determined as false negative. All the 12 isolates of positive carbapenemase gene, which were negative by the mCIM test, included 11 and one isolates of *A. baumannii* and *K. pneumoniae*, respectively. The false negative results missed by mCIM test were linked to *bla*$_{VIM}$ (6/12), *bla*$_{IMP}$ (3/12) *bla*$_{KPC}$ (2/12), and *bla*$_{OXA-48}$ (1/12) carbapenemases.

All the isolates were detected to be positive by the end of 6 h. The negative isolates were incubated overnight, but the result did not change. All the isolates displaying no reduced sensitivity against any of the carbapenems were found to be negative by the mCIM test. The sensitivity and specificity of the mCIM test in the isolates studied was determined to be 90.4% and 100%, respectively. We observed that the mCIM test has a slightly higher sensitivity than the CarbaNP test (90.4%% vs. 80.8%, respectively; p = 0.018).

Detection of carbapenemase genes

All isolates were tested for five carbapenemase genes by PCR. Of all the carbapenem-resistant isolates, PCR results demonstrated that 119 (97.5%) isolates harbored at least a carbapenemase genes, with none of the five carbapenemase genes being detected in three isolates by the PCR method (Table 4). One isolate yielding carbapenemase production by mCIM test was negative for the evaluated genes. The number of isolates that carried these genes is shown in Table 5. The most frequently carbapenemase genes identified were *bla*$_{KPC}$ identified in 59 isolates (48.3%), *bla*$_{VIM}$ identified in 49 isolates (40.1%), followed by *bla*$_{IMP}$ (31, 25.4%) and *bla*$_{OXA-48}$ (9, 7.3%), whereas *bla*$_{NDM}$ was not detected in this study. In addition, a high percentage 54 (70.1%) of *K. pneumoniae* isolates carried *bla*KPC, while only three and two isolates of *A. baumannii* and *E. coli*, respectively, contained this resistant genes. Co-production of 'KPC and VIM', 'KPC and IMP' and 'KPC and OXA-48' was detected in nine (*K. pneumoniae)*, seven (five *K. pneumoniae* and two *E. coli*) and three (*K. pneumoniae*) isolates, respectively.

Discussion

Since the emergence of carbapenem resistance among Gram-negative organisms some years ago, they have become one of the major causes of death among hospital acquired infections. These organisms are also considered as public health threat worldwide [20–22]. This has raised attention towards seeking accurate and rapid methods for detection of carbapenemases via phenotypic or genotypic approaches, which are a chief cause of spread of carbapenem resistance [23]. In this study, we evaluated three different phenotypic methods for confirmatory detection of carbapenemases in 122 carbapenem resistance bacterial isolates of *K. pneumoniae*, *A. baumannii*, and *E. coli*,, using a conventional PCR as a reference method as the most accurate and reliable tool [24, 25]. The results confirmed that 119 isolates harbored one or more carbapenemase genes, with *bla*$_{KPC}$ and *bla*$_{VIM}$ being the most common, in agreement with some studies reporting the high prevalence of *bla*$_{KPC}$ and *bla*$_{VIM}$ carbapenemases [26]. However, *bla*$_{NDM}$ was not detected in this study.

All our isolates were carbapenemase producers as shown by phenotypic methods and almost all were XDR. The majority of these isolates were susceptible to colistin, although these isolates showed high level of resistance to other antibiotics used in this study (Table 1). However, the side effects of colistin such as occasional nephrotoxicity make them the last therapeutic option for the treatment of infections caused by these organisms [27].

Some commercially available and non-commercial phenotypic tests were designated for laboratory detection of carbapenemase activity. The sensitivity and specificity of all phenotypic tests were not found to be 100% in comparison to molecular methods. Although some studies have emphasized the 100% sensitivity and specificity, some other studies have not confirmed these findings [28, 29]. Therefore, proper performance of these methods is necessary under different conditions with various isolates obtained worldwide.

MHT was recommended by the CLSI in 2009 as a screening method for carbapenemase [30]. However, it has been removed from the recent guideline (CLSI 2018) [31]. The advantages of the method include easy to perform and no need for special reagents or media [29]. The sensitivity and specificity of MHT in our study was lower than in the results obtained by two other methods (Table 3).

Carba NP test was developed by Nordmann & Poirel [32] in 2012; however, this method was subsequently modified. Carba NP, with high sensitivity and rapid detection (≤ 2 h), can detect not only all the known carbapenemases, but it identifies also newly emerging carbapenemases, compared to molecular methods [32]. In this study, the Carba NP test had a sensitivity of 80.8%, and a specificity of 100%. This agrees with several studies evaluating the Carba NP test versus gold-standard PCR, which reported variable sensitivities of 72.5%, 88%, 90.2% and 93% and specificity of 100% [33–36]. Many studies have attributed the variable sensitivities and false-negative results encountered with the Carba NP test to many factors, including enzymes with weak carbapenemase activity (e.g. $bla_{\text{OXA-48-like}}$ enzymes and $bla_{\text{GES-5}}$), isolates with mucoid property, size of the bacterial inoculum, early reading before completion of 2 h of incubation, and present but unexpressed carbapenemase genes [33]. In agreement with the current study, several studies reported false-negative Carba NP tests with the same dominating enzymes (NDM, KPC and VIM) and related to mucoid *Klebsiella* strains, besides other factors linked to agar type and ion content for cultivation of the isolate [36]. In this study, the false-negative results missed by Carba NP test were linked to bla_{VIM}, bla_{IMP} and bla_{KPC} carbapenemases.

Recently, the CIM test was introduced and has been shown to be highly sensitive and specific for detection of any type of carbapenemase activity in Gram-negative rods [14]. We believe that although CIM test is not as rapid as Carba NP test (8h vs. 2h), it is a highly sensitive, simple and cost-effective method that can be used in clinical laboratories, particularly those with limited resources, for early detection of carbapenemases. In this study, mCIM results gave a sensitivity of 90.4% and a specificity of 100%. These results are in accordance with many other studies evaluating CIM, which showed low false-negative results with a sensitivity of 98.8% [37–39, 24, 35, 40]. Like our study, these studies reported no false positive results, with a specificity of 100%. Jing et al. [41] introduced the Simplified Carbapenem Inactivation Method (sCIM), which instead of incubating the antibiotic disk in the organism culture media for 4 h as in the mCIM, the organism to be tested was smeared directly onto an antibiotic disk in the sCIM. Based on their results, the sCIM showed high specificity and sensitivity comparable to PCR but has the advantage of being more user-friendly. Yamada et al. [42], was evaluated the detectability of MBL-producing Enterobacterales against three types of MBL inhibitors used in combination with a mCIM. Although inhibitor-combination mCIMs were highly specific (99.0–100 %) for the detection of MBL producers, they found that sensitivity was dependent on the inhibitor's concentration.

Co-production of different carbapenemases in a single isolate is an attention-grabbing entity being reported increasingly for several Enterobacteriaceae species in various studies [43–45]. In our collection, we have identified nine *K. pneumoniae* isolates co-producing 'KPC and VIM', five *K. pneumoniae* and two *E. coli* isolates co-producing 'KPC and IMP' and three *K. pneumoniae* isolate co-producing 'KPC and OXA-48' carbapenemases.

Limitations of this study are that it was carried out in a single center, its design is nonprospective, and a relatively modest (n = 122) collection of isolates was included for evaluation. Of note, broth microdilution is preferable for antimicrobial susceptibility testing compared with disk diffusion, which is considered a point of limitation in this study; we used the disk diffusion method as it is more practical, more affordable and less time consuming.

Conclusions

Antibiotic resistance results are routinely reported to infection control to aid with epidemiological tracking and investigations. In addition, we found both the mCIM and the CLSI Carba NP test to be accurate for detection of carbapenemases among carbapenem-resistant *A. baumannii*, *K. pneumoniae* and *E. coli* isolated from clinical specimens. However, mCIM was superior in being of higher sensitivity compared with the Carba NP test. Overall, mCIM and Carba NP offer the chance for easy implementation of carbapenemase detection in routine laboratories and can be employed to give timely and actionable clinical results. Moreover, they can contribute to better antimicrobial stewardship of carbapenems through prudent control of carbapenemase-producers.

Declarations

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Data availability

All data generated or analyzed during this study are included in this published article [and its tables and figures].

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

The participant has consented to the submission of this article to the journal. We confirm that the manuscript, or part of it, has neither been published nor is currently under consideration for publication. This work and the manuscript were approved by all coauthors.

Code availability

The code is available from the corresponding author upon request.

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Tables

Table 1
Antimicrobial susceptibility testing for 122 nonduplicated isolates of *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Escherichia coli* strains isolated over study period.

	CIP		GM		CAZ		CRO		CTX		AMS		CPM		TZ		CS		IPM		MEM	
Bacteria	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
K. pneumoniae (n = 77)	76	1	76	1	76	1	76	0	76	1	77	0	76	0	76	1	16	61	75	2	75	2
A. baumannii (n = 39)	39	0	39	0	39	0	39	0	39	0	39	0	39	0	39	0	1	38	39	0	39	0
E. coli (n = 6)	6	0	5	1	5	1	6	0	6	0	6	0	6	0	6	0	0	6	6	0	6	0

CRO: ceftriaxone, **CTX**: cefotaxime, **AMS**: amoxicillin, **CPM**: cefepime, **TZ**: tazocin, **CAZ**: ceftazidime, **CIP**: ciprofloxacin, **GM**: gentamicin and **CS**: colistin; **IPM**: Imipenem; MEM: Meropenem

Table 2
The positivity of the different phenotypic test to detection of carbapenemase production by bacteria

	mCIM		CarbaNP		MHT		Total
Bacteria	Positive	Negative	Positive	Negative	Positive	Negative	
K. pneumoniae	74(96.1)	3(3.9)	69(89.6)	8(10.4)	54(70.1)	23(29.9)	77
A. baumannii	8(20.5)	31(79.5)	9(23.1)	30(76.9)	13(33.3)	23(66.7)	39
E. coli	5(83.3)	1(16.7)	6(100)	0	3(50)	3(50)	6
Total							122

Table 3
Performance of three phenotypic methods compared to the molecular detection of carbapenemase-producing isolates in the study

		mCIM (%) (95% CI), n = 122		**CarbaNP (%) (95% CI), n = 122**		**MHT (%) (95% CI), n = 122**	
Bacteria	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	
K. pneumoniae**(n = 77)**	96.25	100	91.66	100	77	100	
A. baumannii**(n = 39)**	79.71	100	56.52	100	75	100	
E. coli**(n = 6)**	85.71	100	100	100	50	100	
Total	90.4	100	80.8	100	75.03	100	

Table 4
PCR carbapenemase screening results of 122 carbapenem resistant clinical isolates of *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Escherichia coli*

Bacteria	**VIM**		**IMP**		**OXA-48**		**NDM**		**KPC**	
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
K. pneumoniae	22(28.6)	55(71.4)	18(28.4)	59(10.4)	6(7.8)	71(92.2)	0	0	54(70.1)	23(29.9)
A. baumannii	26(66.7)	13(33.3)	28(71.8)	11(28.2)	13(33.3)	39(100)	0	0	3(92.3)	36(7.7)
E. coli	1(16.7)	5(83.3)	2(33.3)	4(66.7)	3(50)	3(50)	0	0	2(33.3)	4(66.7)
Total	49(40.1)	74(59.8)	31(25.4)	91(74.5)	9(7.3)	113(92.6)	0	0	59(48.3)	63(51.6)

Table 5
The Results of MHT, Carba NP and CIM for Isolates Tested (n = 122)

			Test result for		
Species (n)	Carbapenemase gene	Meropenem MIC (µg/mL)	MHT	Carba NP	mCIM
Klebsiella pneumoniae (1)	KPC	0.094	-	-	-
K. pneumoniae (1)	VIM + KPC	1	+	-	-
K. pneumoniae (1)	KPC	1.5	+	+	+
K. pneumoniae (1)	KPC + IMP	1.5	+	-	-
K. pneumoniae (1)	KPC	2	+	-	+
K. pneumoniae (1)	KPC	3	+	+	+
K. pneumoniae (1)	VIM	3	+	+	+
K. pneumoniae (1)	KPC	6	+	+	+
K. pneumoniae (1)	VIM + KPC	8	+	+	+
K. pneumoniae (2)	KPC	8	+	-	+
K. pneumoniae (2)	IMP	12	+	+	+
K. pneumoniae (1)	VIM + KPC	12	+	-	+
K. pneumoniae (1)	VIM + KPC + OXA-48	12	+	+	+
K. pneumoniae (1)	VIM + IMP	12	+	+	+
K. pneumoniae (3)	KPC	12	+	+	+
K. pneumoniae (2)	KPC	16	+	+	+
K. pneumoniae (1)	KPC	16	-	-	+
K. pneumoniae (3)	IMP	16	+	+	+
K. pneumoniae (1)	VIM + IMP	16	-	+	+
K. pneumoniae (1)	KPC	24	+	+	+
K. pneumoniae (1)	KPC + VIM	24	+	+	+
K. pneumoniae (8)	KPC	> 32	-	+	+
K. pneumoniae (14)	KPC	> 32	+	+	+
K. pneumoniae (1)	VIM + KPC	> 32	+	+	+
K. pneumoniae (3)	VIM + KPC	> 32	-	+	+
K. pneumoniae (1)	-	> 32	+	+	+
K. pneumoniae (9)	IMP	> 32	-	+	+
K. pneumoniae (5)	VIM	> 32	+	+	+
K. pneumoniae (2)	IMP + KPC	> 32	+	+	+
K. pneumoniae (1)	VIM + IMP	> 32	+	+	+
K. pneumoniae (1)	VIM + KPC + IMP	> 32	+	+	+
K. pneumoniae (1)	VIM + OXA-48 + IMP	> 32	+	+	+
K. pneumoniae (2)	KPC + OXA-48	> 32	+	+	+

			Test result for		
K. pneumoniae (1)	VIM + OXA-48	> 32	+	+	+
Acinetobacter baumannii (1)	IMP + VIM	16	-	-	-
A. baumannii (1)	VIM	16	-	-	-
A. baumannii (2)	KPC	16	+	+	+
A. baumannii (1)	IMP	16	-	-	-
A. baumannii (12)	VIM	> 32	-	-	-
A. baumannii (5)	IMP	> 32	-	-	-
A. baumannii (4)	KPC	> 32	+	+	+
A. baumannii (5)	VIM	> 32	+	-	-
A. baumannii (2)	VIM	> 32	+	+	+
A. baumannii (2)	IMP	> 32	+	-	-
A. baumannii (1)	VIM	> 32	+	+	-
A. baumannii (1)	-	> 32	-	-	-
A. baumannii (2)	VIM + IMP	> 32	-	-	-
Escherichia coli (1)	OXA-48 + KPC	16	+	+	+
E. coli (1)	OXA-48	16	+	+	+
E. coli (1)	OXA-48	> 32	-	+	-
E. coli (1)	OXA-48	> 32	-	+	+
E. coli (1)	IMP	> 32	-	+	+
E. coli (1)	VIM	> 32	+	+	+