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Microbiological and molecular detection of VIM-1 metallo beta lactamase-producing *Acinetobacter baumannii*

S.M. EL-AGEERY, S.S. AL-HAZMI[1]

Medical Microbiology and Immunology Department, Faculty of Medicine, Mansoura University, Egypt; Medical Laboratories Technology Department, Faculty of Applied Medical Sciences, Taibah University, Saudi Arabia
[1]Biology Department, Faculty of Science, Taibah University, Saudi Arabia

Abstract. – BACKGROUND: ***Acinetobacter*** **resistant to carbapenems, is one of the most frequently isolated pathogens in the hospital settings and presents a challenge to the clinician.**

AIM: to detect metallo-β-lactamase in *A. baumannii* by E-test and VIM-1 genes by PCR.

MATERIALS AND METHODS: A four-month prospective study was done on Forty eight carbapenem resistant *A. baumannii* strains that isolated from patients with different types of infection either admitted or attending to the Outpatient Clinics at King Fahd Hospital in Al-Madinah Al-Monawarah. For all collected specimens, microbiological analysis, antimicrobial susceptibility testing using disk diffusion method, metallo Beta-lactamases (MBLs) detection by E-test (Epsilometer test) and VIM-1 metallo β-lactamase detection by PCR (polymerase chain reaction) were performed.

RESULTS: Among the 48 carbapenem resistant *A. baumannii* isolates, 13 strains had MBL detected by E-test and among them VIM-1 gene was detected by PCR in 8 isolates but among the 35 *A. baumannii* isolates that did not produce MBL by E-test, VIM-1 gene was detected in 5 isolates.

CONCLUSIONS: The study revealed that specificity of the E-test is low, thus overestimating the number of MBL-positive isolates while, reduction of blaVIM-1 gene expression, revealing hidden MBL phenotypes. So, all carbapenem resistant isolates should be tested by PCR regardless of whether the conventional MBL testing is performed.

Key Words:
Acinetobacter baumannii, VIM-1 metallo beta-lactamase, E-test, PCR.

Introduction

Acinetobacter (A.) baumannii is a nonfermentive aerobic, opportunistic, catalase-positive and oxidase-negative Gram-negative coccobacillary rod[1]. *A. baumannii* widely distributed in soil and water. *A. baumannii* grows at various temperatures and pH environments and uses a vast variety of substrates for growth[2,3].

A. baumannii is one of the most frequently isolated nosocomial pathogens in the hospital settings specially, intensive care settings[4]. In humans, *Acinetobacter* can colonize on or within skin, wounds, respiratory and gastrointestinal tracts and are also isolated from clinical environment as commensals, such as the skin of hospital staff and patients,under nails of nurses, medical equipments and tools used medical intensive care unit (ICU), surgical ICU, shock-trauma ICU, medical wards, nursery, burn and plastic surgery wards[5].

Being intrinsically, it may not be surprising that prior use of broad spectrum antibiotics seems to drive the development of a multidrug resistance (MDR) phenotype. Today *Acinetobacter* resistant to carbapenems, aminoglycosides and fluoroquinolones presents a challenge to the clinician[6].

Recent reports have indicated that carbapenem hydrolysing-β-lactamases are important cause of resistance[7]. At present, two classes of beta-lactamases, class B (metallo-β-lactamases: MBL) and class D (oxacillin hydrolyzing β-lactamases) have been involved in carbapenem resistance of *A. baumannii*[8]. The most common found transferable MBL families include VIM, IMP, GIM and SIM enzymes, which are located within a variety of integron structures, which they have been incorporated as gene cassettes[9,10]. The VIM "Verona integron-encoded metallo-β-lactamases" family, a second growing family of carbapenemases, was first discovered in *Pseudomonas aeruginosa* in Italy in 1996[11] and includes now 22 members which have a wide geographic dis-

Corresponding Author: Safaa M. El-Ageery, M.Sc, MD; e-mail: safageery@gmail.com

tribution in Europe, South America, the Far East and the United States. Both IMP and VIM are integron associated, sometimes within plasmids and they hydrolyse all β-lactams except monobactams, and evade all β-lactamase inhibitors[12].

The present study aims at isolation, antibiotyping of *A. baumannii* from different patients samples at King Fahad Hospital, Al-Madinah AL – Monawara. The study also directs to detect metallo-β-lactamase in *A. baumannii* by E-test and VIM-1 genes by PCR.

Materials and Methods

Setting and Study Design

A four-month prospective study was done on forty eight carbapenem resistant *A. baumannii* isolates that isolated from patients with different types of infection either admitted or attending to the Outpatient Clinics at King Fahd Hospital in Al-Madinah Al-Monawarah. Samples from patients were collected according to the site of infection.

Microbiological Analysis

Collection,transport and processing of the samples were done according to the standard bacteriological methods[13]. *A. baumannii* were finally identified and tested to the species level using API20E (Bio-Merieux, Marcy l'Etoile, France).

Antibiotypying

The isolated strains were tested for their susceptibilities to 12 antibiotics; amikacin, ampicillin, aztreonam, gentamicin, cefoxitin, cephalothin, cotrimoxazole, ceftazidime, piperacillin, impenem, ciprofloxacin, and neomycin. The inhibition zones were measured and results of disk diffusion method were then reported according the guidelines of the Clinical and Laboratory Standards Institute[14].

Detection of MBL by E-test

E-test MBL strip, consisting of Imipenem (IP)/Imipenem + EDTA (IPI), was used to detect Metallo Beta-Lactamase (MBL). Detection of MBL was performed according to the manufacturer's instructions of the reagents used (Bio-Merieux, Marcy l'Etoile, France). Ratio of IP/IPI of ≥8 or ≥3 log dilutions, Phantom zone, or deformation of the ellipse regardless of the IP/IPI ratio indicates MBL production.

Detection of VIM-1 of Acinetobacter Baumannii by PCR

Genomic DNA was extracted from all isolated strains according to the procedure of Chen and Kuo[15]. One µg of the extracted DNA was amplified in 50 µL of the reaction mixture. Each PCR reaction consisted of Taq Polymerase (Promega, Madison, WI, USA), 2 mM $MgCl_2$, 0.2 mM dNTP (Roche Diagnostics, Mannheim, Germany) and 20 µl of VIM-1 specific oligonucleotide primers (Promega, Madison, WI, USA). The primer sequence used was 5′ATTGGTCTATTTGACCGCGTC, 5′TGCTACTCAACGACTGAGCG and the Size of ampilified product was 780 bp. The samples were overlaid with 100 µL of mineral oil, and subjected to 30 cycles of amplification in the DNA thermal cycler (Bio-Rad Laboratories Inc., Hercules, CA, USA). Parameters for amplification cycles were denaturation for 2 minutes at 94°C, annealing of primers for 1 minute at 55°C, and primer extention for 1 minute at 72°C. After the last cycle, the PCR tubes were incubated for 7 minutes at 72°C. The reaction products were visualized by ultraviolet light transilluminator (Bio-Rad Laboratories Inc., Hercules, CA, USA).

Statistical Analysis

The data were analyzed using Package for Social Sciences 17.0 for Windows" (SPSS-17) software (SPSS Inc., Chicago, IL, USA). Data were

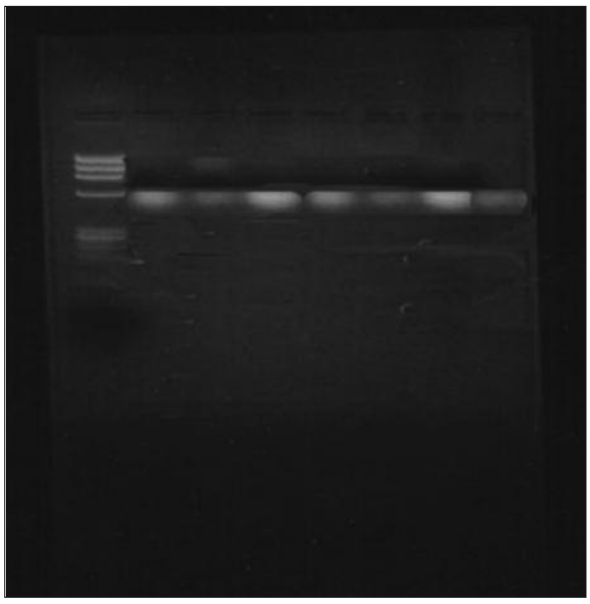

Figure 1. The Figure shows PCR amplification product detecting VIM gene (780 pb).

Table I. Clinical data of patients infected by *A. baumannii*.

Sample	Total no (%)	Sex no (%)		Age group (years) no (%)					
		Male	Female	10-19	20-29	30-39	40-49	50-59	> 60
Wound swabs	17 (35.4)	10 (58.8)	7 (41.2)	0	2 (11.7)	1 (6)	5 (29.4)	2 (11.7)	7 (41.2)
Sputum	13 (27.1)	8 (61.5)	5 (38.5)	1 (7.7)	2 (15.4)	1 (7.7)	2 (15.4)	3 (23.1)	4 (30.7)
Urine	12 (25)	7 (58.3)	5 (41.7)	0	2 (16.7)	1 (8.3)	2 (16.7)	2 (16.7)	5 (41.6)
Blood cultures	6 (12.5)	4 (66.7)	2 (33.3)	1 (16.7)	1 (16.7)	0	0	0	4 (66.6)
Total	48 (100)	29 (60.4)	19 (39.6)	2 (4.2)	7 (14.6)	3 (6.3)	9 (18.7)	7 (14.6)	20 (41.6)

expressed as frequencies, percents. Chi-square used for comparisons of categorical data. A p value < 0.05 was considered statistically significant.

Results

Clinical Data of Patients

Carbapenem resistant *A. baumannii* was isolated from 17 wound swabs, 13 sputum samples, 12 urine samples and 6 blood culture. The prevalence of *A. baumannii* was higher among males more than females The highest prevalence of *A. baumannii* was detected among patients with age more than 60 years as shown in Table I.

In vitro Antibiotic Susceptibility

It was revealed that all isolates were resistant to ampicillin, cephalothin, cefoxitin, imipenem,ceftazidim, neomycin and ciprofloxacin. However, there were different susceptibility patterns to the other tested drugs, as shown in Table II.

Detection of MBL by E-test

Among the 48 *A. baumannii* isolates, 13 strains (27.1%) had MBL detected by E-test as shown in Table III.

Expression of VIM-1 Gene by PCR

Although 13 *A. baumannii* isolates produced MBL detected by E-test, VIM-1 gene was detected by PCR in 8 isolates (61.5%) as shown in Table IV. However, among 35 *A.baumannii* isolates that did not produce MBL detected by E-test, VIM-1 gene was detected by PCR in 5 isolates (14.3%) as shown in Table V.

Discussion

A. baumannii accounts for a substantial proportion of endemic nosocomial infections. Multidrug resistance increasingly reported in these pathogens is posing a threat to hospitalized patients due to the limitation of therapeutic options. The acquisition of multidrug resistance is related

Table II. *In vitro* antibiotic susceptibility of *A. baumannii* isolates.

Antibiotic disc	Disc potency	Sensitivity patterns					
		Sensitive		Intermediate		Resistant	
		no	%	no	%	no	%
Ampicillin	25 µg	0	0	0	0	48	100
Augmentin	30 µg	3	6.3	0	0	45	93.7
Piperacillin	100 µg	14	29.2	0	0	34	70.8
Cephalothin	10 µg	0	0	0	0	48	100
Cefoxitin	10 µg	0	0	0	0	48	100
Ceftazidime	30 µg	0	0	0	0	48	100
Gentamycin	10 µg	9	18.8	0	0	39	81.2
Amikacin	30 µg	5	10.4	0	0	43	89.6
Imipenem	10 µg	0	0	0	0	48	100
Aztreonam	30 µg	2	4.2	0	0	46	95.8
Ciprofloxacin	5 µg	0	0	0	0	48	100
Neomycin	10 µg	0	0	0	0	48	100

Table III. Detection of MBL in *A. baumannii* isolates by E-test.

Total *A. baumannii* isolates		MBL + ve isolates		MBL – ve isolates			
no	%	no	% from total	no	% from total	χ^2	*p*-value
48	100	13	27.1	35	72.9	6.8	0.009

Table IV. Expression of VIM-1 gene among MBL+ve *A. baumannii* isolates.

MBL + ve isolates by E test		VIM-1 gene + ve isolates by PCR		VIM-1 gene – ve isolates by PCR			
Total no	%	no	%	no	%	χ^2	*p*-value
13	100	8	61.5	5	38.5	0.46	0.49

Table V. Expression of VIM-1 gene among MBL – ve *A. baumannii* isolates.

MBL + ve isolates by E test		VIM-1 gene + ve isolates by PCR		VIM-1 gene – ve isolates by PCR			
Total no	%	no	%	no	%	χ^2	*p*-value
35	100	5	14.3	30	85.7	12.6	0.000

to environmental contamination and contact with transiently colonized health care providers. Carbapenems have been the drug of choice for treatment of infections caused by *A. baumannii*. However, in recent years, the number of isolates showing resistance to carbapenems has increased worldwide[10,17,18]. This is mediated by the lack of drug penetration (i.e. porin mutations and efflux pumps) and/or carbapenem hydrolyzing β-lactamase enzymes such as metallo-beta-lactamases[10].

In this work, a total of 48 *A. baumannii* strains were isolated from patients admitted or attending King Fahd Hospital during the study period. Maximum number of *A.baumannii* strains were isolated from wound swabs samples (35.4%) followed by sputum samples (27.1%) urine samples (25%) and blood culture (12.5%). Similarly,it was reported that the highest recovery of *A. baumannii* strains was from wounds infections (25%), and urine samples (25%) followed by blood (12.5%), catheter tips, (10%), bronchial fluid (7.5%) and tracheal aspirates specimens (5%)[19].

In our studied population, the majority of *A. baumannii* occurred among patients with age more than 60 years. Similarly it was showed that the ages of the patients ranged from 3 to 75 years[20]. In addition, it was found that the age more than 55 years was a co factor for the acquisition of *A. baumanni*[21]. Analysis of the relationship between incidence of *A. baumannii* and patients age indicated that there was increase of the pathogen appearance with patients' age, with highest frequency in patients of 61 to 70-years old and mean age of 61.6 years[19].

In this work, the prevalence was highest among males (60.4%) more than females (39.6%). This is in agreement with some authors[22] who found that *A. baumannii* isolates were more among males (58.3%) than female (41.7%).

In our study it was revealed that all isolates were resistant to ampicillin, cephalothin, cefoxitin, imipenem and ciprofloxacin. However, there was different susceptibility patterns to augmentin, piperacillin, gentamycin, amikacin and aztreonam.

Carbapenems are the drugs of choice for nosocomial *Acinetobacter* infections. However, recent study showed high levels of imipenem resistance

among *A. baumannii* isolates[6,23]. Although carbapenem resistance may be caused, in part by impaired permeability, resulting from decreased expression of porins, or by modifications in penicillin-binding proteins[9,10] but, most recent reports have indicated that carbapenem hydrolysing β-lactamases is an important cause of resistance[24].

As regard detection of MBL by E-test; among the 48 *A. baumannii* isolates, 13 strains (27.1%) had MBL (p = 0.009). There are some reports showing failure of phenotypic methods to detect MBL positive isolates, but sometimes no means to avail the molecular methods in resource restricted setup[25].

In the present study, among the 13 *A. baumannii* isolates that produced MBL as detected by E-test,VIM-1 gene was detected by PCR in only 8 isolates (61.5%) (p = 0.49). This is explained by some reports which detected that the specificity of the E-test is low, thus, overestimating the number of MBL-positive isolates[26,27,28]. This also could be attributed to the fact that there are many genes encoding MBL such asVIM-2, IMP, GIM, NDM-1, NDM-2[29,30]. So this, explains why E-test was positive for MBL with negative PCR results for VIM-1 gene in five isolates. Moreover, among 35 *A. baumannii* isolates that did not produce MBL as detected by E-test, VIM-1 gene was detected by PCR in 5 isolates (14.3%) (p = 0.000). This could be explained by reduction of blaVIM-1 gene expression, revealing hidden MBL phenotypes as reported by Ikonomidis et al[25].

Conclusions

In regions where VIM genes *A. baumannii* producers are common, all carbapenem resistant isolates should be tested by PCR regardless of whether the conventional MBL testing is performed. This would be a more expensive and laborious approach. However, these disadvantages might be outweighed by the prevention of horizontal interspecies spread of hidden MBLs.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

Ethical Approval

Ethical Committee of King Fahd Hospital and Scientific Research of Taibah University approved the study.

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