Emergence of carbapenem-resistant *Acinetobacter* in a temperate north Indian State

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ABSTRACT

This study aims to determine drug sensitivity, metallo-β-lactamase (MBL) production and elaboration of bla_{OXA}-type carbapenemases in *Acinetobacter* spp. in a temperate climate area in north India with a heavy influx of tourists. Antimicrobial sensitivity of 165 isolates was performed. Imipenem-resistant isolates were subjected to combined disk (CDT) and double-disk diffusion tests (DDT) for MBL detection. Minimum inhibitory concentration (MIC) and MBL production were tested by Etest. A multiplex polymerase chain reaction (PCR) was performed for the detection of genes encoding bla_{OXA-23} like, bla_{OXA-24} like, bla_{OXA-51} like and bla_{OXA-58} like genes. Ninety-nine (60%) isolates were imipenem-resistant (MICs 2–96 μg/mL). Fifty (50.5%) of the 99 carbapenem-resistant isolates were MBL producers by CDT and 26 (26.3%) by DDT. The majority (77%) of the isolates elaborated bla_{OXA-23} and bla_{OXA-51} like genes. Forty seven of the 50 MBL-positive isolates harboured bla_{OXA-23} like and bla_{OXA-51} like genes. MBL-producing *Acinetobacter* has emerged as a major pathogen in Kashmir with elaboration of bla_{OXA-23} and bla_{OXA-51} related carbapenemases. This poses a significant challenge for healthcare professionals and policy planners, and needs to be addressed immediately. Primary care physicians treating visitors to Kashmir need to be aware of the situation.

KEY WORDS: Acinetobacter.
Carbapenemase.
Carbapenems.
Drug resistance, microbial.

Introduction

Organisms belonging to the genus *Acinetobacter*, believed to be of little significance prior to the 1970s, have recently emerged as a leading cause of nosocomial and a wide range of other healthcare-associated infections (HAIs), especially in intensive care unit (ICU) patients.[1–3] *Acinetobacter baumannii* has emerged as one of the most troublesome pathogens due to its remarkable capability to acquire antibiotic resistance, and is the leading cause of hospital-acquired infections.[2] Mounting evidence indicates that *Acinetobacter* spp. possess a broad range of mechanisms of resistance to all existing antibiotic classes, as well as a prodigious capacity to acquire new determinants of resistance. Multidrug-resistant isolates of *Acinetobacter* are increasingly been reported and are associated with a higher mortality rate and prolonged hospitalisation.[4]

Until recently, the carbapenems remained the ultimate choice for the clinician in treating *Acinetobacter* infections, but the emergence of carbapenem resistance has limited this proposition.[5] Carbapenem-resistant isolates of *Acinetobacter* are usually resistant to most classes of antimicrobial agent, show intermediate resistance to rifampin, while usually retaining susceptibility to tigecycline and colistin.[2,5] Carbapenem resistance is effected through the production of carbapenem-hydrolysing β-lactamases, mediated by Ambler class D β-lactamases or OXA-type carbapenemases, along with Ambler class B metallo-β-lactamases.[2,6] *OXA* genes identified so far in *Acinetobacter* spp. belong to one of four families: bla_{OXA-23} like (bla_{OXA-23}, bla_{OXA-27} and bla_{OXA-49}), bla_{OXA-24} like (bla_{OXA-24}, bla_{OXA-25}, bla_{OXA-26}, bla_{OXA-40}), bla_{OXA-58} and bla_{OXA-51}. Among them, bla_{OXA-51} is typically present in *A. baumannii*.[2,5,7]

A. baumannii is traditionally regarded as a rare coloniser (0.5%) of human skin in temperate climates, being more commonly recognised in hot and humid climates.[8] Recently, *Acinetobacter* was reported as an important emerging pathogen in nosocomial infections from India,[9] but there is a paucity of literature regarding the emergence of drug resistance in *Acinetobacter* from the country, especially the temperate areas.

This study reports the emergence of carbapenem-resistant, metallo-β-lactamase (MBL)-producing *Acinetobacter* spp. in a temperate north Indian state that attracts huge numbers of visitors from across the world and as such is not only of major public health importance but also assumes significance in light of travellers' health.

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Materials and methods

Kashmir is a temperate climate area in northern India which sees a large influx of visitors from across the globe. The current study was conducted at SKIMS, a 600-bed tertiary hospital in the summer capital, Srinagar, from June 2010 to June 2011. *Acinetobacter* spp. grown from various body fluid specimens, obtained from 165 patients (age range: 5–60 years), were included in the study.

Blood, sputum, urine, wound swabs, catheter tips, pus and other body fluids from in-patients and out-patients at SKIMS were obtained and processed according to standard microbiological techniques.[10] Gram-negative coccobacillary

organisms that were catalase-positive, oxidase-negative, non-motile and non-fermentative in characteristic biochemical tests were identified as *Acinetobacter*.

Antimicrobial sensitivity was performed on Muller Hinton agar plates using the Kirby-Bauer disk-diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[11] The following antibiotic disks (Hi-Media, Mumbai) were used: ampicillin 30 µg, gentamicin 10 µg, amikacin 30 µg, ciprofloxacin 5 µg, ofloxacin 5 µg, moxifloxacin 5 µg, gatifloxacin 5 µg, ceftazidime 30 µg, cefotaxime 30 µg, cefipime 30 µg, piperacillin 100 µg and tazobactam 10 µg, imipenem 10 µg, tigecycline 15 µg and polymyxin B, 300 units. In addition, norfloxacin 10 µg and nitrofurantoin 300 µg disks were used for isolates recovered from urine. Isolates were considered to be imipenem-resistant when the zone of inhibition around the disk was ≤13 mm, intermediate when it was 14–15 mm and sensitive if ≥16 mm. *Escherichia coli* ATCC 25922 was used as a control.

For the phenotypic detection of MBL, imipenem-resistant isolates were subjected to the combined disk-diffusion test (CDT), as described by Yong *et al*.[12] and the double disc-diffusion synergy test (DDT), as described by Lee *et al*.[13] For the CDT, an imipenem 10 µg disk was used. In addition, 0.5 mol/L EDTA solution was used, the concentration of which was 750 µg per disk.

An overnight broth culture of the test strain (opacity adjusted to 0.5 McFarland standard) was inoculated on a Mueller Hinton agar plate. An imipenem 10 µg disk was placed on the plate, along with an EDTA-containing imipenem disk. Following incubation at 37°C for 18–24 h, the plates were examined for enhancement of zone sizes. An increase in the zone size ≥7 mm around the imipenem-EDTA in comparison to the imipenem disk alone was recorded as positive for MBL production.

For the DDT, an imipenem 10 µg disk and 0.5 mol/L EDTA poured on blank sterile disks was used. The imipenem 10 µg disk was placed 20 mm (centre to centre) from a blank disk containing 10 µL 0.5 mol/L EDTA. Enhancement of the zone of inhibition in the area between the imipenem and EDTA disks was interpreted as a positive result (Fig. 1).

Table 1. Primers for the detection of genes encoding OXA carbapenemases.

Primer	Primer sequence (5'-3')	Product size (bp)
BlaOXA-23 like-F	GATCGGATTGGAGAACCAGA	501
BlaOXA-23 like-R	ATTTCTGACCGCATTTCCAT	
BlaOXA-51 like-F	TAATGCTTTGATCGGCCTTG	353
BlaOXA-51 like-R	TGGATTGCACTTCATCTTGG	
BlaOXA-24 like-F	GGTTAGTTGGCCCCCTTAAA	246
BlaOXA-24 like-R	AGTTGAGCGAAAAGGGGATT	
BlaOXA-58 like-F	AAGTATTGGGGCTTGTGCTG	599
BlaOXA-58 like-R	CCCCTCTGCGCTCTACATAC	

The MICs of the imipenem-resistant isolates were recorded using the imipenem Etest strips (AbBiodiskSolna, Sweden). These strips consist of a dilution range of imipenem (0.002–32 µg/mL). An MIC ≤ 4 was taken as sensitive, ≥16 as resistant, and 8 as intermediate.[11] Etest MBL strips (AbBiodiskSolna) were used to determine MIC as well as testing for MBL production. These consist of a double-sided seven-dilution range of imipenem IP (4–256 µg/mL) and IP (1–64 µg/mL) overlaid with a constant gradient of EDTA, and are regarded as most sensitive for the purpose.[14]

For both tests, individual colonies were picked from an overnight agar plate and suspended in 0.85% saline (0.5 McFarland opacity standard). The surface of a Muller Hinton agar plate was swabbed (x3) by rotating it approximately 60 degrees. After allowing the excess moisture to dry, the imipenem Etest strip was applied to the agar plate. The plate was incubated at 37°C for 18–24 h, following which the imipenem MIC was read were the inhibition zone intersected the strip. For confirming the production of MBL, the MIC of imipenem plus EDTA was

Table 2. Antimicrobial susceptibility profile of the MBL-positive and MBL-negative *Acinetobacter* isolates.

Antibiotic	MBL+ve (*n*=50)				MBL–ve (*n*=49)				*P* value
	S		R		S		R		
	n	%	*n*	%	*n*	%	*n*	%	
Amikacin	7	14	43	86	10	20.4	39	79.6	0.59
Ciprofloxacin	10	20	40	80	7	14.3	42	85.7	0.58
Ofloxacin	7	14	43	86	9	18.4	40	81.6	0.59
Moxifloxacin	16	32	34	68	20	40.8	29	59.2	0.41
Gatifloxacin	36	72	14	28	40	81.6	9	18.4	0.34
Ceftazidime	1	2	49	98	1	2.04	48	97.9	1.00
Cefotaxime	0	0	50	100	2	4.08	47	95.9	0.24
Cefipime	0	0	50	100	4	8.2	45	91.8	0.056
Gentamicin	4	8	46	92	4	8.2	45	91.8	1.00
Piperacillin/tazobactam	3	6	47	94	1	2.04	48	97.9	0.62
Ampicillin	1	2	49	98	3	6.1	46	93.8	0.36
Imipenem	0	0	50	100	0	0	49	100	1.00
Tigecycline	50	100	0	0	48	97.9	1	2.04	0.49
Polymixin B	50	100	0	0	49	100	0	0	1.00

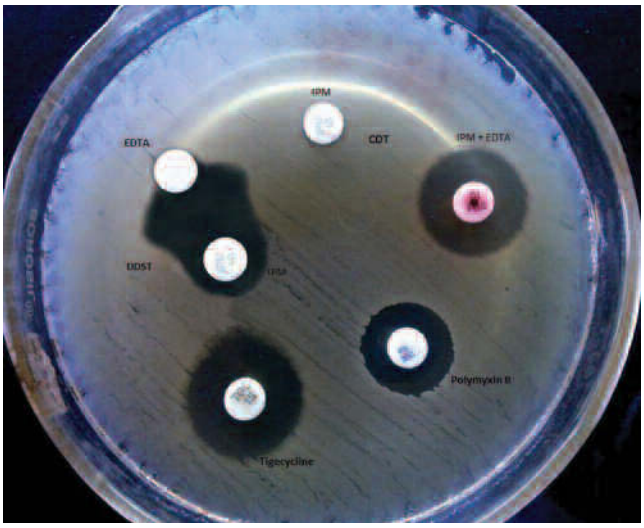

Fig. 1. Plate showing CDT and DDT and also sensitivity to tigecycline and polymixin-B. IPM: imipenem, EDTA: ethylenediaminetetracetic acid, DDT: double-diffusion sensitivity test, CDT: combined diffusion test.

compared to imipenem only. A reduction in imipenem MIC ≥3 (two-fold) in the presence of EDTA was interpreted as being strongly suggestive of MBL production (Fig. 2).

A multiplex polymerase chain reaction (PCR) method was performed to detect OXA-type carbapenemases found in *A. baumannii*. The PCR cycle consisted of initial denaturation at 94°C for 5 min, 33 cycles at 94 °C for 25 sec, 53°C for 40 sec and 72°C for 50 sec, followed by an elongation step at 72°C for 6 min. Primers used in the multiplex PCR for the detection of genes encoding bla_{OXA-23} like, bla_{OXA-24} like, bla_{OXA-51} like and bla_{OXA-58} like genes are shown in Table 1. The PCR products of 501 bp (bla_{OXA-23} like), 353 bp (bla_{OXA-51} like), 246 bp (bla_{OXA-24} like) and 599 bp (bla_{OXA-58} like) were visualised by agarose gel electrophoresis.

Results

A total of 165 non-duplicate *Acinetobacter* strains were examined during the study period. Some 60% (n=99) were resistant to imipenem, with a variable sensitivity to other antimicrobials (Table 2). A high proportion of the isolates were resistant to ampicillin, cefotaxime, ceftaizime, piperacillin-tazobactam, quinolones and aminoglycosides. However, the isolates exhibited uniform sensitivity to tigecycline (97%) and polymixin (100%). Recovery of imipenem-resistant isolates (n=99) was higher from certain wards such as surgical ICU (23.2%), post-operative ward (14.1%), CVTS (12.1%), medical oncology (9%) and plastic surgery (9%). A significantly higher proportion of imipenem-resistant isolates (n=46, 63.8%) compared to imipenem-sensitive (n=26, 36.1%) were recovered from blood samples (n=72). Sixty-five (65.6%) of the 99 isolates were glucose fermenters, whereas 34 (34.3%) were glucose non-fermenters, suggesting that 65 of the carbapenem-resistant isolates belonged to the *Acinetobacter baumanii calcoaceticus* group, and the remaining 34 to other *Acinetobacter* spp.

The MIC for imipenem of 80 representative isolates was performed using Etest strips. The values obtained showed a wide range (2–96 μg/mL). Of the 99 carbapenem-resistant isolates, 50 (50.5%) were MBL producers, whereas 49 (49.5%) were MBL negative by CDT. The DDT method was performed to detect MBL production and was positive in 26 (26.3%) isolates, all of which were MBL-positive by CDT. Out of the 65 glucose-fermenting isolates, 33 (50.7%) were MBL producers.

Table 3. Distribution of the different types of bla*OXA* genes.

OXA type	No of isolates (n=76)
bla_{OXA-23} like only	none
bla_{OXA-24} like only	none
bla_{OXA-51} like only	4
bla_{OXA-58} like only	none
bla_{OXA-23} like and bla_{OXA-51} like	6
bla_{OXA-23} like, bla_{OXA-51} like and bla_{OXA-58} like	4

A higher number (32/50) of MBL-positive isolates were recovered from patients with sepsis, compared to MBL-negative isolates (28/49), although the difference did not reach statistical significance (P>0.05). However, significantly more MBL-positive isolates (n=42, 84%; P=0.0001) were received from patients with intravenous (iv) line catheters. Other risk factors that revealed a trend towards higher isolation of MBL producers compared to MBL non-producers included prior use of β-lactam antibiotics (68% versus 56%, P>0.05), fluoroquinolones (34% versus 20.4%, P>0.05) and imipenem (64% versus 53.1%, P>0.05).

Among the 99 imipenem-resistant isolates, bla_{OXA-23} like and bla_{OXA-51} like were positive in the majority of cases (n=76; 76.8%). Of the other OXA types, bla_{OXA-58} like was seen in four isolates that also possessed the bla_{OXA-23} like and bla_{OXA-51} like genes. On the other hand, bla_{OXA-24} like was not seen in any of the isolates. The distribution of different types of *OXA* genes in the isolates is shown in Table 3. Forty-seven (94%) out of the 50 MBL-positive isolates harboured bla_{OXA-23} like and bla_{OXA-51} like genes. The highest number of MBL-positive isolates carrying the bla_{OXA} gene were recovered from ICU (12/15), followed by CVTS (5/8). Furthermore, almost all of the isolates recovered from blood harboured the bla_{OXA} gene (23/24).

Discussion

A high frequency of carbapenem resistance (60%) and MBL production (50%) among isolates of *Acinetobacter* spp. suggests a significant shift in the antimicrobial spectrum of these microorganisms in the hospital setting, where an earlier study had revealed 98.5% sensitivity to imipenem among a cohort of 258 isolates obtained from patients with nosocomial infections.[9] Prevalence of carbapenem resistance among *Acinetobacter* spp. has ranged from 6% to 52% in Western countries and 2% to 26% in Asia/Pacific countries.[15] Recent reports from Taiwan show an increase in carbapenem-resistant *A. baumannii* (CRAB), from 14.1% in 2003 to 46.3% in 2008, with per 1000 patient day incidence rates for CRAB-associated HAIs increasing from 0.06 in 2003 to 0.12 in 2008, with a 16-fold increase in central Taiwan.[16] While *A. baumannii* is regarded as a rare coloniser (0.5%) of

human skin in temperate climates,[8] carriage is more common in tropical environments.[17] However, the data presented here reveal that not only has the emergence of *Acinetobacter* increased in this temperate climate area in a largely tropical and subtropical country, but drug-resistant strains are increasingly isolated.

Different methods are employed for the detection of MBL.[12–14] The present study used three different methods for the detection of MBL-producing *Acinetobacter* spp.: CDT, DDT and Etest. Of the CDT and DDT, the former was found to more sensitive in detecting MBL-positive isolates. All the isolates that were MBL-positive by DDT were also positive by CDT. Etest for MBL detection was performed on 30 representative samples that were MBL-positive using the CDT, and all showed a reduction in MIC of imipenem ≥3 (two-fold) in the presence of EDTA. The results are in agreement with studies conducted around the world which show that the imipenem-EDTA CDT is a very sensitive method for detection of MBL production.[18,19] Thus, given the cost associated with Etest, a simple and cost-effective screening test like CDT or modified Hodge test can be performed along with routine susceptibility testing in all laboratories.

Most of the *Acinetobacter* spp. were isolated from patients in high-dependency settings such as ICU (16.3%), CVTS ward (9.6%), post-operative ward (9.6%) and plastic surgery (9.6%). Also, all 50 MBL-producing strains were isolated from in-patients, pointing to the fact that MBLs are largely a problem of hospitalised patients who share numerous risk factors. Studies across India have shown maximum recovery of *Acinetobacter* from the ICU setting.[20,21] Forty-two (84%) patients from whom MBL-positive isolates were recovered had iv line catheters, and the difference from non-producers was found to be statistically significant (P=0.0001). The MBL-producing isolates were 100% resistant to cefotaxime and cefipime and showed 98% resistance to ceftazidime and piperacillin plus tazobactam, with 100% sensitivity to polymyxin B and tigecycline. High-level resistance was seen against aminoglycosides and fluoroquinolones.

In the present study *OXA* genes were detected in 76.8% of the carbapenem-resistant *A. baumannii* isolates, with bla_{OXA-23} (n=69) and bla_{OXA-51} (n=76) being the most common OXA carbapenemase isolated. However, bla_{OXA-24} was not seen in any of the isolates, and bla_{OXA-58} was present in four isolates that also had bla_{OXA-23} like and bla_{OXA-51} like genes. Studies have reported that bla_{OXA-23} like carbapenemases are the ones most frequently isolated from carbapenem-resistant *A. baumanni* .[7,22,23] Also, the coexistence of different classes of *OXA* genes in these organisms is well known, especially bla_{OXA-23} like and bla_{OXA-51} like.[24,25]

While bla_{OXA-24} has been reported mainly in Europe and the USA, bla_{OXA-58} has been identified in many countries.[2] However, there are few data on the prevalence and distribution of OXA carbapenemases from India. A recent study of 116 isolates of *A. baumannii* revealed that bla_{OXA-23} like and bla_{OXA-51} like genes are the most common type of OXA carbapenemases in clinical isolates of *A. baumannii*.[26] In another study, bla_{OXA-23} was the most common type in 62 isolates of *A. baumannii*.[27] Although first identified in 1985, the bla_{OXA-23} like cluster is now known to be a major contributor of carbapenem resistance in *A. baumannii*. The bla_{OXA-24} like, bla_{OXA-58} like and bla_{OXA-51} like are the other gene clusters that have been implicated in carbapenem resistance

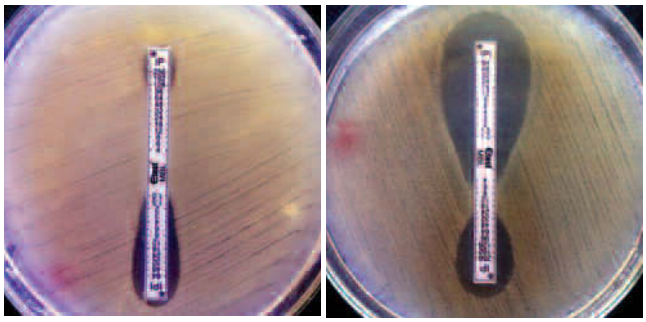

Fig. 2. Plates showing E-test for MBL production.

in *A. baumannii*. While bla_{OXA-23} like, bla_{OXA-24} like and bla_{OXA-58} like enzymes are plasmid/chromosomally encoded, the bla_{OXA-51} like enzymes are chromosomally located and occur naturally in *A. baumannii*. The presence of a unique insertion sequence, ISAba1, in these enzymes has been implicated in causing carbapenem resistance,[2,28] and strains harbouring multiple *OXA* genes have been reported from many parts of the world.[20,29]

In conclusion, MBL-producing *Acinetobacter* spp. have emerged as a major pathogen in north India, with elaboration of bla_{OXA-23} and bla_{OXA-51} related carbapenemases rendering carbapenems largely ineffective against these pathogens. The coexistence of different classes of carbapenemases poses an even greater therapeutic challenge; however, simple microbiological tests such as CDT can be a very useful resource for detection of such strains in order to institute appropriate therapeutic intervention and adopt appropriate infection control practices. □

References

1 Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multidrug resistant *Acinetobacter baumannii. Nat Rev Microbiol* 2007; **5** (12): 939–51.

2 Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin Microbiol Rev* 2008; **21** (3): 538–82.

3 Tsakris A, Ikonomidis A, Poulou A *et al*. Clusters of imipenem-resistant *Acinetobacter baumannii* clones producing different carbapenemases in an intensive care unit. *Clin Microbiol Infect* 2008; **14** (6): 588–94.

4 Sunenshine RH, Wright MO, Maragakis LL *et al*. Multidrug-resistant *Acinetobacter* infection mortality rate and length of hospitalization. *Emerg Infect Dis* 2007; **13** (1): 97–103.

5 Poirel L, Nordman P. Carbapenem resistance in *Acinetobacter baunannii*: mechanism and epidemiology. *Clin Microbiol Infect* 2006; **12** (9): 826–36.

6 Walther-Rasmussen J, Hoiby N. OXA-type carbapenemases. *J Antimicrob Chemother* 2006; **57** (3): 373–83.

7 Mostachio AK, van der Heidjen IV, Rossi F, Levin AS, Costa SF. Multiplex PCR for rapid detection of genes encoding OXA and metallo-beta-lactamases in carbapenem resistant *Acinetobacter* spp. *J Med Microbiol* 2009; **58** (Pt 11):1552–4.

8 Seifert H, Dijkshoorn L, Gerner-Smidt P, Pelzer N, Tjernberg I, Vaneechoutte M. Distribution of *Acinetobacter* species on human skin: comparison of phenotypic and genotypic identification methods. *J Clin Microbiol* 1997; **35** (11): 2819–25.

9 Lone R, Shah A, Kadri SM, Lone S, Shah F. Nosocomial multi-

drug-resistant *Acinetobacter* infections – clinical findings, risk factors and demographic characteristics. *Bangladesh J Med Microbiol* 2009; **3** (1): 34–8 (www.banglajol.info/index.php/BJMM/ article/view/2969).

10 Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC Jr. Guidelines for the collection, transport, processing, analysis and reporting of cultures from specific specimen sources. In: *Color Atlas and Textbook of Diagnostic Microbiology* 6th edn. Philadelphia: Lippincott, Williams and Wilkins, 2006: 68–105.

11 Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: 20th Informational Supplement. CLSI Document M100-S20. Wayne, PA:CLSI, 2010.

12 Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallo-beta-lactamases producing clinical isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J Clin Microbiol* 2002; **40** (10): 3798–801.

13 Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge test and the imipenem-EDTA double disk synergy test for differentiation of metallo-β-lactamase-producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J Clin Microbiol* 2003; **41** (10): 4623–9.

14 Walsh TR, Bolmstrom A, Qwarnstrom A, Gales A. Evaluation of a new Etest for detecting metallo-β-lactamases in routine clinical testing. *J Clin Microbiol* 2002; **40** (8): 2755–9.

15 Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA. Global challenge of multidrug-resistant *Acinetobacter baumannii. Antimicrob Agents Chemother* 2007; **51** (10): 3471–84.

16 Su CH, Wang JT, Hsiung CA *et al*. Increase of carbapenem-resistant *Acinetobacter baumannii* infection in acute care hospitals in Taiwan: association with hospital antimicrobial usage. *PLoS One* 2012; **7** (5): e37788.

17 Chu YW, Leung CM, Houang ET *et al*. Skin carriage of acinetobacters in Hong Kong. *J Clin Microbiol* 1999; **37** (9): 2962–7.

18 Pandya NP, Prajapati SB, Mehta SJ, Kikani KM, Joshi PJ. Evaluation of various methods for MBL production in Gram-negative bacilli. *Int J Biol Med Res* 2011; **2** (3): 775–7.

19 Aybey AD, Aksit F, Oz Y, Kiremitci A, Durmaz G. Evaluation of an automated system for the detection of carbapenem-resistant *Acinetobacter baumannii* and assessment of metallo-β-lactamase production using two different phenotyping methods. *J Microbiol Methods* 2011; **86** (1): 121–3.

20 Mera RM, Miller LA, Amrine-Madsen H, Sahm DF. *Acinetobacter baumannii* 2002–2008: increase of carbapenem-associated multiclass resistance in the United States. *Microb Drug Resist* 2010; **16** (3): 209–15.

21 Anupurba S, Sen MR. Antimicrobial resistance profile of bacterial isolates from intensive care unit: changing trends. *J Commun Dis* 2005; **37** (1): 58–65.

22 Koh TS, Sng LH, Wang GC, Hsu LY, Zhao Y. IMP-4 and OXA beta-lactamases in *Acinetobacter baumannii* from Singapore. *J Antimicrob Chemother* 2007; **59** (4): 627–32.

23 Andriamanantena TS, Rastsima E, Rakotonirina HC *et al*. Dissemination of multidrug-resistant *Acinetobacter baumannii* in various hospitals in Antananarivo Madagascar. *Ann Clin Microbiol Antimicrob* 2010; **9**: 17.

24 Mendes RE, Bell JM, Turnidge JD, Castanheira M, Jones RN. Emergence and widespread dissemination of OXA-23, 24/40 and -58 carbapenemases among *Acinetobacter* spp. in Asia-Pacific nations: report from the SEBTRY Surveillance Program. *J Antimicrob Chemother* 2009; **63** (1): 55–9.

25 Sung JY, Kwon KC, Park JW *et al*. Dissemination of IMP-1 and OXA type-lactamase in carbapenam resistant *Acinetobacter baumannii* (in Korean). *Korean J Lab Med* 2008; **28** (1): 16–23.

26 Amudhan SM, Sekar U, Arunagiri K, Sekar B. OXA beta-lactamase-mediated carbapenem resistance in *Acinetobacter baumannii. Indian J Med Microbiol* 2011; **29** (3): 269–74.

27 Karunasagar A, Maiti B, Shekar M, Shenoy MS, Karunasagar I. Prevalance of OXA-type carbapenemase genes and genetic heterogeneity in clinical isolates of *Acinetobacter* spp. from Mangalore, India. *Microbiol Immunol* 2011; **55** (4): 239–46.

28 Susan B, Amyes S. OXA (beta)-lactamases in *Acinetobacter*: the story so far. *J Antimicrob Chemother* 2006; **57** (1): 1–3.

29 Yang HY, Lee HJ, Suh JT, Lee KM. Outbreaks of imipenem resistant *Acinetobacter baumannii* producing OXA-23β-lactamases in a tertiary care hospital in Korea. *Yonsei Med J* 2009; **50** (6): 764–70.