Original Article

Detection of OXA-181/OXA-48 carbapenemase producing *Enterobacteriaceae* in Bangladesh

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Abstract

Carbapenem resistant *Enterobacteriaceae* (CRE) is becoming a major public health concern globally. Detection of carbapenem hydrolyzing enzyme carbapenemase in *Enterobacteriaceae* is important to institute appropriate therapy and to initiate preventive measures. This study was designed to determine the presence of carbapenemase producers among the CRE isolated from patients at Dhaka Medical College Hospital, Bangladesh. Twenty-nine CRE strains detected by disk diffusion technique were included in the study. Minimum inhibitory concentration of imipenem and tigecycline was determined by agar dilution method. Carbapenemase production was phenotypically detected by Modified Hodge test while MBL producers were detected by combined disk and double disk synergy tests. Genes encoding *bla*NDM-1, *bla*OXA-181, *bla*OXA-48, *bla*KPC, *bla*CTX-M-15, *bla*OXA-1-group were identified by polymerase chain reaction (PCR).

Out of 29 CRE, nineteen (65.6%) were positive for carbapenemase by any of the three phenotypic tests namely MHT, CD or DD tests. Those 19 isolates were also positive either for *bla*NDM-1 or *bla*OXA-181/*bla*OXA-48 by PCR. Of the 19 PCR positive isolates, the rate of positivity for *bla*NDM-1, *bla*OXA-181/*bla*OXA-48 and *bla*NDM-1+ *bla*OXA-181/*bla*OXA-48 was 73.7% (14/19), 57.9% (11/19) and 31.6% (6/19) respectively. Both *bla*OXA-181 and *bla*OXA-48 co-existed. All the carbapenemase producing organisms harboured *bla*CTX-M-15 except one *C. freundii* strain. The rate of resistance to different classes of antibiotics ranged from 63.2% to 100% except colistin and tigecycline. Organisms positive for OXA-181/OXA-48 had a low level of resistance to carbapenem (MIC 1 - 4 ì g/ml) while with NDM-1 had high level resistance to imipenem (MICs 16 - ≥ 32 ì g/ml). Out of 19 carbapenemase positive isolates, 12 (63.16%) were extensively drug-resistant (XDR) and were only sensitive to tigecycline and colistin.

The result of this study showed the presence of *bla*OXA-181/ *bla*OXA-48, *bla*NDM-1 positive strains in Bangladesh and colistin and tigecycline were the most effective drugs against carbapenemase producing *Enterobacteriaceae* (CPE). Epidemiological monitoring of carbapenemase producing organisms in Bangladesh is important to prevent their dissemination.

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Introduction

The emergence of carbapenem resistant *Enterobacteriaceae* (CRE) is a major concern worldwide. This is because of their importance as human pathogens especially within the hospital settings and its high transmissible nature and tendency for rapid spread.[1] This resistance is mediated by the production of carbapenemases or hyper production of Amp C beta lactamase and up regulation of efflux pumps or by their combined mechanisms.[2] Carbapenem hydrolyzing beta lactamases which belong to Ambler classes A, B and D have been reported worldwide in *Enterobacteriaceae*. The most clinically significant

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ones are class A enzymes such as KPC-types, class B enzymes such as IMP, VIM and NDM-1 types and class D enzymes such as OXA-48 and OXA-181 types. The genes encoding them are located on mobile genetic elements, which allow them to spread.[2-4]

The class D, carbapenem hydrolyzing OXA-48 was first identified from a *Klebsiella pneumoniae* in Turkey.[3] These carbapenem hydrolyzing Class D â-lactamases are unusual among carbapenemases for their weak hydrolysis of cephalosporins and their activity is not inhibited by EDTA or clavulanic acid, making them difficult to identify in the laboratory.[4] OXA-181, a variant of OXA-48, was initially reported in India but has been sporadically detected in the United Kingdom, Netherlands, France, New Zealand, Oman, Singapore and Russia.[4-8] In September 2012, CDC reported isolation of *bla*OXA-181positive *K. pneumoniae* in two patients from Bangladesh who were admitted to separate hospitals in Singapore within a short period of time.[7] But there are no reports of *bla*OXA-181 positive isolates in Bangladesh yet. This study was designed to determine the prevalence of carbapenemase encoding genes *bla*KPC, *bla*OXA-181, *bla*OXA-48 and *bla*NDM-1 among CRE at Dhaka Medical College Hospital. Efficacy of colistin and tigecycline against CRE albeit CPE isolates were also evaluated.

Material and Methods

The present cross-sectional study was conducted in the Department of Microbiology of Dhaka Medical College, Dhaka, Bangladesh, during July 2010 to June 2011. The research protocol was approved by the Research Review Committee (RRC) and Ethical Review Committee (ERC) of Dhaka Medical College. Written consent was obtained from each patient or their legal guardian before collection of samples.

***Bacterial isolates and identification*:** Twenty-nine *Enterobacteriaceae* isolates resistant to carbapenem by disk diffusion technique were included in the study. The organisms were isolated from various clinical specimens. The specimens included wound swab, endotracheal aspirate, blood and urine. All the organisms were identified by Gram stain, colony morphology, hemolytic criteria, pigment production and standard biochemical tests.[9]

***Antimicrobial susceptibility test*:** Susceptibility to antimicrobial agents was performed by Kirby Bauer modified disk diffusion technique using Muller Hinton agar plates and zones of inhibition were interpreted according to CLSI guidelines.[10] Antibiotic discs such as ceftazidime (30 μg), cefotaxime (30 μg), ceftriaxone (30 μg), aztreonam (30 μg), amoxiclav (amoxicillin 20 μg & clavulanic acid 10 μg), ciprofloxacin (5 μg), amikacin (30 μg), gentamycin (10 μg), azithromycin (15 μg), piperacillin-tazobactam (100/10 μg), tetracycline (30 μg), imipenem (10 μg), meropenem (10 μg), tigecycline (15 μg) and colistin (10 μg) were used. Isolated organisms which were resistant to at least one agent in three or more antimicrobial classes were considered as multidrug-resistant (MDR) and organisms which were resistant to at least one agent in all but two or fewer antimicrobial classes were considered as extensively drug-resistant (XDR).[11]

***Dteremination of minimum inhibitory concentration*:** Minimal inhibitory concentrations (MICs) of imipenem and tigecycline were determined by agar dilution method and results were interpreted according to CLSI.[10]

***Tests for carbapenamase production*:** CRE were tested for carbapenemase production by Modified Hodge test (MHT), combined disk and double disk synergy tests. PCR was employed to detect specific genes.

***Modified Hodge Test (MHT)*:** A suspension of ATCC *Escherichia coli* 25922, equivalent to 0.5 MacFarland, was diluted 1:10 in sterile saline. This was inoculated on a Mueller Hinton agar plate, dried for 5 minutes and a disc of 10 ì g imipenem was placed in the centre of the agar plate. In a straight line, test organisms were streak from the edge of the disc, to the edge of the plate. The plates were incubated at 37^0 C overnight and were examined next day. They were checked for an enhanced growth around the test organism, at the intersection of the streak and for a zone of inhibition. The presence of an enhanced growth indicated carbapenemases production.[12,13]

***Combined disk test (CDT)*:** Two imipenem disks were placed on an inoculated Mueller-Hinton agar plate. One imipenem disk was supplemented with 5 μl of 0.5 M EDTA and incubated at 37^0 C for 24 hours. An increased zone diameter of $\geq$ 6 mm around the disk

containing imipenem supplemented with EDTA compared to the disk containing imipenem alone was interpreted as MBLs production.[14]

***Double-disk synergy test (DDST)*:** Imipenem disc (10 μg) and a disk containing 20 μl of Tris-EDTA (1.0 M Tris-HCL, 0.1 M EDTA, pH approximately 8.0) and 20 μl of 1:320 diluted 2-mercaptopropionic acid (MPA) were placed 10 mm apart in an inoculated Mueller-Hinton agar plate and incubated at 37^0 C for 24 hours. A clear extension of the edge of the inhibition zone of imipenem disk toward the Tris-EDTA-MPA disk was interpreted as MBLs production.[15]

***Molecular characterization of carbapenemase producers*:** The presence of *bla*NDM-1, *bla*KPC, *bla*OXA-181, and *bla*OXA-48 among CRE were detected by polymerase chain reaction (PCR). In addition, ESBL encoding gene *bla*CTX-M-15 and *bla*OXA-1group were also identified among the CRE by PCR. The primers used are shown in Table 1.[2,16-18] To prepare bacterial pellets, a loop full of bacterial colonies was inoculated into a Falcon tube containing trypticase soy broth. After incubation overnight at 37^0 C, the Falcon tubes were centrifuged at 4,000 g for 10 minutes, after which the supernatant was discarded. A small amount of sterile trypticase soy broth was added into the Falcon tubes with pellets and mixed evenly. Then an equal amount of bacterial suspension was placed into three 1.5 ml microcentrifuge tubes. The microcentrifuge tubes were then centrifuged at 4,000 g for 10 minutes and the supernatant was discarded. The microcentrifuge tubes containing bacterial pellets were kept at -20^0 C until DNA extraction. Bacterial DNA was extracted by boiling method.[17]Multiplex PCR was done for identification of *bla*NDM-1, *bla*OXA-48 and *bla*KPC. Multiplex PCR reaction cycle consisted of preheat at 94^{0}C for 10 minutes followed by denaturation at 94^{0}C for 30 seconds, annealing at 52^{0}C for 40 seconds, extension at 72^{0}C for 50 seconds with a final extension at 72^{0}C for 5 minutes. In case of OXA-181, CTX-M-15, OXA-1-group, PCR reaction consisted of initial denaturation at 95^{0}C for 10 minutes, then 35 cycles of denaturation at 95^{0}C for one minute, annealing at 55^{0}C for 45 seconds, extension at 72^{0}C for one minute and final extension at 72^{0}C for 10 minutes. The amplified DNA were loaded into a 1.5% agarose gel, electrophoresed at 100 volts for 35 minutes, stained with 1% ethidium bromide and visualized under UV light.

Table-1: *Primers used in this study.*[2,16-18]

Target gene	Primer sequence 5'→3'	Product size(bp)
*bla*CTX-M-15	F-CACACGTGGAATTTAGGGACT R-GCCGTCTAAGGCGATAAACA	996
*bla*OXA-1-group	F-ACCAGATTCCAACTTTCAA R-TCTTGGCTTTTATGCTTG	996
*bla*NDM-1	F-GGTTTGGCGATCTGGTTTTC R-CGGAATGGCTCATCACGATC	621
*bla*OXA-48	F-GCGTGGTTAAGGATGAACAC R-CATCAAGTTCAACCCAACCG	438
*bla*KPC	F-CGTCTAGTTCTGCTGTCTTG R-CTTGTCATCCTTGTTAGGCG	798
*bla*OXA-181	F-ATGCGTGTATTAGCCTTATCG R-AACTACAAGCGCATCGAGCA	888

Result

Of the 29 isolated CRE, 9 *Esch. coli*, 8 *Klebsiella pneumoniae*, 3 *Klebsiella oxytoca*, 7 *Citrobacter freundii* and 2 *Entrobacter aerogenes* were identified. Out of 29 CRE, nineteen (65.6%) were positive for carbapenemase by any of the three phenotypic tests namely MHT, CD or DD tests and were also positive either for blaNDM-1 or OXA-181/OXA-48 by PCR (Table 2). Of the 19 PCR positive isolates, the rate of positivity of *bla*NDM-1, *bla*OXA-181/*bla*OXA-48 and *bla*NDM-1+ *bla*OXA-181/*bla*OXA-48 was 73.7% (14/19), 57.9% (11/19) and 31.6% (6/19) respectively (Table 2). All the organisms having blaOXA-181 also contained blaOXA-48. None of the isolate was positive for *bla*KPC. All the carbapenemase producing organisms had co-existing *bla*CTX-M-15 gene except in one *C. freundii* strain. OXA-1 group gene was detected in 47.4% isolates and it co-existed with blaCTX-M-15 gene. Table 3 shows the rate of positivity of CDT, DDST and MHT of 14 NDM-1 and 11 blaOXA-181/blaOXA-48 positive isolates. The rate of positivity for NDM-1 positive isolates was highest by CDT (78.6%) followed by DDST (64.3%) and MHT (35.7%).

Table-2: *Results of phenotypic tests for detection of carbapenemase and distribution of blaNDM-1, blaOXA-181, blaOXA-48 and ESBL genes among the CRE*

Isolates	MIC (μg/ml)		CDT	DDST	MHT	Gene					
	Imipenem	Tigecycline				NDM-1	OXA-181	OXA-48	KPC	CTX-M-15	OXA-1-GRP
Esch. coli	>32	0.5	+	-	+	+	+	+	-	+	+
Esch. coli	16	1	+	+	-	+	-	-	-	+	+
Esch. coli	8	2	+	-	-	+	-	-	-	+	-
Esch. coli	16	2	-	+	-	+	+	+	-	+	+
Esch. coli	2	1	-	-	+	-	+	+	-	+	-
K. pneaumoniae	8	2	+	+	-	+	-	-	-	+	-
K. pneaumoniae	>32	8	+	-	-	+	+	+	-	+	+
K. pneaumoniae	4	1	-	-	+	-	+	+	-	+	-
K. pneaumoniae	16	2	+	+	-	+	-	-	-	+	+
K .pneaumoniae	16	0.5	-	+	+	+	+	+	-	+	+
K. oxytoca	16	1	+	+	-	+	-	-	-	+	-
K. oxytoca	>32	1	+	-	+	+	+	+	-	+	+
C. freundii	16	0.5	+	+	-	+	-	-	-	+	-
C. freundii	2	1	-	-	+	-	+	+	-	+	-
C. freundii	4	0.5	+	+	-	+	-	-	-	-	-
C. freundii	1	1	-	-	+	-	+	+	-	+	+
C. freundii	>32	2	-	+	+	+	+	+	-	+	-
E. aerogenes	8	0.5	+	-	+	+	-	-	-	+	+
E. aerogenes	2	1	-	-	+	-	+	+	-	+	-
Total			11 (57.9)	09 (47.4)	10 (52.6)	14 (73.7)	11 (57.9)	11 (57.9)	0	18 (94.7)	09 (47.4)

MIC range of imipenem: Sensitive ≤ 1 μg/ml, Intermediate 2 μg/ml, Resistant ≥ 4μg/ml; MIC range tigecycline: Susceptible ≤ 2 μg/ml, Intermediate resistant 4 μg/ml, Resistant ≥ 8 μg/ml; NDN-1+ OXA181/OXA48 positive 6/19 (31.6%); 3 strains with intermediate imipenem MIC were positive by MHT and OXA 48 and OXA 181; One C. freundi sensitive to imipenem by MIC was found positive by MHT OXA 181, OXA 48 and CTX m genes. Figure within parenthesis indicate %.

Table-3: *Comparison of phenotypic tests with blaNDM-1 and blaOXA-181/blaOXA-48 positive Enterobacteriaceae*

Genes	Number	Positive by		
		CDT N (%)	DDST N (%)	MHT (%)
*bla*NDM-1 positive	14	11 (78.6)	09 (64.3)	05 (35.7)
*bla*OXA-181/ blaOXA-48 positive	11	03[a] (27.3)	03[a] (27.3)	09 (81.8)

Note: *a= 3 isolates positive by CDT and DDST for MBL wetre also positive for NDM-1.*

The rate of resistance to different classes of antibiotics ranged from 63.2% to 100% except colistin and tigecycline (Table 4). All 19 isolates were sensitive to colistin. The rate of resistance to tigecycline and tetracycline was 5.3% and 63.2% respectively. Organism positive for OXA-181/OXA-48 had a low level of resistance to imipenem (MIC 1 - 4 ì g/ml) while NDM-1 positive organisms had high level resistance to imipenem (MICs 16 - ≥ 32 ì g/ml (Table 2). MIC of tigecycline ranged from 2-0.5 ì g/ml except one had MIC 8 ì g/ml (Table 2). Out of 19

Table-4: *Resistance pattern of CPE to different classes of antibiotics by disc diffusion method*

Antimicrobial agents	*Esch. Coli* (n=5) N (%)	*Klebsialla spp.* (n=7) N (%)	*C. freundii* (n=5) N (%)	*E. aerogenes* (n=2) N (%)	Total *Enterobacteriaceae* (n=19)
Colistin	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0
Tigecycline	0 (0.00)	1 (14.29)	0 (0.00)	0 (0.00)	1 (5.3)
Tetracycline	3 (60.00)	5 (71.43)	3 (60.00)	1 (50.00)	12 (63.2)
Amoxiclav	5 (100.00)	7 (100.00)	5 (100.00)	2 (100.00)	19 (100)
Tazobactam+Piperacillin	4 (80.00)	6 (85.71)	5 (100.00)	1 (50.00)	16 (84.2)
Aztreonam	4 (80.00)	6 (85.71)	5 (100.00)	2 (100.00)	17 (89.5)
Cephalosporins	5 (100.00)	7 (100.00)	5 (100.00)	2 (100.00)	19 (100)
Gentamicin	5 (100.00)	7 (100.00)	5 (100.00)	2 (100.00)	19 (100)
Amikacin	5 (100.00)	6 (85.71)	4 (80.00)	2 (100.00)	17 (89.5)
Ciprofloxacin	4 (80.00)	7 (100.00)	4 (80.00)	2 (100.00)	17 (89.5)

Note: *1. Cepholosporins : Ceftriazone, Ceftazidime, Cefotaxime*
2. Klebsialla spp.: K. pneumoniae (5), K. oxytoca (2).

carbapenemase positive isolates, 12 (63.16%) were extensively drug-resistant (XDR) and were only sensitive to tigecycline and colistin.

Discussion

Generally, OXA-48 carbapenemase and its variants hydrolyze penicillins more effectively, but it is less in can of carbapenems. They show very weak activity against extended spectrum cephalosporins such as ceftazidime and aztreonam. Hence, the *bla*OXA-48 producers' exhibit reduced susceptibility to carbapenems. Their MIC of carbapenems may remain in the susceptible range and their activity is not inhibited by EDTA or clavulanic acid, thus making them difficult to identify in the laboratory.[4,19-22] But the level of resistance to carbapenems is usually higher when ESBL and permeability defects are associated.[19] In such infections, treatment with carbapenems results in adverse outcomes. Their detection is therefore crucial for appropriate therapy and to initiate preventive measures.[2,23]

OXA-181 is a close relative of OXA-48 from which it differs by 4 amino acids.[24] *bla*OXA-181 positive *K. pneumonia* infections were first described in India but imported cases have since been described in Oman, Netherlands and New Zealand.[6,7,20] There are no reports of *bla*OXA-181 positive isolates in Bangladesh. However, this country borders India, which is a source of *bla*OXA-181 positive *Enterobacteriaceae*. These cases highlight potential problems that may arise from the rapidly increasing practice of traveling across international borders to obtain health care. The present study is the first report of the presence of *bla*OXA-181/*bla*OXA-48 genes in *Enterobacteriaceae* in Bangladesh. In this study, eleven OXA-181/OXA-48 producing organisms were isolated. Out of 11 OXA-181/OXA-48 producing organisms 6 were co-harbored with NDM-1 producing gene and showed high level of resistance to imipenem, 5 isolates which harbored only OXA-181/OXA-48 producing genes showed low level of resistance which correlates with one study where it was shown that co-producing NDM-1 and OXA-181 was fully resistant to carbapenem whereas all OXA-181 producing isolates showed an apparent susceptibility to carbapenem.[5] This study evaluated the presence of ESBL encoding genes in CPE. All the CPE harbored ESBL producing gene *bla*CTX-M-15 except one *C. freundii* and nine isolates had *bla*OXA-1-group gene.

In the present study, regarding MIC of tigecycline it was observed that among 29 CRE, 28 (96.55%) isolates were susceptible to tigecycline (MICs 0.5 μg/ml – 2 μg/ml), one *Klebsiella pneumoniae* isolate showed resistance to tigecycline with MIC of 8 μg/ml (Table-2). In a previous study from India reported 95.2% MBL producing *Enterobacteriaceae* as susceptible to tigecycline.[25]

In the present study, CDT was found to be the most sensitive test in detecting carbapenemase producing organisms compared to DDST and MHT. It was reported that, sensitivity of MHT was low for NDM-1 producers (50%) but was increased to 85.7% by adding $ZnSO_4$ (100 μg/ml) in the culture medium.[26] The effect of Zinc might be multiple such as, it acts by increasing stability of the enzyme or/and by modifying porin expression.[27] But in the present study, MHT detected 81.82% OXA-181/OXA-48 producers. It appears that MHT is suitable for OXA-181/OXA-48 producers but not for NDM-1. No phenotypic method is adequate to detect carbapenemase producers. It may be concluded that multiple phenotypic methods and PCR could be the most reliable and acceptable approach for early and accurate identification of carbapenemase producers.

The findings of the current study indicate that *bla*OXA-181/*bla*OXA-48 is an emerging cause of carbapenem resistance in *Enterobacteriaceae* in Bangladesh in addition to *bla*NDM-1. The carbapenemase encoding genes are often harbored along with other resistance genes, resulting into multidrug resistance. Colistin was found as the most effective drug against CPE. But its clinical application is difficult because of its neurotoxicity and nephrotoxicity. Tigecycline having less adverse effects was found to be a highly effective drug against CPE. In conclusion, the study highlights the dissemination of carbapenemase producers, especially NDM-1 and OXA-181/OXA-48 producers in Bangladesh. Most of the carbapenemase producers co-harbored ESBL encoding gene CTX-M-15. Rigorous screening for carbapenemase producers and ESBL producing organisms is warranted for early diagnosis, effective management and prevention of its spread in the hospitals and in community.

References

1. Balm ND, Ngan G, Jureen R, Lin TP, Teo WP. OXA-181-producing *Klebsiella pneumoniae* establishing in Singapore. *BMC Infect Dis* 2013; **13**: 58.
2. Shanthi M, Sekar UMA, Arunagiri K, Bramhne HG. OXA-181 Beta Lactamase is not a Major Mediator of Carbapenem Resistance in Enterobacteriaceae. *Journal of Clinical and Diagnostic Research* 2013; l-**7**(9): 1986-1988.
3. Carrer A, Poirel L, Erakey H, Cagatay AA, Badur S, Nordmann P. Spread of OXA-48 positive carbapenem resistant *Klebsiella pneumonia* isolates in Istanbul, Turkey. *Antimicrob Agents Chemother* 2008; **52**(8): 2950-54.
4. Poirel L, Potron A, Nordmann P. OXA-48-like carbapenemases: the phantom menace, *J Antimicrob Chemother* 2012; **67**(7): 1597-1606.
5. Dortet L, Poirel L, Al Yaqoubi F, Nordmann P. NDM-1, OXA-48 and OXA-181 carbapenemase-producing *Enterobacteriaceae* in Sultanate of Oman. *Clin Microbiol Infect* 2012; 18: E144–E148.
6. Williamson DA, Heffernan H, Sidjabat H, Roberts SA, Paterson DL, Smith M, Freeman JT. Intercontinental transfer of OXA-181-producing *Klebsiella pneumoniae* into New Zealand. *J Antimicrob Chemother* 2011; **66**: 2888–2890.
7. Koh TH, Cao DYH, Chan KS, Wijaya L, Low SBG, Lam MS, Ooi EE, Hsu LY. *bla*OXA-181-positive *Klebsiella pneumoniae*, Singapore. *Emerg Infect Dis* 2012; **18**(9): 1524–1525.
8. Fursova NK, Astashkin EI, Knyazeva AI, Kartsev NN, Leonova ES, Ershova ON, *et al*. The spread of *bla*OXA 48 and *bla*OXA 244 carbapenemase genes among *Klebsiella pneumoniae*, *Proteus mirabilis* and *Enterobacter* spp. isolated in Moscow, Russia. *Ann Clin Microbiol Antimicrob* 2015; **14**: 46: DOI 10.1186/s12941-015-0108-y
9. Cheesbrough M. Microscopical techniques used in Microbiology, culturing bacterial pathogens, biochemical tests to identify bacteria. District Laboratory Practice in Topical Countries, Part 2: Cambridge University Press: 35-70.
10. Clinical and Laboratory Standard Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Twenty-second Informational Supplement Wayne, PA; M100-S22.
11. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012; **18**: 268-281.
12. Orstavik I, Odegaard K. A simple test for penicillinase production in Staphylococcus aureus. *Acta Pathol Microbiol Scand B Microbiol Immunol* 1971; **79**: 855-6.
13. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 19th Informational Supplement. CLSI document. Wayne, PA: Clinical and Laboratory Standards Institute 2009; M100-S19.

14. Qu T, Zhang J, Jiewang J, Tao J, Yu Y, Chen Y, Zhou J, Li L. Evaluation of phenotypic tests for detection of metallo beta-lactamase-producing *Pseudomonas aeruginosa* strains in China. *J Clin Microbiol* 2009; **47**: 1136-1142.

15. Kim SY, Hong SG, Moland ES, Thomson KS. Convenient test using a combination of chelating agents for detection of metallo-beta-lactamases in the clinical laboratory. *J Clin Microbiol* 2007; **45**: 2798-2801.

16. Muzaheed, Doi Y, Adams-Haduch JM, Andimiani A, Sidjabat HE, Gaddad SM, Paterson DL. High prevalence of CTX-M-15-producing *Klebsiella pneumonia* among inpatients and outpatients with urinary tract infection in Southern India. *J Antimicrob Chemother* 2008; **61**: 1393-1394. http://dx.doi.org/10.1093/jac/dkn109

17. Guerra B, Soto SM, Arguelles JM, Mendoza MC. Multidrug resistance is medicated by large plasmids carrying a class 1 integron in the emergent *Salmonella enterica* serotype. *Antimicrob Agents Chemother* 2001; **45**: 1305-1308. *http://dx.doi.org/10.1128/AAC.45.4.1305-1308.2001*

18. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. *Diagonostic Microbiology and Infectious Disease* 2011; **70**: 119-123.

19. Nordmann P, Naas T, Poirel L. Global spread of Carbapenemase producing *Enterobacteriaceae*. *Emerg Infect Dis* 2011; **17**: 1791-1798.

20. Castanheira M, Deshpande LM, Mathai D, Bell JM, Jones RN, Mendes RE. Early dissemination of NDM-1 and OXA-181-producing Enterobacteriaceae in Indian hospitals: report from the sentry antimicrobial surveillance program, 2006–2007. *Antimicrob Agents Chemother* 2011; **55**: 1274–1278.

21. Kalpoe JS, Naiemi N, Poirel L, Nordmann P. Detection of an Ambler class D OXA-48-type b-lactamase in a *Klebsiella pneumonia* strain in the Netherlands. *J Med Microbiol* 2011; **60**: 677–678.

22. Poirel L, Héritier C, Tolün V, Nordmann P. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2004; **48**: 15–22.

23. Paño-Pardo JR, Ruiz-Carrascoso G, Navarro-San Francisco C, Gómez-Gil R, Mora-Rillo M, Romero-Gómez MP. Infections caused by OXA-48 producing *Klebsiella pneumoniae* in a tertiary hospital in Spain in the setting of a prolonged hospital wide outbreak. *J Antimicrob Chemother* 2013; **68**(1): 89-96.

24. Potron A, Nordmann P, Lafeuille E, Maskari Z, Rashdi F, Poirel L. Characterization of OXA-181, a carbapenem-hydrolyzing class D b-lactamase from *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2011; **55**: 4896–4899.

25. Kumar S, Bandyopadhyay M, Mondol S, Pal N, Ghosh T and Banerjee P. Tigecycline activity against metallo-â-lactamase-producing bacteria. *Avicenna J Med* 2013; **3**(4): 92-96.

26. Girlich D, Poirel L, Nordmann P. Value of the Modified Hodge test for detection of emerging carbapenemases in Enterobacteriaceae. *J Clin Microbiol* 2012; **50**(2): 477-479.

27. Conejo MC, Garcia I, Martinez-Martinez L, Picabea L, Pascual A. Zinc eluted from siliconed latex urinary catheters decreases OprD expression, causing carbapenem resistance in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2003; **47**(7): 2313–2315.