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Prevalence, Genotyping of *Escherichia coli* and *Pseudomonas aeruginosa* Clinical Isolates for Oxacillinase Resistance and Mapping Susceptibility Behaviour

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Abstract

In the present study, multi-drug resistant isolates of *Escherichia coli* and *Pseudomonas aeruginosa* were obtained from different clinical specimens and were subjected to molecular typing to detect the genes encoding oxacillinases in these isolates. Subsequently, antibacterial activity of drugs was tested against selected clinical isolates. Two hundred forty six isolates including 98 of *E. coli* and 148 of *P. aeruginosa* were collected from clinical specimens of different centers across India. Out of 246, 123 isolates showed weak synergy for ceftazidime or cefepime and imipenem or clavulanate and were considered as oxacillinase producers. Polymerase chain reaction (PCR) was performed to identify the variants of oxacillinases genes. Our results show a great diversity of occurance of oxacillinase (OXA) genes among clinical isolates. OXA-48 and OXA-10 were more prevalent in both *E. coli* (32.6% OXA-48; 16.3% OXA-10) and *P. aeruginosa* (OXA-48 32.4%; 27.0%) as evident by PCR. The incidence of other OXA genes in *E. coli* and *P. aeruginosa* varied from 4.0 to 12.1%. Of the tested drugs, cefepime plus sulbactam was found to be the most efficacious antibacterial agents with 81.6 to 89.2% susceptibility. Cefepime plus tazobactam was second most active antibacterial agent with 46.9 to 56.7% susceptibility. Surprisingly, imipenem plus cilastatin showed susceptibility to less than 45% isolates.

From the above results, it is evident that cefepime plus sulbactam has enhanced *in vitro* antibacterial activity compared to cefepime alone imipenem plus cilastatin and cefepime plus tazobactam combination in oxacillinases. One significant finding of this study was that cefepime was found to be effective only against the isolates caring OXA-1 and OXA-2 but was found to be resistant to OXA-10, OXA-23, OXA-24, OXA-48, OXA-51 and OXA-58 genes; whereas cefepime in combination with sulbactam was found to be effective against most of these OXA genes in comparison to tazobactam combination.

Keywords: Cefepime; Cefepime plus sulbactam; Oxacillinases; Resistant; Susceptibility

Introduction

The wide spread occurrence and dissemination of extended-spectrum-β-lactamases (ESBLs) among the gram negative organisms has been recognized as major public threats [1]. According to Ambler molecular classification, β-lactamases are divided into four groups naming class A, B, C and D according to their amino acid sequence [2]. Of these classes, class D β-lactamases or OXA-types are of great concern as they are encoded by genes which are transmissible and account for most of the resistance to β-lactams [3,4]. The OXA-type β-lactamases are also known as oxacillinases (OXA β-lactamases) due to their ability to hydrolyze oxacillin much faster than penicillin, benzylpenicillin [5].

Similar to class A and C β-lactamases, OXA type-β-lactamases possess an active-site serine [6,7] and comprise the second large family of β-lactamases, preceded by TEM (came from the patient's name, Temoniera) and SHV (sulfhydryl variable) [8]. OXA type-β-lactamase falls into five groups (groups I–V). The OXA group I includes OXA-5, 7, 10, 13 and its extended spectrum derivatives (OXA-11, 14, 16, 17, 19). Group II includes OXA-2, 3, 15 and 20. Group III includes OXA-1, 4, 30 and 31, whereas, group IV and group V include only OXA-9 and LCR-1 respectively. Earlier most OXA-types belonged to OXA-I or OXA-II derivatives [9]. Later by next dacade higher variants of oxa were reported, where common class D OXA-derivative were derived from OXA-10 by single or double base mutation [10].

Most of the OXA enzymes are embeded in the bacterial chromosome, but many of the oxacillinase genes are part of gene cassettes within class 1 integrons [6,11] which are commonly linked with plasmids or transposons facilitating the spread of OXA genes among bacteria [6]. The main reason behind the spread of OXA gene is associated with the dissemination of a single Incl/M type transferrable conjugative plasmid of 62 kb [12] by which the resistance transfers from one strain to another [13]. Interestingly, not all OXA enzymes are ESBLs, infact some of them are plain oxalinases, some have ESBL phenotype and some are carbapenemases. In recent years, OXA enzyme with carbapenemase activity have increased substantially. Moreover, these enzymes are widely dispersed in some clinically relevant species, such as *Acinetobacter baumannii* [14], *Pseudomonas aeruginosa* [15], *K. pneumoniae* [16] and *E. coli* [5]. However, the OXA type-β-lactamases, oxacillinase predominantly identified in *P. aeruginosa* [17].

The bacterial isolate carring higher variants of OXA exhibit high level of resistance not only to β-lactams including cephalosporins,

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cephamycins, monobactams and carbapenems but also to fluoroquinolone and aminoglycosides [12,18]. Treatment of infections caused by such bacteria harboring oxacillinases is becoming very difficult. As far as authors know, there is scanty information on the frequency of occurrence, prevalence and distribution of OXA in India. In view of increasing resistance and failure of drugs to higher variants of OXA, there is a dire need of alternate therapies, and novel combinations of drugs. Current study was carried out to investigate the prevalence of OXA-β-lactamases among *E. coli* and *P. aeruginosa* clinical isolates collected from different centres across India between March 2011 to September 2013 and to study susceptibility of commonly used antibacterial drugs against these clinical isolates.

Materials and Methods

Antibacterial agents

The antibacterial agents tested included cefepime alone, imipenem plus cilastatin, cefepime plus sulbactam, cefepime plus Tazobactam. All these antibacterial agents were reconstituted with water for injection except cefepime plus sulbactam which reconstituted with solvent provided with the package.

Bacterial strains

One hundred forty eight clinical isolates of *P. aeruginosa* and ninety eight isolates of *E. coli* were collected from a variety of clinical samples of blood, pus, wound, urine and tracheal secretion from various Indian hospitals and identified by conventional methods as described earlier [19]. Prior to use, these isolates were inoculated into cation-adjusted Mueller–Hinton broth (MHB; Hi-Media, Mumbai, India), and incubated at 37°C for overnight. The overnight grown cultures were then adjusted to 0.5 Mac-Farland standard with MHB. All the isolates were subjected for a double disc synergy test using ceftazidime or cefepime and β-lactamase inhibitors (imipenem or clavulanate). The bacterial isolates failed to produce a synergy between ceftazidime or cefepime and imipenem or clavulanate were considered to be probable oxacillinase producers [20].

DNA isolation

DNA from all isolates was extracted as described previously [21]. Five ml of each at concentration of 10^{10} colony forming unit (cfu)/ml was used for the DNA isolation. DNA purity and concentrations were measured with spectrophotometer (260/280).

Characterization of isolates for OXA variants

DNA of all the probable oxacillinase producers of *P. aeruginosa* (74) and *E. coli* (49) isolates was exposed to PCR test to detect presence of different variants of OXA genes using the primers designed earlier. The primers sequence used in this study are shown in Table 1. PCR amplification was performed in a total volume of 20 µl containing 200 pg of DNA, 0.5 mM of dNTPs, 1.25 µM of each primer and 1.5 U of Taq polymerase (Banglore Genei). PCR amplification was done using Eppendorf thermocycler (Germany). The amplicon was analyzed on 1% (w/v) agarose gel supplemented with ethidium bromide.

Antimicrobial susceptibility study

Antimicrobial susceptibility study was carried out according to norms of Clinical and Laboratory Standard Institute guidelines (CLSI) [22]. Results were interpreted based on CLSI break points. *E. coli* (NCTC 13302) served as a control for the susceptibility study.

Results

Bacterial strains

All the isolates were confirmed to be *E. coli* (98) and *P. aeruginosa* (148). Table 2 demonstrates distribution of the isolates in terms of sample sources. This shows that maximum *E. coli* isolates were recovered from tracheal aspirates (36) followed by urine (24), blood (22) and pus (16). For *P. aeruginosa* the greatest number of isolates were identified from pus (54) followed by tracheal aspirates (42) wound (28) and blood (24). Of the 246 isolates, 123 isolates (*P. aeruginosa* 74 and *E. coli* 49) were phenotypically found to be oxacillinase of Ambler class and were subjected to PCR for genotypic typing of OXA.

Prevalence of OXA variants

All isolates were exposed to genotypic typing and results showed that out of 49 *E. coli* isolates, 16 (32.6%) isolates were confirmed to be positive for OXA-48; 8 (16.3%) isolates were OXA-10 positive; 5 (10.2%) of each of isolate were positive for OXA-1, OXA-2, OXA-51 and OXA-58; 3 (6.1%) isolates were OXA-24 positive and 2 (4.0%) isolates were positive for OXA-23. Among 74 isolates of *P. aeruginosa*, OXA-48 was detected in 24 (32.4%) isolates; 20 (27.0%) isolates had OXA-10; 9 (12.1%) isolates were found to carry OXA-1; OXA-23 was evident in 5 (6.7%) isolates. The prevalence of OXA-2, OXA-24, OXA-51 and OXA-58 was 5.4% (Table 3). Overall, OXA-48, followed by OXA-10 was the more prevalent in both *E. coli* and *P. aeruginosa* compared to other OXA variants. All the OXA positive isolates were used for susceptibility study.

Antimicrobial susceptibility study

Cefepime plus sulbactam emerged as the most efficacious antibacterial agents against different variants of OXA producing

Primer	Primer sequences (5'-3')	Amplicon (base pair)	References
OXA-1	F-AGCCGTTAAAATTAA GCC C R- CTTGATTGAAGGGTTGGGCG	908	[38]
OXA-2	F-GCCAAAGGCACGATAGTTGT R-GCGTCCGAGTTGACTGCCGG	700	[2]
OXA-10	F-TCTTTCGAGTACGGCATTAGC R-CCAATGATGCCCTCACTTTCC	760	[39]
OXA-23	F-GATCGGATTGGAGAACCAGA R-ATTTCTGACCGCATTTCCAT	501	[40]
OXA-24	F-GGTTAGTTGGCCCCCTTAAA R-AGTTGAGCGAAAAGGGGATT	246	[40]
OXA-48	F-GCTTGATCGCCCTCGATT R-GATTTGCTCCGTGGCCGAAA	281	[41]
OXA-51	F-TAATGCTTTGATCGGCCTTG R-TGGATTGCACTTCATCTTGG	353	[40]
OXA-58	F-CGATCAGAATGTTCAAGCGC R-ACGATTCTCCCCTCTGCGC	528	[42]

F, sense primer; R, antisense primer

Table 1: Primers for characterization of OXA variants in *E. coli* and *P. aeruginosa* isolates.

Clinical specimens	*E. coli*	*P. aeruginosa*
Blood	22	24
Pus	16	54
Wound	-	28
Tracheal aspirates	36	42
Urine	24	-
Total	98	148

Table 2: Collection of *E. coli* and *P. aeruginosa* from various clinical specimens.

organisms. For *E. coli* 81.6% isolates were susceptible to cefepime plus sulbactam against 46.9% susceptible to cefepime plus tazobactam, 36.7% isolates susceptible to imipenem plus cilastatin and only 20.4% isolates were susceptible to cefepime alone.

Only 8.1% of the isolates were resistant to cefepime plus sulbactam as compared to 20.4% to cefepime plus tazobactam and >59% were resistant to imipenem plus cilastatin and cefepime alone. Of the *P. aeruginosa* 89.2% isolates were susceptible to cefepime plus sulbactam and 56.7% to cefepime plus tazobactam, 44.6% isolates were susceptible to imipenem plus cilastatin with <25% susceptibility to cefpime alone. Similar to *E. coli*, *P. aeruginosa* was also exhibited less activity toward imipenem plus cilastatin or cefepime, and >48% of the isolates demonstrated resistance to both drugs. The detailed susceptibility of each OXA positive isolate is shown in the Table 4.

Discussion

E. coli and *P. aeruginosa* have emerged as important pathogens because they rapidly evolve resistance towards antibacterial agents and pose a threat by limiting the therapeutic options. The pathogens are involved in a variety of nosocomial infections including urinary tract infections (UTI) [23], blood stream infections [24], pneumonia [25,26], lower respiratory tract infections [27] and wounds [28]. The majority of clinically relevant oxacillinases are acquired enzyme whose genes are embedded on plasmids of gram negative pathogens (Pseudomonas, Acinetobacters and Enterobacteriaceae) contained within integeron or transposons and have been reported widely [5-6,11,17]. To date, 121 variants of class D-β-lactamases have been identified of which 45 exhibit carbapenemase activity [15] representing an emerging cause of antibiotic resistance to beta lactams including carbapenem [29]. In contrast, our study reported only two variants were susceptible to imipenem cilastatin out of eight variants studied.

In the present study, the maximum number of *E. coli* isolates were recovered from tracheal aspirates (36.6%) followed by urine (24.5%), blood (22.4%) and pus (16.3%). These results are comparable with an earlier report where 20.4% and 19.8% *E. coli* isolates were recovered from pus and blood, respectively [30]. However, current study shows high prevalence of *E. coli* with OXA variants in tracheal aspirates and low prevalence in urine compared to 18.1% and 41.6% isolates from tracheal aspirates and urine, respectively [30]. Our study showed that the highest number of clinical isolates of *P. aeruginosa* was obtained from pus (36.5%) followed by tracheal aspirates (28.4%), wound (19%) and blood (16.2%). These results showed lower prevalence of *P. aeruginosa* as compared to earlier reports where incidence of *P. aeruginosa* was found to be 47.8%, 23.5% and 29.7% in blood, sputum and pus, respectively [31]. This high variability of recovery of clinical isolates from different clinical specimens is probably due to the variability of number of clinical specimens from where clinical isolates were recovered.

Our PCR results showed that among the oxacillnases, OXA-1, OXA-2, OXA-10, OXA-23, OXA-24, OXA-48, OXA-51 and OXA-58 were found in these isolates. Moreover, the present study identified that the majority of the strains had OXA-48 and OXA-10 which are

Antibiotic resistant	*E. coli*				*P. aeruginosa*			
	Blood	Pus	Urine	Tracheal aspirates	Blood	Pus	Wound	Tracheal aspirates
OXA-1	1	2	1	1	2	3	1	3
OXA-2	1	-	2	2	-	2	1	1
OXA-10	2	2	2	2	3	7	4	6
OXA-23	1	1	-	-	1	2	-	2
OXA-24	1	1	1	-	1	2	-	1
OXA-48	5	-	3	8	4	9	4	7
OXA-51	-	1	2	2	1	1	2	-
OXA-58	-	1	1	3	-	1	2	1
Total	11	8	12	18	12	27	14	21

Table 3: Distribution of OXA variants among clinical specimens of *E. coli* and *P. aeruginosa*.

Antimicrobial agents	Susceptibility patterns	% response behaviour of isolates			
		E. coli	OXA Variants	*P. aeruginosa*	OXA Variants
Cefepime	S	20.5	OXA-1. OXA-2	17.6	OXA-1. OXA-2
	I	16.3	OXA-10	27	OXA-10
	R	63.3	OXA-23,OXA-24, OXA-48, OXA-51, OXA-58	55.4	OXA-23,OXA-24, OXA-48, OXA-51, OXA-58
Cefepime plus sulbactam	S	81.6	OXA-1. OXA-2, OXA-10, OXA-23, OXA-24, OXA-48	89.2	OXA-1, OXA-2, OXA-10, OXA-23, OXA-24, OXA-48
	I	10.2	OXA-51	5.4	OXA-51,
	R	8.1	OXA-58	5.4	OXA-58
Cefepime plus tazobactam	S	46.9	OXA-1. OXA-2, OXA-10, OXA-23, OXA-24	56.7	OXA-1. OXA-2, OXA-10, OXA-23, OXA-24
	I	32.6	OXA-48	32.4	OXA-48
	R	20.4	OXA-51, OXA-58	10.8	OXA-51, OXA-58
Imipenem plus cilastatin	S	36.7	OXA-1, OXA-2, OXA-10	44.6	OXA-1, OXA-2, OXA-10
	I	4.1	OXA-23	6.7	OXA-23
	R	59.2	OXA-24, OXA-48, OXA-51, OXA-58	48.6	OXA-24, OXA-48, OXA-51, OXA-58

Where S= susceptible, I=intermediate, R=resistant

Table 4: Antibiotic susceptibility of *E. coli and P. aeruginosa* clinical isolates.

comparable to the results of other studies [5,10,32-34]. In a study performed in Korea reported the prevalence of OXA-10 was 13.1% in *P. aeruginosa* [33] while in another study the prevalence of OXA-10 was 0.6% suggesting the great variability of OXA in different countries.

Our data showed that majority of the strains were resistant to cefepime (55.4 to 63.3%) and to imipenem plus cilastatin (48.6 to 59.2%). The lack of susceptibility to these two drugs is a cause for concern. It is noteworthy to state that cefepime and imipenem plus cilastatin were found to be effective only to early variants of OXA but showed resistant to higher variants of OXA genes.

It has been reported that the isolates harbouring OXA genes showed resistance to carbapenems but remains susceptible to cephalosporins. Very recently, a study from India reported the reduced susceptibility of carbapenems to OXA isolates [35]. Our study showed that OXA expressing isolates which were earlier resistant to cefepime became susceptible to cefepime after addition of β-lactamase inhibitors, sulbactam or tazobactam. Moreover addition of sulbactam with cefepime was found to be more effective compared with cefepime plus tazobactam against OXA positive isolates. Sulbactam has been reported superior to clavulanic acid and tazobactam and may represent an alternative treatment option for infections due to multiresistant organisms [36]. It has reported that sulbactam has good intrinsic antimicrobial activity against multidrug-resistant Acinetobacter strains at concentrations readily achievable in human serum and may therefore have some therapeutic implications in the treatment of infections caused by multidrug-resistant *A. baumannii* infections whereas tazobactam and clavulanic acid were only moderately active or inactive [37].

Conclusion

From the above results, it is evident that cefepime plus sulbactam has enhanced *in vitro* antibacterial activity compared to cefepime alone, cefepime plus tazobactam, or imipenem plus cilastatin in oxacillinases. One significant finding of this study was that both cefepime and penem was found to be effective only against the isolates carrying OXA-1 and OXA-2 but found to be resistant to higher variants of OXA genes. Overall, extensive surveillance study of OXA-type producing gram-negative organisms should be conducted in India to make the awareness about the prevalence of different variants of OXA which are spreading in India and leading resistance to antibiotics.

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References

1. Balm MN, Ngan G, Jureen R, Lin RT, Teo JW (2013) OXA-181-producing *Klebsiella pneumoniae* establishing in Singapore. BMC Infect Dis 13: 58.
2. Bush K, Jacoby GA, Medeiros AA (1995) A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother 39: 1211-1233.
3. Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, et al. (2007) Global challenge of multidrug-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother 51: 3471-3484.
4. Peleg AY, Seifert H, Paterson DL (2008) *Acinetobacter baumannii*: emergence of a successful pathogen. Clin Microbiol Rev 21: 538-582.
5. Potron A, Poirel L, Rondinaud E (2013) Intercontinental spread of OXA-48 beta-lactamase- producing *Enterobacteriaceae* over a 11-year period, 2001 to 2011. Euro Surveill 18.
6. Alfredson DA, Korolik V (2005) Isolation and expression of a novel molecular class D beta-lactamase, OXA-61, from *Campylobacter jejuni*. Antimicrob Agents Chemother 49: 2515-2518.
7. Hall BG, Barlow M (2004) Evolution of the serine beta-lactamases: past, present and future. Drug Resist Updat 7: 111-123.
8. Bush K, Jacoby GA (2010) Updated functional classification of beta-lactamases. Antimicrob Agents Chemother 54: 969-976.
9. Nordmann P, Guibert M (1998) Extended-spectrum beta-lactamases in *Pseudomonas aeruginosa*. J Antimicrob Chemother 42: 128-131.
10. Sorour AE, Wali IE and El-Hodaky SK (2008) OXA-type-Beta-lactamases among extended-spectrum-cephalosporin non-susceptible *Pseudomonas aeruginosa* isolates collected from a large teaching hospital in Cairo. Egypt J Med Microbiol 17: 565-572.
11. Poirel L, Naas T, Nordmann P (2010) Diversity, epidemiology, and genetics of class D beta-lactamases. Antimicrob Agents Chemother 54: 24-38.
12. Poirel L, Potron A, Nordmann P (2012) OXA-48-like carbapenemases: the phantom menace. J Antimicrob Chemother 67: 1597-1606.
13. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, et al. (2005) Identification of plasmids by PCR-based replicon typing. J Microbiol Methods 63: 219-228.
14. Opazo A, Domínguez M, Bello H, Amyes SG, González-Rocha G (2012) OXA-type carbapenemases in *Acinetobacter baumannii* in South America. J Infect Dev Ctries 6: 311-316.
15. Walther-Rasmussen J, Høiby N (2006) OXA-type carbapenemases. J Antimicrob Chemother 57: 373-383.
16. Lascols C, Peirano G, Hackel M, Laupland KB, Pitout JDD (2013) Surveillance and molecular epidemiology of *Klebsiella pneumoniae* isolates that produce carbapenemases: first report of OXA-48-like enzymes in North America. Antimicrob Agents Chemother 57:130.
17. Weldhagen GF, Poirel L, Nordmann P (2003) Ambler class A extended-spectrum beta-lactamases in *Pseudomonas aeruginosa*: novel developments and clinical impact. Antimicrob Agents Chemother 47: 2385-2392.
18. Kilic A, Aktas Z, Bedir O, Gumral R, Bulut Y, et al. (2011) Identification and characterization of OXA-48 producing, carbapenem-resistant *Enterobacteriaceae* isolates in Turkey. Ann Clin Lab Sci 41: 161-166.
19. Cheesbrough M (2000) Biochemical tests to identify bacteria. In: District laboratory practice in tropical countries. 2nd ed. Cambridge University Press, UK 178-187.
20. Hocquet D, Dehecq B, Bertrand X, Plésiat P (2011) Strain-tailored double-disk synergy test detects extended-spectrum oxacillinases in *Pseudomonas aeruginosa*. J Clin Microbiol 49: 2262-2265.
21. Sambrook J, Russell DW (2001) Molecular cloning: a laboratory manual. New York: Cold Spring Harbor Laboratory Press.
22. CLSI, Clinical and Laboratory Standards Institute (2013) Performance standards for antimicrobial susceptibility testing; twenty-first informational supplement. CLSI document M100-S23. Wayne, PA19087 USA.
23. Kashef N, Djavid GE, Shahbazi S (2010) Antimicrobial susceptibility patterns of community-acquired uropathogens in Tehran, Iran. J Infect Dev Ctries 4: 202-206.
24. Serefhanoglu K, Turan H, Timurkaynak FE, Arslan H (2009) Bloodstream infections caused by ESBL-producing *E. coli* and *K. pneumoniae*: risk factors for multidrug-resistance. Braz J Infect Dis 13: 403-407.
25. Ruiz LA, Zalacain R, Gómez A, Camino J, Jaca C, et al. (2008) *Escherichia coli*: an unknown and infrequent cause of community acquired pneumonia. Scand J Infect Dis 40: 424-427.
26. Tripathi P, Banerjee G, Saxena S, Gupta MK, Ramteke PW (2011) Antibiotic resistance pattern of *Pseudomonas aeruginosa* isolated from patients of lower respiratory tract infection. Afr J Microbiol Res 5: 2955-2959.
27. Tam VH, Chang KT, Abdelraouf K, Brioso CG, Ameka M, et al. (2010) Prevalence, resistance mechanisms, and susceptibility of multidrug-resistant bloodstream isolates of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 54: 1160-1164.
28. Agnihotri N, Gupta V, Joshi RM (2004) Aerobic bacterial isolates from burn wound infections and their antibiograms--a five-year study. Burns 30: 241-243.

29. Verma V, Testero SA, Amini K, Wei W, Liu J, et al. (2011) Hydrolytic mechanism of OXA-58 enzyme, a carbapenem-hydrolyzing class D β-lactamase from *Acinetobacter baumannii*. J Biol Chem 286: 37292-37303.

30. Chaudhary M, Payasi A (2012) Prospective Study for Antimicrobial Susceptibility of *Escherichia coli* isolated from various clinical specimes in India. J Microb Biochem Technol 4:157-160.

31. Chaudhary M, Payasi A (2013) Rising Antimicrobial Resistance of *Pseudomonas aeruginosa* Isolated from Clinical Specimens in India. J Proteomics Bioinform 6: 005-009.

32. Nagano N, Endoh Y, Nagano Y, Toyama M, Matsui M, et al. (2013) First report of OXA-48 carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in Japan from a patient returned from Southeast Asia. Jpn J Infect Dis 66: 79-81.

33. Lee S, Park YJ, Kim M, Lee HK, Han K, et al. (2005) Prevalence of Ambler class A and D beta-lactamases among clinical isolates of *Pseudomonas aeruginosa* in Korea. J Antimicrob Chemother 56: 122-127.

34. Jing-Jou Y, Shu-Huei T, Chi-Luan C and Jiunn WU (2006) OXA-type ß-lactamase among extended- spectrum-cephalosporin-resistant *Pseudomonas aeruginosa* isolated in a university hospital in Southern Taiwn. Microbiol Immunol Infect 36: 130-134.

35. Shanthi M, Sekar U, K A, Bramhne HG (2013) OXA-181 Beta Lactamase is not a Major Mediator of Carbapenem Resistance in *Enterobacteriaceae*. J Clin Diagn Res 7: 1986-1988.

36. Higgins PG, Wisplinghoff H, Stefanik D, Seifert H (2004) *In vitro* activities of the beta-lactamase inhibitors clavulanic acid, sulbactam, and tazobactam alone or in combination with beta-lactams against epidemiologically characterized multidrug-resistant *Acinetobacter baumannii* strains. Antimicrob Agents Chemother 48: 1586-1592.

37. Valcke YJ, Rosseel MT, Pauwels RA, Bogaert MG, Van der Straeten ME (1990) Penetration of ampicillin and sulbactam in the lower airways during respiratory infections. Antimicrob Agents Chemother 34: 958-962.

38. Danel F, Hall LM, Duke B, Gur D, Livermore DM (1999) OXA-17, a further extended-spectrum variant of OXA-10 beta-lactamase, isolated from *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 43: 1362-1366.

39. Lee S, Park YJ, Kim M, Lee HK, Han K, et al. (2005) Prevalence of Ambler class A and D ß–lactamases among clinical isolates of *Pseudomonas aeruginosa* in Korea. J Antimicrob Chemother 56: 122–127.

40. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, et al. (2006) Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. Int J Antimicrob Agents 27: 351-353.

41. Dallenne C, Da Costa A, Decré D, Favier C, Arlet G (2010) Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in *Enterobacteriaceae*. J Antimicrob Chemother 65: 490-495.

42. Poirel L, Marqué S, Héritier C, Segonds C, Chabanon G, et al. (2005) OXA-58, a novel class D {beta}-lactamase involved in resistance to carbapenems in *Acinetobacter baumannii*. Antimicrob Agents Chemother 49: 202-208.