MICROBIOLOGY

SHORT COMMUNICATION

Kazi *et al.*, *Microbiology* 2019;165:246–250

DOI 10.1099/mic.0.000748

Multiplex PCR to detect pAmpC β-lactamases among enterobacteriaceae at a tertiary care laboratory in Mumbai, India

Mubin Kazi,[1] Kanchan Ajbani,[1] Jeffrey A. Tornheim,[2] Anjali Shetty[1] and Camilla Rodrigues[1,*]

Abstract

Drug-resistance due to AmpC β-lactamases represents a growing problem worldwide. In this study, a previously collected sample of 108 cefoxitin-resistant clinical isolates was assessed for AmpC β-lactamase production through routine phenotypic testing and double-disc cefoxitin/cloxcallin (DD-CC), cefoxitin/phenylboronic acid (CDT-PBA) and AmpC disc tests. The same isolates were characterized by a novel multiplex polymerase chain reaction molecular assay to detect the presence of bla_{ACT}, bla_{DHA}, bla_{CIT}, bla_{FOX}, bla_{MIR} and bla_{MOX}. By phenotypic analysis, 56%, 55% and 48 % were detected as being AmpC β-lactamase producers by the CDT-PBA, DD-CC and AmpC disc tests, respectively. By molecular analysis, 57 % were determined to be AmpC β-lactamase producers, including 34 % bla_{FOX}, 8 % bla_{CIT} and 1.6 % bla_{DHA}as mono-AmpC producers. The production of multiple AmpC molecular types was common, including 30 % with both $bla_{CIT+FOX}$ and 1.6 % each of $bla_{CIT+DHA}$, $bla_{ACT+MIR}$, $bla_{ACT+FOX}$, $bla_{ACT+DHA}$ and $bla_{MIR+FOX}$. Molecular characterization of AmpC would help detect the prevalence of AmpC β-lactamase producers, facilitate proper patient management and implement infection control practices.

Drug resistance is a major concern in community and hospital settings worldwide. Resistance can develop through multiple different resistance mechanisms, including bacterial production of compounds such as the AmpC β-lactamases, which inactivate commonly used antibiotics. The presence of an AmpC β-lactamase confers resistance to penicillins, cephalosporins, oxyimino-cephalosporins (ceftriaxone, cefotaxime and ceftazidime), cephamycins (e.g. cefoxitin and cefotetan) and monobactams [1, 2]. AmpC β-lactamases are increasingly implicated as major causes of morbidity and mortality and can be characterized by specific genotypes that are either chromosomally or plasmid-mediated [3]. Several clinically important organisms lack chromosomally encoded AmpC (including *Klebsiella* species, *Proteus mirabilis* and *Salmonella* species), while others (*Escherichia coli*) have only negligible chromosomal expression [4]. As a result, the list of common plasmid-mediated AmpC genotypes affecting these species is relatively short, including ACT, DHA, CIT, FOX, MIR, MOX and ACC [5]. When present, these plasmid-mediated enzymes are expressed at high levels, which allows for rapid transfer both within and between species, but could also allow for rapid detection [6].

Unlike for extended-spectrum β-lactamases (ESBLs) and carbapenemases, standard screening and confirmation methods for AmpC β-lactamases are not provided by the Clinical and Laboratory Standards Institute (CLSI). In practice this leads to the use of numerous detection methods, for isolates resistant to cephamycins and susceptible to cefepime, including proxy assays such as the modified Hodge test, the Tris-EDTA test, boronic acid inhibitor-based testing and rapid chromogenic assays to detect AmpC β-lactamases [4, 7]. These methods are associated with varied sensitivity and specificity for AmpC β-lactamase identification, and are costly in terms of both human resources and time [2, 4, 8].

As a result, many laboratories still do not attempt to detect AmpC β-lactamases [9]. One relatively simple alternative – multiplex polymerase chain reaction (PCR) – has been successfully employed by many laboratories to identify AmpC β-lactamases [5]. In order to evaluate the test characteristics of a multiplex PCR assay to detect AmpC β-lactamases, we performed the test on a previously collected samples of cefoxitin-resistant clinical isolates of *Enterobacteriaceae*.

The study was carried out in the Department of Microbiology of the P. D. Hinduja National Hospital and Medical

Received 24 August 2017; Accepted 2 November 2018; Published 13 December 2018

Author affiliations: [1]Department of Microbiology, P. D. Hinduja Hospital and Medical Research Centre, Veer Sarvarkar Marg, Mahim, Mumbai, India; [2]Division of Infectious Diseases, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

***Correspondence:** Camilla Rodrigues, dr_crodrigues@hindujahospital.com

Keywords: ampC β-lactamases; cefoxitin-resistance; Cefoxtin-Cloxcallin; AmpC disk test; Molecular characterization.

Abbreviations: ACC, Ambler class Cβ-lactamase; ACT, AmpC-type β-lactamase; CDT-PBA, combined disc test (cefoxtin) phenylboronic acid; CMY, cephamycin β-lactamase; DD-CC, double-disc cefoxitin/cloxacillin; DHA, Dhahran Hospital β-lactamase; ESBL, extended-spectrum β-lactamase; FOX, cefoxitin β-lactamase; MIR, Miriam Hospital β-lactamase; MHT (cefoxitin), modified Hodge test cefoxitin; MOX, moxalactam β-lactamase.

Research Centre, a tertiary care centre in Mumbai, India. A total of 108 consecutive, non-duplicate clinical isolates with cefoxitin resistance were collected between January and June 2013 from a variety of clinical specimens. Bacterial species identification was performed according to standard procedures [10]. Susceptibility testing was performed by the Kirby–Bauer disc diffusion method for each isolate using the following drug concentrations: amoxicillin/clavulanic acid (30 µg), ampicillin/sulbactam (10/10 µg), piperacillin/tazobactam (100/10 µg), cefazolin (30 µg), cefuroxime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cefoxitin (30 µg), ceftazidime (30 µg), cefaperazone/sulbactam (50/50 µg/disc), ciprofloxacin (5 µg), cefepime (30 µg), trimethoprim/sulfamethoxazole (25 µg), gentamicin (50 µg), netilmicin (30 µg), amikacin (10 µg), nalidixic acid (30 µg), imipenem (10 µg), meropenem (10 µg) and ertapenem (10 µg) . The zone of inhibition was interpreted as 'susceptible,' 'intermediate' or 'resistant' according to CLSI guidelines. [10]

Two steps were followed to confirm the presence of AmpC β-lactamases: the identification of bacterial isolates that were resistant to cefoxitin with a zone size of $\leq$18 mm and confirmatory testing.

DETECTING AMPC BY PHENOTYPIC METHODS

Phenotypic detection of AmpC β- lactamases was performed by the MHT, as described elsewhere [11]. The formation of a cloverleaf pattern around the cefoxitin zone for the test strain led to a positive report for the production of AmpC β- lactamases, while isolates with no distortion around cefoxitin zone were reported as negative.

Confirmatory testing for cefoxitin-resistant strains was performed using the following three methods.

(a) Detection of class C β-lactamases [double-disc cefoxitin/cloxcallin (DD-CC)]. To confirm the presence of AmpC-β- lactamases, inhibitor-based testing was performed. According to this method, differences in the inhibition zones between cefoxitin (30 µg) alone and in combination with cloxcallin (200 µg) were determined. An increase in the zone size of $\geq$5 mm was reported as positive for AmpC β-lactamase production [12].

(b) Detection of class C β-lactamases (CDT-PBA). Additionally, inhibitor-based testing was performed to detect differences in the inhibition zones between cefoxitin (30 µg) alone and in combination with phenyl boronic acid (300 µg). An increase in the zone size of $\geq$5 mm was reported as positive for AmpC β-lactamase production [12].

(c) Detection of class C β-lactamases (AmpC disc test/Tris EDTA test). The surface of a Mueller–Hilton agar (MH) plate was inoculated with a laboratory standard strain of *E. coli* (ATCC 25922) in a bacterial suspension of 0.5 MacFarland standard. An AmpC disc was rehydrated with 20 µl saline and several colonies of test organism were applied to the disc for inoculation on the plate. Next, a 30 µg cefotixin disc was placed next to the AmpC disc and the plates were incubated at 35° C. The presence of a zone of inhibition around the AmpC disc indicated that the test strain was an AmpC producer, while the absence of a zone of inhibition indicated that the test strain was not an AmpC producer [4, 12].

Genomic DNA was extracted by the heat-boil method. A multiplex PCR assay was developed to identify the following targets from bacterial isolates: bla_{ACT}, bla_{DHA}, bla_{CIT}, bla_{FOX}, bla_{MIR} and bla_{MOX}. The primers employed are listed in Table 1. PCR was completed with the initial denaturation performed at 94 °C for 3 min, followed by 17 cycles each of 94 °C for 30 s, 45.3 °C for 30 s and 72 °C for 30 s, followed by 17 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s, with a final extension of 72 °C for 6 min.

Table 1. Polymerase chain reaction primers used to identify AmpC β-lactamase production

Name	Sequence (5′–3′)	Length	Target gene	Variants coverage
ACT-F	AAC CRT CCG RCA TRA GCR AR	120 bp	bla_{ACT}	1, 2, 3, 4, 6, 10, 13, 38
ACT-R	RGT ARC CCC AG CRT AAR GR			
DHA-F	CTR CAA CAC TRA TRT CCG CT	217 bp	bla_{DHA}	DHA 1–7, 14,15,17,22.
DHA-R	ARTR GTA RTT RCA RTG ACC RGC R			
CIT-F	RAT RCC ARC CAC RTT CARGA	262 bp	bla_{CIT}	LAT 1, 3, 4 and CMY 2, 4, 5, 6, 7
CIT-R	RGT ARC TRC CAA ARC CAC CA			
FOX-F	RAC CCT RTT CRA GAR TRG CT	325 bp	bla_{FOX}	1–10 (except 2 & 9)
FOX-R	ACR ART TRC RGC CAR GTRG AC			
MIR-F	AGC CAC ACT ACT RTA CRCR TCR	401 bp	bla_{MIR}	1–17 (except 14)
MIR-R	ARAGCTCARRCCGRAARGRT			
MOX-F	CAA CRA CAA RCC ARC CRG TR	173 bp	bla_{MOX}	MOX 1 to 8, CMY 1, 8 to 11
MOX-R	CAR CCT TRA RCA CGR CCA C			

R, G or T; S, A or T.

Post-amplification products were visualized on 3 % agarose gel electrophoresis.

A total of 108 cefoxitin-resistant *Enterobacteriaceae* were identified, including 52 samples from urine, 24 from blood, 9 from pus, 4 from sputum, 4 from fluid, 3 from tissue, 3 from bronchoalveolar lavage (BAL) fluid, 2 each from endotracheal secretions, wound swabs, stool and ascitic fluid, and 1 from cerebrospinal fluid (CSF). The majority of samples (69/108, 63.8 %) were referred from other institutions, hospitals, or clinics, while 39 (36.1 %) were obtained from inpatients at our institution. Of these, the majority were *E. coli* (75), followed by *Klebsiella* species (28), *Enterobacter* species (3) and *Proteus* species (2). Each isolate was tested against at least six different classes of antibiotics. A high rate of resistance was observed against ciprofloxacin/ofloxacin, gentamicin and netilmicin. All of the isolates were resistant to all of the cephalosporins and β-lactam/β-lactamase inhibitor combination drugs tested. All isolates were susceptible to carbapenems . Lower rates of susceptibility were identified for amikacin.

Phenotypic testing identified 51 isolates with AmpC β-lactamase production by the MHT (47 %), 61 by the inhibitor-based test (cefoxitin-PBA, 56 %), 60 by the double-disc diffusion test (cefoxitin/cloxacillin, 55 %) and 52 by the AmpC disc test (48 %).

In-house multiplex PCR identified AmpC production among 62 isolates (57 %), with 46 isolates testing negative for all AmpC mechanisms evaluated (42 %). Among the AmpC producers, 35 (56 %) employed a single mechanism and 26 (42 %) produced AmpC through more than one of the evaluated mechanisms. The most common isolates with single AmpC production mechanisms included 29 bla_{FOX} producers (34 %), 5 bla_{CIT} producers (8 %) and 2 bla_{DHA} producer (1.6 %). Among those with multiple AmpC production methods, 21 (30 %) produced AmpC by $bla_{CIT+FOX}$, 1 produced AmpC by $bla_{CIT+DHA}$ (1.6 %), 1 produced AmpC by $bla_{ACT+MIR}$ (1.6 %), 1 produced AmpC by $bla_{ACT+FOX}$ (1.6 %) and 1 produced AmpC by $bla_{MIR+FOX}$ (1.6 %). The genotypic and phenotypic AmpC results are further characterized in Table 2. AmpC production was more commonly identified from urine, blood, BAL fluid and ascitic fluid than from other sample types. The majority of AmpC production was identified among *E. coli* isolates (50), followed by *Klebsiella* species (10), and *Enterobacter* and *Proteus* species (1 each). Compared to phenotypic assays, the sensitivity and specificity of genotypic assay for detection of AmpC β-lactamases was 90 and 87 %, 86 and 85 %, 82 and 73 %, and 78 and 71 %, compared to the CDT-PBA test, the DD-CC test, the Tris-EDTA test and the MHT, respectively (Table 3).

In this study, we evaluated 108 cefoxitin-resistant *Enterobacteriaceae* for AmpC β-lactamase production through a combination of phenotypic methods and an in-house multiplex PCR assay of common plasmid-mediated AmpC β-lactamase production genotypes. Our multiplex PCR test identified AmpC β-lactamase production among 57 % of isolates. In comparison to a composite measure of positive by any test, the CDT-PBA test was found to be highest sensitivity phenotypic assay, while the multiplex PCR assay was 90 % sensitive and 87 % specific compared to that test.

AmpC β-lactamase production is a major contributor to drug resistance worldwide and has been associated with substantial increases in length of inpatient stay and treatment cost [3]. Nearly 250 distinct AmpC β-lactamases have been reported, with different geographical distributions. In this study, phenotypic testing identified AmpC β-lactamase production among 47, 56, 55 and 48 % of isolates by the MHT, the inhibitor-based test, the double-disc diffusion test and the AmpC disc test, respectively. Plasmid-mediated AmpC represents between 8.4 and 77 % of AmpC β-lactamase production worldwide [13] and between 3.3 and 37 % in India [2]. In this study, 57 % of tested isolates were plasmid-mediated AmpC β-lactamase producers, which is consistent with other studies reporting plasmid-mediated production rates of between 53 and 88 % in isolates [4, 8, 14]. The existing testing based on cefoxitin resistance is nonspecific, with 19–28 % of cefoxitin-resistant isolates found not to be AmpC producers, indicating a need for more reliable testing methods. [15, 16]. In this study, 42 % of cefoxitin-resistant isolates were found not to produce AmpC, or at least not to produce it using the mechanisms evaluated by our multiplex PCR assay. Given that between 9.9 and 36 % of isolates that produce AmpC β-lactamases also produce ESBLs, improved detection and specific identification of β-lactamases could have important implications for treatment [8, 14, 16, 17].

Phenotypic detection of AmpC β-lactamases is time- and human resource-intensive, with poor sensitivity and specificity. As a result, while there are several screening assays available [4, 7], no standard guidelines exist to confirm the presence of AmpC β-lactamases. In this study, 43 % of MHT-positive isolates were found to be AmpC β-lactamase producers, suggesting that there are other mechanisms for other positive isolates, such as decreased porin channels or increased efflux pump expression. Furthermore, to confirm the presence of AmpC β-lactamases, the three-dimensional test is the most reliable assay, with good sensitivity and specificity, but it is labour-intensive and cannot be performed on a routine basis. With certain modifications to the three-dimensional test, the AmpC disc test is widely employed and has good sensitivity and specificity across different studies. The AmpC disc test using Tris-EDTA is one of the most commonly employed tests for AmpC production, with sensitivity ranging from 60 to 97% and specificity ranging from 91 to 98 % [4, 6]. In this study, 48 % of isolates were positive by the AmpC disc test, which represented a sensitivity of 83 % and a specificity of 73 % as compared to a composite positive result from all methods. The double-disc test, which is also widely used, performed better against our composite result, with a sensitivity of 86 % and a specificity of 85 %. Both tests, however, demonstrated inferior

Table 2. Summary of AmpC β-lactamase production identified by molecular and phenotypic testing, *n*=108

	No. of isolates	Percentage (%)
Molecular characterization		
AmpC producers	62	57 %
Non-AmpC producers	46	42 %
Mono-AmpC producers	**36**	56 %
FOX	29	34.2 %
CIT	5	8%
DHA	2	1.6%
Multiple AmpC producers	**26**	41.9 %
CIT+DHA	1	1.6 %
ACT+MIR	1	1.6 %
ACT+DHA	1	1.6 %
ACT+FOX	1	1.6 %
MIR+FOX	1	1.6 %
CIT+FOX	21	30 %
Phenotypic AmpC producers		
Screening test		
MHT (cefoxitin)-positive	51	
MHT (cefoxitin)-negative	57	
AmpC producers (MHT)	**47.2** %	
Confirmatory test		
Inhibitor-based test – boranic acid CDT-PBA		
CDT-PBA-positive	61	
CDT-PBA-negative	47	
AmpC producers (CDT-PBA)	**56.5** %	
Double-disc diffusion test – cefotaxime/cloxacillin (DD-CC)		
DD-CC-positive	60	
DD-CC-negative	48	
AmpC producers (DD-CC)	**55.5** %	
AmpC disc test/Tris-EDTA		
Tris-EDTA-positive	52	
Tris-EDTA-negative	56	
AmpC producers (AmpC disc test)	**48.1** %	

POS, positive; NEG, negative.

performance to the CDT-PBA inhibitor test, which had a sensitivity of 90 % and a specificity of 87 %.

Given the limitations of these phenotypic assays, molecular methods are considered to be the gold standard for the identification of AmpC β-lactamases. In this study, the multiplex PCR assay identified 62 isolates as AmpC β-lactamase producers (57 %), with the most common mechanism of AmpC production being bla_{FOX} (34 %), followed by bla_{CIT} (8 %) and bla_{DHA} (1.6 %), which is consistent with the literature [9]. The presence of multiple AmpC β-lactamases in a single isolate has been observed in several previous molecular studies. In this study, 42 % of cefoxitin-resistant *Enterobacteriaceae* produced multiple AmpC β-lactamases, among which $bla_{CIT+FOX}$ (30 %) was the most frequent AmpC producer, although $bla_{CIT+DHA}$, $bla_{MIR+FOX}$, $bla_{ACT+MIR}$, $bla_{ACT+FOX}$ and $bla_{ACT\ +\ DHA}$ were also observed. Even though molecular characterization of AmpC is not widely practised by clinical laboratories, AmpC mechanism typing is crucial as it is correlated with mortality. The presence of the DHA-1 enzyme, for example, is associated with 40 % mortality, while the FOX-7 enzyme is associated with 28 % mortality, and the CMY-1 enzyme is associated with only 14 % mortality [9, 18].

Plasmid-mediated AmpC β-lactamases are common among *Enterobacteriaceae* in India, with 37 % of *E. coli* and 24 % of *Klebsiella* isolates being positive in one study [2]. This study identified AmpC production among 66 % of *E. coli*, 35 % of *Klebsiella* species and 33 % *Enterobacter* species isolates that were resistant to cefoxitin, suggesting a higher rate of AmpC β-lactamase production in our setting. Given such high rates, and the association of different AmpC mechanisms with mortality, it may be worth evaluating bacteria

Table 3. Genotypic test vs Phenotypic test to detect AmpC

n=108	Screening		Confirmatory					
	MHT-POS	MHT-NEG	CDT-PBA-POS	CDT-PBA-NEG	DD-CC-POS	DD-CC-NEG	Tris-EDTA-POS	Tris-EDTA-NEG
PCR-POS	40	16	55	6	52	7	43	15
PCR-NEG	11	41	6	41	8	41	9	41
Total	51	57	61	47	60	48	52	56
Sensitivity	78.4 %		90.1 %		86.6 %		82.6 %	
Specificity	71.9 %		87.2 %		85.4 %		73.2 %	

POS, positive; NEG, negative.

other than *Enterobacteriaceae* for plasmid-mediated AmpC β-lactamases, since plasmids from these bacteria can be transferred to other species, including *Pseudomonas, Acinetobacter*.

The presence of AmpC β-lactamases poses an important epidemiological and clinical challenge that impacts directly on patient outcomes. This study evaluated a new multiplex PCR assay to evaluate AmpC β-lactamase production with high sensitivity and specificity compared to existing tests. Such a test has the potential to provide timely information to improve both specific treatment and infection control measures for resistant Gram-negative bacterial infections in India.

Funding information
This study was funded by the National Health and Education Society, P. D. Hinduja National Hospital and Medical Research Centre. Dr Jeffrey A. Tornheim (J. A. T.) was supported by a Johns Hopkins University Clinician Scientist Award, the UJMT Fogarty Global Health Fellows Program (R25TW009340), NIH/NIAID (R21AI122922), the Ujala Foundation, the Gilead Foundation and the Wyncote Foundation.

Acknowledgements
We thank the National Health and Education Society, P. D. Hinduja National Hospital and Medical Research Centre for their encouragement and support. We also thank the hospital staff of the Department of Microbiology for their support and co-operation. Dr Jeffrey A. Tornheim (J. A. T.) as a native English speaker helped to rewrite the manuscript.

Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. Soha AE, Lamiaa AA. Occurrence and detection of AmpC β-lactamases among *Enterobacteriaceae* isolates from patients at Ain Shams University Hospital. *Egypt J Med Hum Genet* 2015;16:239–244.
2. Manoharan A, Sugumar M, Kumar A, Jose H, Mathai D *et al.* Phenotypic & molecular characterization of AmpC β-lactamases among *Escherichia coli*, *Klebsiella* spp. & *Enterobacter* spp. from five Indian Medical Centers. *Indian J Med Res* 2012;135:359–364.
3. Tamma PD, Girdwood SC, Gopaul R, Tekle T, Roberts AA *et al.* The use of cefepime for treating AmpC β-lactamase-producing *Enterobacteriaceae*. *Clin Infect Dis* 2013;57:781–788.
4. Ingram PR, Vanzetti TR, Murray RJ, Inglis TJJ, Harnett GB *et al.* Comparison of methods for AmpC β-lactamase detection in *Enterobacteriaceae*. *J Med Microbiol* 2011;60:715–721.
5. Pérez-Pérez FJ, Hanson ND. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. *J Clin Microbiol* 2002;40:2153–2162.
6. Barua T, Shariff M, Thukral SS. Detection and characterization of AmpC -lactamases in Indian Clinical Isolates of *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca*. *Univers J Microbiol Res* 2013;1:15–21.
7. Doi Y, Wachino J, Ishiguro M, Kurokawa H, Yamane K *et al.* Inhibitor-sensitive AmpC beta-lactamase variant produced by an *Escherichia coli* clinical isolate resistant to oxyiminocephalosporins and cephamycins. *Antimicrob Agents Chemother* 2004;48:2652–2658.
8. Wassef M, Behiry I, Younan M, El Guindy N, Mostafa S *et al.* Genotypic identification of AmpC β-lactamases production in Gram-negative bacilli isolates. *Jundishapur J Microbiol* 2014;7:e8556.
9. Black JA, Moland ES, Thomson KS. AmpC disk test for detection of plasmid-mediated AmpC beta-lactamases in *Enterobacteriaceae* lacking chromosomal AmpC beta-lactamases. *J Clin Microbiol* 2005;43:3110–3113.
10. CLSI. *Clinical Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Nineteenth Information Supplement*, M100–S23. Wayne, PA; 2013.
11. Yong D, Park R, Yum JH, Lee K, Choi EC *et al.* Further modification of the Hodge test to screen AmpC beta-lactamase (CMY-1)-producing strains of *Escherichia coli* and *Klebsiella pneumoniae*. *J Microbiol Methods* 2002;51:407–410.
12. Gupta G, Tak V, Mathur P. Detection of ampc β lactamases in Gram-negative bacteria. *J Lab Physicians* 2014;6:1-6.
13. Japoni-Nejad A, Ghaznavi-Rad E, van Belkum A. Characterization of plasmid-mediated AmpC and carbapenemases among iranain nosocomial isolates of *Klebsiella pneumoniae* using phenotyping and genotyping methods. *Osong Public Health Res Perspect* 2014;5:333–338.
14. Upadhyay S, Sen MR, Bhattacharjee A. Diagnostic utility of boronic acid inhibition with different cephalosporins against *Escherichia coli* producing AmpC β-lactamases. *J Med Microbiol* 2011;60:691–693.
15. Joseph NM, Mathias S. Challenges in detection of AmpC β-lactamases among *Enterobacteriaceae*. *Indian J Med Res* 2013;137:216–217.
16. Grover N, Sahni AK, Bhattacharya S. Therapeutic challenges of ESBLS and AmpC beta-lactamase producers in a tertiary care center. *Med J Armed Forces India* 2013;69:4–10.
17. Thakar V, Modak M. Comparison of various methods for detection of AmpC β-lactamase enzyme. *J Acad Clin Microbio* 2013;15:45–48.
18. Arena F, Giani T, Becucci E, Conte V, Zanelli G *et al.* Large oligoclonal outbreak due to *Klebsiella pneumoniae* ST14 and ST26 producing the FOX-7 AmpC β-lactamase in a neonatal intensive care unit. *J Clin Microbiol* 2013;51:4067–4072.

Edited by: K. P. Scott and D. Grainger