DOI: 10.7860/JCDR/2013/6013.3293

Letter to Editor

Microbiology Section

A Study on the AmpC Production Amongst the Urinary *Enterobacteriaceae* Isolates

SMITA UDAY SHEVADE[1], GOPAL NANDLAL AGRAWAL[2]

Dear Sir,

The AmpC β-lactamases are clinically important because they confer resistance to the narrow-, expanded- and the broad-spectrum cephalosporins, the β-lactam-β-lactamase inhibitor combinations and aztreonam. The clinical microbiology laboratories should be able to detect the bacterial strains which produce AmpC, since these strains may appear to be susceptible to a particular β-lactam antibiotic in vitro, but may show no clinical response, resulting in a treatment failure with this β-lactam antibiotic [1]. The chromosomally mediated β-lactamase production takes place mainly through the expression of the AmpC gene which is either constitutive or inducible. In most of the genera of the family *Enterobacteriaceae*, AmpC is inducible [2]. The plasmid-encoded AmpC enzymes are almost always expressed constitutively [3].The Clinical and Laboratory Standards Institute (CLSI) does not describe methods for their detection. This study was aimed at detecting inducible and constitutive AmpC production amongst the urinary *Enterobacteriaceae* isolates by using different phenotypic methods.

Three hundred and twenty urinary *Enterobacteriaceae* isolates were screened [4] for their susceptibilities to cefoxitin disks and the strains with a zone diameter of ≤ 18 mm were suspected to be AmpC producers and they were subjected to different phenotypic AmpC detection methods.

Inducible AmpC was tested by the cefoxitin-cefotaxime disk antagonism test (CX-CTX) [4], the ceftazidime-imipenem antagonism test (CAZ-IPM) [1] and the AmpC disk test [5]. In the disk antagonism tests, the test strain was exposed to disks of cefotaxime-cefoxitin or ceftazidime-imipenem which were placed at a distance. Flattening of the radius of the zone of inhibition, which was produced on the side which was nearest the cefoxitin or the imipenem disk indicated inducible AmpC production. In the AmpC disk test, a lawn culture of the *E. coli* ATCC 25922 strain was exposed to a disk which was inoculated with the test organism and a cefoxitin disk, which were placed together. A positive test appeared as a flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disk.

The combined tests which were performed, which detected both inducible and constitutive AmpC were cloxacillin combined disk diffusion test (CCDDT) and double disk synergy test (DDST) [4]. The former test was performed by using cefoxitin and ceftazidime antibiotic disks with and without cloxacillin, which were placed at a distance from each other. An increase of ≥ 5 mm in the zone diameter for either antimicrobial, which were tested in combination with cloxacillin, versus its zone when it was tested alone confirmed the AmpC β-lactamase production. The latter one was performed by using cefotaxime and ceftazidime disks. In between these, a cloxacillin disk was placed at a distance of 10 mm. Synergism between cloxacillin and ceftazidime and/or cefotaxime indicated the presence of AmpC β-lactamase.

Amongst 320 urinary *Enterobacteriaceae* isolates, inducible AmpC production was detected in ten strains (3.1%) by CAZ-IPM, in nine strains (2.8%) by CX-CTX and in seven strains (2.2%) by the AmpC disk test. All the isolates which were detected as positive either by CX-CTX or the AmpC disk test were found to be positive also by CAZ-IPM. Thus inducible AmpC was detected in a total of ten strains.

A combined AmpC production was detected in 10 (3.1%) isolates by CCDDT and in 7 (2.2%) isolates by DDST. CCDDT picked up 3 additional AmpC producers, whom DDST failed to detect. Thus, by both the combined AmpC production tests, ten AmpC producers were detected.

A comparison between the combined AmpC production tests and the inducible AmpC production tests which was done amongst the *Enterobacteriaceae* isolates, is shown in [Table/Fig-1].

Tests		Combined AmpC production tests		Total
		+	–	
Inducible AmpC production tests	+	10	0	10
	-	0	310	310

[Table/Fig-1]: Comparison of combined AmpC production tests and inducible AmpC production tests amongst *enterobacteriaceae* isolates (n = 320)

Amongst the ten AmpC producers, seven (4.1%) were *E. coli*, two (3.5%) were *Klebsiella pneumoniae* and one (16.7%) was *Citrobacter koseri.*

The capability to detect AmpC is important for improving the clinical management of infections and it provides sound epidemiological data, but at present, there are no standardized phenotypic screening methods. The AmpC β-lactamases can be inducible or constitutive. In this study, we employed phenotypic methods which could detect either the inducible AmpC β-lactamases or both the AmpC β-lactamases.

The enzyme extraction methods have traditionally been cited as the optimum phenotypic detection methods for the AmpC activity. However, these are labour-intensive and are not suitable for the routine clinical use. Therefore, in our study, we used disk-based methods to detect the production of the AmpC β-lactamases.

Amongst the *Enterobacteriaceae* isolates, ten (3.1%) isolates showed inducible AmpC production. The inducible AmpC production was maximally detected by the ceftazidime-imipenem disk antagonism test (3.1%), followed by the cefoxitin-cefotaxime disk antagonism test (2.8%) and the AmpC disk test (2.2%). Cefoxitin and imipenem are strong inducers of the AmpC β-lactamases, whereas cefotaxime and ceftazidime are weak inducers.

The AmpC β-lactamases are inhibited by cloxacillin. The other inhibitors of the AmpC enzyme are boronic acid compounds and novel inhibitors such as Syn2190. As boronic acid is costly and as Syn2190 is unavailable, cloxacillin-based methods were preferred. These methods are combined tests which detect both inducible and constitutive AmpC β-lactamases. Amongst the 320

Enterobacteriaceae isolates, combined AmpC production was detected in a total of ten (3.1%) isolates. The combined AmpC production was maximally (3.1%) detected by CCDDT, followed by DDST (2.2%).

If the combined test is positive, it alone cannot deduce whether the test strain had produced inducible AmpC, constitutive AmpC or both. But if the results of both types of tests i.e. if the tests for inducible AmpC production and the combined tests are interpreted together, it may throw some light on type of AmpC which is produced. If a test strain is found to be negative by the inducible AmpC production test and to be positive by the combined test, the strain will most probably be a constitutive AmpC producer. If the strain is positive by both types of tests, it may be an inducible AmpC producer or both an inducible and a constitutive AmpC producer. In this study, as ten isolates were found to be AmpC producers by both types of tests, they may be either only inducible AmpC producers or the producers of both inducible and constitutive AmpC.

In this study, the number of AmpC producing *Enterobacteriaceae* isolates which were studied was less. Hence, further studies with more number of strains are needed. This study is novel in an aspect that it implied as many as five phenotypic methods for the detection of the AmpC production. In our literature search, we have not come across any such study.

It is noteworthy that as much as three percent of the *Enterobacteriaceae* isolates were found to be AmpC producers. Thus, the AmpC production can be tested by simple disk based phenotypic methods which can be performed by resource limited peripheral laboratories. The AmpC detection can avoid treatment failures by the β-lactam antibiotics prescribed for the infections which are caused by AmpC producing organisms thus, AmpC detection can be helpful for a proper management of these infections.

REFERENCES

[1] Cantarelli VV, Everton Inamine E, Brodt TCZ, Secchi C, Cavalcante BC, Pereira FS. Utility of the ceftazidime-imipenem antagonism test (CIAT) to detect and confirm the presence of inducible AmpC beta-lactamases among *enterobacteriaceae*. *Braz J Infect Dis*. 2007; 11: 237-39.

[2] Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for β-lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother*. 1995; 39:1211-33.

[3] Philippon A, Arlet G, Jacoby GA. Plasmid-determined AmpC type β-lactamases. Antimicrob. *Agents Chemother*. 2002; 46:1–11.

[4] Manual of workshop on detection of beta-lactamases: phenotypic and genotypic methods. Organized by Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi. In XXXV National Conference of Indian Association of Medical Microbiologists, MICROCON 2011, Varanasi.

[5] Singhal S, Mathur T, Khan S, Upadhyay DJ, Chugh S, Gaind R et al. Evaluation of methods for AmpC beta-lactamase in gram negative clinical isolates from tertiary care hospitals. *Ind J Med Microbiol*. 2005; 23:120-24.

PARTICULARS OF CONTRIBUTORS:

1. Resident, Department of Microbiology, Indira Gandhi Government Medical College, Central Avenue, Nagpur, Maharashtra-440018, India.
2. Assistant Professor, Department of Microbiology, Indira Gandhi Government Medical College, Central Avenue, Nagpur, Maharashtra-440018, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Gopal Nandlal Agrawal,
Assistant Professor, Department of Microbiology, Indira Gandhi Government Medical College,
Central Avenue, Nagpur, Maharashtra-440018, India.
Phone: 9890450488, E-mail: gnagrawal_1@yahoo.co.in

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Mar 07, 2013**
Date of Peer Review: **Apr 05, 2013**
Date of Acceptance: **May 21, 2013**
Date of Publishing: **Aug 01, 2013**