



## Prevalence and Detection of Amp C $\beta$ - Lactamase Resistance in Gram Negative Uropathogens

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### Abstract

The most common mechanism of resistance in Gram negative bacteria is by the production of  $\beta$  lactamases which inactivate  $\beta$  lactam antibiotics. Among which Amp C  $\beta$  lactamases are more important because they confer resistance to Cephalosporins,  $\beta$  lactam  $\beta$  lactamase inhibitor combinations and Aztreonam. Although reported with increasing frequency, the true rate of occurrence of AmpC  $\beta$ -lactamases remains unknown. The present study was designed to determine the occurrence of AmpC enzyme-harboring Gram-negative uropathogens in a tertiary care hospital. A total of 430 consecutive, non repeat urinary Gram negative isolates were included in the present study. All the 430 isolates were screened for AmpC  $\beta$ -lactamase production by Novel predictor disk placement method. Isolates positive in the screening were further confirmed by inhibitor based Cefoxitin –Cloxacillin disk diffusion test. Sixty eight (16%) isolates were positive for AmpC  $\beta$ -lactamases. Based on the species 36 (14%) *Esch.coli*, 27 (28%) *Klebsiella pneumoniae*, 2 (6%) *Pseudomonas aeruginosa*, 1 (5%) *Citrobacter freundii*, 1 (8%) *Acinetobacter* sp and 1 (33%) *Enterobacter aerogens* harbored AmpC enzymes. All the isolates were sensitive to imipenem. This study has shown the occurrence of Amp C  $\beta$  lactamase producing Gram negative urinary isolates in our hospital. Failure to identify Amp C  $\beta$  lactamase producers may lead to inappropriate antimicrobial treatment. Inhibitor based Cefoxitin -Cloxacillin disk diffusion test is easier to perform routinely to detect Amp C  $\beta$  lactamase production.

**Key words:** Gram negative uropathogens, drug resistance, Amp C  $\beta$ -lactamases

### 1) Introduction

Multidrug resistant Gram negative bacilli have been increasingly responsible for urinary tract infections [1],[2]. Inactivation of  $\beta$  lactam antibiotics by enzymes is a major mechanism of resistance in Gram negative bacteria. Although variety of  $\beta$  lactamases have been described, Extended Spectrum  $\beta$  lactamases (ESBL) and Amp C  $\beta$ -lactamases are most important [3]. Amp C  $\beta$ -lactamases are cephalosporinases, which belong to molecular class

C as classified by Ambler in 1980 and group 1 under classification scheme of Bush et al [4]. These are clinically significant as they may confer resistance to narrow, expanded, broad-spectrum Cephalosporins, 7-  $\alpha$  methoxy cephalosporins,  $\beta$  lactam  $\beta$  lactamase inhibitor combinations and Aztreonam [5]. Earlier the Amp C  $\beta$ -lactamases were presumed to be chromosomally encoded. Recently the plasmid mediated Amp C  $\beta$  lactamase has also arisen through the transfer of chromosomal genes for Amp C  $\beta$  lactamase on to plasmids. This transfer has resulted

in plasmid-mediated Amp C beta lactamases in isolates of *E.coli*, *Klebsiella pneumoniae*, *Salmonella sp* and *Proteus mirabilis* thus providing a new mechanism of resistance for those originally Amp C deficient bacterial strains [6],[7]. With the increasing trend of antibiotic resistance in Gram negative bacilli, the management of urinary tract infection is likely to become more complicated with limited therapeutic options. Many clinical laboratories are not fully aware of the importance of plasmid mediated Amp C  $\beta$ -lactamases. A recently described disk diffusion test is based on comparison of the inhibition zone diameters around a Cefoxitin disk and a Cefoxitin disk supplemented with the beta lactamase inhibitor Cloxacillin. The test was shown to have sensitivity and a specificity of 95% for the detection of AmpC  $\beta$ -lactamase [8],[9]. Prevalence of Amp C  $\beta$ -lactamase resistance mechanism appears to be increasing and has been responsible for nosocomial outbreaks, avoidable therapeutic failures and outbreaks of multidrug resistant Gram negative pathogens that require extensive control efforts. Hence the present study was undertaken to find out the presence of Amp C  $\beta$  lactamase resistance in Gram negative uropathogens by simple, reliable and inexpensive inhibitor based phenotypic method Cefoxitin- Cloxacillin disk diffusion test.

## 2) Material and methods:

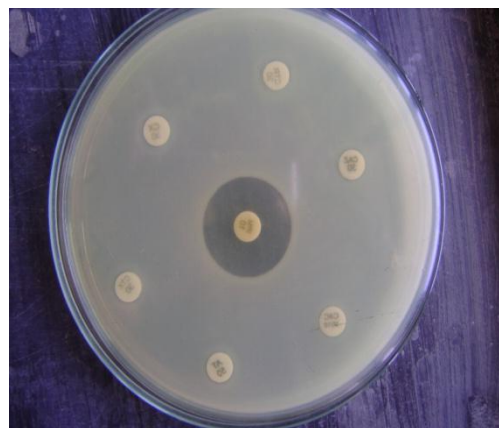
A total of 430 consecutive Gram negative urinary isolates of species *Esch.coli* (n=254), *Klebsiella pneumonia* (n=97), *pseudomonas aeruginosa*(n=34), *Citrobacter freundii*(n=18), *Acinetobacter sp*(n=13), *Proteus mirabilis* (n=6), *proteus vulgaris* (n=5) and *Enterobacter aerogens*(n=3) obtained over a period 6 months from October 2014 to march 2015 at Govt. Thoothukudi medical collage hospital (Tamilnadu) identified by standard method were included in the present study. Susceptibility to antibiotics (concentration in  $\mu$ g) Ampicillin (10), Gentamycin (10), Amikacin(10), Cefotaxime(30), Ceftazidime (30), Ceftriaxone (30) and Ceftazidime (30) (Hi Media) were tested by Kirby Bauer's disk diffusion

method and interpreted as Clinical Laboratory Standard (CLSI) recommendations .

## Screening for Amp C $\beta$ lactamase producing isolates

### 2.1) Novel predictor disk placement method [10],[11]

The disk placement was designed in a novel fashion to assess Amp C  $\beta$  lactamase production. The Ceftazidime and Ceftazidime +Clavulanic acid disks were kept 15-20 mm apart from each other. Imepenam ,an inducer was placed in the centre and on either side of it at a 15 mm distance, Ceftazidime and Cefotaxime (indicators of induction) were placed and in addition another inducer Cefoxitin was placed at 15mm from Cefotaxime, opposite to that of Ceftazidime +Clavulanic acid to avoid any effect of inducible  $\beta$  lactamases on the zone of inhibition of the latter.



**Figure 1:** Novel predictor disc placement method.

### Identification of Amp C

- Resistance to cefoxitin.
- No increase in zone size with addition of an inhibitor
- Inducible Amp C Blunting of zone towards inducers (imepenam. Cefoxitin)
- Screening positive isolates shows Cefoxitin resistance and Ceftazidime plus Clavulanic acid resistance.

## 2.2) Confirmation of Amp C $\beta$ lactamase producing isolates

Isolates positive in the screening were confirmed for Amp C  $\beta$  lactamase production by the cefoxitin-cloxacillin disk diffusion test.

## 2.3) The cefoxitin-cloxacillin disk diffusion test

The cefoxitin-cloxacillin disk diffusion test was performed as described by Tan et al. The test is based on the inhibitory effect of cloxacillin on AmpC. In brief, 30- $\mu$ g cefoxitin disks were supplemented with 200  $\mu$ g cloxacillin. The test strain was inoculated on Mueller-Hinton agar. The diameters of the cefoxitin inhibition zones were compared with and without cloxacillin; if the difference in inhibition was  $\geq 4$  mm, the strain was considered positive for AmpC production. Isolates showing zone diameter around the disk containing CXX (cefoxitin + Cloxacillin)  $\geq 4$ mm than the zone diameter around the disk containing CX alone in the figure below



**Figure 2:** Inhibitor based method for AmpC production:

### Results

Of the 430 isolates tested, 71 were resistant to third generation cephalosporins- (Cefotaxime, Ceftazidime, Ceftriaxone), Cefoxitin, Ceftazidime and Calvulanic acid combination, Aztreonam and sensitive to Imepenam .These isolates were considered as positive in the screening test Novel predictor disc placement method.

**Table 1:** Distribution of Amp C producing strains

Name of the organism	No. of organisms isolated	Screening (Novel disk placement test) positive	Confirmatory (Cefoxitin cloxacillin disk test )Positive
Esch.coli	254	37	36(14%)
K.pneumoniae	97	29	27(28%)
P.aeruginosa	34	2	2(6%)
Citrobacter sp	18	1	1(5.5%)
Acinetobacter sp	13	1	1(8%)
Proteus sp	11	0	0
Enterobacter sp	3	1	1(33%)

Out of 71 screening positive isolates, 68 (16%) were positive for Amp C  $\beta$  lactamase production. Out of 68 Amp C  $\beta$  lactamase positive Gram negative bacteria 36 were (14%) *Esch.coli*, 27 (28%) were *Klebsiella pneumoniae*, 2(6%) were *pseudomonas aeruginosa.*, 1(5%) was *Citrobacter freundii*,1(8%) was *Acinetobacter sp* and1(33%) was *Enterobacter aerogens*

### Discussion

AmpC, beta-lactamases are clinically significant, since they confer resistance to cephalosporins in the oxyimino group (cefotaxime, ceftriaxone, ceftazidime), 7 alpha methoxy cephalosporins (CX) and are not affected by available beta-lactamase inhibitors (clavulanate, sulbactam). Therapeutic options for infections caused by Gram-negative organisms expressing AmpC beta-lactamases are limited except for 4<sup>th</sup> generation cephalosporins such as cefepime and carbapenem. This emphasizes the need for detecting AmpC beta-lactamase harboring isolates so as to avoid therapeutic failures and nosocomial outbreaks.

Currently, CLSI documents do not indicate the screening and confirmatory tests that are optimal for detection of Amp C  $\beta$  lactamases. However, several studies have been done on various test methods namely, the Three Dimensional test, Modified Double Disk test, Amp C disk test, Inhibitor based method employing inhibitors like boronic acids and Cloxacillin, Broth micro dilution method and Cefoxitin Agar method. In spite of many phenotypic tests, isoelectric focusing and genotypic characterization by various molecular methods are considered

gold standard, however these techniques are expensive and the requiring reagents are costly and not easily available<sup>[12]</sup>. Hence simple, reliable and inexpensive inhibitor based phenotypic method Cefoxitin cloxacillin disk diffusion test was used in this study. Helmy et al also reported that Cefoxitin cloxacillin disk test was easier, reliable and rapid method for detection of isolates that harboring Amp C  $\beta$  lactamases<sup>[13]</sup>.

In the present study, Plasmid mediated Amp C  $\beta$  lactamase production was observed in 28% of *Klebsiella pneumoniae* isolates and 14% of *Escherichia coli* isolates. Similar findings were observed by A. Subha et al from Chennai reported that, twenty eight isolates (24.1%) of *Klebsiella spp.* and 12 (37.5%) of *Esch. Coli* were plasmid mediated Amp C  $\beta$  lactamase producers<sup>[14]</sup>. Manchanda & Singh et al from Delhi also reported 20.7 per cent of the clinical isolates were harboring Amp C  $\beta$  lactamases<sup>[15]</sup>. In contrast A.K. Ratna et al from Karnataka reported sixteen (3.3%) isolates were positive for plasmid mediated Amp C  $\beta$  lactamases. Based on the species 9 (3.3%) *Escherichia coli*, 4 (2.2%) *Klebsiella pneumoniae*, 2 (5%) *Citrobacter freundii* and 1 (5.5%) isolate of *Enterobacter aerogenes* harbored Amp C enzymes<sup>[3]</sup>. Differences between these results may be related to the features of the selected isolates (different geographic regions, antimicrobial resistance phenotype of isolates, etc.) or detection methods. Results from a single centre study should be interpreted with care, further population-based prevalence studies are required to observe the true spread of Amp C  $\beta$ -lactamases.

In *K. pneumoniae* Amp C  $\beta$  lactamase production is only plasmid mediated but in *Esch. Coli* hyper production of chromosomal mediated Amp C and plasmid mediated. Cefoxitin resistance in non Amp C producing *Klebsiella pneumoniae* is often due to porin deficient mutants. The interruption of a porin gene by insertion sequences is a common type of mutation that causes the loss of porin expression and increased Cefoxitin resistance in *Klebsiella pneumoniae*. In *Esch. Coli* hyper production of

chromosomal Amp C with OMP F porin loss can produce similar resistance phenotype<sup>[14]</sup>.

### Conclusion

Detection of Amp C  $\beta$  -lactamase producing Gram negative isolates is important for epidemiology and infection control purposes. The inhibitor based confirmatory method used in this study is a practical and efficient method to detect AmpC  $\beta$ -lactamase enzymes. This study has revealed the prevalence of AmpC  $\beta$  -lactamase producing Gram negative urinary isolates in our region. The results are helpful to formulate an empirical therapy in different clinical situations.

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