**ORIGINAL ARTICLE**

**OCCURRENCE OF EXTENDED – SPECTRUM BETA – LACTAMASE & AMP – C BETA – LACTAMASES SUSCEPTIBILITY TO NEWER ANTIMICROBIAL AGENTS IN COMPLICATED URINARY TRACT INFECTION: A CIMS EXPERIENCE**

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**HOW TO CITE THIS ARTICLE:**


**ABSTRACT:** The two most common mechanisms of antimicrobial resistance among gram – negative bacilli are extended spectrum beta lactamases (ESBL) and AmpC Beta – Lactamases. A study was undertaken to know the occurrence of ESBL and AmpC producing strains and their antibiotic susceptibilities to newer agents to guide empirical therapy for complicated urinary tract infections. Over a period of three years (January 2011 to December 2013) Organisms were grown in pure culture & isolated in significant numbers from urine samples, were identified by standard biochemical tests and antibiotic susceptibility determined by disc diffusion method. Gram-negative bacilli that were resistant to third generation cephalosporins, ciprofloxacin and gentamicin/amikacin were defined as highly drug resistant uro-pathogens (HDRU). HDRU were further tested for ESBL and AmpC phenotypes. Uro-pathogens were isolated in significant numbers in 1426 (23.1%) of the total 6074 samples, of which 308 (21.5%) were HDRU. 230 consecutive HDRU isolates were tested for ESBL production and 38.6% were found to be ESBL producers. The highest positivity was found to be in Enterobacter aerogenes. (52.6%), followed by Escherichia coli (44.2%), Klebsiella spp. (34.4%) Proteus Spp. (36.4%) & Pseudomonas aeruginosa (28.8%). The most effective antibiotics for ESBL producers were imipenem & cilastin (5.8% resistance), imipenem (8.6%) piperacillin-tazobactam (10.2%) and ceftazidime-clavulanic acid (26.6%). Among ESBL non-producers, piperacillin-tazobactam (30.08%), ceftazidime-clavulanic acid (47.2%) and imipenem (10%) were less effective, when compared to ESBL producers. Overall, (23.1%) of our isolates were highly drug resistant, while (38.9%) of HDRU isolate were ESBL: producers in this study.

**KEYWORDS:** AmpC β-lactamases – drug resistance – ESBL – uro-pathogens.

**INTRODUCTION:** Antibiotic resistance in uro-pathogens is increasing worldwide in both outpatients as well as hospitalized patients. It depends considerably on appropriateness of antibiotic therapy being instituted in a given geographical area. Understanding the extent and magnitude of drug resistance is of utmost importance in initiating the empirical therapy of most infections, particularly of urinary tract infections.¹² The various mechanisms including extended spectrum beta lactamase (ESBL) production and porin deficiency have been explained as the cause for drug resistant among Gram negative bacilli.³⁶

Amongst the mechanisms of resistance to third generation cephalosporins, production of ESBLs and AmpC β-lactamases, are the most common. AmpC β-lactamases are clinically important because they confer resistance to narrow, expanded and broad-spectrum cephalosporins, β-lactam-β-lactamase inhibitor combinations and aztreonam.
These enzymes are typically associated with multiple antibiotic resistances, posing great therapeutic challenges. Anticipating a high-level drug resistance in this tribal dominated region, where antibiotic policy is seldom followed, we conducted this study to determine the occurrence of ESBL and AmpC producing strains among the highly drug resistance uro-pathogens. In our view, this is the first report on occurrence of β-lactamases and their impact on drug resistance to have come out from the state of Chhattisgarh.

**MATERIAL & METHODS:** A prospective study was conducted over a period of three years (January 2011 to December 2013) at the Department of Medical Microbiology, Chhattisgarh Institute of Medical Sciences, Bilaspur, C.G. India. Urine samples were collected from patients suspected to have urinary tract infection. These included clean catch midstream urine, catheter, suprapubic and nephrostomy samples. Urine (10µl) was inoculated onto MacConkey's agar medium. Organisms grown in pure culture and in significant numbers (>10^5 cfu/ml for midstream urine samples and >10^3 for other types of samples) were identified by standard biochemical tests and antibiotic susceptibility by disc diffusion method. Gram-negative bacilli that were resistant to third-generation cephalosporins (cefotaxime/ceftazidime), ciprofloxacin and gentamicin/amikacin were defined as highly drug resistant uro-pathogens (HDRU). These isolates were further tested for ESBL and AmpC phenotype. Susceptibilities of these HDRUs to newer antibiotics and β-lactam-β-lactamase combinations were also determined.

ESBLs were detected by the confirmatory method recognized by Clinical and Laboratory Standards Institute (CLSI) using cefotaxime (30µg) and ceftazidime (30µg) and a disc of cefotaxime plus clavulanic acid (30 and 10µg) and ceftazidime and clavulanic acid (30/10µg) placed at a distance of 20 mm on a lawn culture (0.5 McFarland inoculums size) of suspected ESBL producing clinical isolate on Mueller-Hinton Agar. Escherichia coli ATCC 25922 was used as the negative control. ESBL production was inferred if the inhibition zone increased by 5 mm towards the cefotaxime plus clavulanic acid disc or ceftazidime plus clavulanic acid disc in comparison to the third generation cephalosporin disc alone.

Screening for the inducible AmpC β-lactamase was done by the disc antagonism test by placing cefoxitin disc (30 µg, Hi-Media, Mumbai) at a distance of 20 mm from ceftazidime (30 µg) on the surface of Mueller-Hinton Agar. Beta-lactamase inducibility was recognized by blunting of the ceftazidime zone adjacent to cefoxitin disc. Briefly, 10-15 mg fresh overnight growth from MHA was taken in a micro-centrifuge tube. Peptone water was added and centrifuged at 800 g for 15 min. Crude enzyme extract was prepared by repeated freeze thawing for five to seven times.

Lawn cultures of E. coli ATCC 25922 were prepared on Mueller-Hinton Agar plates and cefoxitin (30 µg) discs were placed on the plate. Linear slits were cut using a sterile surgical blade 3 mm away from the cefoxitin disc; 10 µg enzyme extract was added to a well-made at the outer edge of the slit. The plates were incubated at 37°C overnight. Quality control was achieved by using known AmpC positive isolate of K. pneumonia ATCC 700603.

Plasmid mediated AmpC beta lactamases production was further confirmed by the AmpC disk test. The test is based on use of tris-EDTA to permeabilize a bacterial cell and release of β lactamases into the external environment. AmpC disks were prepared in-house by applying 20 µl of a 1:1 mixture of saline and 100 x tris-EDTA to sterile filter paper disks.
The surface of a Mueller-Hinton agar plate was inoculated with a lawn of 0.5 McFarland suspensions of cefoxitin susceptible E. coli ATCC 25922. Several colonies of each test organism were applied to a disk. A 30 µg cefoxitin disk was placed on the inoculated surface of the Mueller-Hinton agar. The inoculated AmpC disk was then placed almost touching the antibiotic disk with the inoculated disk face in contact with the agar surface.

The plate was then inverted and incubated overnight at 35°C in ambient air. After incubation, positivity or negativity of the result was obtained by examining the plates for either an indentation or a flattening of the zone of inhibition, indicating enzymatic inactivation of cefoxitin (positive result), or the absence of a distortion, indicating no significant inactivation of cefoxitin (negative result).

Antibiotic susceptibility to newer antibiotics (µg) like imipenem – cilastin (10/10), piperacillin (100), cefepime (30), imipenem (10 µg), aztreonam (30 µg) and beta-lactam beta-lactamase inhibitor combinations such as amoxicillin-clavulanic acid (30/10), ampicillin-sulbactam (10/10), piperacillin-tazobactam (100/10) and ceftazidime-clavulanic acid (30/10) was performed by the NCCLS method. E. coli ATCC 25922 was used as the susceptible control strain.

RESULTS & DISCUSSION: A total of 6074 urine samples (midstream, catheter, suprapubic, nephrostomy) were collected from the same number of patients during the period of study. Among these, uро-pathogens were isolated in significant numbers in 1324 (21.8%) samples, of which 308 (21.5%) were highly drug resistant uро-pathogens. Two hundred and thirty consecutive HDRU isolates were tested for ESBL production and 120 (38.9%) were found to be ESBL producers. The highest positivity was found to be in Enterobacter (52.6%), followed by E. coli (44.2%), Proteus spp. (36.4%), Klebsiella spp. (34.4%) and Pseudomonas aeruginosa (28.8%) These 230 HDU isolates (both ESBL producers and non-producers) were tested for susceptibility to newer antibiotics and beta-lactam-beta lactamase inhibitor combinations.

ESBL producing isolates showed a high degree resistance to piperacillin (92.8%), amoxicillin-clavulanic acid (94.3%), aztreonam (80.2%), cefepime (77.6%), ampicillin-sulbactam (78.6%) and ticarcillin (64.4%). The most effective antibiotics were imipenem-cilastin (5.8% resistance), imipenem (8.6%), piperacillin-tazobactam (10.2%) and ceftazidime-clavulanic acid (26.6%). Similarly, ESBL non-producers also showed a high degree of resistance to piperacillin (92%) and amoxicillin-clavulanic acid (90%), followed by cefepime (79%) aztreonam (76%), and ticarcillin (74.8%). Among ESBL non-producers, ceftazidime-clavulanic acid (52.4%) piperacillin-tazobactam (32.6%), and imipenem (13%) were less effective when compared to ESBL producers.

Of the 230 isolates that were tested for ESBL production, 148 were inhibited by piperacillin-tazobactam. These isolates were tested by placing the piperacillin and piperacillin-tazobactam discs at a distance of 20 mm and a >5 mm increase in the zone towards piperacillin-tazobactam was observed. Fifty three piperacillin and piperacillin-tazobactam positive and 20 negative isolates were further tested for AmpC production.

It was found that all the 53 positive isolates were also positive by modified 3-D test for AmpC β-lactamase and the disk test using tris-EDTA. In this study we focused on the multi-drug resistant uро-pathogens and their sensitivity pattern to newer antibiotics. Overall, 22.1% of our isolates were highly drug resistant and E. coli (32.6%), P. aeruginosa (28.5%), Proteus (24.6%), Enterobacter aerogenes (16.6%) and accounted for the most resistant isolates.
The guidelines for detection and interpretation of ESBLs in routine laboratory are well entrenched (NCCLS 2000, CLSI 2011). In the absence plausible guidelines for detection of AmpC lactamases, it has become difficult to screen them routinely. However, various methods have been described for testing AmpC enzymes in Gram-negative bacterial isolates.

In our study both ESBL producers and non-producers showed similar level of resistance to the antibiotic tested, except to piperacillin and piperacillin-tazobactum. This can be explained by the fact that AmpC β-lactamase producers are more susceptible to tazobactam as compared to clavulanic acid. Both ESBL producers and non-producers showed high level resistance to cefepime. AmpC producers were susceptible to fourth generation cephalosporins like cefepime, while, ESBL producers were variably resistant to fourth-generation cephalosporins. This is in consonance with other reported studies.

In conclusion, routine screening for ESBL and AmpC production has become essential for all uro-pathogens causing complicated urinary tract infection. There is an urgent need to formulate a policy for empirical therapy, enforcement of intervention strategies, and resistance surveillance measures, especially in high risk units, where highly drug resistant uro-pathogens have become menacingly common.

REFERENCES:


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