



## **A COMPARATIVE STUDY ON PHENOTYPIC DETECTION OF AMP-C BETA LACTAMASES AND EXTENDED SPECTRUM BETA LACTAMASES IN GRAM NEGATIVE BACTERIAL URINE ISOLATES IN A TERTIARY CARE HOSPITAL**

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### **Abstract**

The prevalence of multidrug resistant Gram negative bacteria has increased in the past few years and the bacterial strains producing Amp - C Beta Lactamase and Extended Spectrum Beta Lactamase are of particular concern. With the increase in occurrence and different types of these multiple  $\beta$ -lactamase enzymes, early detection is crucial. The benefits of which include implementation of proper antibiotic therapy and to frame infection control policy. Amp - C Beta lactamase confer resistance to Aminopenicillins, Cephalosporin, Oxyimino - Cephalosporins (Cefoxitin & Cefotetan) and Monobactams and are not affected by Clavulanic acid like Extended Spectrum Beta Lactamase producers. The objective of the present study was to screen and phenotypically detect Amp-C Beta Lactamase and Extended Spectrum Beta Lactamase in Gram negative bacterial urine isolates. Out of the 100 Cefoxitin and Ceftazidime resistant strains taken for study, 71 % were found to produce Amp - C Beta lactamase 81 % strains were found to produce ESBL. Totally 17 % of strains were resistant to both Cefoxitin - Cloxacillin combination disc and Ceftazidime - Clavulanic acid disc. They are subjected to meropenem sensitivity and the results were analysed.

**Key words:** ESBL, Amp – C, Beta lactamase and Combination disc.

### **1. Introduction**

Amp - C Beta Lactamases were classified as class C  $\beta$ -lactamases Ambler classification and in functional classification of Bush, they were assigned to Class C. They confer resistance to Aminopenicillins, Cephalosporin, Oxyimino - Cephalosporins (Cefoxitin & Cefotetan) and Monobactams. The ESBLs belong to Bush class A  $\beta$ -lactamases, that can hydrolyse the Penicillin's, Oxyimino - Cephalosporins and Monobactams and were inhibited by Beta lactamase inhibitors like Clavulanic acid, Sulbactam and Tazobactam (Sahid *et al.*, 2004)

Amp - C Beta Lactamases were inhibited by Cloxacillin and 3 - amino phenyl boronic acid but they were not affected by clavulanic acid. In Gram negative bacteria, Amp-C Beta Lactamases production was either plasmid or chromosomal mediated. These enzymes were often produced by *Escherichia coli*, *Klebsiella* spp., *Proteus mirabilis* and *Salmonella* spp. They were associated with multiple antibiotic resistances and have only few therapeutic options. Different phenotypic screening methods were reported for detection of Amp-C Beta Lactamases. This disc diffusion test is based on comparison of the inhibition zone diameters around Cefoxitin (30  $\mu$ g) and Cefoxitin disc supplemented with inhibitor Cloxacillin (200

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µg). This test has sensitivity of 100 % and specificity 98 % (Bloemberg *et al.*, 2011).

## 2. Materials and Methods

A total of 756 urine samples were received for culture in our laboratory from February to August 2012. From these specimens 613 consecutive and non-duplicate isolates of Gram negative bacteria were screened for Amp - C Beta lactamase and ESBL production. The isolates are speciated by standard biochemical methods (Monica Cheesbrough, 2016). The 100 Cefoxitin resistant (zone size <18 mm) Gram negative bacterial isolates (*Proteus* spp., *Escherichia coli*, *Klebsiella* spp. and *Pseudomonas* spp.) were taken for study. They have reduced susceptibility to Ceftazidime (30 µg), Cefotaxime (30 µg) and Cefoxitin (30 µg). Antibiotic susceptibility testing was performed using susceptibility test discs and interpretation was done according to CLSI guidelines 2011 ATCC *Escherichia coli* 25922 used as a control strain.

### Screening method for Amp - C Beta Lactamases

The Cefoxitin - Cloxacillin disc (30 µg/200 µg) diffusion test was performed. It is based on inhibitory effect of Cloxacillin on Amp - C Beta Lactamases. A culture suspension of the test isolates was adjusted to 0.5 McFarland's standard and inoculated by using a sterile cotton swab on the surface of a Mueller Hinton Agar plate, incubated at 37 °C, for 18 to 24 hrs (Monica Cheesbrough, 2016). The inhibition zones diameters were compared with and without Cloxacillin, if the difference in inhibition zone was > 4 mm (Bloemberg *et al.*, 2011), the strain was considered to be positive for Amp-C Beta lactamases.

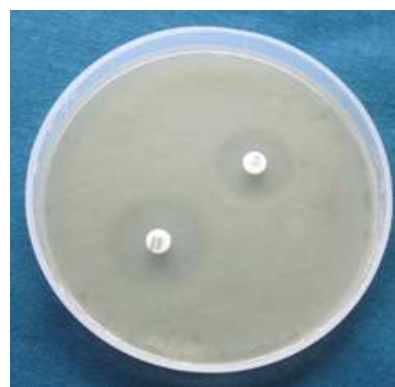
### Detection of Extended spectrum Beta Lactamase

Isolates resistant to Ceftazidime (zone size <27 mm) were selected for confirmation of ESBL production. It was done by phenotypic confirmatory test. (Double Disc Synergy Test) A culture suspension of the test isolates was adjusted to 0.5 McFarland's standard and inoculated by using a sterile cotton swab on the surface of a Mueller Hinton Agar plate.

Amoxicillin and Clavulanic acid (10 µg) disc were kept 30 mm distance from centre to centre. After incubating overnight at 37 °C, the presence of enhanced zone of inhibition towards clavulanic acid disc indicates synergy and was interpreted as positive for ESBL production. Positive control *Klebsiella pneumoniae* ATCC 700603 (Hi-media Laboratories, Mumbai, India) and Negative control *Escherichia coli* ATCC 25922 were used as control strains. Seventeen strains which were resistant to both combination discs were subjected to meropenem sensitivity. Twelve strains were sensitive to Imipenem and five strains were resistant.

## 3. Results

One hundred Gram negative bacterial isolates which were resistant to Ceftazidime and Cefoxitin isolates were screened for Amp - C Beta Lactamases and ESBL production. Many strains of *Proteus* sp. which produces Amp - C Beta lactamase also found to produce ESBL. Out of 100 Cefoxitin resistant strains 71 % were observed to produce Amp - C Beta lactamase and 29 % were resistant to the combination disc (Cefoxitin + Cloxacillin) (Fig - 1). 81 % strains were found to produce ESBL and 19 % were resistant to Amoxicillin + Clavulanic acid combination disc (Fig - 2). Resistant to both of these combination drugs may be due to alteration of outer membrane protein. Seventeen strains were resistant to both Cefoxitin - Cloxacillin combination disc and Amoxicillin - Clavulanic acid disc. They are subjected to Meropenem sensitivity and the results were analysed.



**Figure - 1: Demonstration of Amp - C Beta Lactamase Production**

The inhibition zones diameters for Cefoxitin were compared with and without Cloxacillin, if the difference in inhibition zone was > 4 mm, the strain was considered to be positive for Amp - C Beta lactamases.



**Table – 1: Sensitivity of Antibiotics against bacterial pathogens**

Organisms N = 100	Number of Isolates N=100	Cefoxitin + Cloxacillin		Ceftazidime + Clavulanic acid		Both combination drugs	
		Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
<i>Proteus</i> spp	42	29 (69 %)	13 (31 %)	40 (95 %)	2 (5 %)	21 (56 %)	6 (14 %)
<i>Klebsiella</i> spp.	20	12 (60 %)	8 (40 %)	17 (85 %)	3 (15 %)	15 (75 %)	4 (2 %)
<i>Escherichia coli</i>	11	7 (64 %)	4 (36 %)	10 (90 %)	1 (10 %)	6 (54 %)	3 (27 %)
<i>Citrobacter freundii</i>	10	9 (90 %)	1 (10 %)	8 (80 %)	2 (20 %)	4 (40 %)	1 (10 %)
<i>Pseudomonas</i> spp.	8	6 (75 %)	2 (25 %)	2 (25 %)	6 (75 %)	2 (25 %)	2 (25 %)
<i>Acinetobacter</i> spp.	6	5 (83 %)	1 (7 %)	1 (17 %)	5 (83 %)	1 (16 %)	1 (16 %)
<i>Enterobacter</i> spp.	3	3 (100 %)	0 (0 %)	3 (100 %)	0 (0 %)	3 (100 %)	0 (0 %)
Total	100	71	29	81	19	43	17

**Figure – 2: Double Disc Synergy Test (DDST)**

Amoxicillin/Clavulanic acid (20 µg/10 µg) and Ceftazidime (CAZ) discs were placed at a distance of 20 mm from centre to centre on lawn cultures on Muller Hinton agar plates. The plates were incubated at 37 °C overnight. Any enhancement in zone of inhibition of Cephalosporins towards the Amoxicillin/Clavulanic acid disc was considered as a positive result.

#### 4. Discussion

Urinary Tract Infection is the most common Hospital Acquired Infection accounting for 35 % of nosocomial infections. High degree of resistance was observed in organisms causing nosocomial infections. Despite the discovery of ESBLs and Amp - C Beta lactamase at least a decade ago, there remains a low level of awareness of their importance. Techniques to identify Amp C beta-lactamase producing isolates are available but are still evolving and are not yet optimized for the clinical laboratory, which probably now underestimates this

resistance mechanism. We should not miss the Amp - C Beta lactamase producing strain because they were resistant to Cephalosporins, Beta Lactamase inhibitors and Monobactams. This combination drug test was found to be simple and cost effective in the presumptive screening of Amp - C Beta lactamase. Hence, in the routine microbiological diagnostic methodology, Amp - C Beta lactamase producers should be screened. Early detection of this Amp - C Beta lactamase producing isolates in a routine laboratory could help to avoid treatment failure. Although, ESBL production is the predominant antibiotic resistance mechanism observed in Gram negative bacteria isolates, the present study clearly indicates that Amp - C Beta lactamase production is also of equal importance. Our findings showed the need to do screen to detect Amp - C Beta lactamase producers and ESBL producers. The early detection of such resistant strains may curtail the spread of these strains, as the infections due to such resistant bacteria will lead to therapeutic failure.

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