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Original Article

# Detection of ESBL and AmpC production among MDR uropathogens in a tertiary care centre in Kerala

**Neethu John*[a], Girija K.R[b], Lancy J[c]**

[a]*Assistant Professor, Department of Microbiology, Govt. Medical College, Ernakulam*
[b]*Professor, Department of Microbiology, AIMS, Kochi*
[c]*Additional Professor, Department of Microbiology, Govt. Medical College, Trivandrum*

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**ABSTRACT**

UTI is a very common community as well as healthcare acquired infection. MDR organisms are now increasingly found in these infections. So a study was carried out on these MDR uropathogens to detect their antibiotic resistance mechanisms and to study the local antibiograms. Aim:1.To detect the presence of ESBL & AmpC beta-lactamases in the multidrug resistant urinary isolates 2. To study the susceptibility patterns of ESBL and AmpC producing isolates. Methods: ESBL production is detected by double disc diffusion synergy test & phenotypic confirmatory test. AmpC production is detected using combined disc diffusion test using phenyl boronic acid as the inhibitor. Results and conclusion:Out of 150 MDR uropathogens included in the study, 136 (91%) isolates were ESBL positive and 14 (9%) were negative. Among the 150 MDRs, 16 (10.67%) were found as AmpC producers by combined disk diffusion test using phenyl boronic acid as inhibitor with cefoxitin. ESBL and AmpC co-carriage was found in two isolates.



## Introduction

Worldwide about 150 million people are diagnosed with urinary tract infection each year [1]. UTI is very common both in community and hospitalized patients. Empirical antibiotic therapy is usually applied in the treatment of UTI. So a good knowledge of the common uropathogens and their susceptibility pattern to commonly used antibiotics is essential.

Beta-lactamase production is the most common mechanism of resistance in Gram-negative bacteria[2] which are also the most common etiological agents in UTI. Extended spectrum beta-lactamases(ESBLs) and AmpC beta-lactamasesare produced by many Enterobacteriaceae. 'Plasmids' carrying ESBL and AmpC genes often carry multiple other resistance genes, thus leading to multi drug resistance in clinical isolates[3, 4]. Incidence of these MDR organisms is being continuously increasing throughout the world with limited treatment alternatives. Therefore it becomes necessary to know the prevalence of these organisms and to formulate treatment policy.

Detection of ESBL producing organism from samples such as urine may be important because this represents an epidemiologic marker of colonization and therefore there is potential for transfer of such organisms to other patients [5]. Considering all these facts, a study is undertaken to detect the presence of ESBLs and AmpC β-lactamases in the uropathogens isolated from Microbiology department in a tertiary care hospital in central Kerala.

## MATERIALS AND METHODS

A random 150 MDR E.coli and Klebsiella pneumoniae isolates from urine samples received in the Microbiology lab in a tertiary care centre in central Kerala during the period from August 2011 to July 2012formed the study group. Multi drug resistance (MDR) was defined as resistance to three or more antimicrobial classes[6].

The 150 MDR E.coli and Klebsiella pneumoniae urinary isolates included in the study were tested for the presence of ESBL production by double disk diffusion test described by Jarlier et al [7] and phenotypic confirmatory test recommended by CLSI 2010 [8].

Among the 150 MDR isolates, cefoxitin resistant isolates were considered as potential AmpC producers and those isolates were further tested for the presence of AmpC β-lactamase by combined disc diffusion test which used phenyl boronic acid as the inhibitor [6].

The susceptibility pattern of the 150 MDR isolates to the following antibiotics was noted as per CLSI guidelines (2010) - Ampicillin, Gentamicin, Amikacin, Ciprofloxacin, Cotrimoxazole, Nitrofurantoin, Cephalexin, Ceftazidime, Cefotaxime, Ceftriaxone, Cefepime, Aztreonam, Cefoxitin, Piperacillin-tazobactam and Imipenem.

* Corresponding Author : **Dr.Neethu John**
Assistant Professor
Department of Microbiology
Govt. Medical College
Ernakulam
E-mail: neethujohn1980@gmail.com

## Result

Among the 150 MDR urinary isolates, 134 (89%) isolates were found as ESBL positive and 16 (11%)isolates as negative by double disk diffusion synergy test. PCT was positive for 136 (91%) and negative for 14 (9%) isolates [Figure 1, 2]. Thus a slightly higher rate of ESBL detection was found with phenotypic confirmatory test.

Out of the 150 MDR study isolates, 37 (25%) were found to be cefoxitin resistant by Kirby Bauer disk diffusion method. Of these 37 isolates, 16 were found as AmpC producers by combined disk diffusion test using phenyl boronic acid as inhibitor with cefoxitin [Figure 3]. ESBL and AmpC co-carriage was found in two isolates.

The antimicrobial susceptibility patterns of 14 antimicrobial agents were studied. Imipenem showed least resistance (4%) followed by Nitrofurantoin (8.7%), Amikacin (10%) and Piperacillin-tazobactam (31.3%). Resistance pattern is summarized in the figure given below [Figure 4].

**Figure 1**

Double disc diffusion test showing synergy indicative of ESBL production

AMC – Amoxicillin-clavulanate, AO – Aztreonam, CTX – Cefotaxime, CAZ - Ceftazidime

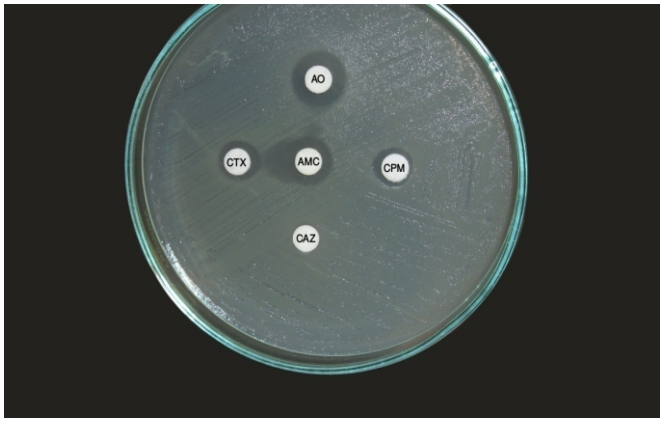



**Figure 2**

PCT (Phenotypic Confirmatory Test) showing ESBL production by the isolate - A ≥ 5 mm increase in zone diameter is seen with ceftazidime-clavulanate (CAC) and cefotaxime clavulanate(CEC) than with Ceftazidime (CAZ) or cefotaxime (CTX) alone

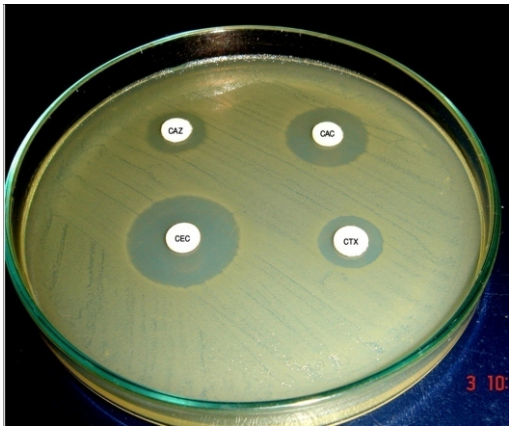



**Figure 3**

Combined Disc Diffusion with Phenyl boronic acid as inhibitor with cefoxitin - An increase of >5mm in zone diameter in the presence of phenyl boronic acid compared with cefoxitin tested alone considered positive for AmpC β-lactamase. CX – Cefoxitin, PBA – Phenyl boronic acid

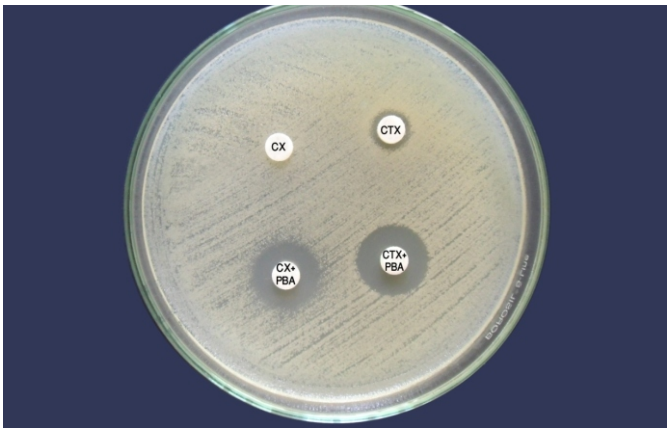
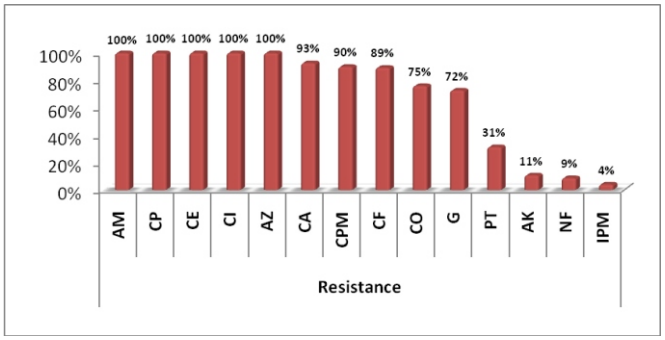



**Figure 4**




Resistance percentage exhibited by the MDR uropathogens

AM – Ampicillin, CP – Cephalexin, CPM – Cefepime, CF – Cefoxitin, CO – Cotrimoxazole, G – Gentamicin, PT – Piperacillin-tazobactum, AK – Amikacin, NF – Nitrofurantoin, IPM - Imipenem

**Table 1**

| | ESBL | Non-ESBL | AmpC | Non- AmpC |
|---|---|---|---|---|
| E.coli | 107(91.5%) | 10 (8.5%) | 12(10.3%) | 105( 89.7%) |
| K.pneumoniae | 29(88%) | 4 (12%) | 4(12.1%) | 29(87.9%) |

Percentage of ESBL and AmpC production in MDR E.coli and MDR K. pneumonia isolated from urine

## DISCUSSION

Out of 117 E.coli, 107 (91.5%) were ESBL positive by PCT and 10 (8.5%) were ESBL negative. Among the 33 K. pneumoniae isolates included in the study, 29 (88%) were ESBL positive and 4 (12%) were ESBL negative [Table 1]. A very high rate of ESBL production is noted in the isolates in this study. Moreover, a higher rate of ESBL production is noted in E.coli than in K.pneumoniae.

A large survey of 1610 Escherichia coli and 785 Klebsiella pneumoniae isolates from 31centers in10 European countries found that the prevalence of ESBL in these organisms ranged from as low as 1.5% in Germany to as high as 39- 47% in Russia, Poland and Turkey [9]. Previous studies from India have reported ESBL production varying from 6% to 87% [10]. Tankhiwale et al from Nagpur has noted 48.3% of cefotaxime resistant Gram negative bacilli to be ESBL producers [11]. They also noted that 90.5% of the ESBL producing isolates were multidrug resistant. All the 150 samples in this study were multidrug resistant. This might explain the very high percentage of ESBL production in this study group.

Out of the 150 MDR isolates, 16 (11%) samples were AmpC phenotype positive. Out of 117 E.coli isolates, twelve(10.3%)were AmpC positive. Among the 33 K.peumoniae isolates, four (12.1%) were AmpC positive [Table 1].

The prevalence of plasmid mediated AmpC varied widely in different parts of the world from 2% to 46% [12, 13]. In Indian studies, the prevalence of AmpC ranged from 8% to 47% [14, 15]. In a study of ESBLs among Enterobacteriaceae from South India, 15.1% of the Enterobacteriaceae isolates were found as Amp C producers by modified three dimensional test [16]. AmpC β-lactamases production was found to be 3.3% and 8% by Ratna et al from Karnatakaand Hemalatha et al [17, 18]. In contrast to the above studies, a higher percentage of AmpC detection of 45.61%, 43% and 47% is mentioned in studies by Patel et al from Ahmedabad, Singhal et al and Manchanda et al respectively [19, 4, 20].

In another study by Manoharan et al on prevalence of AmpC β-lactamases in India which included 909 consecutive Gram-negative isolates from five Indian Medical Centers, Amp C phenotypes were found in 12.5 percent isolates and 36.5% of the cefoxitin resistant isolates were confirmed to have AmpC phenotype [6]. They have also noted that ESBL co-carriage and multidrug resistance was high among the AmpC isolates suggesting plasmid mediated spread. The percentages of AmpC detection in this study matches with those found by Manoharan et al but we could not detect a very high ESBL-Amp C co-carriage. Plasmid-mediated AmpC genes are of special interest because their mobility allows them to emerge in one genus or species and spread to different organisms.

Resistance percentage was noted among the 150 study samples [Figure 6].Antibiotic susceptibility pattern of the isolates vary between regions and institutions. In our institution a high resistance rate was noted for ciprofloxacin and co-trimoxazole which are commonly used as empirical treatment of UTI. Nitrofurantoin, amikacin and piperacillin-tazobactam were found as more susceptible. Most sensitive antimicrobial found was carbapenems. But even the carbapenems showed 4% resistance, which is an alarming situation.

## CONCLUSION

The present study highlights the increasing presence of β-lactamases among the multidrug resistant gram negative bacilli in UTI. It also reflects the limited treatment options available for these notorious pathogens. In this study, the uropathogens showed highest sensitivity to carbapenems, be it an ESBL producer or an AmpC producer. The next best alternatives were nitrofurantoin followed by amikacin. The commonly used antibiotics for empirical therapy of UTI such as ciprofloxacin and cotrimoxazole showed a high percentage of resistance as 88.7% and 75% respectively. These findings point to the need for a change in empirical treatment of UTI in our area.

Routine detection of ESBL and AmpC producing microorganisms should be done by each laboratory by the standard detection methods. This will help to formulate institutional antibiotic policies which in turn will help to control the spread of these MDR infections.

Periodic surveillance of antibiotic resistance patterns and efforts to decrease irrational empirical antibiotic therapy would go a long way in addressing some of the problems associated with ESBLs and AmpCs. Strong decision has to be established regarding the antibiotic policies for UTI and stringent measures have to be taken to ensure the effectiveness of the same.

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