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Characterization and management of β- lactamases producing *Escherichia coli* causing complicated UTIs in an era of antimicrobial resistance

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Introduction

Urinary tract infections (UTIs) are among the most common infectious diseases in the community as well in healthcare settings.[1] A complicated UTI is defined as an infection of urinary tract that is associated either with structural or functional genito-urinary tract abnormalities or in the presence of an underlying factors like foreign bodies (such as calculi, indwelling catheters), obstructions, immune-suppression, renal failure, pregnancy etc., which increases the risk of acquiring an infection or of failing therapy. Complicated UTIs (cUTIs) may occur in both the sexes and in all age groups.[2,3] There is a broad range of pathogens that can cause cUTI and *Escherichia coli* remains the most common. In the past few years, the number of cases of cUTI due to resistant *Escherichia coli* has risen, mainly due to spread of β-lactamase producing bacteria posing a significant therapeutic challenge.[4]

β-lactamases are heterogenous bacterial enzymes that catalyze the hydrolysis of the β-lactam ring of penicillins and cephalosporins and inactivate them. Major risk factors for colonization or infection with β-lactamase producing organisms are long-term antibiotic exposure, prolonged hospital stays, high rates of cephalosporins use and use of invasive devices (urinary catheters, endotracheal tubes, and central venous lines) for a prolonged duration.[5] The pressure generated by indiscriminate use of β- lactam antibiotics has induced a dynamic and continuous production and mutation of β-lactamases in the bacteria expanding their resistance leading to the selection of variety of mutant forms of β-lactamases such as the ESBLs, AmpC β-lactamases and metallo-β-lactamases.[10] Extended spectrum β-lactamase (ESBL) are typically plasmid mediated and beta lactamase inhibitors susceptible enzymes that hydrolyze the penicillins, expanded spectrum cephalosporins and aztreonam.[6]

Since late 1970s, there was increasing recognition and clinical concern of gram negative isolates showing resistance to even extended spectrum beta lactams and beta lactamase inhibitors due to the production of newer beta lactamase enzymes: AmpC β-lactamases. Amp C beta lactamases belong to molecular class C in the Ambler classification and group 1 in the functional classification by Bush et al. These confer wide range of resistance to

penicillins and most cephalosporins apart from the fourth-generation agents cefepime and cefpirome. These enzymes are even resistant to inhibition by beta lactamse inhibitors like clavulanic acid.[7]

Carbapenems are last resort antibiotics for such infections. Use of carbapenems has led to production of carbapenemases. Carbapenemases are diverse enzymes that have the ability to hydrolyze carbapenems and other β-lactams. They are often associated with extensive, sometimes total, antibiotic resistance.[8] β-lactamases producing infections are of grave concern to the medical world as these are associated with increased morbidity and mortality even in infections like UTIs. So this study was aimed to know the incidence of ESBL, AmpC β-lactamases and carbapenemases producing *Escherichia coli isolated from cUTI cases and to know the effective antimicrobial therapy for management of such infections based upon their antimicrobial resistance pattern.*

Materials And Methods

The present study was conducted in the Microbiology department of the Guru Gobind Singh Medical College and Hospital, Faridkot. A total of 210 consecutive, non-duplicate strains of *Escherichia coli* isolated from urine samples of cUTI cases (January 2017 to December 2017) were taken for the study. Detailed history of the patient and related data was recorded. The study was undertaken after ethical committee approval.

Significant bacterial growth ($>10^5$cfu/ml from non catheterized & $\geq 10^3$ cfu/ml from catheterized patients) obtained on MacConkey agar after overnight incubation was identified by colony characteristics, gram staining, motility and various biochemical reactions.[9,10] The antibiogram of the isolates to various antibiotics such as ampicillin (2µg), ceftazidime (30µg), cefotaxime (30µg), ceftriaxone (30µg), cefepime (30µg), cefoxitin (30µg), amikacin (30µg), ciprofloxacin (5µg), norfloxacin (10µg), nitofurntoin (300µg), trimethoprim (5µg), imipenem (10µg), meropenem (10µg), fosfomycin (200µg), amoxicillin-clavulanic acid (20+10µg), was determined by the Kirby-Bauer's disk diffusion method according to Clinical Laboratory Standards Institute (CLSI) guidelines.[6] *Escherichia coli* ATCC 25922 was used as control strain.

Detection of ESBL by combination disc diffusion technique[6]:- The isolates resistant to one or more of third generation cephalosporins as per CLSI guidelines were considered as suspected ESBL producers and further processed for confirmation by combination disc diffusion technique.

Ceftazidime (30µg) alone as well in combination with clavulanic acid (30/10µg) and Cefotaxime (30µg) alone as well in combination with clavulanic acid (30/10µg) were used for the test. ≥5mm increase in the zone diameter for ceftazidime or cefotaxime in combination with clavulanic acid (30/10µg) than that for ceftazidime/ cefotaxime were taken positive for ESBL production.

Modified three dimensional test for detection of AmpC[11]:- Isolates with zone of inhibition ≤ 18 mm towards cefoxitin disc were taken to be putative AmpC producers and were processed for confirmation by Modified three dimensional test.

Fresh overnight growth (10-15mg) was suspended in peptone water and centrifuged at 3000rpm for 15 minutes. Crude enzyme extract was prepared by repeated freeze-thawing of the bacterial pellet. Lawn culture of *Escherichia coli* ATCC 25922 was prepared on MHA plate with cefoxitin disc (30µg) placed in the centre. Linear slits (3cms) were cut using sterile blade, 3mm away from the cefoxitin disc. 30 µl of the test enzyme extract was loaded into each slit. Plates were kept upright

for 5-10 minutes until the liquid dries and then incubated at 37° C overnight. Enhanced growth of the surface organism at the point where the slit intersects the zone of inhibition of cefoxitin disc, causing a clear distortion was interpreted as positive for AmpC enzyme.

Modified Hodge Test to detect carbapenemases[6]:- 0.5 McFarland suspension of *E. coli* ATCC 25922 was prepared and was inoculated on an MHA plate as for the routine disk diffusion procedure. The plate was dried for 5–10 minutes. Meropenem disks were placed on the plate. 3–5 colonies of test organism grown overnight were taken and inoculated in a straight line (25-30mm) out from the edge of the disk. MHA plate then incubated overnight at 37°C. The MHA plate was examined for enhanced growth around the test organism streak at the intersection of the streak and the zone of inhibition.

Enhanced growth = positive for carbapenemase production.

No enhanced growth = negative for carbapenemase production.

Results

Urine samples collected from 500 patients with cUTI were taken for the study. Out of which 314 (62.8%) were culture positive. Among the culture positive samples *Escherichia coli* was the most common organism (210; 66.9%) isolated followed by Citrobacter sp. (54; 17.2%), Enterococcus sp. (17; 5.4%), Klebsiella sp. (15; 4.7%), Pseudomonas sp. (14; 4.4%), Proteus sp. (4; 1.3%). The information from the patients regarding the underlying causes for cUTI is given in table 1. The most common cause was found to be urologic/renal disease including renal stones. About 2/3rd (143/210) strains of *Escherichia coli* were isolated from indoor patients while 1/3rd (67/210) were from outdoor patients. The average age of women diagnosed with cUTI was 54.5 years (range 25–84) and the average age of men was 65.5 years (range 38–93). Figure 1 shows antibiotic susceptibility pattern of *Escherichia coli* isolates. Maximum resistance was observed against ampicillin (88%), ciprofloxacin (83.7%), norflox (75.3%) and third generation cephalosporins. Lower resistance was seen towards amoxyclav (35.2%), amikacin (14.7%), nitrofurntoin (13.3%), imipenem (5.2%) and meropenem (4.3%). Of the 210 *Escherichia coli* isolates, 111(52.8%) were ESBL producers, followed by 29(13.7%) AmpC producers and 6(2.8%) were carbapenemases producers. (Figure 2)

Table 1: Additional information related to patients with cUTI

Factor present	Number (210)	Percentage
Functional/anatomic abnormalities	7	3.3
Diabetes mellitus	92	43.8
Urologic/renal disease Including renal stones	106	50.4
Obstructions to urine flow	12	5.7
Immuno-suppression	18	8.5
Indwelling urinary catheter	27	12.8
Pregnancy	9	4.3

Multiple answers were permitted;

Discussion

We evaluated a total of 500 patients with symptoms and sign of cUTI. Out of this, 314 (62.8%) yielded significant

bacteriuria. *E. coli* (66.9%) was the most common isolate from cUTI cases in the study. *Escherichia coli* is the major aerobic organism residing in the intestine and is the most commonly reported cause of UTI being a common faecal contaminant. The predominance of *Escherichia coli* isolates among UTI cases is supported by our finding as well that of other researchers.[12,13] About two third (143/210) isolates in our study were from indoor patients and one third (67/210) from outdoor patients. Fem et al have also reported more frequent (84%) isolation of *Escherichia coli* from patients admitted in hospital.[14] In contrast, a study from Spain reported more isolates (55.7%) from community as compared to admitted patients (44.3%).[15] The mean age for cUTI in both the sexes was above 50s in our study. Complicated UTI can occur in any gender and at any age but is most common after the fifth decade in both men and women voiding abnormalities related to other conditions are common.[16] In our study maximum isolates shown resistance to most of the commonly used antibiotics like ampicillin (88%), ciprofloxacin (83.7%), norflox (75.3%) and third generation cephalosporins probably because of their misuse or overuse. Lower resistance was seen towards amoxyclav (35.2%), amikacin (14.7%), nitrofurntoin (13.3%), imipenem (5.2%) and meropenem (4.3%). Kidwai, Saera Suhail et al also reported that the most sensitive antibiotics were Imipenem, meropenem, tazobactam, gentamicin and amikacin.[17] Shifali and Gupta proved proved maximal susceptibility pattern of pathogens to amikacin and nitrofurantoin in the study done on females.[18] The prevalence of ESBL, AmpC β-lactamases among common isolates like *Escherichia coli* is increasing worldwide.[19] The present study highlights burden of ESBL, AmpC and carbapenemases producing *Escherichia coli* strains from cUTI cases. In the study of the 210 *Escherichia coli* isolates, 111(52.8%) were ESBL producers, followed by 29(13.7%) AmpC producers and 6(2.8%) were carbapenemases producers. In India, the prevalence rate of ESBL producing *Escherichia coli* varies in different institutions from 28 to 84%[20,21] and that of AmpC varies from 3.4% - 47.8%.[22-25] Carbepenems are the main stay of treatment for these resistant strains. Carbapenem resistance due to carbapenamase production was first discovered in the year 1988 leaving us with very few treatment options. In the present study the rate of carbapenemase producers was 2.8%. While in studies done by Wadekar *et al*. and Oberoi *et al*. higher rates of carbapenemase producers was seen 18% and 10.9% respectively.[26,27]

Conclusion

The rapid and worldwide spread of *Escherichia coli* resistant to beta lactam drugs (mostly the first line therapy) causing cUTI both in hospitalized and non-hospitalized patients has made the selection of empirical therapy more difficult. Clinicians must frequently face the dilemma between choosing one of those agents, which might not be active in some patients, or a drug with a very broad spectrum, that may further fuel the spread of resistance. Carbapenems are considered the drugs of choice for complicated infections due to ESBL or AmpC-producing strains, traditionally rendered as 'last-resort' antibiotics. If these last resort antibiotics are misused then we will be the same as in the pre antibiotic era.

Therefore, the decisions about therapy must be according to the susceptibility profile and, if available, the resistance mechanism. There is a need for rapid testing to characterize the mechanisms of resistance, the development of new drugs, and better clinical studies investigating the efficacy and safety of old active drugs and combinations.

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