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Prevalence of ESBL Producing Enterobacteriaceae in Patients with UTI in A Rural Tertiary Care Hospital

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Abstract

Background and Objective: *Urinary tract infection (UTI) is the most common bacterial infection in human population leading to significant morbidity and health care costs. Expanded spectrum beta lactamase producing enterobacteriaceae hydrolyse expanded spectrum cephalosporins like ceftazidime, cefotaxmie. They play a significant role in UTI primarily in hospital admitted patients causing morbidity and mortality all over the world. Appropriate diagnosis and treatment are always challenging. An attempt was made to study retrospectively in the Department of Microbiology, Index Medical College about the prevalence of ESBL producing uropathogens belonging to enterobacteriaceae, isolated from patients with suggestive symptoms of urinary tract infection.*

Method: *Urinary isolates from symptomatic UTI cases were identified by conventional methods. A total of 1244 enterobacteriaceae isolated during October 2015 to October 2017. Antimicrobial susceptibility testing was done by Kauber-Bauer Disc Diffusion method following CLSI guidelines. Isolates resistant to cefotaxime (<_19mm) were tested for ESBL production by double disc synergy method.*

Result: *The most common isolated species was E.coli 943(76%) followed by Klebsiella spp. 227(18%) and other enterobacteriaceae 74(6%). Of all enterobacteriaceae, 673 (57%) among 1244 were ESBL producer. ESBL production was detected in 564(84%) E.coli, 92(14%) Klebsiella spp. and other enterobacteriaceae17(2%). More than 90% of the isolates were sensitive to amikacin, cefoperazone-sulbactam, imipenem and 36% isolates were sensitive to norfloxacin.*

Conclusion: *In the present study a large number of uropathogens were found to be ESBL producer. Monitoring ESBL production and antimicrobial susceptibility testing are necessary to avoid treatment failure in patients with urinary tract infection.*

Keywords: *Antibiotic susceptibility, ESBL, Urinary tract infection, Uropathogens.*

Introduction

Urinary tract infections (UTIs) are one of the most prevalent extra intestinal bacterial infections. Nowdays, it represents one of the most common diseases encountered in medical practice affecting people of all ages from the neonate to geriatric age group.[1]

The Extended Spectrum beta lactamase (ESBL) producing bacteria are increasingly causing urinary tract infections. ESBLs have been found most commonly in uropathogens, like *Escherichia coli* and *Klebsiella pneumoniae*. Other enterobacteria and non- fermenting Gram negative rods also produce ESBLs but to a lesser extent.[2,3]

Extended Spectrum beta lactamases (ESBLs) were first described in 1983. They are able to hydrolyseoxyimino-cephalosporins (for example cefotaxime, ceftazidime and ceftriaxone) and monobactams (for example aztreonam), but not cephamycins or carbapenems. A variety of beta lactamases have been classified into class A, B, C and D according to their amino acid homology. ESBLs are class A enzymes which are inhibited in vitro by beta lactamases inhibitors such as clavulanic acid, sulbactam and tazobactam where those belonging to class B, C and D are not affected.[4]

ESBL producing uropathogens making therapy of UTI difficult and promoting greater use of expensive broad spectrum antibiotics such as carbapenems. Detection of ESBLs using conventional antimicrobial susceptibility methods and delay in the detection and reporting of ESBLs production by Gram negative bacilli are associated with prolonged hospital stay, increased morbidity, mortality and health care costs.[5]

The distribution of uropathogens and their susceptibility pattern to antibiotics vary regionally. Therefore the knowledge on the frequency of the causative microorganisms and their susceptibility to various antibiotics are necessary. Hence, the present retrospective study of the uropathogens belonging to enterobacteriacae family and their susceptibility pattern in patients with UTI in a rural tertiary care hospital has been taken.[6,7]

Material and Methods

A total of 1244 clinical isolates of enterobacteriaceae from symptomatic UTI patients were studied retrospectively for ESBL activity from October 2015 to October 2017. No mixed infections were taken. Only one specimen per patient was studied.

Urine specimens from patients diagnosed clinically to be having UTI on the basis of symptoms (fever, dysuria and increased frequency of urination) were inoculated on Blood agar and MacConkey agar plates, which were incubated aerobically at 37^0c overnight.[8]

Plates showing growth suggestive of significant bacteriuria, with colony counts exceeding 10^5cfu/ml were subjected for standard biochemical tests for identification.[8]

Inclusion Criterion- The samples which yielded enterbacteriaceae were included in the study.

Exclusion criterion- The samples which donot yield enterobacteriaceae were excluded in the study.

Antimicrobial agents- The antibiotics tested for both ESBL and non ESBL pathogen were Amikacin (30 mcg), Cefoperazone- Sulbactam (75/30 mcg), Cefepime (30 mcg), Norfloxacin(30 mcg), Imipenem (10 mcg).

Antibiotic susceptibility testing- Antibiogram of the isolates was done by Kirby Bauer method [9] using antibiotics from Hi Media, Mumbai. The above mentioned antibiotics were used. The results were interpreted as per National Committee for Clinical Laboratory Standard Recommendations (NCCLS).[10]

Double disc synergy test-The organisms that were suspected as ESBL producers were tested for ESBL production by double disc synergy test (DDST). The test inoculums (0.5 McFarland's turbidity) was spread onto Mueller- Hinton agar by using a sterile cotton swab. A disc of augmentin (20 mcg amoxicillin + 10 mcg clavulanate) was placed on the surface of the

Mueller Hinton agar, then disc of cefotaxime (30mcg) was kept 15mm apart from the augment-in disc. The plate was incubated at 37^0c overnight. The enhancement of the zone of inhibition of the cephalosporin disc towards the clavulanic acid disc was inferred as synergy and the strain was considered as an ESBL producer.[11]

Quality Control- Simultaneous tests with non ESBL producing organism (*Escherichia coli* ATCC 25922) and an ESBL producing organism (*Klebsiella pneumoniae* ATCC 700603) were performed.

Results

The present study was conducted in the Department of Microbiology at the Index Medical College, Indore, Madhya Pradesh from October 2015 to October 2017, to know the prevalence of ESBL producing enterobacteriaceae.

A total of 1244 *Enterobacteriacae spp*. were identified from urine samples of patients showing symptomatic UTI. Data was analyzed and compiled in Microsoft Excel word. Figure 1 and 2 shows, different members of family enterobacteriacae isolated from urine samples. *E. coli* was the most common isolate 943(76%) followed by *Klebisella spp. 227(18%*) and other *Enterobacteriacae spp.*74 (6%) which include, *Proteus spp.*, *Enterobacter spp.*, *Citrobacter spp.* and *Morganella morganii.*

A total of 673 isolates were ESBL producers, based on phenotypic confirmation which was carried out by disk diffusion assay as per the recommendations of NCCLS.[10]The zone of inhibition of the antibiotic alone was compared with the zone of inhibition in combination with clavulanic acid. According to NCCLS recommendations a difference of ≥5 mm increase in zone diameter for either agent tested in combination with clavulanic acid versus its zone diameter when tested alone confirms the presence of ESBLs. *E.coli* was the most common ESBL producer 564 (84%), followed by *Klebsiella spp* 92 (14%) and others 17(2%) as shown in the figure 2.

The antibiotic sensitivity pattern of *E.coli* showed that 73% were sensitive to imipenem, amikacin and cefoperazone-sulbactam while 26% to norfloxacin and 25% to cefipime. *Klebsiella spp.* showed the similar pattern, 98% sensitive to imipenem, 94% to amikacin and cefoperazone-sulbactam while 54% to norfloxacin and 50% to cefipime. Other *Enterobacteriacae spp*. followed the similar sensitivity pattern. The overall antibiotic susceptibility pattern of the isolates showed that 98% were sensitive to imipenem, 95% were sensitive to amikacin and cefoperazone – sulbactam and 39% were sensitive to norfloxacin and cefepime (as shown in the table 1 and figure 3)

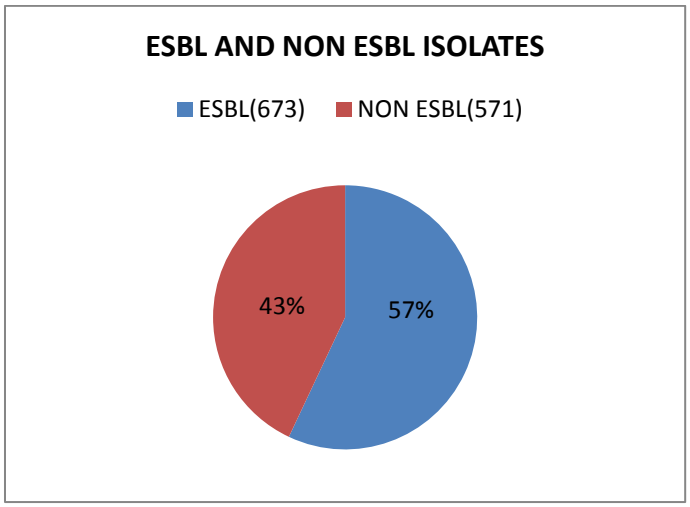

Figure 1- Percentage of ESBL and Non-ESBL isolates

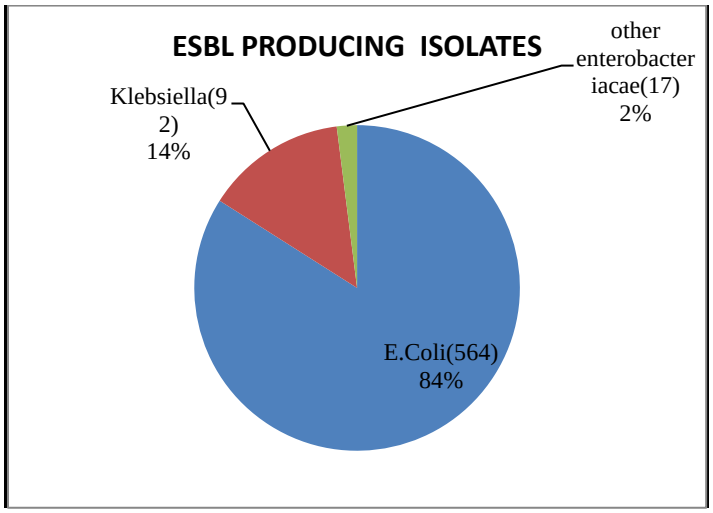

Figure 2- Percentage of ESBL producing isolates belonging to Enterobacteriacae family

Table 1- Antimicrobial drug susceptibility of urinary Enterobacteriaceae isolates in patients with UTI at a tertiary care hospital

Drugs ⇒ Isolates ⇓	Amikacin	Norfloxacin	Cefoperazone-sulbactam	Cefipime	Imipenem
E.coli	910(73%)	322(26%)	907(73%)	318(25%)	910(73%)
Klebsiella spp.	212(94%)	124(54%)	212(94%)	114(50%)	222(98%)
Proteus spp.	22(88%)	16(64%)	22(88%)	16(64%)	24(96%)
Enterobacter spp.	19(86%)	16(72%)	20(90%)	14(63%)	22(100%)
Citrobacter spp.	22(100%)	18(81%)	22(100%)	18(81%)	22(100%)
Morganella morganii	4(80%)	4(80%)	5(100%)	4(80%)	5(100%)

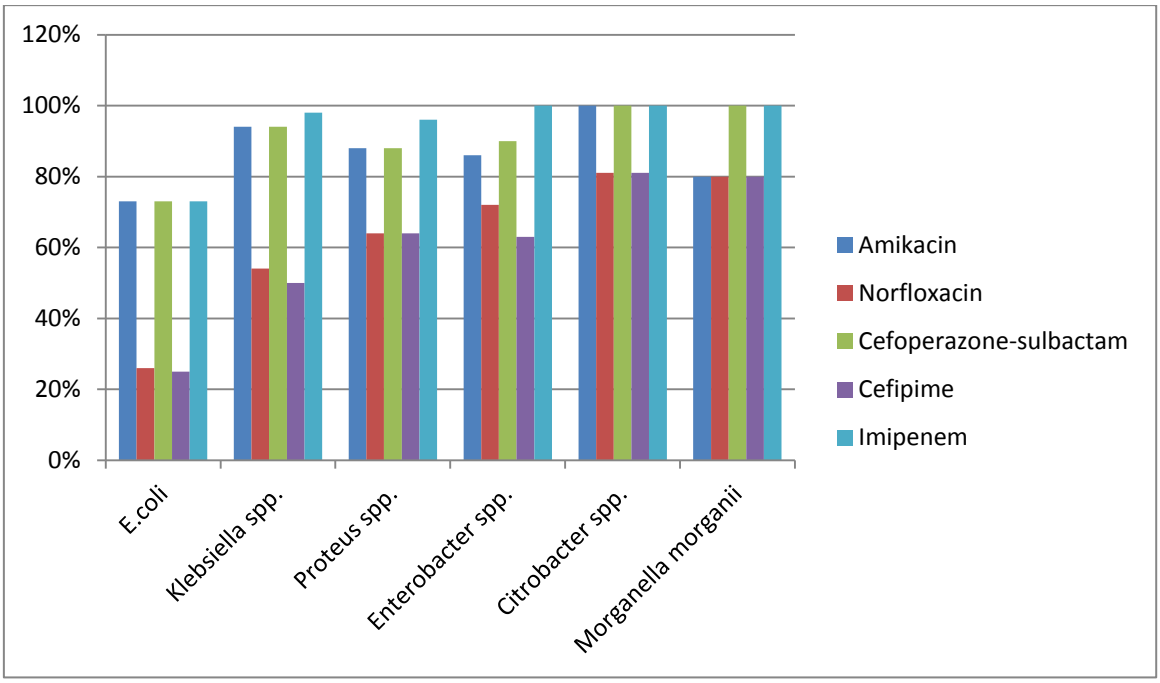

Figure 3-Percentage showing antibiotic sensitivity pattern of Enterobacteriacae spp.

Discussion

Antibiotics are usually given empirically before laboratory results of urine culture are available. To ensure appropriate therapy current knowledge of the organism that cause UTI and their antibiotic susceptibility is mandatory.[12]

The spread of ESBL producing bacteria has been strikingly rapid worldwide, indicating that continuous monitoring is required. The extended spectrum beta lactamases are mutant, plasmid mediated beta lactamases which are derived from the broad spectrum β lactamases.[13]

These enzymes are the result of the mutation of the TEM1 and TEM2 and the SHV-1 enzymes. All of the beta lactamase enzymes are commonly found in enterobacteriaceae family. Normally the TEM-1, TEM-2 and the SHV-1 enzymes confer a high level of resistance to the early penicillins and a low level resistance to the first generation cephalosporin. The wide spread use of the third generation cephalosporin and aztreonam is believed to be the major cause of the mutations in these enzymes, that has led to the emergence of the ESBLs. Because of their greatly extended substrate range, these enzymes were called as the extended spectrum beta lactamases.[14]

In this study, *Escherichia coli* (76%) was the most common pathogen followed by *Klebsiella spp.*

(18%) and others (6%).Many studies from different parts of India also reported *E.coli* and *K. pneumoniae* as the most common enterobacteriaceae causing urinary tract infection. Gunti et.al[15] from Andhra Pradesh reported 85% *E.coli* and 14% *K. pneumoniae*, Selvakumar et.al [16] from Tiruchirappalli reported 44% *E.coli* and 14% *K. pneumonia*, Babypadmini et.al [17] from Chennai reported 49% *E.coli* and 8% *K. pneumoniae* and Tankhiwale et.al [18] from Nagpur too reported the prevalence of 49.8% *E.coli* and 37.8% *K. pnuemoniae* in urinary tract infection, which is well comparable to the reports from our study. Studies from the neighbouring countries Nepal and Pakistan also reported *E.coli* as the most common pathogen followed by *Klebsiella spp*. Shakya et.al from Nepal showed *E.coli* (80.9%) as the most common pathogen followed by *Klebsiella spp.* (4.5%).[23] Hassan et.al from Pakistan also reported *E.coli* (80%) as the most prevalent bacteria causing urinary tract infection.[24] So reports from the neighbouring countries is also well comparable to this study.

The prevalence of ESBL producing organisms in this study was found to be 57%. Previous studies from India have reported the prevalence of ESBL producers to be 6.6% to 91%. The wide variation in the prevalence is probably due to the variation in the risk factors and in the extent of antibiotics use. Subha et.al [19] reported 6.6% ESBL prevalence among *K. pneumoniae* in children from Chennai whereas Wattal et.al[20] observed 91.7% higher incidence of ESBL production from New Delhi. Tankhiwale et.al[18] also reported 48.3% overall ESBL production which was similar to our study.

The occurrence of ESBL production in the present study was 84% in *E.coli* and 14% in *K. pneumoniae* and 2% in others. Two studies from Andhra Pradesh reported ESBL production in *E.coli* was high followed by *K.pneumoniae*, include studies from Kumar et.al[21]and Gunti et.al[15]which is well comparable to the present study. Babypadmini et.al[17] reported ESBL productivity in *E.coli* 41% and in *K. pneumoinae* 40% whereas Selvakumar et.al[16]reported 42.37% *E.coli* and 15.25% *K. pneumoinae* to be ESBL producer. Studies from Nepal, Pakistan and Srilanka also reported ESBL production in *E. coli* was high.[23,24,25]

According to Mandel et.al[22] reports from India, *E.coli* as the commonest cause of UTI and antibiotic resistance was high among the strains, which emphasizes the need for the judicious use of antibiotics. Certain virulence factors like haemolysin production and presence of fimbriae in *E.coli* may be associated with urovirulence.

Antibiotic susceptibility pattern of enterobacteriaceae was studied by choosing one antibiotic each from amino glycoside group (amikacin), fluoroquinole group (norfloxacin), fourth generation cephalosporin (cefepime), carbapenem group (imipenem) and combined therapy (cefoperazone- sulbactam). Susceptibility to amikacin and cefoperazone-sulbactam was found to be 95% and with imipenem 98%. Only 39% isolates were sensitive to norfloxacin and cefepime. Babypadmini et.al[17]reported 90.75% isolates were sensitive to amikacin and 100% isolates were sensitive to imipenem which was similar to our study. Shakya et.al[23], Hassan et.al[24], Tillekeratne et.al[25], Somashekara et.al[26], Basavaraj C et.al [27]and Selvakumar et.al [17] also observed higher sensitivity of uropathogens belonging to enterobacteriacae family to amikacin and imipenem.

So, present study advocates the use of carbapenem antibiotic as a therapeutic alternative in the patients of urinary tract infection showing increasing resistance rates which were observed with the conventional beta lactam and non-beta lactam antibiotics.

Conclusion

This retrospective study furnished the details about common uropathogens belonging to enterobacteriacae family and their antibiotic sensitivity pattern. From the study, it is clear that, *E. coli* is still the most common bacteria causing urinary tract infection. Imipenem and amikacin are most effective and may be prescribed for the

treatment of infections caused by ESBL strains. The increased clinical threat of ESBL prevalence is creating significant therapeutic problems. There is an immediate need to formulate strategic policy initiatives to reduce their prevalence and there should be a manual of antibiotic policy of the individual institute. This will avoid the indiscriminate use of antibiotics and prevent the further development of antimicrobial resistance.

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