

PHENOTYPIC DETECTION OF EXTENDED-SPECTRUM BETA LACTAMASES (ESBLs) PRODUCING ORGANISMS AMONG ENTEROBACTERIACEAE AND THEIR ANTIBIOTIC RESISTANCE PATTERN IN URINARY TRACT INFECTION (UTI) AT KUMAUN REGION, UTTARAKHAND

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ABSTRACT

BACKGROUND

Urinary tract infection (UTI) is one of the major health problems affecting all age groups. Extended Spectrum β -Lactamases (ESBLs) producing Enterobacteriaceae exhibits a resistant mechanism, which challenges the strategies of broad-spectrum antibiotics used in treatment of UTI, thus limiting therapeutic options. Knowing resistance pattern of such multidrug resistant pathogens will help in the appropriate usage of antimicrobial agents.

The study was carried out to determine the prevalence of ESBL among the Enterobacteriaceae uropathogens and their antimicrobial resistance pattern.

MATERIALS AND METHODS

All the uropathogenic isolates obtained from symptomatic UTI cases were identified by conventional methods. The prevalence of potential ESBL producers among Enterobacteriaceae isolates was explored. Antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method and the results were interpreted according to Clinical Laboratory Standards Institute 2014 guidelines. Study Design- Descriptive study.

Place and Duration of Study- Department of Microbiology, Government Medical College and Hospital, Haldwani from November 2013 to September 2015.

RESULTS

A total of 695 clinical isolates were obtained from 2438 urine samples. Out of these 695 isolates 454 (65.32%) isolates were from Enterobacteriaceae family, out of which a total of 188 (41.4%) isolates were ESBLs producing Enterobacteriaceae which predominantly comprised of *Klebsiella pneumoniae* (65.71%) followed by *Escherichia coli* (40.56%) and *Proteus* species (34.28%). A high degree of resistance to ampicillin (98.4%) and cotrimoxazole (93.08%) were found among these ESBL producing Enterobacteriaceae isolates followed by gentamicin (58.51%) and ciprofloxacin (55.31%). Nitrofurantoin and Amikacin were found to be most effective drugs.

CONCLUSION

A large number of uropathogens were found to be ESBL producers in the present study. Most of the ESBL producing isolates were multidrug resistant. Monitoring of ESBL production, judicious use of antibiotics and infection control measures are necessary to avoid treatment failure in patients with UTI.

KEYWORDS

Prevalence, Urinary Tract Infections, Extended Spectrum β -Lactamases, Enterobacteriaceae, *Klebsiella Pneumoniae*, *Escherichia Coli*.

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BACKGROUND

Extended-spectrum β -lactamase (ESBLs) enzymes are plasmid-mediated enzymes with capacity to hydrolyse and thus inactivate broad-spectrum β -lactam antibiotics, which in turn confer a decreased susceptibility against commonly used antibiotic drugs, such as penicillins and extended-spectrum cephalosporins.^[1]

A wide range of Enterobacteriaceae family members express Extended-spectrum β -lactamase (ESBLs) enzyme. The prevalence of extended spectrum beta-lactamase (ESBL) producing organisms among clinical isolates vary greatly worldwide and is rapidly changing over time. Urinary tract infection is a common bacterial disease, often contributes to a frequent cause of morbidity in outpatients as well as hospitalised patients.^[2] Although, UTIs occur in both men and women, clinical studies suggest that the overall prevalence of UTI is higher in women. The introduction of antimicrobial therapy has contributed significantly to the management of UTIs. Still the major problem with routine antibiotic therapies is the rapid emergence of antimicrobial resistance in clinical settings and the community.^[3] The resistance pattern of community acquired uropathogens has not been extensively studied in the Indian subcontinent.^[4-6] Detection of ESBL producing organism from samples such as urine may be important, because this represents an epidemiologic marker

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of colonisation and therefore there is potential for transfer of such organisms to other patients.^[7] The study was carried out to determine the prevalence of ESBL among the Enterobacteriaceae uropathogens and knowing resistance pattern of such multidrug resistant pathogens will help in the appropriate usage of antimicrobial agents and prevent further emergence of resistant strains.

MATERIALS AND METHODS

A Descriptive study of total 2438 urine samples were processed from symptomatic UTI cases attending or admitted to the Government Medical College and Hospital, Haldwani from November 2013 to September 2015. Urine samples were inoculated onto cystine lactose electrolyte-deficient agar (CLED agar) using a fixed volume loop (0.01 mL). Culture plates were aerobically incubated at 37°C for 18 to 24 hrs. and examined for growth of pathogenic microorganisms. Colony count of $\geq 10^5$ CFU/ mL was considered to be significant. The antibiotic discs (concentrations in μg) included in the panel for Enterobacteriaceae isolates were ampicillin (10 μg), gentamicin (10 μg), amikacin (30 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), norfloxacin (10 μg), nitrofurantoin (300 μg) and cotrimoxazole (1.25/ 23.75 μg). Ceftazidime (30 μg) and ceftazidime/clavulanic acid (30 $\mu\text{g}/10 \mu\text{g}$) were included with antibiotics panel to screening as well as for detection of ESBL producing Enterobacteriaceae. E-test strip was applied for confirmation of ESBL producing Enterobacteriaceae isolates. The discs were provided by Hi-Media Laboratories Pvt. Ltd., Mumbai.

Tests for ESBL Production in Members of Family Enterobacteriaceae

Isolates of family Enterobacteriaceae that were considered to be positive for ESBL production by the phenotypic confirmatory disc diffusion test (PCDDT) were subjected to the E-test-

1. **CLSI Phenotypic Confirmation Test[®]**- The ceftazidime (30 μg) discs alone and in combination with clavulanic acid (ceftazidime + clavulanic acid, 30/10 μg disc) were applied onto a plate of Mueller-Hinton Agar (MHA), which was inoculated with the lawn culture of the test strain. The plates were incubated overnight at 37° C. An increase of ≥ 5 mm in the zone of inhibition of the combination disc in comparison to the ceftazidime disc alone was considered to be a marker for ESBL production.
2. **E-test**- Minimum inhibitory concentration (MIC) was determined for the ESBL Enterobacteriaceae isolates by E-test using ESBL strips (HiMedia Laboratories Pvt. Ltd., Mumbai). The ESBL E-strip is a plastic drug-impregnated strip, strips were impregnated with cefotaxime (CTX) at one end and cefotaxime + clavulanic acid (CTX+) at another end. A lawn culture of the test organism was inoculated on Mueller-Hinton Agar (MHA). The E-test ESBL strip was placed on the centre of the plate. The plates were incubated aerobically at 37°C for 16 - 18 hours. The MIC was interpreted as the value at the intersection of the growth ellipse with the strip. The isolate was confirmed to be an ESBL producer when the ratio of the MIC value of cefotaxime to the MIC value of

cefotaxime in combination with clavulanic acid was more than 8. The ESBL production was also confirmed when no zone was obtained for cefotaxime, but zone was observed in cefotaxime and clavulanic acid combination.

Control

Escherichia coli ATCC 25922 were used as the negative control and Klebsiella pneumoniae ATCC 700603 was used as the positive control.

Date Entry and Analysis

Data entry will be done in MS Excel and Statistical analysis was done. Among the Enterobacteriaceae isolates obtained from sample, screening for ESBL producing Enterobacteriaceae was done and quantified in percentages and tabulated. Antibiotic resistance pattern of ESBL producing Enterobacteriaceae isolates obtained was quantified in percentage and tabulated.

RESULTS

A total of 695 clinical isolates were obtained from 2438 urine samples. Out of these 695 isolates 454 (65.32%) isolates were from Enterobacteriaceae family, out of which a total of 188 (41.4%) isolates were ESBLs producing Enterobacteriaceae.

Among the ESBL producing Enterobacteriaceae, Escherichia coli was found in maximum number of isolates. Whereas the higher prevalence detected in Klebsiella pneumoniae (65.71%) followed by Escherichia coli (40.56%), Klebsiella oxytoca (37.5%), Proteus species (34.28%), Citrobacter freundii (29.41%) and Providencia rettgeri (25%). A high degree of resistance to ampicillin and cotrimoxazole were found among these ESBL producing Enterobacteriaceae isolates (98.4% and 93.08% respectively). High degree of resistance was also seen with fluoroquinolones and gentamicin. The most susceptible drug against these strains was nitrofurantoin and amikacin, which showed 15.95% and 25% resistances respectively.

Organisms	Enterobacteriaceae Isolates (n)	ESBL Producing Enterobacteriaceae	Percentage (Enterobacteriaceae Isolates/ Gram Negative Bacilli)
E. coli	355	144	40.56%
Klebsiella pneumoniae	35	23	65.71%
Klebsiella oxytoca	8	3	37.5%
Proteus mirabilis	23	10	43.47%
Proteus vulgaris	12	2	16.66%
Citrobacter freundii	17	5	29.41%
Providencia rettgeri	4	1	25.0%
Total	454	188	41.4%

Table 1. Screening of ESBL producing Enterobacteriaceae Isolates

Antibiotics	Resistance Isolates (n)	Percentage (%)
Ampicillin	185	98.4%
Gentamicin	110	58.51%
Amikacin	47	25%
Levofloxacin	77	40.95%
Ciprofloxacin	104	55.31%
Norfloxacin	130	69.14%
Nitrofurantoin	30	15.95%
Cotrimoxazole	175	93.08%

Table 2. Antibiotic Resistance Pattern of ESBL producing Enterobacteriaceae Isolates

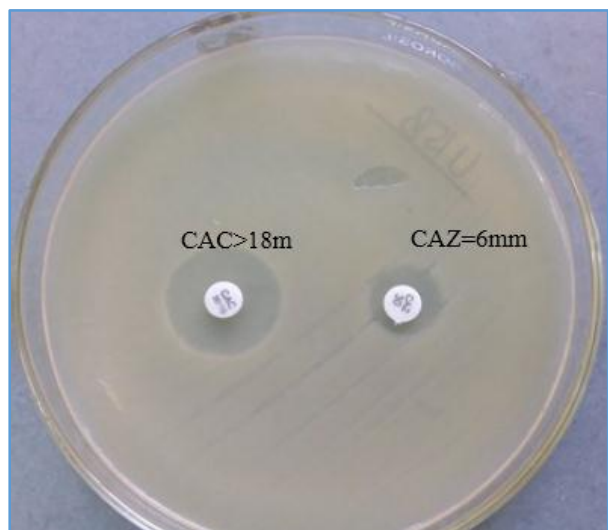


Figure 1. A Photograph showing Production of ESBL by Phenotypic Confirmatory Disc Diffusion Test, Ceftazidime Clavulanic Acid (CAC) and Ceftazidime (CAZ)

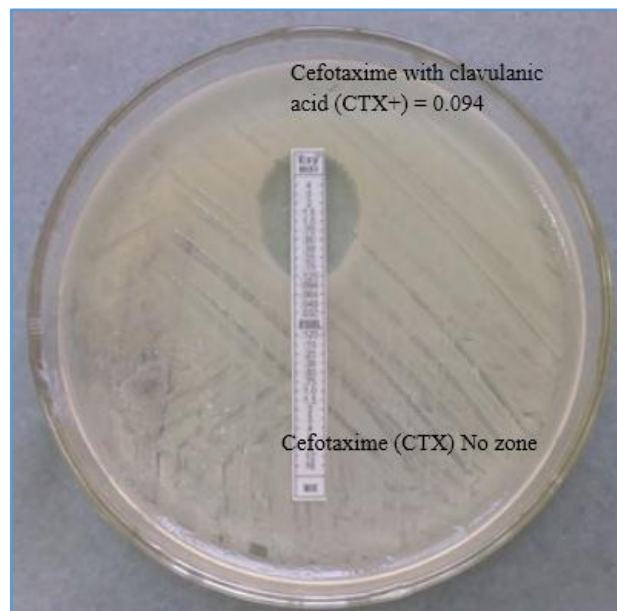


Figure 2. A Photograph showing Production of ESBL by E-Test

DISCUSSION

In the current era, extended-spectrum β-lactamases (ESBLs) organisms are a major global problem in the clinical and community settings due to the increasing use of broad-spectrum antimicrobial agents. The total prevalence of ESBL

producing Enterobacteriaceae was found to be 41.4% in our study, which was approximately equal to Tillekeratne et al^[9] and Metri et al,^[10] which reported the ESBL prevalence of 40.2% and 39.1% respectively in urine specimen among Enterobacteriaceae. Hanan A et al^[11] and Shashwati et al^[12] also reported 51% and 50% prevalence respectively. Mohanty et al^[13] reports the higher prevalence of 68.78% for ESBL producing Enterobacteriaceae organisms. This study showed the degree of ESBL prevalence is high among Klebsiella pneumoniae (65.71%) followed by E. coli (40.56%), Proteus spp. (34.28%), Citrobacter freundii (29.41%) and Providencia spp. (25%). Afridi F et al^[14] study showed highest frequency for ESBL production in Klebsiella species (84.61%) followed by Escherichia coli (68.55%), Enterobacter species (36.84%) and Proteus mirabilis (28.57%). Ahmed et al^[15] reported frequency rates among Klebsiella species (40%) followed by Escherichia coli (30%), Proteus spp. (16%) and Enterobacter species (14%). Khurana et al^[16] reported Klebsiella species prevalence of 38.5% followed by 24.7% of Escherichia coli in urinary isolates from hospitalised patients, while Mathur et al^[17] reported 80% prevalence of Klebsiella species as a most frequent ESBL producing organism. In a study concluded by Shobha et al,^[18] she states that Citrobacter spp. was the third most common urinary pathogen and 30% of the isolates were extended spectrum beta lactamase (ESBL) producers. The prevalence of ESBL producers was found to vary greatly in different areas of India. ESBL producing strains often arise in focal outbreaks. Regional and local estimates are probably more useful than are more global assessments in clinical decision making and for infection control purpose.^[14] All isolates were applied for antibiotics sensitivity testing and nitrofurantoin was found to be the most effective drug. It showed 15.95% resistance to all isolates, whereas Amikacin was the second most effective drug which showed 25% resistance. Resistance to ampicillin was 98.4% followed by cotrimoxazole (93%), norfloxacin (69.14%), gentamicin (58.51%), ciprofloxacin (55.31%) and levofloxacin (40.95%). Garcia A et al^[19] reported nitrofurantoin was a most effective drug, it showed 10.9% resistance. This study also reported 62%, 84.8% and 37% resistance to cotrimoxazole, ciprofloxacin and gentamicin respectively. Shashwati N et al^[12] showed 50% resistance against gentamicin, 87.5% to ciprofloxacin and 94.65% to trimethoprim/ sulfamethoxazole. This study suggests that the use of these antibiotics for common illness should be avoided and the drug should be reserved as a second line drug. Nitrofurantoin, which is excreted primarily in urine and used also to treat UTI in pregnancy had also been found as the most effective drug against these isolates. Among other antibiotics, resistant isolates against norfloxacin and ciprofloxacin were found to be more than the aminoglycosides antibiotics. Therefore, aminoglycosides antibiotics can be used to treat the urinary infections on the empirical basis. ESBL producing Enterobacteriaceae are on rise and there are various factors that attribute to such kind of resistance. In this study, ESBL producing Enterobacteriaceae were studied in urinary samples and factors that can lead to rise in this type of resistance in urinary tract infections include old age, prolong bladder catheterisation, recurrent infection, long hospital stay, irrational use of antimicrobial agents, non-compliance of patient and presence of comorbidities.

CONCLUSION

Multidrug resistance was significantly higher in ESBL positive isolates. Knowledge of the prevalence of ESBLs and resistance pattern of bacterial isolates in a geographical area is of utmost importance. The sensitivity pattern of microorganisms to various antibiotics varies over time and among different geographical locations. Therefore, continuous analysis of the antibiotic resistance pattern acts as a guide in initiating the empirical treatment of UTI and the therapy must be started. Only urine culture and sensitivity have been done. It helps in avoiding the treatment failure.

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