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Antimicrobial Activity of Multi Drug Resistance Escherichia Coli Causing Urinary Tract Infection in Tertiary Care Hospital from Central India.

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KEYWORDS

Extended-spectrum beta-lactamases, AmpC beta-lactamases, Urinary tract infections

ABSTRACT:

Background: Escherichia coli can cause disease and is responsible for most community-acquired (as opposed to hospital-acquired) urinary tract infections (UTIs). Treatment can be complicated by antimicrobial resistance, particularly against extended-spectrum Cephalosporin's. Increasingly, enzymes, such as AmpC beta-lactamases (ACBLs) and extended-spectrum beta-lactamases (ESBLs), have been reported in pathogenic bacteria, including E. coli. Resistance to beta-lactams is also associated with multidrug resistance.

Aim: The present study was conducted to evaluate the role of antimicrobial activity for UTI patients caused by ESBL and AmpC producing E. coli isolate.

Materials and Methods: This observational hospital-based study was conducted in the Department o f Microbiology, Index Medical College, Hospital and Research Centre Indore MP,

India. The study population included 1000 of all age. Clinically suspected cases of urinary tract infection were included. Antimicrobial susceptibility of isolates was tested for uropathogens by using Kirby Bauer disk diffusion method according to CLSI guide lines.

Results: Out of (83.4%) Escherichia coli sensitivity 19 (54.3%) were major ESBL producer. And 16 (45.7%) was the major AmpC producer Escherichia coli.

Conclusion: In c o n c l u s i o n , t h e p r e s e n t results suggest that the increasing drug-resistant isolates of ESBL producing E. coli to commonly used antibiotics like fluoroquinolones, cephalosporins and other β-lactams. This trend of rise in isolation of MDR uropathogens poses a challenge to the current armamentarium for the

treatment of UTIs.

INTRODUCTION

Escherichia coli is a commensal microorganism found both inside and outside the mammalian large intestine and is commonly used as an indicator of fecal contamination.[1]

E. coli can cause disease and is responsible for most community-acquired (as opposed to hospital-acquired) urinary tract infections (UTIs).[2]

Treatment can be complicated by antimicrobial resistance.[3] particularly against extended-spectrum cephalosporins.[4]

Increasingly, enzymes, such as AmpC beta-lactamases

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(ACBLs) and extended-spectrum beta- lactamases (ESBLs), have been reported in pathogenic bacteria, including E. coli. Resistance to beta- lactams is also associated with multidrug resistance.[5]

MATERIALS AND METHODS

This observational hospital-based study was conducted in the Department of Microbiology, Index Medical College, Hospital and Research Centre Indore MP, India.

The study population included 1000 of all age group and either sex whom was sample was received in microbiology laboratory. Clinically suspected cases of urinary tract were included in the study.

The samples from patients with ongoing antibiotic, repeat samples of the same patient and samplesthat are not labelled properly and are contaminated were excluded from the study.

Sample was processing semi quantitative culture of sample was done within two hours of receipt.Using a 4 mm calibrated nichrome loop, a 0.001 mL loopful of urine was inoculated on Cystine- Lactose Electrolyte Deficient (CLED) agar.

The inoculated plates were then aerobically incubated for 18- 24 hours at 37°C. Growth on CLED was assessed for significant bacteriuria with colony-forming units ≥105/mL of pure growth of single isolates.[6]

The isolated organisms *E. coli* was confirmed by using standard biochemical tests. Vitek-2 Compact (BioMerieux, France) was employed for identification of the isolates and their Antimicrobial Susceptibility Testing (AST).

The ESBL and AmpC isolates were verified by the CLSI 2019 guidelines using the Advanced Expert System of VITEK-2 automated system; based on analysis of MIC patterns.[7]

Antimicrobial susceptibility of isolates was tested for uropathogens by using Kirby Bauer disk diffusion method according to Clinical Laboratory Standards Institute (CLSI) guide lines.

A standard inoculum adjusted to 0.5 McFarland is swabbed on to Muller-Hinton agar; antibiotic disc is dispensed after drying the plate for 3–5 min and incubated at 37°C for 18- 24 hours.

The CLSI advocates use of cefotaxime (30 μg) or ceftazidime (30 μg) disks with or without clavulanate (10 μg) for phenotypic confirmation of the presence of ESBLs in *Escherichia coli.*

STATISTICAL ANASYSIS

Microsoft Excel was used in creating the database and producing graphs, while the data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 23.0 for Windows.

RESULT

From the 1000 non-duplicate samples were received in the Department of Microbiology for culture and antibiotic sensitivity. Out of 1000 cases, majority (69.3%) of patients were in age group 20-49 years followed by 50-80 years that constituted 18.3% of study population.12.1% were ≤19 years and least 0.3% were >80 years of age [Table-1].

Table 1: Classification of studied patients on the basis of age group

Age group	Frequency(n)	Percentage (%)
≤19	121	12.1
20-49	693	69.3
50-80	183	18.3
>80	3	0.3
Total	1000	100.0

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In present study, out of 1000 studied samples, in 738 (73.8%) cases organism were unidentified.Escherichia coli was the highest scorer and isolated in 162 (16.2%) of cases.

Acinetobacter 25 (2.5%), YLC 7 (0.7%), YLC resembling Candida 20 (2.0%), Pseudomonas aeruginosa 20 (2.0%), Klebsiella pneumonia 17 (1.7%) were major isolates.

Some other isolates included Citrobacter freundii 3 (0.3%), Proteus mirabilis 3(0.3%), Proteus vulgaris 3 (0.3%), Klebsiella oxytoca 1 (0.1%) and Citrobacter koseri 1 (0.1%) [Table-2].

Table 2: Distribution of isolated Uropathogens in the study

Isolated organism	**Frequency(n=262)**	**Percentage (%)**
Acinetobacter	25	9.5
Citrobacter freundii	3	1.1
Citrobacter koseri	1	0.4
Escherichia coli	162	61.8
Klebsiella oxytoca	1	0.4
Klebsiella pneumoniae	17	6.5
Proteus mirabilis	3	1.1
Proteus vulgaris	3	1.1
Pseudomonas aeruginosa	20	7.6
YLC	7	2.7
YLC resembling Candida	20	7.6

In this table we noted that the ESBL producing in 54.3% *E. coli* culture and AmpCproducing in 45.7% *E. coli* culture [Table-3].

Table 3: Shows the uropathies that were AmpC producers and ESBL producers

MBL Producer	***Escherichia coli***	
	Frequency (n)	**Percentage (%)**
ESBL	**19**	**54.3**
AmpC	**16**	**45.7**

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Total	**35**	**100.0**

Below table highlights the drug sensitivity of various drugs on isolated uropathogens. Number of drug like piperacillin/tazobactam (94.4%), cefoperazone-sulbactam (92.6%),

amikacin (87.7%), polymyxin-B (98.1%), colistin (88.9%), imipenem (84.0%), ertapenem (85.2%), meropenem (97.5%) and nitrofurantoin (95.7%) sensitive against *E coli*. While ampicillin (78.4%), norfloxacin (66.0%), ciprofloxacin (61.1%), ceftriaxone (58.0%) and ceftazidime (56.8%) are the most resistance drug in UTI infection organism *E. coli*. Few urine sample cases were also seen intermediate stage in this condition there was more exposures to get result [Table- 4].

Table 4: Percentage distribution of drug sensitivity by pathogens *Escherichia coli.*

Antibiotic Drug	**Sensitive (%)**	**Intermediate (%)**	**Resistance (%)**
Ampicillin	35 (21.6)	0 (0.0)	127 (78.4)
Ceftriaxone	68 (42.0)	0 (0.0)	94 (58.0)
Ceftazidime	70 (43.2)	0 (0.0)	92 (56.8)
Piperacillin/Tazobactam	153 (94.4)	0 (0.0)	9 (5.6)
Cefoperazone-Sulbactam	150 (92.6)	4 (2.5)	8 (4.9)
Ciprofloxacin	59 (36.4)	4 (2.5)	99 (61.1)
Norfloxacin	52 (32.1)	3 (1.9)	107 (66.0)
Gentamicin	114 (70.4)	1 (0.6)	47 (29.0)
Amikacin	142 (87.7)	5 (3.1)	15 (9.3)
Polymyxin-B	159 (98.1)	1 (0.6)	2 (1.2)
Colistin	144 (88.9)	16 (9.9)	2 (1.2)
Imipenem	136 (84.0)	2 (1.2)	24 (14.8)

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Ertapenem	138 (85.2)	8 (4.9)	16 (9.9)
Meropenem	158 (97.5)	1 (0.6)	3 (1.9)
Tigecycline	128 (79.0)	27 (16.7)	7 (4.3)

DISCUSSION

Urinary tract infection (UTI) is one of the most common infectious diseases diagnosed in outpatients, with a high rate of annual global incidence.

The incidence of UTI is more common among boys until the age of 12 months; however, the pooled prevalence rate of febrile UTI in females is about 3-fold of circumcised males and UTI occurrence increases among uncircumcised male infants.

Escherichia coli (E coli) is by far the most commonly isolated organism in pediatric UTI with prevalence ranging from 80% to 90% followed by others such as Enterococcus species (spp.), Enterobacter spp., Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, and Staphylococcus spp. [8]

Antibiotic sensitivity and resistance pattern vary over time and places. High resistance to antimicrobials like ampicillin, co-trimoxazole and nalidixic acid and a possible reason could be these antibiotics were in general use for a long period.[9]

We have opted for the cross-sectional study as we have to check the prevalence of the identified organism's concomitant with urinary tract infection in clinically suspected patients attending the in- patient and out-patient department of tertiary care unit. A similar study was performed by **Gupta K etal. [10]** to analyze the antimicrobial resistance among uropathogens. **Raghuvanshi BR et al. [9]**

has opted for the cross-sectional study to analyze the patients while **Pouladfar G et al. [8]** also studied the cross-sectional study to analyze the antibiotic susceptibility pattern of uropathogens among children. Other than the studies as mentioned above mostly were the case reports in whichone or two patients were analyzed.

We haven't gone for case and control study because it was not feasible for us to ask a healthier personwithout any concrete reason for taking the sample. This implies that observational study was ideal for performing in this particular topic.

In conventional Clinical Laboratory Services processing, urine samples were cultured in five percent blood agar and Mac Conkey's agar. Similar tools were used by **Raghuvanshi BR et al. Sabharwal ER [9&11].** also used Mac Conkey's agar and antibiotic sensitivity testing was done by the Kirby Bauer disc diffusion method according to the CLSI guidelines. Also, **Pouladfar G et al. [8]** reported that Samples were cultured on blood agar or MacConkey agar by using a standard. calibrated loop. This implies that Mac Conkey's agar was used as a gold standard for urine sample culture process in the present as well as in previous studies **Betty AF et al. [12]** used the bacterial identification by colony morphology, Gram staining, and standard biochemical tests. Antimicrobial susceptibility test was performed by Kirby-Bauer disc diffusion technique. **Mathew AW et al [13].** different antimicrobial panels were used for different groups of microorganisms and second line antimicrobials were used only when necessary, following the Clinical and Laboratory Standards Institute (CLSI) guidelines.

The proportion of resistant organisms was calculated by dividing the number of isolates that were resistant to each antimicrobial agent by the number of organisms that were tested against that antimicrobial agent.

The primary outcome of interest was the proportion of organisms that were resistant to each antimicrobial agent nationally and within geographic regions for patients. The significance of differences in resistance according to geographic region or age was determined by means of the x^2 testfor independence.[14] Because

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of the considerable number of isolates in the database, most differences in the prevalence of resistance that we tested were significant (P <0.05), even if they varied by only 2%. The resulting sample size was 1000 cases as 85 cases were found to be unsuitable for the study or were dropouts. The clinical features of these cases were reviewed, and statistical analysis was performed.

Table 5: Sample Size Comparison

Study	Year	Sample Size	Inference
Seifu WD *et al*[15]	2018	384	Urinary tract infection was highly prevalent in the study area and all uropathogens isolated developed a resistance against mostly used antibiotics.
Poulad far G et al8	2017	202	The efficacy of the third generation of the cephalosporins was reduced because of the high rate of production of ESBL and drug resistance. These results inform the physician as to which antibiotics are appropriate to prescribe for the patient, as well as urine culture reports and following the patient's clinical response so that high antimicrobial resistance is not developed at the community level.
Sabharwal ER et al11	2012	250	therapy in cases of suspected UTI.
Prakasam AKC *et al*[17]	2012	200	The prevalence rate of uncomplicated UTI in general practice is high among young females in reproductive age groups. *E. coli* was found to be the most a common cause of UTI in all age groups. These isolates show resistance to commonly used antibiotics and susceptible only to injectable antibiotics.

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LIMITAITON OF THE STUDY

- Generalization of the study result to all of the patients with UTI is not free from bias.
- The study did not provide any information about the causality
- The study did not provide any information about the time course of different variables

CONCLUSION

- Urinary tract infection is the most common problem throughout the world, particularly in developing countries.
- UTI mainly occurred in the middle-aged persons
- Females were more likely to be affected than the males
- E. coli is the most common microorganism causing UTI. Antimicrobial susceptibility pattern varies in different regions and according to time
- The association of isolated pathogens with the gender was found to be statistically insignificant ($p>0.05$)
- The highest sensitivity was Escherichia coli (83.4%)
- **Escherichia coli 19 (54.3%) were major ESBL producer**
- Escherichia coli 16(45.7%) was the major AmpC producer

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