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(RESEARCH ARTICLE)

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Prevalence of extended spectrum β-lactamase (ESBL) and AmpC β-lactamase producing bacteria in urinary tract infection patients visiting tertiary care hospital in Central India

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Abstract

Antimicrobial resistance showed by different uropathogens is one of the barricades that might hinder the successful treatment (Bajpai *et al.,* 2019). Detection of extended beta–lactamase (ESBL) production among uropathogens is an important marker of endemicity.

Aim: This study was undertaken to determine the prevalence of pathogens in urinary tract and their antimicrobial susceptibilities, based on extended-spectrum beta-lactamase (ESBL) and AmpC beta-lactamase production in Central India. Methodology and results: The prevalence of pathogenic microorganisms in urinary tract and their antimicrobial resistance patterns were identified in 400 isolates from patients with urinary tract infections. Combined disc diffusion was performed to identify the presence of ESBL-producing strains. Moreover, disc approximation assay, disc potentiating test and double disc synergy test were performed to determine the presence of AmpC beta-lactamase producing bacterial strains. This study demonstrated a higher prevalence of UTIs in females (81.25%) than in males (18.75%). The most common pathogen was found *Escherichia coli* (44.5%), followed by *Klebsiella pneumoniae* (20%), *Pseudomonas aeruginosa* (12.5%) *Enterobacter species* (5%) and *Proteus species* (2.5%). ESBL and AmpC beta-lactamase production occurred more frequently in *E. coli* (17.5%) and *Klebsiella pneumonia* (5.5%) respectively.

Significance and impact of study: The result of this study would provide physicians with important information which help them to make a judicious choice of antibiotics for therapeutic purposes. However, it is emphasized that continuous surveillance of antibiogram of medically important organisms causing UTI is necessary for adopting a rational antibiotic policy in the country.

Keywords*:* Urinary tract infection; ESBL; Bacteria; Antibiotic resistance

1. Introduction

Urinary tract infection (UTI) is one of the most prevailing infectious diseases of the community along with the hospital settings (Rashedmarandi *et al.*, 2008). It is an important cause of morbidity and mortality not only in developing but also in developed countries of the world, affecting diverse age and sex groups (Akram *et al.*, 2007; Alipourfard and Nili, 2010; Dogra *et al.*, 2012 Mitu *et al.*, 2019). β-lactams have been used extensively to treat such type of infections, However, the widespread use of antibiotics poses a selective pressure leading to the selection of resistant bacteria. Extended-spectrum β-lactamases (ESBLs) belonging to class A and AmpC β-lactamases belonging to class C. Ambler classification are the two prevalent β-lactamases. Both ESBLs and AmpC β-lactamases confer resistance to a broad-

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spectrum of β-lactams includes Penicillins and Cephalosporins. But, unlike ESBLs, plasmid-encoded AmpC β-lactamases are effectively active against cephamycins and are not inhibited by a β-lactamase inhibitor such as Clavulanic acid. Harbouring these enzymes is usually associated with multiple antibiotic resistance (MDR) means that there are fewer antibiotic options available to treat. Knowing the epidemiology of ESBLs andAmpC producing organisms from the sample such as urine is important to ensure effective therapy, as well as infection control measures. Therefore, this study was designed to compare frequency of various bacterial urinary pathogens, identification of ESBL and AmpC production among Uropathogens as well as study their antibiotic resistance profile (Bajpai *et al.,* 2019).

2. Material and method

The present study was carried out from October 2021 to September 2023 in the Microbiology laboratory of Centre for Biotechnology and Microbiology studies, A.P.S. University Rewa and SGM Hospital associated with S.S.Medical College Rewa, (M.P.). The study protocol was approved by the Institutional Ethical Committee. A total of 400 non-repetitive midstream urine samples were collected in sterile containers. Within 2-4 hours of collection, the urine samples were transferred in cooler boxes to the Microbiology Lab for further processing and studies. All samples were cultured on the blood agar, Cystine-lactose-electrolyte deficient (CLED) agar, and Mac Conkey agar and were incubated further at 37°C for 18 h. More than 10^5 cfu/mL colony count for urine specimens was considered as significant bacteriuria for UTI. Bacterial identification for positive urine cultures was performed using standard Biochemical tests used in Microbiology lab.

Antibiotic sensitivity testing (AST) was performed by the Kirby-Bauer disc-diffusion method on Mueller-Hinton agar (Bauer *et al.,* 1966). For this test, a culture medium, specifically the Mueller-Hinton agar, was uniformly and aseptically inoculated with the test organism and then filter paper discs, which were impregnated with a specific concentration of a particular antibiotic, were placed on the medium. If the organism was susceptible to a specific antibiotic, there was no growth around the disc containing the antibiotic. Thus, a "zone of inhibition" was observed and measured to determine the susceptibility to an antibiotic for that particular organism. The following antibiotics were tested: Ampicilin (10mcg), Cefoxitin (30mcg), Ceftazidime (30mcg), Ceftriaxone (30mcg) Cefepime (30mcg), Norfloxacin (10mcg), Gentamicin (10mcg) and Meropenem (10mcg) All disks were supplied from (HiMedia Labs, India). Diameter of zone of inhibitions were measured and recorded in millimeters with the help of sliding calipers and organism was labeled as sensitive, resistant or intermediate as per CLSI 2012 guidelines.

Detection of ESBL and AmpC beta-lactamase producing strains:

- ESBL and AmpC beta-lactamase detection of all gram-negative uropathogens were performed using phenotypic methods to CLSI guidelines.
- ESBL confirmation
- ESBL confirmation was done by the double disk diffusion method where a ≥ 5mm increase in a zone diameter for Ceftazidime clavulanate (30\10 µg) compared to ceftazidime (30ug) alone confirms ESBL production. (Ahmad I, Aqil F,2007)

All isolates were inoculated in peptone water and adjusted to 0.5 Mc Farland unit then isolates were swabbed on to MHA. A 30 µg disk of ceftazidime and 30/10 µg disk of ceftazidime-clavulanic acid were placed on the same plate by keeping a minimum distance of 30 mm between them. Plates were further incubated for overnight at 37°C. Zone size of more than 5 mm around ceftazidime-clavulanic disk compared to ceftazidime disk alone was considered positive for ESBL production. Control strains *Escherichia coli* ATCC 25922 were used for the procedure. (Malik *et al*., 2019)

2.1. Screening for AmpC beta-lactamases producer

The organism that is resistant to cefoxitin and AmpC production by the isolates was first screened by a disc-diffusion method using cefoxitin disk with zone size of <18 mm was considered possible producer.

2.2. Confirmation of AmpC Producer

It was further confirmed by using cefoxitin-cloxacillin double-disc synergy test where 4-5 mm or greater zone around the disk containing cefoxitin and cloxacillin compared to the disk containing cefoxitin alone was considered as AmpC producer (Polsfuss *et al*.,2011).

Phenotypic AmpC confirmatory test by cefoxitin-cloxacillin double-disc synergy test:

30 µg disk of cefoxitin and the combination of cefoxitin and 200 µg of cloxacillin were used for this study. All strains of 0.5 McFarland units were inoculated on MHA and further kept for overnight incubation at 37°C. Equal or more than 4 mm zone size difference between both the disks was indicative of AmpC production (Polsfuss *et al.*,2011).

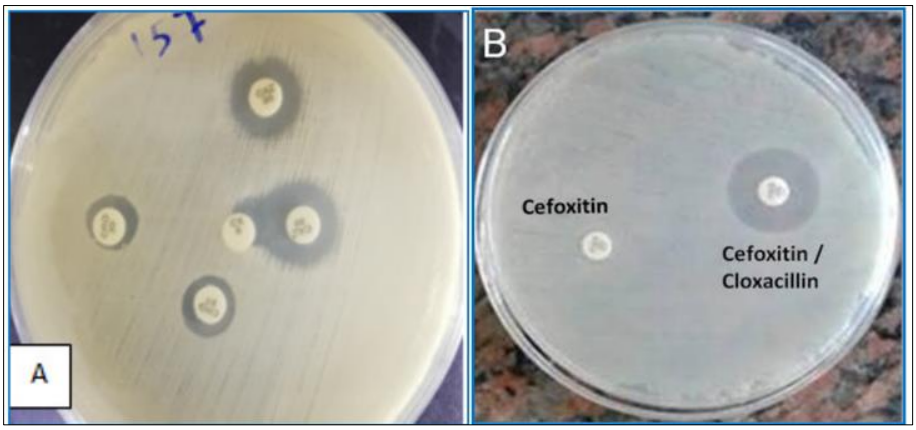

Figure 1 Screening for ESBL production(A) and Amp C production(B)

3. Results

3.1. Age and sex distribution of patients

During the study period, a total of 400 patients of different age and sex who showed culture positive urine were included in this study. 400 cases, 75(18.75%) were males and 325 (81.25%) were females. Most of the patients (43%) were between 61-70 years of age. In each age group, the percentage of female patients was high (Table 1).

Table 1 Age and sex distribution of study population

Distribution of case by age	**Distribution of cases by sex**		**Total**	**Percent**
	Male	Female		
0-14years	20	58	078	19.5%
15-40years	18	27	045	11.25%
41-60years	15	90	104	26.25%
≥61	22	150	172	43%
	75(18.75%)	325(81.25%)	400	

3.2. Identification of Uropathogens

Culture of 400 urine samples yielded a total of 240 (60%) of *E. coli*, 80 (20%) of *Klebsiella pneumonia*, 50(12.5%) *Pseudomonas aeruginosa* followed by *Enterobacter species* 20(5%) and *Proteus species*10 (2.5%) (Table 2).

Table 2 Distribution of Uropathogens

Uropathogens	**Total(400)**
Escherichia coli isolates	240 (60%)
Klebsiella pneumoniae	80(20%)
Pseudomonas aeruginosa	50(12.5%)
Enterobacter species	20(5%)
Proteus species	10(2.5%)

3.3. Prevalence of ESBL-producing organisms and AmpC producing isolates.

Identified isolates were further tested for the production of ESBL (Figure 1). 50(12.5%) of the total 240 *E. coli* were identified as positive for ESBL, 20(5%) were AmpC producing strains while 170 (42.5%) were negative. Out of 80 isolates *K. pneumoniae* 15(3.75%) positive for ESBL,7(1.75%) AmpC producing strains, 58(14.5%) were negative, 10 (2.5%) *P. aeruginosa* isolates, 4(1%) *Enterobacter species* and 03 (0.75%) out of 50,20 and 10 isolates respectively were positive for ESBL producing, and 40(10%), 16(4%) and 07(1.75%) were ESBL negative while none were AmpC positive among these species. In the present study ESBL positive and AmpC producing isolates were mainly detected with *E. coli* 12.5%and 5% respectively.

3.4. Antibiotic resistance pattern in bacterial isolates

In the present study, it is observed that *E. coli* and other Uropathogens were 94.5% resistant for Ampicilin, 88.5% resistant for Gentamicin. Third generation cephalosporins like Ceftazidime and Ceftriaxone were resistant between 79.5% and 70.5% for all isolates. Whereas, only 2% isolates were resistant for Meropenem. (Table 3)

Figure 2 MDR Strains of *E. coli* Sensitive Strains of *E. coli*

Table 3 Antibiotic Resistance Pattern of Bacterial Isolatesby Disc Diffusion Method

Antibiotic	***E.coli***	***K.pneumoniae***	***P.aerogenosa***	***Enterobacter sp.***	***Proteus sp.***	**Total**
	N=240	N=80	N=50	N=20	N=10	N=400
Ampicilin (10µg)	230(95.8%)	72(90%)	47(94%)	19(95%)	10(100%)	378 (94.5%)
Cefoxitin (30 µg)II	117(48.8%)	34(42.5%)	30(60%)	10(50%)	03(30%)	194 (48.5%)
Ceftazidime (30 µg)III	180(75%)	68(85%)	43(86%)	19(95%)	08(80%)	318(79.5%)
Ceftriaxone(30 µg)III	167(69.6%)	49(61.3%)	42(84%)	15(75%)	09(90%)	282(70.5%)
Cefexime (30 µg)IV	153(63.8%)	51(63.8%)	45(90%)	18(90%)	09(90%)	276(69%)
Norfloxacin (10 µg)	110(45.8%)	47(58.8%)	50(100%)	13(65%)	07(70%)	227(56.8%)
Gentamicin (10 µg)	208(86.7%)	76(95%)	50(100%)	20(100%)	08(80%)	354(88.5%)
Meropenem (10 µg)	02(0.83%)	00(00%)	00(00%)	00(00%)	00(00%)	02(0.5%)

4. Discussion

UTIs have already been very common around the world with diverse pattern. The findings of this study have disclosed the relationships between sex, age, isolated bacterial strains and antibiotic resistance of UTIs. Our study demonstrated a high prevalence of UTIs in females (81.25%) than in males (18.75%) which correlates with other findings which declared that the frequency of UTIs is higher in females as compared to males (Mitu *et al*., 2019, Rajalakshmi and Amsaveni, 2011). The reason behind this higher frequency of UTI in females may be due to close proximity of the external urethral orifice to the anus, smaller urethra, incontinence, sexual intercourse, and bad toilet (Aiyegoro *et al.*, 2007, Orrett and Davis, 2006). Most of the studies showed that *E. coli* is the most common uropathogen worldwide (Dimitrov *et al.*, 2004; Abubakar, 2009). Other studies showed that *Klebsiella* spp. (Aboderin *et al.*, 2009) and *Pseudomonas aeruginosa* (Ehinmidu *et al.*, 2004) are the most prevalent uropathogens. *Citrobacter* spp. was either the second most prevalent uropathogens (Baral *et al.*, 2012) or the third most prevalent urinary isolates (Shobha *et al.*, 2007). However, we have found *K. pneumoniae* 7.5%, *P. aeruginosa* 5.5% and *Citrobacter* spp. only 0.5% in our clinical samples. Uropathogens with antibiotic resistance has become a major public health concern in India. In the current study the antimicrobial resistance was high compare to the first line antimicrobial agents such as ampicillin and gentamicin. This may due to excessive use of these drugs temporarily because they are comparatively cheap and also easy to administer orally. A total of 27.25% isolates were found to produce ESBL and AmpC producing detected by the double disc diffusion test. The prevalence of ESBL was reported 22% and 64% respectively in earlier studies. (Agrawal *et al.*, 2008; Singhal *et al.*, 2005). Among the five most frequent UTI pathogens, *E. coli* (17.5 %) and *Klebsiella pneumoniae* (5.5 %) were most prevalent ESBL and AmpC producers similar with a study in Bangladesh *E. coli* (25.84 %) and *Klebsiella pneumoniae* (6.6 %) (Mitu *et al*., 2019). More studies are required to know the exact magnitude of the problem in Central India.

Other emerging resistance mechanisms such as AmpC enzymes are also being increasingly found in members of family *Entero-bacteriaceae.* AmpC beta-lactamase is one type of cephalosporinase which is either not inhibited, or weakly inhibited by clavulanic acid. It can hydrolyze not only cephamycins also extended spectrum cephalosporins. High level resistance to cefepime had been showed by both ESBL producers and non-producers while AmpC producers are very susceptible to fourth generation cephalosporins including cefepime (Livermore, 1995). In our study out of 400 Gram negative organisms, 27 samples were AmpC beta-lactamases producing organisms. Of them 5%*E. coli* and 1.75%*K. pneumoniae* are AmpC beta-lactamases positive. In this study overall AmpC prevalence was 6.75% compared to two studies in India reporting 27% (Subha *et al.*, 2003) and 47.3% (Hemalatha *et al.*, 2007). Current study has demonstrated the drug resistance among uropathogens. On the basis of our findings, it is very urgent to analyze and follow-up the antibiotic resistance pattern continuously. In addition, development of regional surveillance programs is also necessary to provide information which would then enable the development of UTI guidelines.

5. Conclusion

In conclusion, our study showed that among the urine isolates, AmpC and ESBLs production were prevalent in Gram negative bacteria. Majority of these organisms were resistant to common antibiotics used for treatment of UTI. Further drug resistance surveillance in the hospitals and molecular characteristics of ESBLs and AmpC isolates in Central India is necessary. This study is important for strict antibiotic policy implementation in hospitals to estimate the impact of higher drug resistance in bacteria and to take steps for reducing this resistance. The findings of the present study recommend that the UTI should be treated by selective antibiotics obtained from culture and sensitivity tests to minimize increasing trend of drug resistance. To eradicate multi drug resistant strains, new guidelines of antibiotic therapy for UTI may be necessary through more evaluation.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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