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Isolation and identification of various Extended Spectrum Beta-Lactamase producing uropathogens in a tertiary care hospital, Chhattisgarh

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ABSTRACT

Extended Spectrum B-Lactamase producing bacteria has become a worldwide problem and made the treatment very much complicated due to their resistance ability against the wide number of antimicrobial drug. This study was undertaken to measure the emergence of ESBL producing bacteria in urine collected from the patient Chhattisgarh Institute of Medical Science, Bilaspur, C.G. A total of 141 *Eterobactericeae* groups isolates and identified. A Total of 81 ESBL producing uropathogens were identified. The most prevalence of ESBL producing bacteria was *E. coli* 59(41.84%) followed by *Klebsiella* species 12(8.51%), and *Enterobacter species* were 06(4.26%). ESBL producers are confirmed by the Phenotyping confirmatory disc diffusion test. Antimicrobial susceptibility testing by Kirby-Bauer method showed most resistance were ampicillin (88.9%), Ciprofloxacin (69.1%), amoxycillin clavulanic acid (76.5%) resistance to ESBL producers and least resistant to imipenem 15(18.5%). We are confident that this research will be helpful in human beings and further medical microbiological study.

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INTRODUCTION

Urinary tract infections are the most common bacterial infection obtained in a community and hospital setting (Foxman, 2010). This is a world-wide problem, and it is calculated that about 150 million people each year infected by UTIs and costing the global economy more than 6 billion US dollars (Gonzalez and Schaeffer, 1999) UTI can occur in all age groups in both male and female (Huh, 2016). But females are more infected than the male because of their short urethra, pregnancy and contraception (Harrington and Hooton, 2000). It was estimated that about 50-60% of females suffer from at least one episode of UTI during their lifespan (Foxman, 2002). Because of UTI, Hospital admission and economy cost are increasing commonly (Foxman, 2002; Cove-Smith and Almond, 2007).

A large number of organisms that is responsible for urinary tract infection. The common gram-positive uropathogens are *Staphylococcus saprophyticus, Enterococcus faecalis, Staphylococcus aureus* (Kline

and Lewis, 2016) and common gram-negative uropathogens are *Escherichia coli, Psuedomonas spp. Proteus sp., Klebsiella pneumoniae and Enterobacter species* (Foxman, 2010; Griebling, 2005; Wilson and Gaido, 2004). But the most common uropathogen is *Escherichia coli* (Chang and Shortliffe, 2006).

Antibiotics resistance in bacteria is increasing worldwide. The bacteria are becoming highly drug-resistant (HDR) to conventionally used antibiotics (Taneja *et al.*, 2008). There is multiple drug resistance cases documented in both outdoor patients and admitted patients. These varies are according to geographical circumstance erroneous use of antibiotics and play a serious role in the emergence of the drug-resistant uropathogens (Tadesse *et al.*, 1950; Rockville, 2008). Mainly, ESBLs is a group of plasmid-encoded enzymes that provide resistance to third-generation cephalosporins group antibiotic drugs (Paterson and Bonomo, 2005; Stedt *et al.*, 2015) ESBLs are presented into groups that are TEM, SHVand CTX-M genes.CTX-M enzymes are the most common extended beta-lactamase (Lahlaoui *et al.*, 2014; Malloy and Campos, 2011).

Aims

The aim of this study is to know the prevalence of different uropathogens, their antibiogram and ESBL resistance among uropathogens.

MATERIALS AND METHODS

This study was conducted at the period of September 2018 to February 2019. Early morning, Clean-catch, Mid-stream urine sample was collected in a sterile, screw capped, leak proof container from patient as per standard operating guidelines. Urine sample was collected from both OPD and catheter patient and transport to microbiology laboratory.

Samples were inoculated on the MacConkey Agar (HiMedia) and Cysteine Lactose Electrolyte Deficient medium (CLED Agar, HiMedia)by semi-quantative method Kass and incubated for 24 hr. At 37°C temperature. If culture was negative, then we further re-incubated for next 24 hours. In culture-positive cases colony count in $\geq 10^5$CFU/ml and identification of organisms done by the gram staining and panel of a biochemical test as per the standard microbiological procedure.

The antimicrobial susceptibility test was done on Mueller Hinton Agar (HiMedia) by Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI, 2011). All *Enterobactericeae* organisms were further tested by combined disk test for the ESBL detection Ceftazidime (30μg) and Ceftazidime Clavunic acid (30/10μg) were placed on the MH Agar and incubated at 37°C for 24hrs. Ceftazidime Clavunic acid zone size was $\geq$5mm increase comparison to the Ceftazidme considered as ESBL is present (CLSI, 2012) (*Escherichia coli* ATCC 25922 strains, used as negative control), Figure 1.

Double disk synergy test (DDST): Five antibiotics were applied for detection of ESBL producer, ceftazidime-clavulanic acid (AMC/20/10μg) placed in center of the MH agar and around ceftazidime clavulanic acid, azteronam (ATZ/30μg), cefotaxime (CTX/30μg), ceftriaxone (CTR/30μg) and ceftazidime (CAZ/30μg) were placed at the distance of 1.5cm and incubated for 37°C for 24hrs. After incubation increased zone size were considered as ESBL producer.

RESULTS AND DISCUSSION

Total 618 urine samples were processed from the OPD and IPD patients of CIMS hospital. In this study, out of the 618 samples, 247(39.96%) urine samples had significant bacteremia. Among the UTI cases 164(66.4%) females and 83(33.6%) were male patients (Table 1). So, female:male ratio was (1.97:1). As shown in (Table 1) UTI was more prevalent in the age (year) group of 21-30, as 65 out of 247 patients (26.3%) and followed by 45 above $\geq$61 years (18.2%) and 19 children below $\leq$10 years (7.7%).

Total of 267 uropathogens were isolated. Where we found more than one organism in 20 samples. Among the uropathogens Gram-negative 178 (66.67%) followed by Gram-positive bacteria were 89 (33.33%) and *Non-albicans candida* 05 (1.87%). The most common uropathogens was *Escherichia coli* 111 (41.57%) followed by *Enterococcus sp.* 49 (18.4%) and *Pseudomonas sp.* 29 (10.9%), as shown in (Table 2).

Among the gram-negative uropathogens *E. coli* was highly resistant to ampicillin 87 (78.4%) and ofloxacin 72 (64.9%), whereas imipenem 15 (13.5%) was least resistant (as shown in (Table 3).

Among the gram-positive organisms, *Enterococcus spp.* were highly resistant to ciprofloxacin 30 (61.2%) followed by Ampicillin 22 (44.9%) and least resistant were vancomycin and linezoilid 02 (4.08%) followed by piperacillin tazobactam 10 (20.4%) and high level gentamycin (HLG) 20 (40.8%) as shown in (Table 4).

Among the *Enterobacteriaceae,* out of 141, 81 (57.45%) were ESBL producers. Among all the

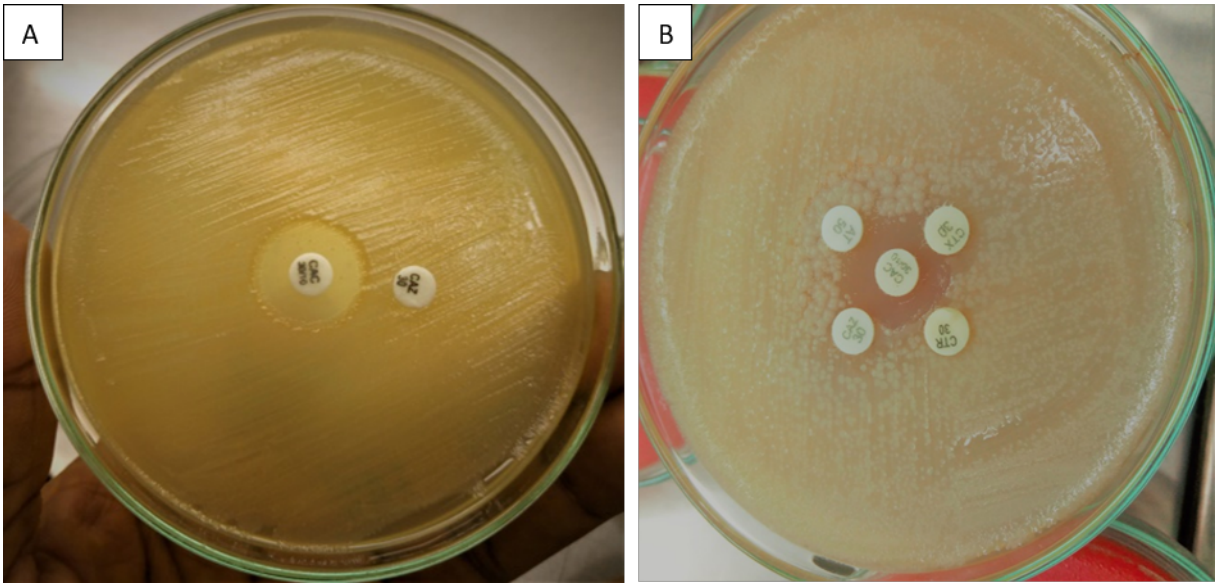

Figure 1: ESBL detection: (A) ceftazidime (CAZ 30mcg) (right) and ceftazidime-clavulanic acid (CAC 30/10mcg) (left). (B) Positive DDST ceftazidime, ceftriaxone (CTR 30mcg), cefotaxime (CTX 30mcg), aztreonam (ATZ30mcg), and ceftazidime clavulanic acid (CAC 30/10mcg) at the center

Table 1: Age and Gender wise distribution of uti patients(n=247)

Age in years	Female N=164	Male N=83	Total (N=247)
Below 10	15(6.07%)	4(1.62%)	19(7.7%)
11-20	21(8.5%)	7(2.83%)	32(13.0%)
21-30	50(20.2%)	15(6.07%)	65(26.3%)
31-40	24(9.72%)	12(4.86%)	36(14.6%)
41-50	17(6.88%)	12(4.86%)	29(11.7%)
51-60	11(4.45%)	10(4.05%)	21(8.5%)
≥61	22(8.91%)	22(8.91%)	45(18.2%)

Table 2: Distribution of isolated uropathogens (n=267)

Gram Neagative Organisms		Gram positive Organisms	
Escherichia coli.	111 (41.7%)	Enterococcus sp.	49 (18.4%)
Pseudomonas sp.	29 (10.9%)	Staphylococcus aureus	25 (9.36%)
Klebsiella pneumoniae	16 (5.99%)	Staphylococcus saprophyticus	04 (1.5%)
Acinetobacter sp.	08(3.00%)	Staphylococcus epidermidis	06 (2.25%)
Enterobacter sp.	07 (2.62%)	Non-albicans candida	05 (1.87%)
Proteus sp.	04 (1.5%)		
Citrobacter sp.	03 (1.12%)		
	178		89

Table 3: Antibiotic resistance pattern of gram-negative uropathogens (n=178)

Uroathogen N=178	Antibiotics									
	AMC	AMP	GEN	CTX	OF	NIT	NX	IPM	PIT	CIP
E. coli (111)	59(53.2%)	87(78.4%)	25(22.5%)	53(47.7%)	72(64.9%)	39(35%)	47(42.3%)	15(13.5%)	20(18%)	53(47.8%)
Klebsiella pneumoniae. (16)	11(68.8%)	-	5(31.3%)	7(43.8%)	6(37.5%)	12(75%)	7(43.8%)	4(25%)	6(37.5%)	7(43.8%)
Enterobacter species (07)	-	-	3(42.9%)	3(42.9%)	3(42.9%)	3(42.9%)	2(28.6%)	1(14.3%)	2(28.6%)	3(42.9%)
Proteus Spp (04)	2 (50%)	2(50.0%)	4 (100%)	3(75.0%)	4 (100%)	2 (50%)	04 (100%)	01 (25%)	1 (25%)	2(50.0%)
Citrobacter spp(03)	-	-	02(66.67%)	02(66.7%)	02(66.7%)	1(33.3%)	02(66.77%)	0(00%)	00%	1(33.3%)
Pseudomonas species (29)	-	-	17(58.6%)	-	16(55.2%)	17(58.6%)	20(69%)	05(17.2%)	17(58.6%)	14(48.3%)
Acinetobacter species (08)	-	-	2(25%)	-	6(75%)	3(37.5%)	2(25%)	03(37.5%)	04(50%)	6(75%)

AMC=amoxycilin clavulanic acid (20/10 mcg), FOS= fosfomycin (200 mcg), GEN= genatamicin (10mcg), CIP= ciprofloxacinoxacin (5 mcg), OF= ofloxacin (2 mcg), NIT=nitrofurantoin (300 mcg), CTX=cefotaxime (30 mcg), IPM= imipenem (10 mcg), PIT=piperacillin tazobactam (100/10 mcg), AMP= ampicillin (30 mcg)

Table 4: Antibiotic resistance pattern of gram positive uropathogens (n=84)

Uropathogens	Antibiotics							
	GEN	AMP	HLG	NIT	LZ	VA MIC	CIP	PIT
Staphylococcus aureus (25)	05(20%)	-	-	18(72%)	05(20%)	02 (8.0%)	09(36.0%)	-
S. saprophyticus (04)	01(25%)	-	-	01(25%)	0(0.0%)	0(0.0%)	01(25%)	-
Staphylococcus epidermidis (06)	01(16.7%)	-	-	01(16.7%)	04(66.7%)	01(16.7%)	02(33.3%)	-
Enterococcus spp. (49)	-	22(44.9%)	20(40.8%)	33(67.3%)	02(4.08)	02(4.08%)	30(61.2%)	10(20.4%)

HLG=HighLevel Gentamycin (100 mcg), LZ= Linezolid(30 mcg), VA MIC= Vancomycin Minimal Inhibitory Concentration

Table 5: ESBL producers in *enterobactericeae* out of n=141

Uropathogens	No. of Isolates	No. of non-ESBL producers	No. of ESBL producers
Escherichia coli	111	52 (36.9%)	59 (41.84%)
Klebsiella pneumoniae	16	04 (2.84%)	12 (8.51%)
Enterobacter species	07	01 (0.71%)	06 (4.26%)
Proteus Spp	04	02 (1.42%)	02 (1.42%)
Citrobacter spp.	03	01 (0.71%)	02 (1.42%)
	141	60 (42.55%)	81 (57.45%)

Table 6: Resistance antibiogram pattern in ESBL producing uropathogens (N= 81)

Uroathogens	Antibiotics								
	AMC	AMP	GEN	CTX	OF	NIT	IPM	PIT	CIP
E. coli (59)	47(79.7%)	53(89.8)	20(33.9)	48(81.4%	51(86.4%)	22(37.3)	11(18.6)	12(20.3)	46(78.0%)
Klebsiella pneumoniae (12)	7(58.3%)	10(83.3)	5(81.7)	9(75.0%)	8(66.7)	5(41.7%)	3(25.0%)	4(33.3%)	5(41.7%)
Enterobacter species (06)	4(66.7%)	05(83.3%)	3(50.0%)	4(66.7%)	4(66.7%)	2(33.3%)	1(16.7%)	4(66.7%)	4(66.7%)
Proteus Spp (02)	2(100%)	2(100%)	1(50.0%)	2(100%)	2(100%)	2(100%)	0(0.0%)	0(0.0%)	1(50.0%)
Citrobacter spp.(02)	2(100%)	2(100%)	2(100%)	2(100%)	1(50.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
Total Resistant	76.5%	88.9%	38.3%	82.7%	81.5%	38.3%	18.5%	24.7%	69.1%

ESBL producers *E. coli* 59 (41.84%) was the most frequent uropathogens observed, followed by *Klebsiella spp.* 12 (8.51%), *Enterobacter spp.* 06 (4.26%), as shown in (Table 5).

Among the ESBL producer, *E. coli* 53 (89.8) was the highest resistant to ampicillin followed by ofloxacin 51 (86.4%), cefotaxime 48 (81.4%) and ciprofloxacin 46 (78.0%). Least resistance was observed to imipenem 11 (18.6%) and piperacillin tazobactam 12 (20.3%) as shown in (Table 6).

Urinary tract infections (UTIs) are the most common bacterial infection in the world. UTIs can occur in all age groups. ESBL (Extended Spectrum Beta lactamase) and multiple drug resistance producing bacteria have made the treatment difficult. In our study UTI was most common at the age 21-30, 65(26.3%) shown in (Table 1), UTI cases were more common in adult than other age groups (Alanazi *et al.*, 2018). In this study, UTI cases was most common in female 164(66.4%) as shown in (Table 1) because of the short urethra, use of contraceptive, poor hygiene, pregnancy and sexual (Agbo, 2016) . In these cases, gram-negative bacteria 178(66.67%) is common than gram-positive bacteria 89(33.33%). Another study also showed the same frequency of uropathogens (Chaudhary and Mahadeva, 2013).

The *E. coli* 111(41.7%) is the most common uropathogens found in our study. Similar to our finding in other studies, the *E. coli* was the most common uropathogens 56.5%, 52.4% and 53.8% respectively (Chaudhary and Mahadeva, 2013; Yusuf *et al.*, 2017; Patel *et al.*, 2012) *Enterococcus sp.* was the second most common uropathogens 49(18.4%) whereas 16.91% and 3.03% observed in Moradabad (Kumar *et al.*, 2016) . In another study, *Klebsiella pneumoniae* was the second most common uropathogens, 12.1% (Srinivas, 2014).

In our study 81(57.45%) ESBL producers found whereas, in other studies, the prevalence of ESBL producers has been documented, (33%), (38.6%), (64.7%) and 34.71% respectively (Alqasim *et al.*, 2018; Murthy *et al.*, 2018; Bhandari *et al.*, 2016; Aruna and Mobashshera, 2012).

Among the ESBL producers, *E. coli* 59 (41.84%) was the most frequent organisms, as shown in (Table 6). In similar studies, the prevalence of *E. coli* was most prevalent in Singapore (33%), Hong Kong (48%) and India 60% as reported by (Hsueh *et al.*, 2011) 37.87% Saudi Arabia (Alqasim *et al.*, 2018).

Klebsiella spp. 12(8.51%) was second most common ESBL producer in our study, in around Bilaspur

, Chhattisgarh, whereas reported in south Mumbai *Klebsiella pneumoniae* (27.5%) (Mathur *et al.*, 2002) and in Channai *Klebsiella pneumoniae* (37%) (Gururajan *et al.*, 2011) were commonest.

An increasing rate of ESBL producing bacteria which are causing serious UTI in the society, hospitals even world-wide because of the limit to treatment choice and increases the disease. In our study, antibiogram pattern resistance were found to more in ESBL producers as a comparison to Non- ESBL producers. In our study, *E.coli* was most multi-drug resistant found among the *Enterobacteriaceae.* Among ESBL producers, the most resistance were ampicillin 72(88.9%) and ciprofloxacin 20(69.1%) as shown in (Table 6), whereas around 90% - 72.05% resistance and (Aruna and Mobashshera, 2012) and least resistance to imipenem 15(18.5%) whereas in other studies 3.6% and 09% were respectively reported (Bhandari *et al.*, 2016; Hassan *et al.*, 2011).

CONCLUSION

We observed that *E. coli* is the most common uropathogens and ESBL producing organisms and responsible for the complicated UTI and treatment difficulty. This study the high drug resistance were amoxicillin clavulanic acid, ampicillin and ciprofloxacin least imipenem resistance. Need to take an urgent action and make new strategies against the ESBL producers.

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