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Detection of Extended Spectrum Beta Lactamase Producing Escherichia Coli and Klebsiella Species Isolated from Urine Samples of UTI Patients by Phenotypic and Genotypic Methods

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Abstract **Original Research Article**

***Background*:** *Escherichia coli* and *Klebsiella* spp. are the most common organisms causing urinary tract infection (UTI) and commonly responsible for extended spectrum beta lactamase (ESBL) production. This study was carried out to detect ESBL producing uropathogenic *Escherichia coli* and *Klebsiella* spp. by phenotypic and genotypic method. Rapid and accurate detection of ESBL producing *E. coli* and *Klebsiella* spp. has an important role to avoid treatment failure. ***Materials and Methods*:** This was a cross sectional observational study and carried out in department of microbiology in Chittagong Medical College, Bangladesh from January to December 2017. Urine was collected from suspected UTI patients and standard microbiological and biochemical tests were carried out. ESBL producing *E. coli* and *Klebsiella* spp. were identified by phenotypic confirmatory disc diffusion test (PCDDT). Polymerase chain reaction (PCR) was performed by using standard protocol with specific primers. ***Results*:** 448 urine samples were collected. Among them, 140 showed bacterial growth; 72 were *E. coli* and 35 were *Klebsiella* spp. Among *E. coli* 38(52.8%) and in *Klebsiella* spp. 15(42.9%) were detected as ESBL producers by PCDDT respectively. Among *E. coli*, 41(56.9%) and in *Klebsiella* spp. 21(60%) strains produced ESBL genes by PCR. Out of 38 phenotypically positive *E. coli*, 7 strains do not carry any detectable genes. Similarly, out of 15 phenotypically positive *Klebliella* spp. 3 isolates did not produce any detectable gene. On the other hand, 10 *E. coli* isolates and 9 *Klebsiella* spp. carry detectable genes although these were not phenotypically ESBL producers. Moreover, ESBL producing *E. coli* and *Klebsiella* spp. showed more multidrug resistant than Non-ESBL producing *E. coli* and *Klebsiella* spp. ***Conclusion*:** This study revealed that large portion of *E. coli* and *Klebsiella* spp. was ESBL producers. PCR can be used along with phenotypic method to detect some additional cases of ESBL producing isolates. So, PCR can be used along with phenotypic method to detect ESBL producing organism.

Keywords: Uropathogenic bacteria, Extended Spectrum Beta Lactamase (ESBL), *Escherichia Coli*, *Klebsiella*, UTI, Phenotypic method, Genotypic method.

INTRODUCTION

Urinary tract infections (UTIs) are frequent bacterial infection worldwide and the most commonly encountered bacterial infection, particularly in women. It is estimated that about 150 million cases of UTI occur worldwide as many as 50% of women and 12% of men experience at least one symptomatic UTI during their lives and about 25% of affected women have recurrent UTI [1].

In Bangladesh, Rahman *et al.*, showed the prevalence of UTI is 24.14% [2]. One study in Iran conducted by Hossein *et al.*, in 2017 showed that prevalence of UTI in female is 75.1% and male is 24.9%. Islam *et al*., in Dhaka Medical College showed that prevalence of UTI was 41.2% [3, 4].

E. coli, K. pneumonia, P. mirabilis, P. aeruginosa, S. aureus, E. faecalis and S. saprophyticus are most common bacteria causing UTIs in human.

Among the uropathogenic bacteria, *Escherichia coli* is predominant in both community and nosocomial UTI [5].

Extended spectrum beta-lactamases (ESBLs) are the enzymes which offer resistance to penicillins, cephalosporins and aztreonam by hydrolysis of the antibiotics. These enzymes catalyze the hydrolysis of β-lactam ring of antibiotics, thereby destroying their antimicrobial property [6]. *Klebsiella pneumoniae* and *Escherichiacoli* remain the major ESBL-producing organisms isolated worldwide, but these enzymes have also been identified in several other members of the Enterobacteriaceae family [7].

The ESBL producing bacteria are increasingly causing urinary tract infections both in hospitalized and outpatients. This is making therapy of UTI difficult and promoting greater use of expensive broad-spectrum antibiotics [8].

Several phenotypic methods for detection of ESBL have been proposed including Double disc synergy test, Phenotypic confirmatory disc diffusion test, E-test ESBL strips, Three-dimensional test, Vitek system. Clinical laboratory and standard institute recommended phenotypic confirmatory disc diffusion test as phenotypic methods for detection of ESBL and it is easy to perform [9]. Recent reports have shown that a rapid and alarming dissemination of CTX-M-15 type ESBL in certain countries including India and have become the most prevalent ESBL-type worldwide. The majority of them are now recoverd from *Escherichia coli* isolates causing urinary tract infection [10].

Bacteria producing CTX-M-15 often associated with carriage of other beta lactamase such as TEM-1 and OXA-1. One study in Dhaka Medical College conducted by Begum *et al*., in 2016 reported that 80% ESBL producing bacteria were positive for *bla*CTX-M-15 and 40% were positive for *bla*OXA-1 in urine samples [11]. There was a lack of information on molecular characterization of ESBL producing organisms in Chittagong. Therefore, the objective of this study was the detection of UTI by conventional method, perform antimicrobial sensitivity of isolated organisms, detect extended spectrum beta lactamase producing uropathogenic *Escherichia coli* and *Klebsiella* species by phenotypic and genotypic method and compare between phenotypic and genotypic method in Chittagong Medical College Hospital.

MATERIALS AND METHODS

Study Design and Study Subject

It is a cross sectional study, carried out in the department of microbiology, Chittagong medical college, during the period of January to December 2017. Before conducting the study, approval from ethical review committee of Chittagong medical college was duly taken.

Study Samples

Urine samples were collected from patients of outpatient department of Chittagong medical college hospital, Chittagong. Total 448 samples were collected from both sex and different age groups.

Laboratory Procedure

Labatory procedures are performed by following the standard international protocols.

Specimen Collection

Samples were collected from both sexes and different age groups using all aseptic precautions after taking informed written consent from patient or his/her legal guardian. The clean catch mid-stream urine samples of about 15-20ml were collected in a sterile container by standard technique for microscopy, culture and sensitivity test. Each sample in the container was properly labeled with patient's name, ID number etc. The specimens were then transferred to the laboratory as quickly as possible and processed within 2 hours after collection.

Microscopic Examination

Five ml of urine sample was poured into a sterile container of 15 ml centrifuged tubes and centrifuged at 3000 RPM for 5 minutes. The supernatant fluid was discarded and one drop of sediment was transferred to a clean glass slide, covered with a clean cover slip and then was examined under light microscope using 10x and 40x magnifications. On the basis of finding of pus cell >5/HPF urine samples was included in this study.

Culture

Urine samples contain >5 pus cell/ HPF were inoculated in Blood agar, MacConkey agar and CLED agar media by calibrated wire loop (0.001ml). Then, that inoculated media was aerobically incubated at 37^0C for 24 hours. After overnight incubation, plates were checked for presence of any suspected pathogens [12].

Isolation and Identification of Organisms

A presumptive identification of the isolates were made on the colony colour and morphology on CLED agar, Blood agar and MacConkey agar plates and were confirmed by standard laboratory methods of identification such as staining characteristics, lactose fermentation, pigment production, oxidase reaction, citrate utilization test, motility test, indole production, urease production and reaction in TSI media. Gram positive bacteria were identified by catalase test, coagulase test and novobiocin sensitivity test. Prior to above test for detection of urinary pathogen from plate, colony count was done by calibrated wire loop

(0.001ml) method. The number of colonies grown were counted and interpreted as CFU/ml of urine by multiplying the colonies grown by 1000. Colony counts $\geq 10^5$ CFU/ml were taken as significant bacteriuria [12].

Antimicrobials used for Susceptibility Testing:

For *Staphylococcus saprophyticus*- Amoxycillin/Clavulanic acid (AMC), Co-trimoxazole (SXT), Oxacillin (O), Ciprofloxacin (CIP), Ceftriaxone (CRO), Cefotaxime (CTX), Ceftazidime (CAZ), Nalidixic acid (NA NET), Nitrofurantoin (F), Imipenem (IPM), Gentamicin, Azithromycin, Vancomycin and Novobiocin.

For *Enterobacteriaceae* - Amoxycillin/Clavulanic acid (AMC), Co-trimoxazole (SXT), Ciprofloxacin (CIP), Ceftriaxone (CRO), Ceftazidime (CAZ), Cefotaxime (CTX), Nalidixic acid (NA), Nitrofurantoin(F), Cefuroxime, Gentamicin, Azithromycin and Imipenem (IPM).

For *Pseudomonas* Species – Amoxycillin/Clavulanic acid (AMC), Ciprofloxacin (CIP), Ceftriaxone (CRO), Ceftazidime (CAZ), Cefotaxime (CTX), Nitrofurantoin (F), Nalidixic acid (NA) Gentamicin, Azithromycin and Imipenem (IMP), were used.

Quality Control

A representative disc from each batch was standardized by testing against reference strains of *E.coli* ATCC 25922 and *S.aureus* ATCC 25923; zones of inhibition were tested with standard value.

Antibiotic Sensitivity Testing

All bacterial isolates were tested for antimicrobial sensitivity by Kirby-Baurer disc diffusion technique against different antimicrobial agents.

Media for Antibiotic Sensitivity Testing

Mueller-Hinton agar media were used for antibiotic sensitivity testing for all the bacteria.

Methods of Sensitivity Testing

3-5 isolated colonies of similar appearance were taken with a sterile loop from a pure culture in a tube containing 5 ml sterile normal saline. Turbidity of the organisms in the tube was adjusted by adding more bacteria or more saline to turbidity equivalent to that of 0.5 McFarland's standard which approximately corresponds to 1.5 X 10^8 organisms/ml. Within 15 minutes after standardization of inoculums, a sterile cotton swab stick was immersed into bacterial suspension. The excess suspension was removed by rotating the swab with firm pressure against the inner side of the tube above the fluid level. The plates were dried in an incubator at 37^0c for 30 minutes before use. The swab was then streaked evenly on the surface of freshly prepared media in three different planes (by rotating the plate 60^0 each time) to get a uniform distribution of inoculums. The plates were left at room temperature for 10-15 minutes with lid closed to allow the inoculums to dry. The antimicrobial sensitivity discs were then placed on the inoculated surface by sterile fine forceps 15 mm away from the edge of the petridish and having 20-25 mm gap between the discs. The plates were then inverted and incubated at 37^0c for 18-24 hours.

Reading of Sensitivity Tests

Each plate was examined after overnight incubation (18-24 hours) and diameter of the zone of inhibition was measured in mm with the help of scale placed on the underneath of the petridish without unfastening the lid. Zone of inhibition was measured in two directions at right angles to each other through the center of the disc and average of the two reading was taken.

Interpretation of Zone Size

Zone of inhibition produced by each organism was considered into three susceptibility categories namely Sensitive (S), Intermediate and Resistant (R) that were interpreted according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) [9].

Screening for ESBL Production

The isolates were screened for presumptive ESBL production by testing their susceptibility to Ceftazidime (30µg) and Cefotaxime (30µg) by using Kirby Bauer disk diffusion method. The inhibition zone sizes were interpreted as per the CLSI guidelines. All the isolates with an inhibition zone diameter of less than 21 mm in case of Ceftazidime and less than 26 mm in case of Cefotaxime were considered as screen positive for ESBL. These screen positive isolates were selected for confirmatory test.

Phenotypic Confirmatory Disc Diffusion Test (PCDDT)

PCDDT is based upon the resistance of ESBLs to Cefotaxime and Ceftazidime & the ability of Beta–lactamase inhibitor (Clavulanic acid) to block the resistance. Clavulanic acid has become a useful supplement to penicillin that are susceptibile to hydrolysis by plasmid-mediated Beta-lactamases. This property of clavulanic acid forms the basis of phenotypic confirmatory disc diffusion test [13]. This test requires use of both Cefotaxime (30µg) and Ceftazidime (30µg) disc alone and in combination with Clavulanic acid (10µg).

Materials Required: Mueller-Hinton agar**, 0**.5 McFarland standards.

ESBL- Producing Bacterial Strains used- *E. coli, Klebsiella* spp.

Quality Control Organisms used for ESBL Detection

ESBL producer *E.* coli was used as positive control and *E.coli* ATCC25922 was used as negative control for quality control of ESBL test.

Antimicrobial Agent used

Cefotaxime (CTX) (30µg), Ceftazidime (CAZ) (30µg), Combined (Ceftazidime (30µg) +Clavulanic acid (10µg) and Cefotaxime (30µg) +Clavulanic acid (10µg) were obtained from Oxoid, UK.

Detection of ESBL Genes by PCR

PCR was performed by using standard protocol with specific primers for detection of ESBL genes. Procedure of PCR for detection of ESBL from culture was as follows.

Documentation of the DNA Samples

After electrophoresis, the gel was taken out carefully from the gel chamber and the gel gently placed on the UV transilluminar in the dark chamber of the image documentation system. The UV light of the system was switched on and the image was viewed on the monitor at 996 bp CTX-M-15 & at 598 bp OXA-1 by transilluminar and primed on glossy paper.

Data Collection

Data collection was done by using structural questionnaire and checklist.

Data Analysis

Data was collected and recorded in a predesigned data sheet. The results of the experiments were recorded systematically and statistical analysis was done by standard statistical procedure.

RESULT

Total 448 patients clinically suspected as UTI were studied. Among them, 206 (45.9%) had >5 pus cells/HPF and the remaining 242 (54.1%) patients had pus cells <5/HPF.

Among the 206 samples (each was collected from 206 suspected UTI patient), 140 (67.9%) shows significant bacteriuria ($\geq 10^5$ CFU/ml) in culture and the remaining 66 (32.1%) samples shows no growth or non-significant bacteriuria ($<10^5$ CFU/ml) in culture.

A total of 140 (67.9%) culture positive isolates, *E. coli* 72(51.4%) was the most prevalent isolates followed by *Klebsiella spp.* 35(25%). Other gram negative isolates were Pseudomonas spp. 17 (12.1%), *Proteus* spp. 12 (8.6%), and gram positive cocci were *Staphylococcus saprophyticus* 4 (2.9%).

Age & Sex Distribution

Among 140 culture positive samples. It showed frequency of UTI in case of male patients was the highest (13.6%) in the age group of >61 years followed by 6.4% in 51-60 years. In females, the majority (22.9%) of UTI occurred in the age group of 21-30 years followed by 15% in 31-40 years. The frequency of UTI in different sex and age groups was significantly different ($p<0.001$).

Table 1: Age and sex distribution among culture positive samples (n=140)

		Sex				Total	
		Male		Female			
		No.	%	No.	%	No.	%
Age Group	0-20 years	3	3.6%	20	14.3%	23	16.4%
	21-30 years	2	1.42%	32	22.9%	34	24.3%
	31-40 years	2	1.42%	21	15%	23	16.4%
	41-50 years	3	2.1%	17	12.1%	20	14.3%
	51-60 years	9	6.4 %	4	2.9%	13	9.3%
	>61 years	19	13.6%	8	5.8%	27	19.3%
	Total	38	27.1%	102	72.9%	140	100.0%

Mean Age =35.26±20.11, M:F=1:2.7

Table 2: Antimicrobial sensitivity pattern of isolated pathogens

Antibiotics	**Sensitivity patterns**	***E coli*(n=72)**	***Klebsiella* spp.(n=35)**	***Pseudomonas* spp.(n=17)**	***Proteus* spp.(n=12)**	***Staphylococcus saprophyticus*(n=4)**
Amoxyclav	S	6(8.4%)	4(11.4%)	1(5.8%)	1(8.3%)	3
	R	66(91.6%)	31(88.6%)	16(94.2%)	11(91.7%)	1
Ciprofloxacin	S	20(27.8%)	15(42.8%)	6(35.3%)	4(33.3%)	3
	R	52(72.2%)	20((57.2%)	11(64.7%)	8(66.7%)	1
Ceftazidime	S	29 (40.3%)	14(40%)	5(29.4%)	6(50%)	2
	R	43(59.7%)	21(60%)	12(70.6%)	6(50%)	2
Cefotaxime	S	28(38.8%)	13(37.1%)	3(17.6%)	5(41.7%)	2

Antibiotics	**Sensitivity patterns**	***E coli*(n=72)**	***Klebsiella* spp.(n=35)**	***Pseudomonas* spp.(n=17)**	***Proteus* spp.(n=12)**	***Staphylococcus saprophyticus*(n=4)**
	R	44(61.2%)	22(62.9%)	14 (82.4%)	7(58.3%)	2
Ceftriaxone	S	24 (33.3%)	13(37.1%)	4 (23.5%)	8(66.7%)	3
	R	48(66.7%)	22(62.9%)	13(76.5%)	4(33.3%)	1
Cefuroxime	S	17(23.6%)	15(42.8%)	8(47.1%)	8(66.7%)	0
	R	55(76.4%)	20(57.2%)	9(52.9%)	4(33.3%)	4
Azithromycin	S	20(27.7%)	9(25.7%)	7(41.2%)	7(58.3%)	0
	R	52(72.2%)	26(74.3%)	10(58.8%)	5(41.7%)	4
Cotrimoxazole	S	20(27.7%)	12(34.28%)		6(50%)	2
	R	52(72.2%)	23(65.7%)		6(50%)	2
Nalidixic acid	S	19(26.3%)	9(25.7%)	3(17.6%)	3(25%)	2
	R	53(73.6%)	26(74.3%)	14(82.4%)	9(75%)	2
Nitrofurantoin	S	63(87.5%)	28(80%)	12(70.6%)	9(75%)	-
	R	9(12.5%)	7(20%)	5(29.4%)	3(25%)	-
Gentamycin	S	57(79.1%)	31(88.6%)	9(52.9%)	9(75%)	4
	R	15(20.8%)	4(11.4%)	8(47.1%)	3(25%)	0
Imepenem	S	69(95.8%)	31(88.6%)	13(76.5%)	11(91.7%)	4
	R	3(4.2%)	4(11.4%)	4(23.5%)	1(8.3%)	0
Vancomycin	S	-	-	-	-	4
	R	-	-	-	-	0
Oxacillin	S	-	-	-	-	3
	R	-	-	-	-	1
Novobiocin	S	-	-	-	-	0
	R	-	-	-	-	4

Table 2 shows the antimicrobial sensitivity pattern of isolated pathogens. Total 52 resistant *E.coli* isolates and 27 *Klebsiella* spp detected as possible ESBL producers were subjected to phenotypic test by (Cefotaxime + Clavulanic acid) and (Ceftazidime + Clavulanic acid) combined disc test. Among the 52 screening positive *E. coli*, 38 were ESBL producers and in 27 screening positive *Klebsiella* spp. isolates 15 were ESBL producers which were identified by PCDDT (Phenotypic confirmatory disc diffusion test).

Table 3: Distribution of ESBL producing *E. coli* and *Klebsiella* spp.identified by PCDDT

Name of bacteria	**No. of screening positive**	**ESBL producers by PCDDT**	
		PCDDT positive	**PCDDT negative**
E. coli (n=72)	52	38 (52.8%)	14 (19.4%)
Klebsiella (n=35)	27	15 (42.9%)	12 (34.3%)

PCR was done in total 72 *E.coli* and 35 *Klebsiella* sppisolates. *E.coli* produced 41(56.9%) and *Klebsiella* spp produced 21(60%) genes. In *E.coli* 14 (19.4%) *bla*OXA-1,10 (13.9%) *bla*CTX-M-15, 17 (23.6%) *bla*CTX-M-15+*bla*OXA-1 were detected by PCR. In*Klebsiella* spp. 8 (22.9%) *bla*OXA-1, 8(22.9%) *bla*CTX-M-15 and 5 (14.3%) *bla*OXA-1+*bla*CTX-M-15 were detected by PCR. No *bla*TEM-1 genes were detected by PCR.

Table 4: Result of PCR

Name of bacteria	***bla*OXA-1**	***bla*CTX-M-15**	***bla*OXA1+ *bla*CTX-M 15**	***bla*TEM-1**	**Total**	**Absent**
E.coli (n=72)	14(19.4%)	10(13.9%)	17(23.6%)	0(00%)	41(56.9%)	31(43.1%)
Klebsiella spp. **(n=35)**	8(22.9%)	8(22.9%)	5(14.3%)	0(00%)	21(60%)	14(40%)

In this study, phenotypically ESBL positive *E.coli* (PCDDT Positive) was detected 38, but genotypically 31 (43%) strains was detected by PCR. In 34 Non ESBL (PCDDT negative + Ceftazidime and Cefotaxime sensitive) strains 10 (13.9%) produced genes.

Table 5: Resistant genes detected by PCR among *E.coli* identified by PCDDT

Total isolates	PCDDT	*bla*OXA-1	*bla*CTX-M-15	Both (*bla*OXA-1 &*bla*CTX-M-15)	Total	Absent
E.coli(n=72)	ESBL (38)	11(15.3%)	7(9.7%)	13(18%)	31(43%)	7(9.7%)
	Non-ESBL(34)	3(4.2%)	3(4.2%)	4(5.5%)	10(13.9%)	24(33.3%)

Non ESBL-PCDDT Negative + Ceftazidine and Cefotaxime Sensitive

In case of *Klebsiella* spp., phenotypically ESBL positive (PCDDT Positive) was 15, but 12 strains was detected by genotypically. In 20 Non-ESBL (PCDDT negative + Ceftazime and Cefotaxime sensitive) strains *Klebsiella* spp. 6(17.1%) produced *bla*OXA-1, 2(5.7%) produced *bla*CTX-M-15 and 1(2.8%) produced both *bla*OXA-1 and *bla*CTX-M-15.

Table 6: Resistant genes detected by PCR among *Klebsiella* spp.identified by PCDDT

Total isolates	PCDDT	*bla*OXA-1	*bla*CTX-M-15	Both(*bla*OXA-1 &*bla*CTX-M-15)	Total	Absent
Klebsiella spp (n=35)	ESBL (15)	2(5.7%)	6(17.1%)	4(11.4%)	12(34.3%)	3(8.5%)
	Non-ESBL(20)	6(17.1%)	2(5.7%)	1(2.8%)	9(25.7%)	11(31.4%)

Non ESBL-PCDDT Negative + Ceftazidine and Cefotaxime Sensitive

Table 7: Comparison of PCDDT and PCR among the screening positive *E. coli* (n=52)

PCDDT	PCR		
	PCR Positive	PCR Negative	Total
PCDDT Positive	31 (59.6%)	7 (13.5%)	38
PCDDT Negative	4 (7.7%)	10 (19.23%)	14
	35	17	52
$\chi^2 = 11.32$, df = 1, p = 0.023			

Table-7 shows the comparative result between PCR and PCDDT in Cefotaxime and Ceftazidime resistant *E. coli* isolates. The differences in result were statistically significant (p<0.05).

Table 8: Comparison of PCDDT and PCR among the screening positive *Klebsiella* spp. (n=27)

PCDDT		PCR		
		PCR Positive	PCR Negative	Total
PCDDT Positive	15	12 (44.4%)	3 (11.1%)	15
PCDDT Negative	12	3 (11.1%)	9 (33.3%)	12
	27	15	12	27
$\chi^2 = 4.18$, df = 1, p = 0.045				

Table-8 shows the comparative result between PCR and PCDDT in Cefotaxime and Ceftazidime resistant *Klebsiella* spp. isolates. The differences in result were statistically significant (p<0.05).

DISCUSSION

In the present study, a total of 448 samples were collected and of which 206 (45.9%) showed pus cells>5/HPF. This result is closely related to that of Begum *et al.*, in Dhaka Medical College, Dhaka who found 40% samples containing pus cells>5/HPF [11]. Another study in Nepal conducted by Kettel *et al.*, found 36% samples contain pus cells>5/HPF which were dissimilar to our result [14].

Among the 206 samples containing pus cells>5/HPF, 140 (67.9%) samples yielded growth which was not comparable with the other study by Sharmin *et al.*, and Haque *et al.*, in Bangladesh where 42.5% and 42.66% were culture positive respectively [15, 16]. The probable reason is that they inoculated all the collected samples but in the present study only the samples containing pus cells>5/HPF were inoculated.

Amongest the isolates in our study, the majority were *E.coli* 72 (51.4%) followed by *Klebsiella* spp 35 (25%), *Pseudomonas* spp. 17 (12.1%), *Proteus* spp.12 (8.6%) and *Staphylococcus* spp. 4 (2.9%). Similarly Begum *et al.*, of Dhaka and Kulkarni *et al.*, in India showed *E.coli* and *Klebsiella* spp. were the most prevalent isolates from urine samples [11, 17]. But Rajivgandhi *et al.*, found *Proteus mirabilis* (25%) was the second predominant bacteria after *E.coli* (30%) [18].

In this study, among 140 culture positive samples of UTI patients 38 male and 102 were female with male to female ratio of 1:2.7. The result shows that

UTI is more prevalent in female than males. The present study correlates well with the studies conducted by Chander *et al.*, Lina *et al.,* and Shakya *et al.,* where male to female ratio were 1:2.4, 1:3 and 1:2.96 respectively [19-21]. In Haque *et al*., and Akram *et al.,* male to female ratio was 1:1.46, 1:1.2 respectively which is significantly different from our study [16, 22]. In our study, UTI were most commonly found in the age group between 21-30 years in female and >61 years in male, The result is closely related to Moue *et al.,* who reported significant bacteriuria in females in the age group between (21-40) years and male were between (41-60) years [23]. But another study conducted by Sivaleela and Pragathi found UTI is most common in 31-40 years in females and 36-40 years in males which is contrast to our study [24].

Among 72 *E. coli* 38(52.8%) were ESBL producers confirmed by phenotypic confirmatory disc diffusion test (PCDDT). Closely similar to the present study Ejaj *et al.,* of Pakistan detected 57.4% ESBL producer by PCDDT and Kulkarni *et al.,* of India detected 51.04% by PCDDT [8, 17]. On the other hand, Dissanayake, Fernando and Chandrasiri in Sri Lanka found that 29% of *E coli* were detected by PCDDT [25]. Similarly, among 35 *Klebsiella* spp isolates 15(42.9%) were ESBL producers confirmed by PCDDT. This finding is similar to Latifpur *et al.,* in India who found 48% and Raei, Eftekhar and Feizabadi in Iran found 46.9% by PCDDT [26, 27]. In contrast to our study Shakya *et al.,* in Iran showed 15% *Klebsiella* spp isolates were detected by PCDDT [21].

Present study observed that, out of 72 isolated *E.Coli* 41(56.9%) isolates produced ESBL encoding genes which were detected by PCR. Of them 14(19.1%) were positive for *bla*OXA-1 and 10 (13.9%) were positive for *bla*CTX-M-15. 17(23.6%) strains simultaneously produced both *bla*OXA-1 and *bla*CTX-M-15. Similarly in 35 *Klebsiella* spp. isolates 21(60%) ESBL encoding genes were detected by PCR. Among them 8(22.9%) were positive for *bla*OXA-1 and *bla*CTX-M-15 each. 5(14.3%) strains simultaneously produced both *bla*OXA-1 & *bla*CTX-M-15. Previous study in DMCH reported 47(52.2%) ESBL encoding genes were detected in 90 *E.coli* isolates. Out of them 34.4% were positive for *bla*CTX-M-15 and 17.8% were positive for *bla*OXA-1. In 20 *Klebsiella* spp. isolates 20% produced *bla*CTX-M-15 and 10% produced *bla*OXA-1.

Present study observed that out of 72 ESBL producing *E.coli,* 38 were phenotypically positive. In phenotypically positive *E. coli* strains 31(43%) ESBL encoding genes were detected by PCR. Of them 11(15.3%) were positive for *bla*OXA-1, 7(9.7%) for *bla*CTX-M-15 and 13(18%) for both *bla*OXA-1 and *bla*CTX-M-15. Similarly out of 35 *Klebsiella* spp. 15 were phenotypically ESBL producers. In phenotypically ESBL positive *Klebsiella* spp strains 12(34.3%) ESBL encoding genes were detected by PCR. Among them 2(5.7%) were positive for *bla*OXA-1, 6(17.1%) for *bla*CTX-M-15 and 4(11.4%) strains for both *bla*OXA-1 and *bla*CTX-M-15. Previous study in DMCH reported in 90 ESBL positive *E.coli* strains 31(34.4%) *bla*CTX-M-15 and 16(17.7%) *bla*OXA-1 and in 6 ESBL positive *Klebsiella* spp. 4(20%) *bla*CTX-M-15 and 2(10%) *bla*OXA-1 genes were detected by PCR. The result is dissimilar to our study. Another study in Morocco, conducted by Barguigua *et al.,* found 1.4% combined genes (*bla*OXA-1 + *bla*CTX-M-15) which also contrast to our study [28]. The possible explanation behind the variation of results from different studies may be that there are geographical and institutional variations of prevalence of ESBLs production.

In 34 Non-ESBL (PCDDT negative +Ceftazidime and Cefotaxime sensitive) *E.coli* strains 10(13%) genes were detected by PCR. No detectable genes were produced in remaining 24(33.3%) strains. Similarly, in 20 Non-ESBL *Klebsiella* spp. 7(20%) genes were detected by PCR. No detectable genes were produced in remaining 14(40%) strains which were truly sensitive. Similar study conducted by Bajpai, *et al.,* found 52.6% pheonotypically Non ESBL isolates were genotypically positive [29]. This may mean that the presence of ESBLs can be masked by the expression of chromosomal or plasmid mediated AMP C beta lactamases. So, ESBL producing strains with AMP C beta lactamases may cause a false negative ESBL production. The similar mechanisms of drug resistance can be occurred in case of carbapenemase production.

Presence of genes in phenotypically ESBL negative isolates indicates higher sensitivity of genotypic methods. The same presentations during phenotypic tests can be disguised due to various reasons but can often be acknowledged genotypically. However, those that lacked *bla*OXA-1 or *bla*CTX-M-15 may have actually been negative or might have carried some other "hidden" gene for ESBL production.

CONCLUSION

This study revealed that large portion of *E. coli* and *Klebsiella* spp. was ESBL producers. ESBL detection by phenotypically only confirm whether an ESBL is produced but it cannot detect the subtype of ESBL and cannot detect the genes whose expression is obscured. PCR can detect some additional cases of ESBL producing isolates among the phenotypically sensitive species. Therefore, the genotypic method is superior to the phenotypic method. So, PCR can be used along with phenotypic method to detect ESBL producing organism.

REFERENCES

1. Foxman, B. (2010). The epidemiology of urinary tract infection. *Nat Rev Urol*, 7, 653-660.
2. Rahman, F., Chowdhury, S., Rahman, M. M., Ahmed, D., & Hossain, A. (2009). Antimicrobial resistance pattern of gram-negative bacteria causing urinary tract infection. *Stamford journal of pharmaceutical sciences*, *2*(1), 44-50.
3. Hossein, K., Sedighi, S., Behruz, M., Mehrnoush, F., & Mokhtari, M. (2017). Community Acquired Urinary Tract Infections' Etiological Organism and Antibiotics Susceptibility Patterns. *Nephro-Urol Mon*, *9*, 448-452.
4. Islam, M. S., Yusuf, M. A., Begum, S. A., Sattar, A. A., Hossain, A., & Roy, S. (2015). Extended-spectrum-beta-lactamase producing uropathogenic Escherichia coli infection in Dhaka, Bangladesh. *African Journal of Bacteriology Research*, *7*(1), 1-7.
5. Shatlov, A. (2015). Prevalence and Antibiotic Resistance Pattern of *Escherichia coli* and *Klebsiella pneumonie* in Urinary Tract Infection at the La Paz Medical Centre, Malabo, Equatorial Guinea. *Open Journal of Medical Microbiology*, *5*, 177-183.
6. Iadarilin, W., Dutta, T. K., Lalzampuia, H., & Chandra, R. (2015). Detection and characterization of extended-spectrum β-lactamases (blaCTX-M-1 and blaSHV) producing *Escherichia coli*, *Salmonella spp*. and *Klebsiella pneumoniae* isolated from humans in Mizoram. *Research Article*, 8(5), 599-604.
7. Pitout, J., & Laupland, K. (2008). Extended – spectrum-β-lactamase-producing Enterobacteriaceae: an emerging public-health concern.*Lancet Infect Dis*, *8*, 159–166.
8. Ejaz, H., Haq, I., Zafar, A., Mahmood, S., & Javed, M. M. (2011). Urinary tract infections caused by extended spectrum β-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumonia. African Journal of Biotechnology*, *10*(73), 16661-16666.
9. Clinical and Laboratory Standard Institute. (2016). CLSI Document M100-S22, *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard.* 26th Edn, Wayne, Pennsylvania, USA, 32 (3).
10. Azap, Ö. K., Arslan, H., Şerefhanoğlu, K., Çolakoğlu, Ş., Erdoğan, H., Timurkaynak, F., & Senger, S. S. (2010). Risk factors for extended-spectrum β-lactamase positivity in uropathogenic Escherichia coli isolated from community-acquired urinary tract infections. *Clinical microbiology and infection*, *16*(2), 147-151.
11. Begum, N., & Shamsuzzaman, S. M. (2016). Emergence of CTX-M-15 producing *E. coli* O25b-ST131 clone in a tertiary care hospital of Bangladesh. *Malaysian J Pathol*, *38*(3), 241 – 249.
12. Collee, J. G., Duguid, J. P., Fraser, A. G., Marmion, B. P., & Simon, S. (1996). Laboratory strategies in the diagnosis of infectious syndromes. In: Collee, J. C., Marmion, B. P., Fraser, A. G., and Simons, A. eds. *Mackie and McCartney Practical Medical Microbiology.* 14th Edn.New York: Churchill Llivingstone, pp. 53-95.
13. Bush, K. (1989). Classification of beta-lactamases: Groups 1, 2a and 2b. *Antimicrob Agents Chemother*, 33, 264-270.
14. Kattel, H. P., Mishra, S. K., Acharya, J., Shah, A. S., Rijal, B. P., & Pokhre, B. M. (2009). Relationship between Pyuria and Bacteriuria in Suspected Urinary Tract Infection.*Journal of Nepal Association for Medical Laboratory Sciences*, *10*, 19-21.
15. Sharmin, S., Alamgir, F., Begum, F., & Jaigirdar, M. Q. H. (2010). Use of Chromogenic Agar Media for Identification of Uropathogen. *Bangladesh J Med Microbiol*, *04*(01), 18-23.
16. Haque, R., Akter, L., & Salam, A. (2015). Prevalence and susceptibility of uropathogens: a recent report from a teaching hospital in Bangladesh. *BMC Res Notes*, *8*, 416-422.
17. Kulkarni, D. M., Bardapurkar, S. A., Nilekar, S. L., More, S. R. (2016). Prevalence of extended spectrum β -lactamase (ESBL) producing *E. coli* and *Klebsiella* species in urinary isolates. *IOSR Journal of Dental and Medical Sciences*, *15*(6), 26-29.
18. Rajivgandhi, G., Maruthupandy, M., Ramachandran, G., Priyanga, M., & Manoharan, N. (2018). Detection of ESBL genes from ciprofloxacin resistant Gram negative bacteriaisolated from urinary tract infections (UTIs). *Frontiers in laboratory Medicine* vailable from:http//doi.org/10.1016/j.flm.2018..01.001 [Accessed 20 May, 2018].
19. Chander, A., & Shrestha, D. C. (2013). Prevalence of extended spectrum beta lactamase producing *Escherichia coli* and*Klebsiella pneumonia* urinary isolates in a tertiary care hospital in Katmandu, Nepal. *BMC research notes*, *6*, 487-492.
20. Lina, T. T., Rahman, S. T., & Gomes, D. J. (2007). Multiple-Antibiotic Resistance Mediated by Plasmids and Integrons in Uropathogenic *Escherichia coli* and *Klebsiella Pneumoniae*. *Journal Microbial*, 24, 19-23.
21. Shakya, P., Shrestha, D., Maharjan, E., Sharma, K. V., & Paudya, R. (2017). ESBL Production Among*E. coli* and *Klebsiella spp*. Causing Urinary Tract Infection: A Hospital Based Study. *The Open Microbiology Journal*, *11*, 23-30.
22. Akram, M., Shahid, M., & Khan, A. U. (2009). Etiology and antibiotic resistance pattern of community-acquired urinary tract infections in J N M C Hospital Aligarh, India. *Annals of clinical microbiology and antimicrobials*, *6*(4). Available

from:http://www.ann-clinmicrob.com/content/6/1/4 [Accessed on 15 May 2018].

23. Moue, A., Aktaruzzaman, S. A. Q. M., Ferdous, N., Karim, M. R., Khalil, M. M. R., & Das, A. K. (2015). Prevalence of urinary tract infection in both outpatient department and in patient department at a medical college setting of Bangladesh. *International Journal of biosciences*, 7(5), 146-152.
24. Sivaleela, C., & Pragathi, E. (2014). Etiology and antibiogram of community acquired urinary tract infection in Hyderabad. *World Journal of Pharmaceutical Research*, *3*(6), 902-913.
25. Dissanayake, D. M. B. T., Fernando, S. S. N., & Chandrasiri, N. S. (2012). The distribution and characteristics of Extended-Spectrum β-Lactamase (ESBL) producing *Escherichia coli* and *Klebsiella* species among urinary isolates in a tertiary care hospital. *Sri Lanka Journal of Infectious Diseases*, *2*(2), 30-36.
26. Latifpour, M., Gholipour, A., Mohammad, S., & Damavandi, M. S. (2016).Prevalence of Extended Spectrum Beta Lactamase Producing *Klebsiellapneumoniae* Isolatesin Nosocomial and Community-Acquired Urinary Tract Infections. *Jundishapur J Microbiol*, *9*(3), 417-422.
27. Raei, F., Eftekhar, F., & Feizabadi, M. M. (2014). Prevalence of Quinolone Resistance among Extended-Spectrum β -Lactamase Producing Uropathogenic *Klebsiella pneumonia*. *Jundishapur J Microbiol*, *7*(6), 222-228.
28. Lautenbach, E., Patel, J. B., Bilker, W. B., Edelstein, P. H., & Fishman, N. O. (2001). Extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae: risk factors for infection and impact of resistance on outcomes. *Clinical infectious diseases*, *32*(8), 1162-1171.
29. Bajpai, T., Bhatambare, G. S., Pandey, M., & Varma, M. (2018). Prevalence of TEM, SHV, and CTX-M Beta-Lactamase genes in the urinary isolates of a tertiary care hospital. *Avicenna Journal of Medicine*, *7*, 6-12.