Research article

Antibiotics resistance of extended spectrum beta lactamases uropathogenic *Escherichia coli* in Peshawar-Pakistan

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Abstract

Urinary tract infection (UTI) is predominantly caused by *Escherichia coli* (*E. coli*). *E. coli* is the major extended-spectrum beta-lactamases (ESBL) producing uropathogenic bacteria, leading to serious complications and limiting therapeutic management. This study was undertaken to measure the proportion of ESBL producing and antibiotic susceptibility pattern of uropathogenic *E. coli* in Peshawar. During the study duration, a total of 214 isolates of uropathogenic *E. coli* with urine samples were collected from patients. The urine samples were inoculated on the recommended media for the growth of bacteria. The bacteria growth was identified based on clinical laboratory standard institute (CLSI) protocol. The antibiotic susceptibility testing (AST) was carried out for *E. coli* using various discs of antibiotics. The ESBL producing *E. coli* were identified by double discs diffusion method. Among the studied isolates, 46.3% were collected from male patients. The highest percentage of patients was identified in the age group of 21-30 years. The female patients were observed more resistant to antibiotics. Similarly, ESBL producing isolates were more resistant than non-ESBL producing isolates. The *E. coli* isolates were more resistant to few antibiotics including nalidixic acid, cefuroxime sodium, cephadrine, and ofloxacin while least to imipenem, fosfomycin, and meropenem. Conclusively, MDR and ESBLs producing *E. coli* isolates were observed more challenging in the studied region. Therefore, the early identification of ESBL producing and MDR *E. coli* isolates may be helpful to prevent further resistance in patients and will be helpful for better management of UTIs.

Keywords: Antibiotics; *E. coli*; ESBL; Multi-drug resistant; Peshawar

Introduction

Urinary tract infection (UTI) is the most common nosocomial and community bacterial infection and significantly

responsible for health care cost and morbidity rate [1, 2]. The un-diagnosed UTIs leads to damage renal structure and function (kidney failure) [3]. The UTIs are responsible for

almost 35% of nosocomial infections [4, 5]. UTIs are the prevalent infectious diseases affecting about 0.15 billion individuals worldwide each year. Affecting all age groups of both sexes unanimously but the proportion of females is more as compared to males [6, 7]. Among 100 healthy females, every 5th female is suffering from uncomplicated UTIs cases annually while about twenty percent of cases occurring in males [7].

The gram-negative bacteria are the most common etiological agent of UTIs [8] especially the *Escherichia coli* (*E. coli*) is identified as the predominant causative bacterial isolates responsible for more than 80% of uncomplicated UTIs cases [9]. Other gram-negative isolates like *Klebsiella species* (spp.), *Pseudomonas spp.*, *Proteus spp.* and *Enterobacter spp.* and gram-positive isolates including *Staphylococcus spp.*, *Streptococcus spp.* and *Enterococcus spp.* account from 5-15% of UTIs cases [10, 11]. The UTIs treatment is a challenging task due to antibiotics resistance but frequently treated with broad-spectrum fluoroquinolones (ciprofloxacin), cephalosporins (ceftazidime, cefepime) and aminoglycosides (gentamicin, amikacin). The fluoroquinolones are inhibitors of DNA topoisomerase and gyrase, cephalosporins are antibiotics to inhibits cell wall and aminoglycosides can inhibit the protein synthesis of bacteria [12]. Among these antibiotics, beta (β) lactam antibiotics are frequently used as a therapeutic agent for UTIs particularly related to gram-negative bacterial infections [13].

The antibiotics resistance increased all over the world because of the production of extended beta spectrum lactamases (ESBL) producing by bacteria due to the frequent use of beta-lactam antibiotics in the last two decades [14-16]. The ESBL producing bacterial isolates are associated with greater morbidity and fatality than non-ESBL producer strain of *E. coli* [17]. The *E. coli*

producing ESBL is possibly one of the important mechanisms of resistance to cephalosporins and penicillins which may be chromosomally mediated or plasmid-mediated β -lactamases [18, 19]. The treatment of *E. coli* with beta-lactam antibiotics in the presence of ESBL production is useless [20]. Moreover, the ESBL producing *E. coli* strains are usually related to multi-drug resistance (MDR). The coexistence of MDR and ESBL production worsens the condition and complicates the treatment particularly in developing countries [16].

The ESBL producing *E. coli* strains have been significantly determined from various regions of the world. Inter-regional antibiotics susceptibility variation is high in ESBL producing *E. coli*. It is important to know and understands the common ESBL producing bacteria such as *E. coli* for necessary management. Therefore, the current study was undertaken to find the percentage of uropathogenic *E. coli* producing ESBL and its antibiotic susceptibility profile in Peshawar, Pakistan.

Materials and methods

Study design, duration and study setting

This cross-sectional descriptive study was performed in the microbiology department of a well-reputed private laboratory (Pak Medical Center and Laboratory) in Peshawar from January to June 2019.

Study population

Inclusion criteria

The patients visited clinics were included in the study population. The patients suspected UTIs with symptoms such as an intense urge to urination; fever/chills, abdominal pain (back or lower), and burning micturition were included.

Exclusion criteria

The UTI patients received antibiotic history in the last one week, have urinary catheterization, and samples without labeling or held for more than 2 hours without any

preservative or at temperature 0-8°C were excluded from the study. The isolates were non-repetitive (single sample per patient).

Ethical consideration

The permission of the study was obtained from authorized committee members of the Pak Medical Center and Laboratory.

Sample collection and processing

The midstream fresh urine samples (5-10 ml) were collected in screw-capped, sterile container aseptically and were further processed for culture and sensitivity within two hours of collection. The urine samples were streaked on cysteine lactose electrolyte deficient agar (CLED), MacConkey agar (MA) and Blood agar (BA) (Oxide, Ltd England) plates according to the clinical laboratory standard institute (CLSI) guidelines [21]. The culture Petriplates were aerobically incubated at 37°C for overnight. The culture plates were checked periodically for bacterial growth after 18-24 hours. The gram-negative bacteria were identified by the morphological appearance of colonies, characteristics of gram staining, and biochemical tests (lactose fermentation, triple sugar iron, indole reaction, and citrate utilization) as per CLSI protocol [21]. The *E. coli* growth with more than 10 colony-forming units per milliliter (ml) was considered clinically significant [22].

Antibiotics susceptibility testing

The antibiotic susceptibility testing for recovered *E. coli* isolates was performed on Mueller Hilton Agar (MHA) by Kirby Bauer disk diffusion method. A total of 18 commercially available antibiotic discs (Oxide, Ltd England) were used with their appropriate concentrations: amikacin (30ug), amoxicillin (10ug), augmentin: amoxicillin-clavulanic acid (30ug), azithromycin (15ug), aztreonam (30ug), cephadrine (30ug), cefotaxime (30ug), cefoxitin (30ug), ceftazidime (30ug), ceftriaxone (30ug), cefuroxime (30ug), ciprofloxacin (5ug), fosfomycin (50ug), gentamicin(10ug),

imipenem (10ug), meropenem (10ug), nalidixic acid (30ug), nitrofurantoin (30ug), norfloxacin (10ug), ofloxacin (5ug), piperacillin-tazobactam (40ug) and cotrimoxazole: trimethoprim-sulphamethoxazole (25ug). The zone of inhibition around antibiotics discs formed by *E. coli* was measured in millimeter (mm) and interpreted according to the CLSI standards. *E. coli* strain American Type Culture Collection (ATCC) 25922 was used as a reference control strain [21, 22].

ESBL identification

All the recovered *E. coli* strains were tested for phenotypic detection of ESBL production. The ESBL producing *E. coli* isolates were initially identified by a zone of inhibition of cephalosporins (cefotaxime, cefoxitin, ceftazidime, and ceftriaxone) which was the further process for confirmation and were verified as ESBL producer by double disc diffusion method (cephalosporins and monobactam). The clear zone of inhibition (≥ 5 mm) of cephalosporins extended towards the aztreonam was considered as ESBL producing *E. coli*. The *E. coli* strain was considered as multi-drug resistant if it was resistant to three or more antibiotics of various groups/classes of antibiotics [21, 22].

Data analysis

The data were analyzed by statistical package for social sciences (SPSS version 21). The frequency and percentages were calculated for all categorical variables such as gender, microorganism, and ESBL number. The mean and standard deviation was computed for the age of patients.

Results and discussion

During the study period, a total of 214 confirmed *E. coli* isolates were collected from urine samples with UTIs patients. Out of total (214), males were 46.3% (n=99) and females patients were 53.7% (n=115). The prevalence of females (53.7%) was more than male (46.3%) in present findings. This

predominance in female to male is concordance the study of Polse et al. from Iraq [23]. The study conducted by Jamil et al., from Swabi, Pakistan revealed 66.3% prevalence in females [24]. Our finding also supports other previously conducted studies with the same proportion of female to male urinary tract infections cases caused by *E. coli* [14, 15]. The high number of female patients could be due to anatomic posture of females including urethral opening, the proximity of the urethra to anus, short urethra, and during pregnancy physiologically leads to urinary tract infections [16].

The mean age of male patients was 45.1 ± 19.4 years in which majority male patients were noted in 31-40 years of age group (19.2%, n=19), followed by 21-30 years (18.2%, n=18) and 51-60 years (17.2%, n=17). On the other hand, frequent cases of females were observed in the age group of 21-30 years (24.3%, n=28), followed by 51-60 years and 61-70 years (16.5%, n=19 each). The highest number of urinary tract infections cases found in the age group of 21-30 years with 21.5% (n=46) (Table 1). It is observed in our study that UTIs caused by *E. coli* were more frequently occur in 11-50 years of age with the highest number of cases identify in the age group of 21-30 years (21.5%). Our findings in line with the results of different researchers which reveals a high prevalence of UTI caused by *E. coli* at the age of 21-30 years [12, 17]. The frequent cases at a young age maybe because of sexual activity and using certain kinds of contraceptives can raise the risk at the age of 21-30 years [18].

The present study shows that 88.3% (n=189) strains were resistant to three or more classes of antibiotics and are known as the multi-drug resistant in which highest proportion of patients were occurred in the age group of 21-30 years (22.2%, n=42), followed by 51-60 years (16.9%, n=32) and 31-40 years (15.3%, n=29) (Table 1). The highest number of

MDR found in ESBL producing *E. coli* infected patients with 90.1% (n=145) as compared to non-ESBLs (83.0%, (n=44) (Table 2). Furthermore, a large proportion of MDR isolates were observed in males (89.9%, n=89) than female patients (87%, n=100) (Table 3). Almost similar MDR prevalence (81.9%) was reported by Shakhathreh et. al from Jordan [25]. A previous study reports showed 82%, 65.5% and 65% MDR isolates of uropathogenic *E. coli* [14, 20, 23]. Additionally, the current study shows that 76.7% of MDR isolates identified in ESBL producing *E. coli*. Ullah et al., reported 83% MDR isolates in ESBL producing *E. coli* [26]. Moreover, this study indicated that drug resistance was high among ESBL producing *E. coli* than the non-ESBL producer. The production of beta-lactamases enzymes may be a plasmid or chromosomal origin. Plasmid origin is occurred by transferring genetic materials from one to other organisms which leads to antibiotics resistance [25]. Therefore, the MDR isolates are probable to be common in *E. coli* producing ESBL isolates.

The ESBLs phenotype was detected in 75.2% (n=161) *E. coli* isolates in which highest number of isolates observed in age group of 21-30 years (22.3%, n=36), followed by 61-70 years (19.8%, n=32) and 51-60 years (13.6%, n=22) (Table 1). The percentage of ESBLs producing *E. coli* was found higher in males (76.8%, n=76) as compared to females (73.9%, n=85) (Table 3). The percentage of *E. coli* producing ESBL was 75.2% which is similar to the study conducted at by Ahn et al., and Al-Mously, et al. which is 71.7% and 75.1% respectively [27, 28] while high with the study conducted at Jordan by Shakhathreh et. al (49.9%) [25]. In contrast to the report of Jamil et al., where ESBLs producing *E. coli* proportion is 33.3% [24]. Another study reported 64.9% ESBL producing *E. coli* isolates which nearly similar to our study [23]. Ahmed et al., from Pakistan reported

53.4% ESBL producing isolates of *E. coli* which is lower than the present study [29]. Additionally, multi-drug resistant and ESBL producing *E. coli* cases was high in male

(MDR=46.3% & ESBL=47.2%) as compared to female patients (MDR=53.7% & ESBL=52.8%) that agreed with previous findings [27].

Table 1. According to the age and gender of patients, MDR, and ESBL producing isolates of *E.coli* are distributed

Age (Years)	Male % (n)	Female % (n)	Total % (n)	MDR % (n)	ESBL % (n)
0-10	01.0 (01)	0.90 (01)	0.90 (02)	1.10 (02)	1.10 (02)
11-20	09.1 (09)	11.4 (13)	10.3 (22)	10.6 (20)	10.5 (17)
21-30	18.2 (18)	24.3 (28)	21.5 (46)	22.2 (42)	22.3 (36)
31-40	19.2 (19)	10.4 (12)	14.5 (31)	15.3 (29)	13.0 (21)
41-50	12.1 (12)	13.0 (15)	12.6 (27)	13.8 (26)	11.7 (19)
51-60	17.2 (17)	16.5 (19)	16.8 (36)	12.2 (23)	13.6 (22)
61-70	15.1 (15)	16.5 (19)	15.9 (34)	16.9 (32)	19.8 (32)
>70	08.1 (08)	7.0 (08)	07.5 (16)	7.90 (15)	08.0 (13)
Total	46.3 (99)	53.7 (115)	100 (214)	88.3 (189)	75.2 (161)

The *E. coli* showed higher resistance to nalidixic acid (93.9%), followed by cefuroxime sodium (93.5%), cephadrine (90.2%) and ofloxacin (90.2%) while least resistance was found against to imipenem (25.7%), fosfomycin (36.0%) and

meropenem (38.8%). However, antibiotics susceptibility analysis illustrates that all ESBL producing *E. coli* strains have a higher resistance to almost all tested antibiotics except cephadrine and gentamicin as compared to non-ESBL strains (Table 2).

Table 2. Comparison of antibiotic resistance *E. coli* isolates between ESBL and Non-ESBL

Antibiotics	ESBL % (n=161)	Non-ESBL % (n=53)	Total Resistance % (n=214)
MDR % (n)	76.7 (145)	23.3 (44)	88.3 (189)
Amikacin	60.9 (98)	47.2 (25)	53.7 (115)
Augmentin	87.6 (141)	77.4 (41)	83.6 (179)
Aztreonam	100 (161)	60.4 (32)	79.9 (171)
Ceftazidime	93.2 (150)	67.9 (36)	84.6 (181)
Cefotaxime	93.8 (151)	75.5 (40)	86.4 (185)
Ceftriaxone	94.4 (152)	75.5 (40)	88.3 (189)
Cefuroxime sodium	96.3 (155)	88.7 (47)	93.5 (200)
Cephadrine	93.2 (150)	94.3 (50)	90.2 (193)
Ciprofloxacin	91.9 (148)	83.0 (44)	84.6 (181)
Fosfomycin	32.9 (53)	26.4 (14)	36.0 (77)
Gentamicin	68.9 (111)	71.7 (38)	68.7 (147)
Imipenem	31.7 (51)	18.9 (10)	25.7 (55)
Meropenam	42.2 (68)	24.5 (13)	38.8 (83)
Nalidix acid	96.9 (156)	88.7 (47)	93.9 (201)
Nitrofurantoin	69.6 (112)	60.4 (32)	70.6 (151)
Ofloxacin	93.8 (151)	83.0 (44)	90.2 (193)
Piperacillin/tazobactam	64.6 (104)	41.5 (22)	59.3 (127)
Sulbactam/Cefoperazone	63.4 (102)	45.3 (24)	58.4 (125)

It was also observed that approximately all antibiotics besides few (ceftriaxone, gentamicin, nalidixic acid, and nitrofurantoin) have a better response to uropathogenic *E. coli* in males as compared to female. The carbapenem and fosfomycin were most effective against uropathogenic *E. coli* while the least effective was nalidixic acid, cefuroxime sodium, cephradine, and ofloxacin. Our finding was consistent with the previous studies that reported high resistance to nalidixic acid and cefuroxime [14, 19]. Sharma et al., from India mentioned that uropathogenic *E. coli* showed higher sensitivity to imipenem that is consistent with the finding of this study [28]. In a recent study, researchers revealed similar imipenem sensitivity as the current study [13, 17, 23].

Furthermore, this study showed that almost all tested antibiotics were less resistant against non-ESBLs than ESBLs producing *E. coli* except cephradine and gentamicin. A similar finding was reported by the previous report that is agreed with the present study [17].

In this study, the male showed higher resistance than female to various antibiotics including amikacin, augmentin, aztreonam, ceftazidime, cefotaxime, cefuroxime sodium, cephradine, ciprofloxacin, fosfomycin, imipenem, meropenem, ofloxacin, piperacillin/ tazobactam, and sulbactam/ cefoperazone whereas few antibiotics (ceftriaxone, gentamicin, nalidixic acid, and nitrofurantoin) showed higher resistance in female as compared to male (Table 3).

Table 3. Comparison of antibiotic resistance between male and female infected patients

Antibiotics	Male% (n) 99	Female % (n) 115
MDR	46.3 (89)	53.7 (100)
ESBL	47.2 (76)	52.8 (85)
Amikacin	59.6 (59)	55.6 (64)
Augmentin	85.9 (85)	84.3 (97)
Aztreonam	91.9 (91)	87.8 (101)
Ceftazidime	87.9 (87)	86.1 (99)
Cefotaxime	92.9 (92)	86.1 (99)
Ceftriaxone	88.9 (88)	90.4 (104)
Cefuroxime sodium	99.0 (98)	92.2 (106)
Cephradine	96.0 (95)	91.3 (105)
Ciprofloxacin	90.9 (90)	87.8 (101)
Fosfomycin	32.3 (32)	30.4 (35)
Gentamicin	59.6 (59)	78.3 (90)
Imipenem	36.4 (36)	19.1 (25)
Meropenem	43.4 (43)	33.0 (38)
Nalidixic acid	92.9 (92)	95.7 (110)
Nitrofurantoin	65.7 (65)	77.4 (89)
Ofloxacin	96.0 (95)	87.0 (100)
Piperacillin/tazobactam	61.6 (61)	56.5 (65)
Sulbactam/Cefoperazone	94.9 (94)	53.9 (62)

This study has some limitations; the study was limited to a single center with a small sample size and also could not confirm the ESBLs producing *E. coli* at a molecular level. This study does not reveal the picture of the

whole country; hence longitudinal and prospective study is necessary to a cover wide range of population to get a geographical, ethnic, and seasonal variation of *E. coli* and their susceptibility pattern of

antibiotics. Although, there were few limitations we believe that this study provides better information for selecting an empirical therapy and will help clinicians in the future to control the continuous resistance of antibiotics.

The higher number of MDR and ESBL producing isolates in our regions is a great public problem. This indicates that continuous monitoring, infection control measurement, and proper administration of antibiotics are required to reduce the frequency of ESBL. Additionally, antibiotics should be selected for the treatment of urinary tract infections based on culture and sensitivity reports of urine.

Conclusion

Our study highlights the frequency of ESBL producing *E. coli* along with MDR isolates and their antibiotics susceptibility pattern in our regions. The higher percentages of MDR were observed in male and ESBL producing *E. coli* isolates. These findings suggest further molecular studies regarding the MDR and ESBL strains of *E. coli*. Moreover, it is strongly recommended that appropriate antibiotics therapy and periodical surveillance can play a better role in the control of the antibiotics resistance strains along with ESBL producers. Furthermore, novel drugs and their selection against particular isolates are need of today.

Authors' contributions

Conceived and designed the experiments: A Muhammad, S Ali & MJ Khan, Performed the experiments: A Muhammad, S Ali, SL Shuaib, IQ Afridi & W Saeed, Analyzed the data: A Muhammad, N Ali & W Saeed, Contributed materials/ analysis/ tools: N Ali, W Saeed & MJ Khan, Wrote the paper: A Muhammad, N Ali, S Ali & SL Shuaib
Critical Review: N Ali, SL Shuaib, IQ Afridi and MJ Khan

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