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Molecular prevalence and antibiotics resistance pattern of class A bla CTX-M-1 and bla TEM-1 beta lactamases in uropathogenic *Escherichia coli* isolates from Pakistan

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Background/aim: Extended spectrum beta lactamase (ESBL) production among *E. coli* is one of the principal mechanisms that augment resistance to antibiotics. In the current study the molecular detection of class A beta lactamases among uropathogenic *Escherichia coli* was evaluated.

Materials and methods: A total of 355 urine samples were collected from a tertiary care hospital in Peshawar. The ESBL production among *E. coli* isolates was detected by using the disc synergy diffusion method. Moreover, the molecular detection of bla TEM-1 and bla CTX-M-1 ESBLs, the antibiotic resistance pattern, and the minimum inhibitory concentrations (MICs) were also documented.

Results: Among the 355 urine samples, 157 isolates were *E. coli*, and 23.56% of the isolates were ESBL *E. coli*. Among phenotypic ESBL producers, bla CTX-M-1 and bla TEM-1 were found in 59.45% and 40.54% of the isolates, respectively. A high resistance rate was observed against aztreonam (97.29%), while the lowest resistance was observed against imipenem (2.7%). The MICs of ESBLs *E. coli* for ceftriaxone, ciprofloxacin, and gentamicin was >512 µg/mL, 4 µg/mL to 128 µg/mL, and 1 µg/mL to 14 µg/mL respectively.

Conclusion: The present study showed that bla CTX-M-1 ESBL production is more prevalent in our clinical *E. coli* isolates. More often the ESBLs were resistant to commonly used antibiotics.

Key words: Urinary tract infection, *E. coli*, ESBLS, bla CTX-M-1, bla TEM-1, antibiotics profile

1. Introduction

Urinary tract infection (UTI) is the invasion and inflammation of the urinary tract structures caused by microbial agents in human (1,2). About 150 million people worldwide are diagnosed with UTI each year (3). Bacterial agents like Enterobacteriaceae are frequently isolated from UTI samples (84.3%) (4). Among Enterobacteriaceae, *Escherichia coli* is the most common causative agent of UTI (5).

UTIs are mainly treated with beta lactam antibiotics; however, acquired resistance to these antibiotics in UTI pathogens is commonly augmented by bacterial enzymes, and leads to the emergence of extended spectrum beta lactamases (ESBLs) (6). ESBL producing pathogens of bla TEM and bla SHV types were primarily hospital acquired and were usually produced by several enteric bacterial pathogens; however, this scenario has been changed (6,7). Among bla TEM, type TEM-1 was early described and is able to hydrolyze penicillin and early generation cephalosporins (7).

CTX-M ESBLs are another type of beta lactamases that have been widely reported in *E. coli* (6–8). CTX-M-1 type beta lactamases confer resistance to expanded-spectrum cephalosporins (8). As far as literature mining is concerned, we have not found any study about the occurrence of bla CTX-M-1 and bla TEM-1 among uropathogenic *E. coli* in Pakistan.

The present study documented the molecular prevalence of bla CTX-M-1 and bla TEM-1 ESBL producing *E. coli* causing urinary tract infections. Furthermore, the isolates were tested for antibiotic resistance pattern against commonly used antibiotics. The findings of the current study will be helpful for the control of antibiotic resistance among *E. coli* isolates from UTI patients.

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2. Materials and methods

2.1. Samples

This study was conducted at the Department of Microbiology, Kohat University of Science and Technology, Kohat, Pakistan, from April 2013 to January 2014. A total of 355 urine samples were collected from suspected UTI patients at a tertiary care hospital in Peshawar. Written consent was taken from each patient included in the study. The departmental ethical review board approved the study.

2.2. Processing of samples

All urine samples were inoculated on cystine lactose electrolyte deficient (CLED) agar (Oxoid, UK). These plates were incubated overnight at 37 °C aerobically. Only those samples were processed for ESBL production that showed significant growth and were identified as *E. coli* on the basis of culture and biochemical characteristics.

2.3. Determination of ESBL *E. coli*

ESBL production of *E. coli* isolates was investigated as described by Jarlier et al. (9). Briefly, 0.5 McFarland dilution of the test isolate in nutrient broth was swabbed on Mueller Hinton agar (MHA). An amoxicillin/clavulanic acid (AMC, 20/10 µg) disc was placed at the center of the MHA plate while discs of aztreonam (ATM, 30 µg), ceftazidime (CAZ, 30 µg), cefotaxime (CTX, 30 µg), and ceftriaxone (CRO, 30 µg) were placed in close proximity of 15 mm distance. The inhibition zone of cephalosporin towards the AMC disc was interpreted as an ESBL producer. *E. coli* ATCC 25922 strain was used as quality control with disc diffusion and tube dilution assays.

2.4. Molecular detection of bla (CTX-M-1) bla (TEM-1) ESBLs

Polymerase chain reaction (PCR) was employed to detect genes responsible for the ESBL producers. DNA amplification of the $bla_{CTX\text{-}M\text{-}1}$ coding region (780 bp) was performed using forward (5'-CGTCACGCTGTTGTTAGGAA-3') and reverse (5'-ACGGCTTTCTGCCTTAGGTT-3') primers, while a 966 bp fragment corresponding to the $bla_{TEM\text{-}1}$ entire coding region was amplified by using a forward (5'-TCGGGGAAATGTGCG-3') and a reverse primer (5'-TGCTTAATCAGTGAGGCACC-3') (10). Briefly, DNA was extracted by NucleoSpin tissue kit (Macherey-Nagel, Germany). For PCR amplification, 1 µL of template DNA was added to 25 µL of the master mixture having 1.5 µL of DNTP mixture (0.2 mM of each), 2.5 µL of PCR buffer , 0.5 µL of Taq polymerase, 0.5 µL of each primer stock solution (50 pmol/µL), and 0.5 µL of $MgCl_2$, and the remaining 18 µL volume was filled by nuclease free water (Fermentas, USA). The amplified product was resolved on agarose gel and visualized under a UV transilluminator.

2.5. Antibiotics susceptibility patterns of ESBLs *E. coli*

The antibiotic sensitivity pattern of ESBL *E. coli* isolates was determined by the standard Kirby–Bauer method (11). Briefly, a 0.5 McFarland dilution of the already refreshed bacterial isolate in nutrient broth was swabbed on MHA. Antibiotic discs (Oxoid, UK) of known concentration were placed on the MHA plates and then incubated overnight at 35 ± 2 °C. Following incubation, the zone of inhibition was measured and interpreted as discussed in the Clinical Laboratory Standard Institute (CLSI) guidelines (12). Susceptibility (minimum inhibitory concentration (MIC)) to colistin sulfate (CT-10 µg) and tigecycline (TGC-15 µg) was measured by the broth dilution method.

2.6. Determination of MICs

MICs were determined using the standard two-fold tube dilution method for ceftriaxone, ciprofloxacin, and gentamicin (13).

3. Results

Approximately one-third of the 157 *E. coli* isolates showed ESBL activity as shown in Figure 1. Thus, the overall prevalence was 23.56% (n = 37), while non-ESBL producers were 76.43% (n = 120).

ESBL *E. coli* isolates (n = 37) were subjected to PCR to identify the presence of bla CTX-M-1 and bla TEM-1 genes. A total of 33 (89.18%) isolates were positive for bla CTX-M-1 and bla TEM-1 genes. Individually, bla CTX-M-1 was detected in 22 (59.54%) isolates and bla

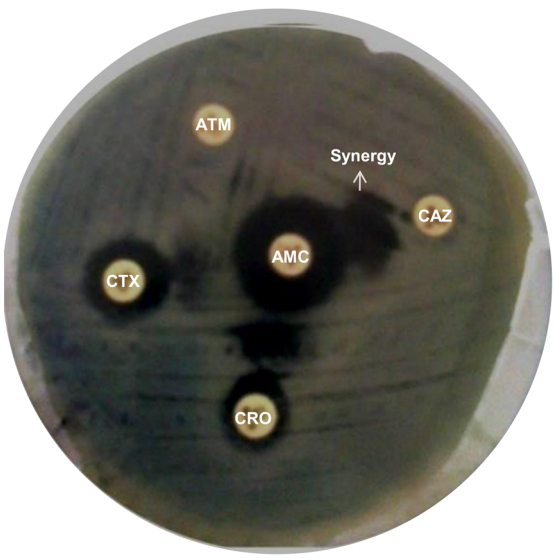

Figure 1. Phenotypic detection of ESBL producing *E. coli*. The AMC (amoxicillin/clavulanic acid) disc was placed at the center of the agar plate, while the discs of ATM (aztreonam), CAZ (ceftazidime), CRO (ceftriaxone), and CTX (cefotaxime) were placed in close proximity. The resistance of the *E. coli* isolate to all cephalosporin and aztreonam, and the synergy between AMC and CAZ, phenotypically confirmed the ESBL production.

TEM-1 was detected in 15 (40.54%) isolates of ESBL *E. coli*. The size of the amplified bla CTX-M-1 and bla TEM-1 genes was 780 bp and 971 bp, respectively (Figure 2).

Moreover, the antibiotic susceptibility pattern of ESBL *E. coli* was determined. ESBL producing *E. coli* were resistant to aztreonam (n = 36; 97.29%) and ceftriaxone (n = 35; 94.59%). Nineteen (51%) ESBL producing *E. coli* were susceptible to piperacillin/tazobactam, while 18 (49%) were susceptible to fosfomycin (FOS50); however, imipenem showed excellent activity (n = 35; 94.59%) against ESBL producing *E. coli* (Table).

MICs were determined for selected drugs commonly used against ESBL *E. coli*. It was found that the MIC of ceftriaxone was >512 µg/mL against ESBL *E. coli* isolates, followed by ciprofloxacin, which ranged from 4 µg/mL to 128 µg/mL, and gentamicin, which ranged from 1 µg/mL to 14 µg/mL.

4. Discussion

UTIs are caused by a microbial attack and succeeding multiplication in the urinary tract (14). It is the most common form of infection, mainly caused by *E. coli*. In the

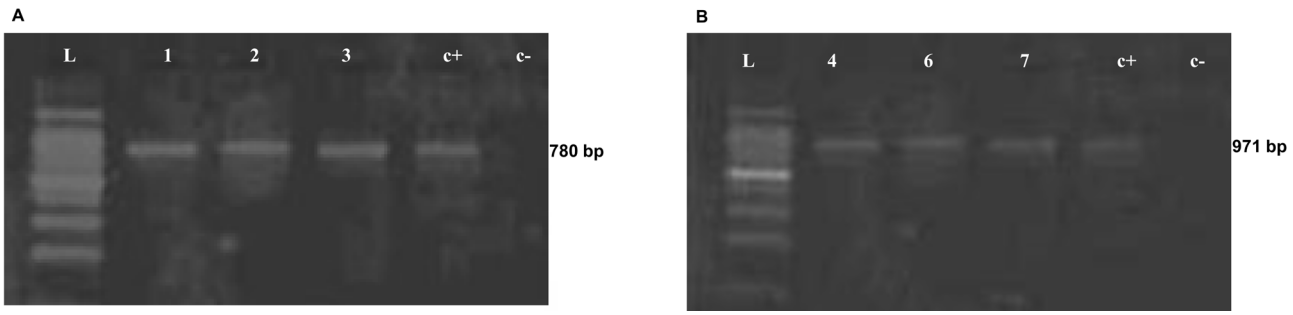

Figure 2. Molecular detection of bla CTX-M-1 and bla TEM-1 ESBLs producing *E. coli*. (A) bla CTX-M-1 gene amplification. Product size: 780 bp. L: Ladder (100 bp). 1–3: samples. c+: positive control (*Pseudomonas aeruginosa* having CTX-M-1); c^-: negative control (*Bacillus* spp. lack CTX-M-1). (B) bla TEM-1 gene amplification. Product size: 971bp. L: Ladder (100 bp). 4, 6, 7: samples. c+: positive control (*Pseudomonas aeruginosa* having TEM-1); c^-: negative control (*Bacillus* spp. lacking TEM-1).

Table. Antibiotic susceptibility pattern[a] of ESBL producing *E. coli* isolates.

Antibiotics (Abb.)	Disc content (µg)	Susceptible isolates n (%)	[a]Intermediate isolates n (%)	[a]Resistant isolates n (%)
[b]Colistin sulfate (CST)	10	5 (0)	0 (0)	32 (86.48)
Aztreonam (ATM)	30	0 (0)	I (2.70)	36 (97.29)
Ceftriaxone (CRO)	30	0 (0)	2 (5.40)	35 (94.59)
Sulfamethoxazole/trimethoprim (SXT)	1.25/23.75	4 (10.81)	5 (13.51)	28 (75. 67)
Nitrofurantoin (F)	300	4 (10.81)	2 (5.40)	31 (83.78)
Ciprofloxacin (CIP)	5	6 (16.21)	1 (2.70)	30 (81.08)
[c]Tigecycline (TGC)	15	13 (35.13)	8 (21.62)	16 (43.24)
Gentamicin (CN)	10	11 (29.72)	10 (27.02)	16 (43.24)
Fosfomycin (FOS)	200	18 (48.64)	4 (10.81)	15 (40.54)
Piperacillin/tazobactam (TZP)	100/10	19 (51.35)	1 (2.70)	17 (45.94)
Imipenem (IPM)	10	35 (94.59)	1 (2.70)	1 (2.70)

Abb.: abbreviation.

[a] = as per CLSI guidelines (12).

[b] = Susceptibility was measured by the broth dilution method and interpreted as sensitive (S) if MIC ≤ 2 µg/mL), and resistant (R) if MIC ≥ 4 µg/mL) (32, 33).

[c] = Susceptibility was measured by the broth dilution method and interpreted sensitive (S) if MIC ≤ 2 µg/mL), and resistant (R) if MIC ≥ 8 µg/mL) (34).

present study, among the suspected cases of UTIs, 44.3% of the positive cultures were *E. coli*, which is in agreement with findings reported by a previous study (15).

Complications in UTIs have been amplified because of the occurrence of ESBL producing *E. coli*. ESBLs are the result of the overuse of third generation cephalosporins and monobactams (16). It is difficult to craft a valid similarity of the prevalence of ESBLs because of differences in the study design (17). Several studies from Pakistan in 2005 reported a 40% ESBL production rate, and two other studies in 2009 reported a 43% and a 58.7% production rate, respectively (18,19); in 2011 the production rate was 64% (10). In our study ESBL prevalence was 23.56% and similar results were reported by another study (20). In Pakistan the prevalence and distribution of ESBLs differ geographically (21). The inconsistency in the reported results has increased the need for an improved method of ESBL detection and its incorporation into routine laboratory procedures.

From literature mining we have not found any study about the prevalence of bla CTX-M-1 and bla TEM-1 among uropathogenic *E. coli* in Pakistan. Therefore, in the current study, we examined the occurrence and molecular detection of ESBL producing *E. coli* by amplifying bla CTX-M-1 and bla TEM-1 genes (7). In this study bla CTX-M-1 (59.45%) and bla TEM-1 genes (40.54%) originated from phenotypically confirmed ESBL producing *E. coli* isolates. Shafiq et al. (20) in their study in 2014 reported similar to our results. Epidemiological data reveal that some enzymes are more regularly reported than others, but the main enzyme type differs by country and the varied CTX-M types are frequently reported within a particular country (22).

A wide range of resistance has been reported in Pakistan among Enterobacteriaceae and other organisms isolated from urine to commonly used antibiotics like ampicillin, tetracycline, and cotrimoxazole (14). Nowadays, organisms with multiple antibiotic resistance genes are becoming more prevalent (23). In this study aztreonam and amoxicillin/clavulanic acid were found 90%–100% resistant; this is in agreement with other studies (18,24,25). Third generation cephalosporins have been used for gram negative bacterial treatment (26). In the present study *E. coli* was found to be 94% resistant to ceftriaxone (24). This high rate of resistance may be due to the lack of antibiotic policy and the irrational use of third generation cephalosporins, mainly ceftriaxone, in the hospital (27). However, in the current study, *E. coli* was highly resistant to ciprofloxacin (81%), which is consistent with the results of previous findings (90.9%) reported from Bangladesh (28).

Aminoglycosides show good action against gram negative rods (29). In the present study 43% of the isolates were resistant to gentamicin, which is similar to previous studies (24,25). In 2012, gentamicin resistance in Pakistan and Bangladesh was reported as 60% and 55.5%, respectively (18,25,28). This peculiarity may be due to the nonjudicious use of gentamicin in different regions (30). Carbapenems are drugs of choice for several infections caused by bacterial pathogens (18). Imipenem showed the best results and *E. coli* resistance was only 2.7%, suggesting that this antibiotic can still be used for UTI treatment (31).

MICs for ceftriaxone, ciprofloxacin, and gentamicin ranged from 1 to 1024 μg/ mL, 4 to 128 μg/ mL, and 1 to 32 μg/mL, respectively. High MICs for third generation cephalosporins make them inappropriate for empirical therapy. The antibiotic resistance in *E. coli* isolated from UTIs indicates the need for careful examination and antibiotic recommendation following culture sensitivity tests.

In short, the overall prevalence of ESBLs was 23.56%, while bla CTX-M-1 production was more prevalent in *E. coli*. It was found that imipenem exhibited potential activity against resistant *E. coli* and may be an appropriate choice for the treatment of UTI. These findings will be helpful for prescribing appropriate antibiotics for UTI patients infected with *E. coli*.

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