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Susceptibility Pattern of Extended Spectrum *β-lactamases* Positive *Escherichia coli* isolated from a Tertiary Care Hospital of Peshawar, Pakistan

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Abstract: The emergences of extended spectrum β-*lactamases* (ESBLs) are on the rise in hospital settings across the world. This study was carried out in order to isolate and identify the ESBLs producing *Escherichia coli* in the urine samples and to also find antibiotics susceptibility pattern in the clinical isolates. *E. coli* is the most common pathogen isolated from urine that exhibit the production of ESBLs that hydrolyse oxyimino beta lactam drugs and challenge the management of infections. A total of 195 urine samples were collected from the UTI suspected patients admitted at Lady Reading hospital, a tertiary care unit in Peshawar, Khyber Pakhtoonkhawa (KPK), Pakistan. The organisms were isolated, morphologically screened and biochemical characterized following standard microbial protocols. The isolated *E. coli* were screened for ESBLs production by double disk synergy method. Out of 68 positive samples 58 (85.2%) were *Escherichia coli* while, *Streptococcus faecalis*6 (8.8%), *Enterobacteraerogens* 2 (2.9%), *Staphylococcus epidermidis*1 (1.4%) and *Proteus mirabilis* 1 (1.4%) were also identified. Thirty eight out of 58 *E. coli* samples were identified as ESBLs producers. Frequency of ESBLs producer *E. coli* was high in females 24 (63.2%) in comparison to males 14 (36.8%). Furthermore, prevalence of ESBLs producing *E. coli* was high (47.3%) in age group >50 years. ESBLs producing *E. coli* were multi drug resistant. Finally,Meropenem, Tazobactum+Pipracilline, Salbactum+Cefaperazone and Amikacin are treatment of choice for urinary tract infections caused by ESBLs producing *E. coli*.

Key words: Extended Spectrum β-*lactamases* · *Escherichia coli* · Double Disk Synergy Method · Urinary Tract Infections

INTRODUCTION

Urinary tract infections (UTIs) are of worldwide prevalence [1]. It is estimated that about 150 million UTIs occur per year worldwide affecting both genders but the prevalence of UTIs is greater in females than male with every 50 out of 1000 healthy women reporting uncomplicated UTIs each year. It is estimated that about 50% of women experience one episode of UTI at some point in their lifetime and about 20% to 40% of women have recurrent episodes, while approximately 20% UTIs occur in men.UTIs may lead to chronic renal failure, renal dialysis and even renal transplantations [2].

E. coli found to be the most prevalent clinical isolates in UTIs that cause nearly 60-70% uncomplicated cases [3]. While other Gram negative bacteria including *Citrobacter* spp., *Klebsiella* spp., *Pseudomonas* spp., *Proteus* spp. and *Enterobacter* spp. and Gram positive bacteria such as *Staphylococcus* spp. and *Streptococcus* spp.contribute from 5 to 15% in causing urinary tract infections [4, 6].

UTIstreatmentfacing great challenges, because of increasing level of antimicrobial resistance, that leads to a rise in urological complications due to lack of detection and improper treatment [5]. The resistance towards conventional drugs for uropathogen is increasing globally [6].

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The common antimicrobial agents used in treatment of UTIs include cell wall inhibitors like Penicillin, third generation cephalosporins (cefotaxime, cephradine, ceftazidime and cefaclor), DNA gyrase inhibitors like floroquinolones (ciprofloxacin, ofloxacin, sparfloxacin and enoxacin) and aminoglycosides (amikacin, gentamycin and kanamycin) that are protein synthesis inhibiters [1]. The most common antibiotics used for the treatment of bacterial infection are the β-lactam antibiotics, but the production of β-*lactamases* make the pathogen resistant to these drugs [7]. β-lactamases are extra cellular enzyme produced by large number of bacteria causing breakage of amide bond of β-lactam ring of penicillins and also capable of inactivating oxyimino-cephalosporins and aztreonam but are inactive against cephamycins and carbapenem [8]. Most of these ESBLs are encoded on plasmid that transfer horizontally among different bacterial genera and have greater aspect regarding to its spreading and are responsible for nosocomial infections [9]. Increasing risk factors for spread of ESBLs are the potential of disease; prolong catheterizations, intubations, prolonged hospitalization and excessive and indiscriminate use of broad spectrum antibiotics like cephalosporin, aminoglycosides and quinolones [10].

Unfortunately ESBLs producer strains identification is not done routinely in many laboratories in developing countries that result in misreporting and treatment failure [10]. A rapid testing of ESBLs producing bacteria is not only essential for effective treatment plan but also to prevent further spread of such bugs. Keeping in view this situation, this study was conducted to determine prevalence and antibiotic susceptibility pattern for ESBLs producing *E. coli* isolated from urinary tract infections and to manage the treatment failure caused by ESBLs producing strains.

MATERIALS AND METHODS

Bacterial Strains: Between April and June 2012, one hundred and ninety five fresh mid-stream urine samples (5 to 10 ml volume) from 95 male and 100 female eligible admitted patients at Lady Reading Hospital, Peshawar, KPK, Pakistan, were collected aseptically in sterilized labelled containers and were brought to the Microbiology Section, City Medical Laboratory, Peshawar. The isolates were not repetitive (One sample per patient) in this study.

Isolation and Identification of Pathogens: The pathogens were isolated by following standard protocol using sterile bacteriological media, including blood agar, MacConky agar and CLEDagar. The specimen were aseptically inoculated on the plates and incubated aerobically at 37 °C for 24 hours. Identification of the organisms were done on the basis of Gram's staining and routine biochemical tests including lactose fermentation, ability to produce indole, reaction on triple sugar iron (TSI) producing acids and citrate utilization test. Bacterial growth, only for strains of *E. coli* with clinically significant growth ($>10^5$CFU/ml) were included in this study.

Antimicrobial Susceptibility Testing: All isolates of *E. Coli* -were subjected to *in-vitro* testing for determination of their susceptibilities to various antibiotics using disk diffusion method (Kirby Baur's method) on Muller Hinton agar according to the Clinical Laboratory Standard Institute standards, CLSI [11]. A total of twelve antibioticswere used for the study, which included tazobactum+pipracilline(30+10ug), ceftazidime (30µg), cefotaxime (30µg), norfloxacin (30µg), moxifloxacin (30µg), salbactum+cefaperazone (30+10µg), amoxicilline+clavulanicacid(30+10µg),ceftriaxone(30µg), nalidixic acid (30µg), ciprofloxacin (5µg), meropenem (10µg), amikacin (30µg) disks were obtained from Oxide, UK. The zone of inhibition showed by bacterial pathogens around the discs is measured in mm and susceptibility interpretation made according to the CLSI [11].

Detection of ESBLs Production: Identification of ESBLs was done on Muller Hinton agar plates inoculated with the test strains as recommended for standard disc diffusion susceptibility test. Discs containing ceftazidime (30µg), cefotaxime (30µg), were placed 25mm (centre to centre of the disc) apart from the amoxicillin+clavulanic acid (20+10µg) that were placed in the center of the same plate, after 24 hrs incubation at 37 °C a zone of inhibition around one or more Cephalosporin discs was extended on the side nearest to the amoxicillin+ clavulanic acid. This synergy of the organism was considered ESBLs positive [12].

RESULTS

Out of 195 samples 68 (34.8%) samples were culturally positive for different uropathogens that included *E. coli, S.faecalis, E.aerogens, S.epidermidis* and *P.mirabilis.* From the 68 isolates, 58 (85.2%) were *E. coli.* The ESBLs phenotype was detected in 38(65.5%) of the *E.coli* isolates. The frequency of ESBLs producing *E. coli* was higher in female (63.2%) than male (36.8%) as shown in (Table 1).

Table 1: Gender wise distribution of ESBLs and non-ESBLs producing *E. coli*

Gender (samples)	Total *E. coli* Isolates	ESBL (*E. coli*)	Non- ESBL (*E. coli)*
Male *(n = 95)*	23 (39.6%)	14 (36.8%)	09 (15.5%)
Female *(n = 100)*	35 (60.3%)	24 (63.2%)	11 (18.9%)

Table 2: Age wise distribution and percentage of ESBLs producing *E. coli*

Age group	Number of ESBLs positive *E. coli*	Percentage (%)
< 1 year	04	10.5
1 – 10 years	01	2.6
11 – 20 years	03	7.89
21 – 30 years	06	15.7
31 – 40 years	03	7.9
41 – 50 years	03	7.9
> 50 years	18	47.3

Table 3: Antibiotics susceptibility pattern of ESBLs producing *E. coli.*

Antibiotics	Sensitive Isolates (%)	Resistant Isolates (%)	Intermediate Isolates (%)
Ceftazidime (CAZ)	01 (2.63)	37 (97.3)	00 (0.00)
Cefotaxime (CTX)	02 (5.26)	36 (94.7)	00 (0.00)
Norfloxacin (NOR)	04 (10.52)	34 (89.4)	00 (0.00)
Moxifloxacin (MXF)	04 (10.52)	33 (86.8)	01 (2.63)
Salbactum+Cefaperazone (SCF)	36 (94.7)	02 (5.26)	00 (0.00)
Amoxycillin+Clavulanic acid (AMC)	02 (5.26)	36 (94.7)	00 (0.00)
Ceftriaxone (CRO)	02 (5.26)	36 (94.7)	00 (0.00)
Nalidixic acid (NEG)	00 (0.00)	38 (100.0)	00 (0.00)
Ciprofloxacin (CIP)	02 (5.26)	36 (94.7)	00 (0.00)
Amikacin (AK)	35 (92.1)	03 (7.89)	00 (0.00)
Meropenem (MEM)	38 (100.0)	00 (0.00)	00.(0.00)
Tazobactum+Pipracilline (TZP)	38 (100.0)	00 (0.00)	00 (0.00)

The age wise distribution showed (Table 2) the highest occurrence in age group > 50 years (47.3%) while prevalence in other age groups was; less than one year (10.5%), 1-10 years (2.63%), 11-20 years (7.89%), 21-30 years (15.7%), 31-40 years (7.9%) and 41-50 years (7.9%). It was also observed that children at the age group 01-12 months have also a high risk of urinary tract infections caused by ESBLs producing *E. coli.*

A high resistance in ESBLs producing *E. coli* (Table 3) towards nalidixic acid (100%) ceftazidime (97%), ciprofloxacin (94%), cefotaxime (94%), ceftriaxone (94%) and norfloxacin (89%) were observed. However, antibiotics susceptibility analyses revealed that all ESBLs producing *E. coli* isolates were sensitive more than 94% to meropenem, tazobactum+pipracilline and salbactum+cefaperazonewhileamikacin showed susceptibility of 92.1%.

DISCUSSION

Urinary tract infection caused by *E. coli* is one of the most common and prevalent infections of the genitourinary tract [6]. Production of ESBLs by the strain of this organism causes inactivation of large number of antibiotics and present major therapeutic dilemma since the choice of antibiotics is limited [13].

In the present study the frequency of *E. coli* (85.2%) was higher than the report of Chutterjee *et al*. [14], who reported 45% prevalence. This disagreement may be due to species variation of uropathogen on geographical basis.

The ESBLs production by *E. coli* inPakistan has been reported [1, 10] and this report conforms those reports that indicates the persistence of this hazardous bug posing serious health threat tofading treatment options. Moreover, prevalence of ESBLs producing *E. coli* isolated from the female urine samples (63.2%)was higher than male (36.8%), which is in agreement with a previous report from this region [1], thatgave (71.2%) in females and (28.9%) in males for ESBLs producing *E. coli* in admitted patients. The frequency of ESBLs producing *E. coli* in females were higher than male which may be attributed to sexual activity, pregnancy, short urethral length and lack of estrogens that may happen as consequence of menopause [1].

The age of the patient included in this study ranged from less than a year to > 50 years. The highest percentage of ESBLs producing *E. coli* were noted among the age group >50 years was (47.3%) which is in conformity to previous report of Mumtaz *et al*. [10]in Pakistan that gave a 51.6% prevalence for this age group. The patients of age group 51 – 70 years are more frequently affected by the ESBLs producing *E. Coli* [15] and high frequency of ESBLs producer*E.coli* in old ages may be attributed to catheterization, long stay in hospitals, excess use of broad spectrum antimicrobial agents and any underlying disease that are common consequence of 50 years plus group [10].

High frequency of ESBLs producing *E. coli* was observed among the uropathogen in the present study is not in agreement with previous report,Tankhiwale *et al*. [16] whichshowedESBLs production prevalence in 25.6*% Klebsiella species* followedby 18.5% *E. coli* whereas 65.5% ESBLs producing *E. coli* that are observed in the presentstudy can be attributed towards factors like species variation among uropathogen causing urinary tract infections and differences among prevalence at different geographic locations.

In our study ESBLs producing *E. coli* were multi drug resistant (showing resistance to six or more than six antibiotics) which is in agreement to previous findings of Mekki *et al*. [17] in Khartoum, Sudan that reported resistance to more than five antibiotics. The drugs of choice used for the treatment of urinary tract infection against ESBLs producer *E. coli and Klebsiella* spp. were reported [8] to be imipenemand amikacinwith sensitivities of 100% and 86%, respectively. While in the current study meropenem and amikacin showed 100% and 92.1% responses, respectively, for ESBLs producing *E. coli*. In our study high resistance to cephalosporin group of antibiotics was indicated. Third generation cephalosporins are commonly used in treatment of UTIs, were included in this study and resistances of >90% were observed for ceftazidime and cefotaxime by ESBLs producing *E. coli* which is in similarity with previous report of Behroozi *et al*. [18] in Indiawhorecorded resistances of 88 and 99.9% for ceftazidime and cefotaxime, respectively by ESBLs producing *E. coli.* High resistance pattern was observed for quinolone group of antibiotics,ciprofloxacin (94.7%), norfloxacin (89.4%) and nalidixic acid (100%) that were included in this study on the reason that they are routinely and extensively prescribed medicines of this region in case of UTI's. The resistance pattern data has similarity with the report of Chitterje *et al*. [14] that observed resistances of 91% and 94% for ciprofloxacin and norfloxacin, respectively while it also gave sensitivities of 90% and 84% for meropenem and amikacin respectively, which is also in agreement with this study.

The most common drug used in this region to treat different bacterial infections including urinary tract infections is amoxycillin+clavulanic acid, which interestingly showed high resistance (94.7%) by ESBLs producing *E. coli.* This percentage is nearly 10% higher than another resistance report from central region of Pakistan [19] this can be reasoned towards misuse along with over use of antibacterial drugs at community and as well as hospitals levels. One another factor for the rise of resistance can be the free availability and self-prescription of drugs of these POMs (Prescription only medicines) in this region.

To limit microbial drug resistance it is highly suggested to generate strict policy for rationale use of antibiotics, so as to ensure their proper selection and minimize their misuses. Furthermore, clinicians shall consider prescription of antibiotics after obtaining antibiotic sensitivity report and especially for ESBLs producing organisms, for which double disc synergy test (DDST), which is a simple, non-expensive, reliable and convenient modification, can be easily done along with normal susceptibility test.

CONCLUSION

It is concluded that *E.coli* is the most common pathogen causing urinary tract infections and incidence of ESBL producing strains isolated from atertiary care hospital of Peshawar, Pakistanare more prevalent in the age group >50 years. Imipenem and synergestic combination of penicillin + β-lactamase inhibitor is suggested therapy for such indication.

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