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Extended spectrum beta lactamases (ESBL):Preference for age and gender: A hospital based study

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ABSTRACT

Background: Antibiotic resistance is a challenge to treat. Extended Spectrum Beta Lactamases (ESBL) is one of the phenomenon by which the uropathogen express resistance to the commonly used antibiotic. **Objectives:** To determine the frequency of ESBL in uro-pathogens and ESBL preference for age and gender. **Methods:** This cross sectional study was conducted in Qazi Hussain Ahmed Medical Complex Nowshera from 1st Mar 2019 to 31st Jan 2020. A total of 315 urine samples were received for culture and sensitivity out of which 77 cases reported to be ESBL positive (24.44%). Relevant information's were recorded in SPSS version 25. **Results:** A total of 77 cases with established ESBL phenomenon were studied. Fourty percent were males and 60% females. The frequency of ESBL positive isolates was (24.44%). In 71(92%) cases of ESBL, *Escherichia coli* strain was reported, *Klebsiella pneumonia* in 5(6.5%) and *Proteus mirabilis in* 1.3% cases. The difference in means ±SD of age of the male gender (24.25years±9.6) was statistically significant with female gender (31.16years±14.49) (*p-value 0.02*). The probability of ESBL infections was 2.16 times higher in female gender (*p-value: 0.01*, OR:2.16). The sensitivity pattern of the ESBL producing *Escherichia coli* was; Imipenem(IMP) 100%, meropenem (Mem)100%, piperacillin tazobactam (TZP) 92%, nitrofurantoin (F)-85% . While imipenem & meropenem were drugs of choice for ESBL-*Klebsiella pneumonia and* ESBL -*Proteus mirabilis*. **Conclusion:** The frequency of ESBL isolates was 24.44%. Female gender reported more ESBL infection as compared to male gender. *Escherichia coli ESBL* was most common. The common sensitivity pattern *to* ESBL was: Imipenem(IMP) 100%, meropenem (Mem)100% and piperacillin tazobactam 95%.

Introduction

Treating uropathogenic *Escherichia coli* (UPEC) is a challenge for the doctors of the present era. It has led to the elimination of treatment option for urinary tract infections UTIs)(when reshapes to resistant type extended spectrum beta lactamases ((ESBL) [1]. Uropathogenic *Escherichia coli* are associated with increased rate of expression of ESBL gene. Extended spectrum beta lactamases contain many plasmid-mediated derivatives. Extended spectrum beta lactamases, called CTX-M (i.e., "active on CefoTaXime, first isolated in Munich"), has been reported in 2000 is major type prevalent worldwide for causing ESBL types of infections [2]. Resistance to commonly used antibiotics in clinical practice has resulted in evolution of wide range of ESBL producing strains, a global public health concern resulting in wastage of huge loss of economy on simple infections that has now become resistant due to ESBL production by the microbial [3].

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At the same time with the irrational use of second and third generation cephalosporins for the treatment of simple UTIs, has led to the development of resistant organism and production of enzymes called ESBLs that results in the development of multidrug resistance [4]. A study from Saudi Arabia reported that the ESBL phenotype was detected in 351 of 1151 isolates (30.5%). They further reported that the highest proportion of ESBL producing microbials were isolated from the urine samples (62.5%) [5]. **Tipelli et al.** [6] reported that (25%) of the isolates were resistant to at least one cephalosporin according to the recommendations of Clinical and Laboratory Standards Institute (CLSI) criteria and (43.75%) of the resistant isolates were ESBL. From Nepal limited data published in this regard showing the prevalence of ESBL as 28% [7].

Extended spectrum beta lactamases are inhibited in vitro by clavulanic acid. There are many genotypes of ESBL. The important clinically manifesting genotypes of ESBL are SHV, TEM, and CTX-M types. Other less important types are VEB, PER, BEL-1, BES-1, SFO-1, TLA, and IBC [8] ESBL has been reported in other species of bacteria beside the *Escherichia coli* that includes *Klebsiella pneumonia and Proteus mirabilis* [1].

Present study was designed as to determine ESBL frequency in uro-pathogens and its preference for age and gender using inferential statistics.

Material and Methods

This cross sectional study was performed in the Pathology Department of Qazi Hussain Ahmed Medical Complex Nowshera from 1st March 2019 to 31st Jan 2020. A total of 315 urine samples were received for culture and sensitivity out of which 77 cases reported to be phenotypically ESBL positive (24.44%).

Sample size of 315 was calculated in Open-epi software on the assumption that the anticipated proportion of the ESBL was taken as, 28%**,** keeping the absolute precision at 5% and confidence interval of 95% [8].

The inclusion criteria were patients suffering from UTI and their midstream urine was collected irrespective of age and gender. Exclusion criteria were urine samples received in the laboratory 24hour after collection, patient already on the antibiotic therapy and improperly collected urine.

Ethical Approval was taken from ethical review board of Nowshera Medical College Nowshera. Verbal consent was taken from all the respondents that their confidentiality shall be maintained. The Code of Ethics of the World Medical Association (Declaration of Helsinki) [9] for experiments involving humans were strictly followed.

The urine specimens were received in the pathology section collected under strict aseptic conditions and with educating patients on midstream urine sample collection. Culture media were prepared according to CLSI.

The the urine samples were inoculated on CLED (Cystien Lactose Electrolyte Deficient) media. Then the specimens were incubated under ambient air35 $\pm$2 C for 24 hours. Species identification was done on the basis of colony morphology, biochemical tests (and API (Analytical prophylactical index) where ever required.*Escherichia coli* was identified by colony morphology (shiny metallic with intact edges), biochemical test (Yellow colored TSI, Urease negative, indol positive) and motile. *Klebsiella pneumonia* was identified by mucoid colonies, Urease positive, indol negative and non motile. *Proteus mirabilis* identified by swarming colonies on blood agar, blackening of TSI, urease positive and highly motile.

The growing colony was inoculated on Mueller Hinton agar for sensitivity testing. For urine samples the antibiotic disks (OXOID-UK) used were; Ak- amikacin, Mem-meropenem, IPM-imipenem, CAZ-ceftazadime,CTX-cefotaxime, TZP- piperacillin tazobactam, SCF- cefperazone sulbactum, CT- colistin sulphate, F-nitrofruantoin ,AMC- amoxicillin clavolinic acid Fos-fosfomycin , SXT- co-Triamaxazole , Levo-levofloxacin & CIP-ciprofloxacin. Plates were incubated for 18-20 hours and then zone of inhibition were calculated on caliper, including the size of the disk. Zones were compared with CLSI Guidelines 2015 [10] for sensitivity to be reported as sensitive, intermediate and resistant.

Disk of AMC-amoxicillin clavolinic acid was placed at a distance of 25mm center to center to any of the following antibiotic; CRO-ceftrixone-CTX-cefotaxime- CAZ-ceftazadime- ATM-Aztreonam. Phenotypically, enhancement of zone of any of the indicator antibiotics (CRO-ceftrixone-CAZ-ceftazadime- ATM-aztreonam) towards AMC-amoxicillin clavolinic acid was considered to be positive for ESBL phenomenon **(Figure 1)**.

Figure 1. ESBL Phenomenon.

Statistical analysis

Data was entered in SPSS Version 25 using descriptive statistics for scale variables like age, gender and frequency of uropathogens. Inferential statistics like p-value for significance in group variables using independent t-test and binary logistic regression analysis for probability testing.

Results

Out of total 313 urine samples received, 77 showed the ESBL phenomenon.. The ESBL positivity rate was (24.44%). Fourty percent were males and 60% females with male to female ratio of 1:1.5 In 92% cases ESBL phenomenon was observed in the *Escherichia coli*, 6.5% in *Klebsiella* ESBL and 1.3% in *Proteus mirabilis* **(Table 2)**. Mean age was 28.6 years with standard deviation of 13.3years. Age ranged from 3 to 63 years of age **(Table 3)**. Mean age with SD for males was 24.25±9 and females 31.14±13 years.

On population pyramid when analyzed the distribution of patients of both genders. It was noted that *Escherichia coli* were the major ESBL producers followed by *Klebsiella pneumonia* **(Figure 2)**.

We cross tabulated the age categories with frequency of virulent uropathogens. It was observed that frequency of the cases pertaining to all types of ESBL uropathogens was high in the age category 21-30 years of age (27,4,0) followed by the age range 31-45 years age (21,1,0). *Proteus mirabilis* was reported in age category less than 20 years of age **(Table 4)**.

The difference in means ±SD of age of the male gender (24.25years±9.6) was statistically significant with the mean ±SD of age of female gender (31.16years±14.49) with p-value of *0.02* (Independent sample test) in acquiring ESBL infections **(Table 5)**. Many factors can contribute to preference of females for ESBL type of infections as compared to males.

In binary logistic regression analysis of gender with age categories we observed that probability of acquiring ESBL infections increases by 2.16 times in female gender (*p-value:0.01*, OR:2.16) **(Table 6)**.

We observed that the sensitivity pattern to the ESBL- *Escherichia coli* was; *Imipenem*(IMP) 100%, meropenem(Mem) 100%, amikacin(AK) 79%, piperacillin tazobactam (TZP) 92%, nitrofurantoin (F)-85%, cefperazone sulbactum (SCF) 79%, fosfomycin (Fos) 82%,co-trimaxazole (SXZ) 24% and 3-4% to quinolones.

The sensitivity pattern to the ESBL- *Klebsiella pneumonia* was; IMP & Mem 100%, AK 80%, SCF 80%,TZP 80%, F-80%, Fos 40,SXZ 20%. Imipenem & meropenem were only drugs of choice for *Proteus mirabilis*-ESBL **(Table 7)**.

Table 1. Gender wise categorical distribution of cases.

		Culture			
		***E. coli* ESBL**	***Klebsiella* ESBL**	***Proteus mirabilis***	**Total**
Gender	**Male**	26	1	0	27
	Female	45	4	1	50
Total		71	5	1	77

Table 2. ESBL frequencies.

Type of ESBL producing bacteria	Frequency	Percent	Cumulative Percent
***Escherichia coli* ESBL**	71	92.2	92.2
***Klebsiella pneumonia* ESBL**	5	6.5	98.7
Proteus mirabilis	1	1.3	100.0
Total	77	100.0	

Table 3. Age statistics

Number	77
Mean	28.65
Median	27.00
Mode	18
Std. Deviation	13.3
Range	60
Minimum	3
Maximum	63

a. Multiple modes exist. The smallest value is shown

Table 4. Age categories and culture crosstabulation (*p-value 0.30*)

	Culture	**Total**

		Escherichia coli ESBL	*Klebsiella pneumonia* ESBL	*Proteus mirabilis-ESBL*	
Age categories	**Age <20 years**	17	0	1	18
	21-30 years	27	4	0	31
	31-45 years	20	1	0	21
	>46 years	7	0	0	7
Total		71	5	1	77

Table 5. Difference in means ±SD of age in gender groups.

	Gender	**Number**	**Mean**	**Std. deviation**	**Std. error mean**
Age	**Male**	28	24.25	9.659	1.825
	Female	49	31.16	14.495	2.071
Independent samples test		**df**	**Sig. (2-tailed)**	**95% Confidence Interval of the difference**	
				Lower	**Upper**
Age	**Equal variances assumed**	75	0.027	-13.031	0.795

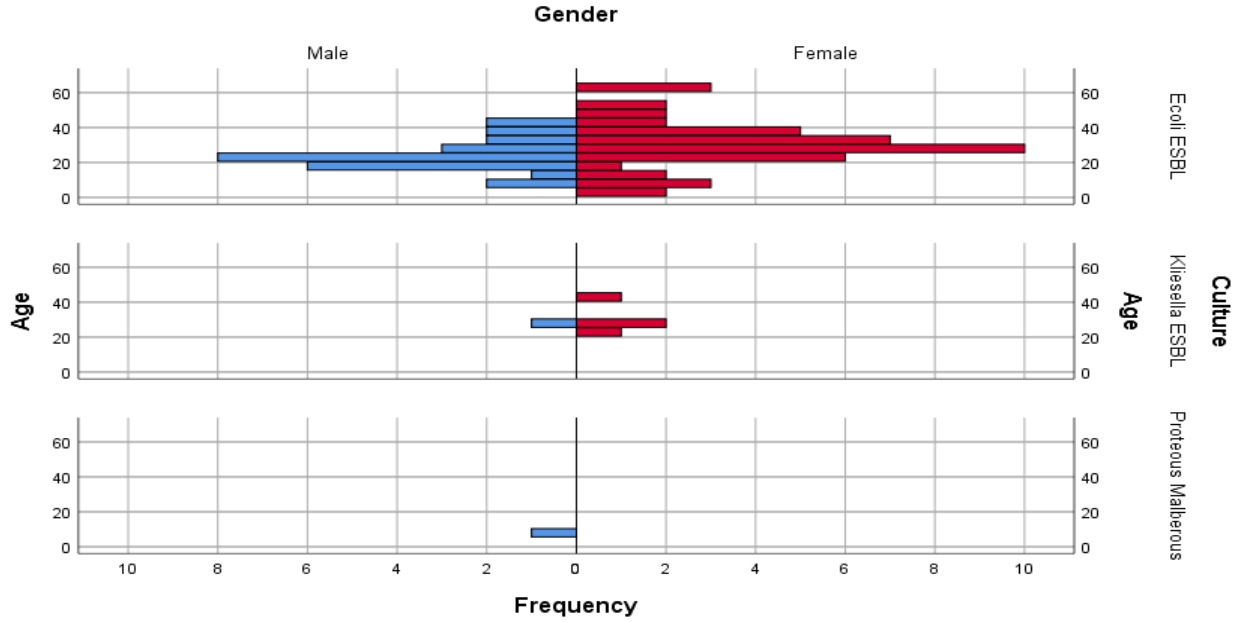
Table 6. Regression analysis to show probability of ESBL in gender groups.

Observed		**Predicted**		
		Gender		
		Male	**Female**	**Percentage correct**
Gender	**Male**	10	18	35.7
	Female	8	41	83.7
Overall Percentage				66.2
Regression	**B**	**df**	**Sig.**	**Exp(B)**
Age categories	0.773	1	0.01	2.167

Table 7. Sensitivity pattern to ESBL.

Sensitivity Pattern	**AK**		**IMP**		**Mem**		**SXZ**		**TZP**		**SCF**		**F**		**Fos**		**Lev/Cip**		**Fox**	
Organism	YES	%	YES	%	YES	%	YES	%	YES	%	YES	%	YES	%	YES	%	YES	%	YES	%
Escherichia coli	56	79	71	100	71	100	17	24	65	92	56	79	60	85	58	82	3	4.2	0	0
Klebsiella pneumonia	4	80	5	100	5	100	1	20	4	80	4	80	4	80	2	40	0	0	0	0
Proteus mirabilis	0	0	1	100	1	100	0	0	1	100	1	100	1	100	1	100	0	0	0	0

Figure 2. Population pyramid.



Discussion

The prevalence of ESBL producing *Escherichia coli* is rapidly increasing in our part of the world because of poor implementation of the rules and regulations pertaining to the rational use of antibiotics and increased in the quackery associated with medical professional, minimal or negligible role of the drug regulatory authority and health care commission. As per noticed clinical practice in our scenario the treatment of UTI is started without the culture and sensitivity with empirical antibiotics, which is essential prior to start any treatment to attain the desired objectives from the antibiotic therapy. This results in production of ESBL by the microorganism and the infection goes toward a resistant one [11].

In present study the ESBL positive rate was (24.44%) which is quite alarming. Many studies have reported a diverse distribution of ESBL production rate ranging from 17% to 70% in their isolates [8,12,13]. **Shakya et al.** [1] reported (20.4%) samples with ESBL matching our findings. A locally reported study from Swabi Pakistan reported that 25 (33.3%) isolates were positive for ESBL [14].

We analyzed the distribution ESBL in types of bacteria, it was noted that ESBL main reservoir is *Escherichia coli* 71(92%) followed by *Klebsiella pneumonia* 5(6.5%). **Shakya et al.** [1] found that *Escherichia coli as* the highest ESBL producers in their study 33 (91.7%) and followed by *Klebsiella* pneumoniae i.e. 3(8.3%) [1] that strongly matches our findings.

Frequency of the cases pertaining to all types of ESBL producers (*Escherichia coli, Klebsiella* and *Proteus*) the age category 21-30 years of age frequency was (27, 4,0 respectively) followed by the age range 31-45 years with (21,1,0) cases. **Jamil et al.** [14] also reported that *Escherichia coli* was the most common uropathogen recorded in 23 (30.67%) causing UTIs in age range 21-30 years., second modal age range was 31-40 years with frequency of 17 (22.67%) cases.

The difference in means ±SD of age of the male gender (24.25years±9.6) was statistically significant with the mean ±SD of age of female gender(31.16years±14.49) with p-value of *0.02* (Independent sample test) in acquiring ESBL infections. Another study reported the mean age of patients with ESBL infections was 35.07 years (±13.30 SD) [14]. Using logistic regression analysis of gender with age categories we observed that probability of acquiring ESBL infections increases by 2.16 times in female gender and with increase in age (*p-value: 0.01*, OR:2.16). Similar findings have been reported by other studies [1,14,15]. Many explanations could justify this fact including the short urethra in female, shorter distance between anus and urethra in female gender and sexual intercourse etc.

The sensitivity of ESBL-*Escherichia coli* was; imipenem (IMP)100%, meropenem (Mem) 100%, amikacin 79%, piperacillin tazobactam (TZP)

92%, nitrofurantoin (F)-85%, cefoperazone sulbactam (SCF) 79%, Fosfomycin (Fos) 82%, co-trimoxazole (SXZ) 24% and 3-4% to quinolones.

Almost similar to the sensitivity of the *Klebsiella pneumonia* stand ESBL. However, imipenem & meropenem were only drug of choice for *Proteus mirabilis*.

Many other studies have reported the sensitivity pattern matching to our findings for *Escherichia coli* ESBL isolates; Imipenem (99.54 %), ampicillin-sulbactam (97.48%), piperacillin-tazobactam (96.86%), Fosfomycin (94.51%), amikacin (92.26%) and nitrofurantoin (90.68 %) [14,16].

Another locally reported study carried out in 2015, reported the UTI caused by ESBL mostly affecting the age range 21-30 years [17] with sensitivity of E. coli-ESBL isolates to Amikacin (76%) followed by imipenem (66.67%), ofloxacin (64%), ciprofloxacin (58.67%), norfloxacin (56%) comparing to our study where the sensitivity to imipenem was 100% [18].

Our findings are supported by other researchers that ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* were highly sensitive to meropenem and imipenem and a variable pattern of sensitivity to other antibiotics like aminoglycosides and fluoroquinolones and reduced or nil susceptibility to β-lactam β-lactamase inhibitors [19].

Conclusion

In present study *Escherichia coli* was found to be the most important multidrug pathogen in cases of UTI. The prevalence of the ESBL – *Escherichia coli* was higher as compared to other types of uro-pathogens. Sensitivity of ESBL producing uro-pathogens was excellent to meropenem and *imipenem* antibiotics. Similarly, piperacillin tazobactam, Nitrofurantoin, cefoperazone sulbactam and Fosfomycin (Fos) can be used as alternatives. Aminoglycosides, fluoroquinolones, co-trimoxazole and β-lactam antibiotics are of least clinically importance in cases of ESBL infections.

A comprehensive strategy using advocacy, communication social mobilization and CME events can help in understanding healthcare provider in proper selection of antibiotics for treatment of ESBL type resistant infections.

There is need for multidisciplinary approached at national, international levels to control the unlawful and similarly irrational use of antibiotics

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