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Original Article

FREQUENCY OF EXTENDED SPECTRUM BETA LACTAMASES IN ENTEROBACTERIACEAE IN URINARY ISOLATES RELATED TO AGE AND GENDER

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ABSTRACT

Objective: To determine frequency of extended spectrum beta lactamase (ESBL) producing Enterobacteriaceae in urinary isolates with reference to age and gender of patients. **Study Design:** Observational study. **Methodology:** Urine samples were collected from a tertiary care hospital with details of age and gender of patient between October 2015 to December 2015. Urine samples were centrifuged. All urine samples showing pyuria were inoculated on Cysteine Lactose Electrolyte Deficient (CLED) medium and Blood Agar and incubatged overnight 37°C. Organisms were identified by standard biochemical procedures in cultures showing significant growth. Detection of ESBLs was carried out in members of Enterobacteriaceae by double disk diffusion technique. **Results:** Out of 209 isolates belonging to Enterobacteriaceae, 67 (32%) were ESBL +ve. ESBL positivity was highest in Enterobacter isolates (47.8%) followed by Klebsiella species (40%) and E.coli (29.5%). Male to female ratio of patients was 40:60. Mean age of patients with ESBL producing organisms was 47±19.4. Positivity was maximum in age group above 55 (45.5%) followed by age group 1-18 (38.8%) and age group 37-54 (28.3%) with least incidence in age group 19-36 (21.8%). **Conclusion:** Highest frequency was observed in Enterobacter species. Female patients had higher prevalence. Age group above 55 and below 18 showed higher prevalence.

Key words: Extended Spectrum beta lactamases, Enterobacteriaceae, Enterobacter, Klebsiella, Escherichia coli, Frequency, age distribution.

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INTRODUCTION

Beta lactamases are amongst the most commonly used class of antibiotics world over[21]. Bacteria counter antibacterial activity of these compounds by hydrolyzing their b-lactam bond through action of b-lactamases[27]. First b-lactamase was identified in culture of *E.coli* even before clinical use of penicillin (first b-lactam compound) had started[1]. When penicillin

was first introduced in clinical practice in early 1940s, most of *Staph. aureus* isolates (common nosocomial pathogen) were penicillin-sensitive except 5% of b-lactamase producing isolates which were penicillin resistant[21].

Reports of S.aureus isolates becoming penicillin resistant started pouring in shortly, and within half a decade, most of S.aureus isolates in major hospitals had developed penicillin resistance indicating gene transfer and strain selection[5,6,7,12,13]. And by the end of decade of its introduction, laboratories across the world were reporting S.aureus resistance to penicillin[20].

Scientists were continuing research on penicillin molecule and succeeded in introducing semi-synthetic penicillins and early cephalosporins by 1960. These newer b-lactams were effective against gram negative bacilli (GNB)[26]. GNB developed resistance to newer b-lactams during 1960s and 1970s by producing beta-lactamases, commonest amongst them being *TEM* and SHV[15]. *TEM* was first plasmid-mediated enzyme identified in *E.coli* isolated from blood culture of patient named Temoneira in Athens in 1965 (and was named so after that patients name)[4]. SHV identical to PIT-2 was identified by Pitton in 1972[18].

Oxyimino-cephalosporins were specifically designed to overcome prevalent bacterial resistance. They were first used in life-threatening infections in 1980[4]. Bacteria were quick enough to respond to this onslaught by producing SHV2 (a mutant of SHV1) identified in *K*lebsiella pneumonia and K.ozenae in 1984 and CTX-1 (later named TEM-3) identified in K. pneumoniae[24]. Because of their enhanced spectrum of activity, they were named Extended Spectrum b-Lactamases (ESBLs) and more than 200 such enzymes have been identified[25]. ESBLs are resistant to all b-lactams (except cephamycins and carbapenems) and other classes of antibiotics limiting treatment options of both nosocomial and community acquired infections resulting in increased morbidity and mortality[25].

Epidemiology

Development of antimicrobial resistance, a localized phenomenon initially has changed into one of global dimensions contributed by movement of people across countries resulting from traveling, trade and re-location of populations. Significant increase has been reported in ESBL production across most of countries of world by international surveillance studies. Tigecycline Evaluation and Surveillance Trial (TEST) reported ESBL positivity being 5.8% for E.coli and 13.8% for K. pneumonia in 2004 increased to 15.5% and 18.5% respectively for two organisms in 2013 at global level[14]. Study for Monitoring Antimicrobial Resistance Trends (SMART) reported significantly increase prevalence of ESBLs in urinary pathogens between 2009 to 2011 in Asia (P value 0.002) and Middle East (P value 0.0001)[24].

Objective

Purpose of this study was to determine frequency of extended spectrum beta lactamase in urinary isolates with reference to age and gender of patients.

METHODOLOGY

This observational study was conducted in Biotechnology Department of University of Karachi during the period from October 2015 to December 2015. 253 urine samples were collected from different wards of Jinnah Postgraduate Medical Centre, Karachi with details of age and gender of patients. Urine samples were centrifuged and sediments examined for red cells, pus cells, casts etc. All samples showing many pus cells were inoculated on CLED medium and blood agar medium with standard calibrated loop. Plates were incubated at 37°C in ambient air for 24 hours. Samples showing 10^5 CFU or more were considered to have significant bacteriuria. Organisms were identified by Gram staining and standard biochemical procedures[10]. Out of 253 organisms, 209 belonged to family Enterobacteriaceae. Eschericia coli American Type Culture collection (ATCC) 25922 and Klebsiella plneumonia ATCC 700603 were used as controls

Antimicrobial susceptibility was performed on Muller Hinton Agar medium using modified Kirby Bauer disk diffusion method according to CLSI guidelines[9]. ESBLs were detected using double disk diffusion technique. Amoxicillin-clavulanic acid disk (20/10µg) was placed in centre of plate with disks of Aztreonam (30µg), Ceftazidime (30µg), Cefotaxime (30µg) and Ceftriaxone (30µg) placed at a distance of 25-30 mm from central disk. Plates were incubated at 37°C for 24 hours. ESBL production was indicated by extension in zone of inhibition of surrounding disk towards central disk.

Statistical analysis

Data was analysed using standard statistical methods. Frequency and percentages calculated for categorical variables (micro-organisms, gender, ESBL positivity). Distribution of ESBL Positivity in various species of

organisms shown by bar diagram. Mean value calculated for age of patients. Age wise distribution of ESBL positive patients shown by bar diagram. SD calculated by formula:

$$\text{S } (x_1\text{-}x)^2$$
$$\text{Ö } (n\text{-}1)$$

x = Sample mean, x_1 = ith element of sample, n = Number of elements in sample

RESULTS

Out of 253 urine sample onoculated, 209 were infected with organisms belonging to family Enterobacteriaceae which were processed for ESBL detection.

Of 209 organisms belonging to Enterobacteriaceae, 67 (32%) were ESBL positive while 142 (68%) were

FIGURE 1.
Prevalence of ESBLs in *E. coli*, K. pneumonia, K. oxytoca and P. mirabilis from urinary-tract infections by region from SMART 2009 to 2011 (Morrissey I et al., 2013).

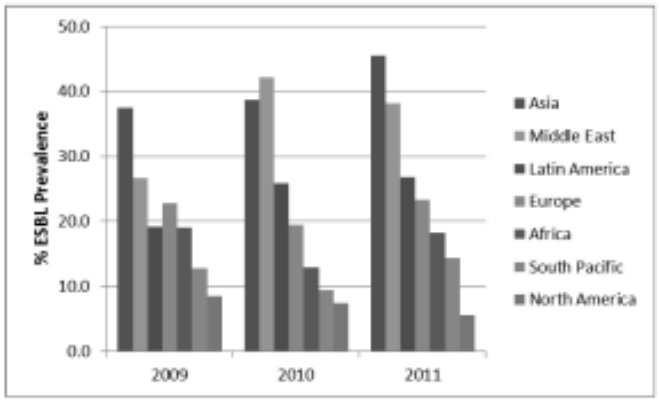


TABLE I
FREQUENCY OF ESBL-PRODUCING ENTEROBACTERIACEAE

Isolates belonging to Enterobacteriaceae	ESBL positive isolates	ESBL negative isolates
209	67 (32%)	142 (68%)

TABLE II
FREQUENCY OF ESBL-POSITIVE ISOLATES IN VARIOUS SPECIES

Species	Total No.	ESBL positive
E.coli	176	52 (29.5%)
K. pneumoniae	10	04 (40%)
Enterobacter species	23	11 (47.8%)

TABLE III
GENDERWISE DISTRIBUTION PATIENTS SHOWING ESBL-POSITIVE RESULTS

Total No.	Male	Female
67	27 (40%)	40 (60%)

TABLE IV
AGE WISE DISTRIBUTION OF PATIENTS

Age (Years)	Total No. of isolates	ESBL positives isolates
1-18	18	07 (38.8%)
19-36	55	12 (21.8%)
37-54	81	23 (28.3%)
Above 55	55	25 (45.5%)

Mean age of patients with ESBL producing organisms was 47±19.4. ESBL positivity was more in patients above 55 years of age followed by younger age group of below 18 years.

FIGURE 2
FREQUENCY OF ESBL-POSITIVE ISOLATES IN VARIOUS SPECIES

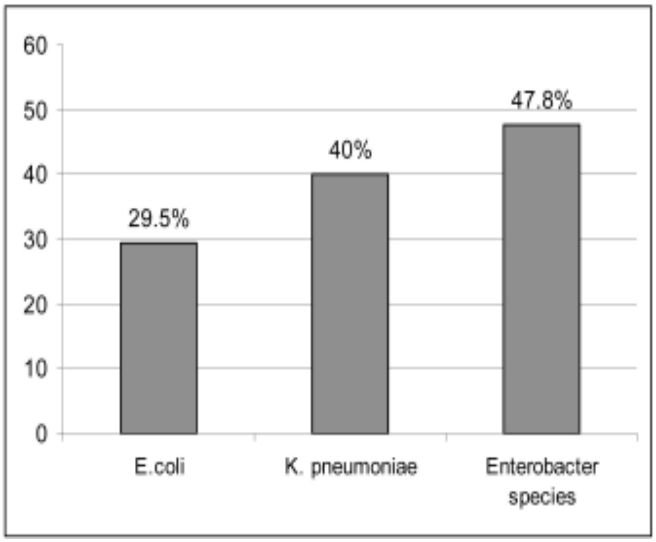
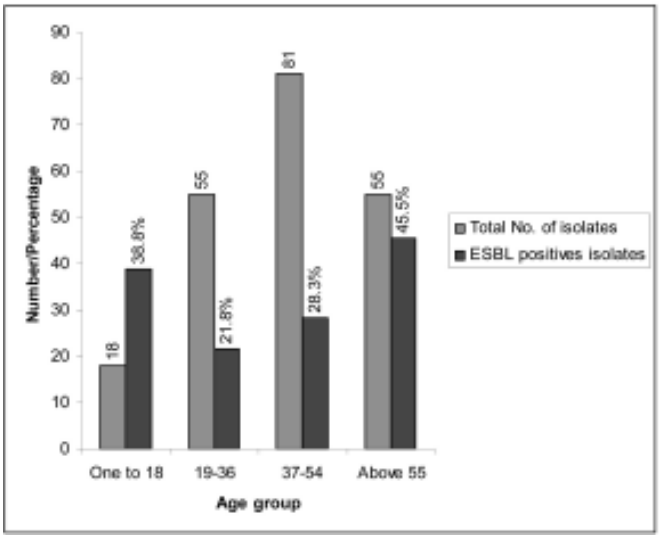


FIGURE 3
AGEWISE DISTRIBUTION OF PATIENTS



ESBL negative (Table I). Of 209 isolates, 176 were E.coli with 52 (29.5%) being ESBL positive, 10 were identified to be Klebsiella pneumoniae with 4 (40%) being ESBL positive and remsining 23 were Enterobacter species with 11 (47.8%) being ESBL positive (Table II, Figure 2).

Of the 67 patients infected with ESBL producing organisms, 27 (40%) belonged to male gender and remaining 40 (60%) were female (Table III). Maximum number of ESBL positive patients 25 (45.5%) were above 55 years of age followed by 07 (38.8%) in age group below 18 years. Incidence was 23 (38.3%) in age group 37-54 and lowest incidence of 12 (21.8%) was seen in age group 19-36 (Table IV, figure 3). Mean age of patients with ESBL producing organisms is 47±19.4.

DISCUSSION

ESBL producing organisms being MDR cause serious infections requiring prolonged hospital stay with financial implication and increased morbidity and mortality. These infections are associated with difficulties in early diagnosis and limited choices of treatment. All these factors make ESBL producing organisms important for medical profession. In our study, 67 (32%) isolates were ESBL- producing out of 209 uropathogens belonging to family Enterobacteriaceae. Different authors have reported different figures for ESBL prevalence in their studies. Our figure of 32% is almost similar to ones reported by Habib and others[16] as 31.8% in E.coli, Hafeez et al.[17] 35.5%, Jabeen et al.[19] 40%, Babypadmini et al.[8] 40% and 41% in K. pneumoniae and E.coli isolates respectively from India. Another study from India by Taneja et al.[30] reported prevalence of 36.5%.

Higher figures of ESBL prevalence are reported by Afridi et al.[2] i.e. 67.5%, Akram et al.[3] 61%, Mathur et al.[22] from India 68% and Saboor et al.[28] 70%. Our study showed highest incidence of ESBL positivity in Enterobacter species (47.8%) followed by Klebsiella species (40%) and E.coli (29.5%). Jabeen et al.[19] also reported highest figures for Enterobacter species (50%). Babypadmini et al.[8] reported figures of 40% and 41% for K.pneumoniae and E.coli respectively. These differences in prevalence may be attributed to differences in population under study, sample size and type of clinical isolates.

In our study, gender wise distribution of patients with ESBL producing organisms was 60% females and 40% males almost similar to findings of Mumtaz et al.[23] reporting females 64.3% and males 35.7%. Different figures are reported by Shah et al.[29] with female and male percentages being 34.67% and 65.33% respectively. Difference may be result of difference in sample size.

The mean age of patients in this study was 47 years similar to one reported by Jabeen et al.[19] while another study reported mean age as 58.69[2]. Majority

of ESBL producing isolates in our study were detected in patients over the age of 55 (45.5%) which is similar to observations of Shah et al.[29] being 48% for patients above 50 years of age and that of Mumtaz et al.[23] being 16.9% for patients above 50 and 27.9% for patients above 60 (Cumulative incidence above 50 years of age being 16.9+27.9=44.8%).

CONCLUSION

High prevalence of ESBLs in our isolates support
i) Urgent need for regular screening/detection of ESBLs for these organisms by clinical laboratories.
ii) Review on policy on antibiotic use.

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Authors contribution:
* Concept and design** Critical review of the paper
*** Collection and assembly of data **** Final Approval

Conflict of interest: Non Declareed