

# Frequency of Extended Spectrum Beta Lactamase Producing Enterobacteriaceae Among Urinary Pathogen Isolates

Faisal Iqbal Afridi<sup>1</sup>, Badar Jahan Farooqi<sup>1</sup> and Arif Hussain<sup>2</sup>

## ABSTRACT

**Objective:** To determine the frequency of extended-spectrum beta lactamase (ESBL) producing Enterobacteriaceae in urinary isolates.

**Study Design:** Observational study.

**Place and Duration of Study:** Ziauddin University Hospital, Karachi, from February to October 2008.

**Methodology:** All members of Enterobacteriaceae isolated from urinary samples of in-patients were included and identified using standard biochemical tests. Urinary samples from out-patients were excluded. Detection of ESBL was carried out by double disk diffusion technique. Statistical analysis was performed by SPSS version 10.

**Results:** A total of 289 isolates of Enterobacteriaceae were identified during the study period. Of those 190/289 (65.7%) of the isolates were found to be ESBL producing. ESBL positivity within individual organism group was highest in *Klebsiella* species 84.16%, followed by *Escherichia coli* 68.55%, *Enterobacter* species 36.84%, and *Proteus mirabilis* 28.55%. Mean age of patients with ESBL producing organisms was 58.69 ± 18.97 years. ESBL production was almost similar in all age groups.

**Conclusion:** A high frequency of ESBL producing organisms especially *Klebsiella* species and *Escherichia coli* amongst the hospital obtained urinary isolates was documented particularly in the older age group. The data points towards an urgent need for regular screening and surveillance for ESBL producing organisms in this region.

**Key words:** Age distribution. Extended-spectrum beta-lactamase. Enterobacteriaceae. *Klebsiella*. *Escherichia coli*. Urinary tract infections.

## INTRODUCTION

Extended-spectrum beta-lactamases (ESBLs) are cephalosporinases that induce resistance to oxyimino-cephalosporins and create serious therapeutic problems.<sup>1</sup> Due to their spectrum of activity against oxyimino-cephalosporins (e.g., Cefotaxime and Ceftazidime), these enzymes became known as ESBLs. Large numbers of outbreaks of infection due to ESBL producing organisms have been described in every continent of the globe except Antarctica.<sup>2</sup>

ESBL producing organisms are distributed worldwide and their prevalence is increasing. Because of the difficulties encountered in their detection, true prevalence of ESBLs is not known. ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* were reported in 3.5% and 1.8% of isolates, respectively in the Canadian National Intensive Care Unit study.<sup>3</sup> From 10,000 bacterial isolates from ten centres widely distributed throughout South America in SENTRY antimicrobial surveillance programme it was noted that 45% of *Klebsiella pneumoniae* and 8.5% of *Escherichia coli* expressed

an ESBL.<sup>4</sup> From Asia, ESBL mediated resistance in *Klebsiella* species was 48.8% in Korea and ranged from 20-40% throughout Southeast Asia, China, and Japan.<sup>5</sup> A large study from India has reported high frequencies (68.78%) of ESBL producing organisms.<sup>6</sup> A study from Pakistan showed that the prevalence of ESBL producing Enterobacteriaceae in nosocomial isolates was found to be 35%.<sup>7</sup> Another study from a tertiary care hospital of Karachi reported 40% ESBL producing isolates.<sup>8</sup> While, a study from a tertiary care hospital of Lahore documented a frequency of 35.5% ESBL producing gram negative bacilli among clinical isolates.<sup>9</sup>

There is a rising incidence of urinary tract infection (UTI) with ESBL producing bacteria.<sup>10</sup> In hospitalized patients with positive cultures for ESBL producing *Escherichia coli*, the majority of the isolates are attributed to a clinical infection rather than colonization. The commonest clinical specimen to yield the organism was urine, which was positive in 57.8% of patients.<sup>11</sup>

Fast and adequate detection of ESBL is crucial for implementing infection control measures and selecting the choice of antimicrobial therapy.<sup>12</sup> It is essential that ESBL positivity rates are monitored and appropriate measures are taken in light of the local data.

The purpose of this study was to determine the frequency of ESBL-producing Enterobacteriaceae in urinary isolates and their age wise distribution.

Department of Microbiology<sup>1</sup> / Pathology<sup>2</sup>, Dr. Ziauddin University Hospital, Karachi.

**Correspondence:** Dr. Faisal Iqbal Afridi, A-79/6 Block No. 14, Gulistan-e-Jauhar, Karachi-75290.

E-mail: afridi03@hotmail.com

Received January 18, 2011; accepted October 25, 2011.

## METHODOLOGY

This descriptive study was conducted from February to October, 2008 in the Department of Clinical Microbiology of Ziauddin Medical University Hospital. All Enterobacteriaceae isolated from urine samples of in-patients were included in the study and studied for ESBL production. All urinary samples from out-patients were excluded from the study. Approval from the institutional ethical committee was obtained. Informed consent was taken from either the patient or any other patient's relative.

Urine samples from in-patients were received in a sterile container supplied from the microbiology laboratory. These samples were inoculated on Cystein Lactose Electrolyte Deficient (CLED) medium (Oxoid Ltd., England) and incubated at 37°C in ambient air for 24 hours, using standard microbiological techniques.<sup>13</sup> Enterobacteriaceae isolated in urine samples were identified using routine biochemical tests.<sup>13</sup>

Antimicrobial susceptibility testing was performed on Mueller Hinton agar (MHA) medium (Oxoid Ltd., England) using modified Kirby Bauer disk diffusion method according to clinical and laboratory standards institute (CLSI) guidelines.<sup>14</sup> Detection of ESBL was carried out by double disk diffusion technique by using disks containing Amoxicillin/Clavulanic acid (20/10 µg) placed in the centre of the sensitivity plate with the disks of Aztreonam (30 µg), Ceftazidime (30 µg), Cefotaxime (30 µg), and Cefipime (30 µg) that were applied around the Amoxicillin/Clavulanic acid disk (20/10 µg) 25-30 mm apart.<sup>15</sup> The plates were incubated overnight at 37°C in an ambient air incubator. After incubation, ESBL production was detected by the presence of expansion, augmentation, or window formation of Cephalosporins, or Aztreonam disc zone by the Clavulanate. *Escherichia coli* American Type Culture Collection (ATCC®) 25922 and *Klebsiella pneumoniae* ATCC® 700603 were used as controls.

Data analysis was performed by using Statistical Package for Social Sciences (SPSS) version-10. Frequency and percentages were computed for presentation of all categorical variables like micro-organisms, gender and ESBL positivity. Mean values and standard deviation was calculated for quantitative variables like age of patients. Test of significance was calculated using independent sample T-test to compare mean ages and chi-square test for the ESBL positivity in various age groups. A p-value of less than 0.05 was considered statistically significant.

## RESULTS

Urine samples of 4492 in-patients were processed for culture and antimicrobial sensitivities during the study period. From these urine samples a total of 289 isolates of Enterobacteriaceae were identified. Predominantly these isolates were from female patients 206/289

(71.28%), while isolates from male patients were 83/289 (28.71%). Male to female ratio was 1:2.48. Out of 289 Enterobacteriaceae isolates, 229 (79.2%) were *Escherichia coli*; 26 (9%) were *Klebsiella* species; 19 (6.6%) were *Enterobacter* species; 14 (4.8%) were *Proteus mirabilis*; and only 1 (0.3%) was *Proteus vulgaris*. In these isolates of Enterobacteriaceae the overall ESBL producing organisms were 190/289 (65.7%). Frequency of ESBL positivity within individual organism group was highest amongst *Klebsiella* species (84.61%) followed by *Escherichia coli* (68.55%), *Enterobacter* species (36.84%), and *Proteus mirabilis* (28.57%) as shown in Figure 1.

Mean age of patients with ESBL producing organisms was 58.69 ± 18.97 years versus 56.23 ± 20.48 years for patients with non-ESBL organisms. There was no statistically significant difference with regards to the mean age of patients between ESBL with non-ESBL groups (p=0.310). ESBL production among various age group range from 60.34% to 70.51%. However, there was no statistically significant difference among the age groups with respect to ESBL positivity (p=0.669). Age wise breakup of ESBL positivity rate is shown in Table I.

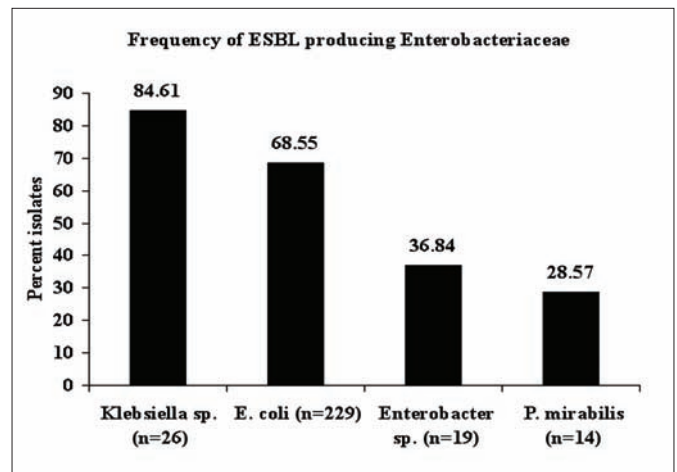


Figure 1: ESBL positive isolates within individual organism divided by total isolates of that organism represent percent ESBL isolates.

Table I: Age wise distribution of ESBL producing Enterobacteriaceae in different age groups.

Age group (years)	Total number of isolates (n=289)	ESBL positive (n=190)	Percent ESBL positive	p-value
< 15 years	09	06	66.66%	
15-45 years	58	35	60.34%	0.669*
46-60 years	78	55	70.51%	
> 60 years	144	94	65.27%	

\*P > 0.05 (not significant).

## DISCUSSION

ESBL producing organisms set unique challenges to clinical microbiologists, clinicians, and infection control professionals. These organisms are among the emergent problem in the area of infectious diseases with worldwide distribution and their prevalence is increasing.

UTI is the most common nosocomial infection, comprising about 35% of such occurrences in both hospitals and nursing homes.<sup>16</sup> More than 95% of UTIs are caused by a single bacterial species and *Escherichia coli* is by far the most frequent infecting organism in acute infection.<sup>17</sup> The spectrum of uropathogens isolated from urinary samples in this study is not too different from that reported in the literature. In this study, *Escherichia coli* predominated followed by *Klebsiella* species, *Enterobacter* species with least common organisms are *Proteus* species. In a study of urinary isolates from India, Gupta *et al.* reported that *Escherichia coli* was also the most common isolate followed by *Klebsiella* species, *Enterobacter* species, and *Proteus* species among Enterobacteriaceae in in-patients group which is similar to the present results.<sup>18</sup> Baqai *et al.* also reported *Escherichia coli* (50%) was the predominant organism in diabetic patients causing UTI.<sup>19</sup> In those studies the prevalence of *Escherichia coli* was less as compared to this data which indicates that the prevalence of *Escherichia coli* is rising.

Prevalence of ESBL producing strains of Enterobacteriaceae varies from country to country and from species to species in Asia.<sup>2</sup> In a large study carried out in India, Mohanty *et al.* reported the overall prevalence of 68.78% for ESBL producing organisms which is almost similar to our data which shows the overall prevalence of 65.7%.<sup>6</sup> Two other studies from Pakistan have also reported high prevalence of ESBLs among Enterobacteriaceae.<sup>7,8</sup> Zaman *et al.* reported the overall prevalence of 35% for ESBL producing Enterobacteriaceae.<sup>7</sup> While Jabeen *et al.* reported the ESBL prevalence of 40% among Enterobacteriaceae.<sup>8</sup> Hafeez *et al.* reported a frequency of 35.5% among gram negative bacilli in clinical isolates.<sup>9</sup> This shows that the prevalence of ESBL producing Enterobacteriaceae varies not only from country to country but also from hospital to hospital and this prevalence of ESBL producing Enterobacteriaceae is increasing.

Due to this great variation in prevalence of ESBL producing organisms from one place to another and even over time for a given place. Because ESBL producing strains often arise in focal outbreaks, regional and local estimates are probably more useful than are more global assessments in clinical decision making and for infection control purpose. This study showed highest frequency for ESBL production in *Klebsiella* species (84.61%) followed by *Escherichia coli* (68.55%), *Enterobacter* species (36.84%), and *Proteus mirabilis* (28.57%). Jabeen *et al.* reported highest frequency of ESBL positivity among *Enterobacter* species (50%) followed by *Escherichia coli* (41%) and *Klebsiella* species (36%).<sup>8</sup> Zaman *et al.* reported highest frequency of ESBL production in *Klebsiella* species followed by *Enterobacter* species and *Escherichia coli*,<sup>7</sup> and Shah *et al.* reported

similar but higher frequency rates among *Klebsiella* species (70%) followed by *Enterobacter* species (33.33%) and *Escherichia coli* (28.57%) in nosocomial isolates.<sup>20</sup> While Hafeez *et al.* reported *Escherichia coli* (44.8%) as a commonest ESBL producing organism followed by *Klebsiella pneumoniae* (38.6%).<sup>9</sup> In the Southeast Asian perspective, two studies from India reported a high frequency of ESBL producing organisms; *Klebsiella* species emerged as the top most ESBL producing organism. Khurana *et al.* reported 38.5% of *Klebsiella* species followed by 24.7% of *Escherichia coli* in urinary isolates from hospitalized patients<sup>21</sup> while Mathur *et al.* reported 80% of *Klebsiella* species as a most frequent ESBL producing organism.<sup>22</sup> The SENTRY surveillance programme from Asia Pacific and South Africa also reported that the most common ESBL producer was *Klebsiella* species.<sup>23</sup>

The present study revealed that overall there was a female preponderance of UTI occurrence (the ratio of male to female patients was 1:2.48); these findings are almost similar to those reported elsewhere.<sup>24</sup> Most of the patients in this study were elderly females and UTI in women vastly out-numbered men. Among males, although the increase rate of UTI was much less, the trend of infection was similar, in that the UTI was high in the elderly population.

In this study, the mean age of patients were  $58.69 \pm 18.97$  years from which ESBL producing organisms were isolated which is almost similar to other study in which the mean age of patients was 58.<sup>25</sup> Another study from Pakistan reported a mean age of 47 years.<sup>8</sup> This study exhibited that ESBL producing organisms were prevalent among all age groups and there was no statistically significant difference among various age groups, while Jabeen *et al.* reported that ESBL producing organisms were significantly more in patients less than 5 years of age and more than 60 years of age.<sup>8</sup> This increasing prevalence of ESBL producing organisms as shown by this data in all groups draw the attention toward the overall increase in ESBL producing organisms load and this thought is also supported by other surveillance studies from Asian Pacific region and South Africa reporting an alarming increase in ESBL positivity.<sup>23</sup>

## CONCLUSION

A high frequency of ESBL producing organisms was found especially among *Klebsiella* species and *Escherichia coli* in urinary isolates. This is suggestive of a need for regular screening and surveillance for ESBL producing organisms in our region. ESBL positivity in isolates from patients in all age groups also reflects high and non-judicious use of antimicrobials among all the age groups in this setup. Patients with these organisms should be nursed with contact precautions to avoid cross-infection among patients admitted in hospital.



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