EXTENDED SPECTRUM β-LACTAMASES IN ENTERIC GRAM-NEGATIVE BACILLI: RELATED TO AGE AND GENDER

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Background: Extended Spectrum β-Lactamases (ESBLs)-producing strains of Enterobacteriaceae have emerged as a major problem in hospitalized as well as community based patients. Infections due to ESBLs-producers range from uncomplicated urinary tract infection to life threatening sepsis .The objective of this study was to find out the prevalence of ESBLs-producing Gram- negative bacilli among clinical isolates. **Methods**: This descriptive study was conducted at the Microbiology department of Fauji Foundation Hospital, Rawalpindi over a period of two years (March 2004–April 2006). Six hundred and nine isolates of Enteric Gram-negative rods from various samples were tested for ESBLs- production by double disc synergy test. In 176 ESBLs-producing isolates, source of samples in term of indoor/outdoor was analyzed. In 165 ESBLs-producing isolates, patients' gender and age was analysed from 3 months to 70 years. **Results**: The ESBLs-producing isolates were more commonly isolated from indoor patients (88.1%) as compared to outdoor patients (11.9%). Escherichia coli was found to be most prevalent organism in indoor patients while Klebsiella pneumoniae, was the most prevalent organism in outdoor patients. ESBLs were most commonly isolated from female patients (64.3%) suffering from urinary tract infections ((41.5%), as compared to male patients (35.7%) in which the organisms were most commonly isolated from pus samples (54.2 %). ESBLs-producing Enteric Gram-Negative rods were most frequent at later part of life where they were most common (27.9%) at 61–70 years, followed by 41–50 years of age group (20.0%). Another peak (13.3%) was also seen at younger age group (11–20 years). The least prevalence (5.5%) was seen in two age groups (0–10 and 31–40 yrs). In case of female patients, ESBLs-producing EGNR were most frequently (29.2%) isolated from middle age group (41–50 years) followed by later age groups (51–60 and 61–70 years, (15.1% and 25.5%). **Conclusions:** Considering the high prevalence of ESBLs in Enteric Gram-negative rods, it is suggested that all such isolates should be tested for the production of ESBLs in the routine microbiology laboratory.

Key Words: Extended-Spectrum Beta-lactamases, Enterobacteriaceae, Enteric Gram-Negative rods, Escherichia coli, Klebsiella pneumoniae, Enterobacter spp.

INTRODUCTION

Beta-lactamases of Gram-negative bacteria are the most important mechanism of resistance against β-lactam drugs. These enzymes destroy the β-lactam ring of the β-lactam antibiotics. They bind to and prevent the action of penicillin binding proteins (PBPs), which are responsible for building and maintenance of peptidoglycan layer.[1] The β-lactam agent become so changed in its chemical structure that it is no longer recognized by the enzymes responsible for making the peptidoglycan layer of the bacterial cell wall.[2]

Two types of β-lactamases can confer resistance against 3rd generation cephalosporins; inducible chromosomal β-lactamases of Enterobacteriaceae, which are not inhibited by clavulanic acid and plasmid-mediated β-lactamases, which are inhibited by clavulanic acid. The latter are called extended-spectrum β-lactamases (ESBLs). They are defined as a rapidly evolving group of β-lactamases which share the ability to hydrolyze 3rd generation cephalosporins and aztreonam yet are inhibited by clavulanic acid,[3] and can confer resistance against all β-lactam drugs except Carbapenems and cephamycins.[4]

ESBLs-producing strains of Entero-bacteriaceae have emerged as a major problem in hospitalized as well as community based patients. These strains have been isolated from abscesses, blood, catheter tips, lungs, peritoneal fluid, sputum, and throat culture.[5,6] Infections due to ESBL-producers range from uncomplicated urinary tract infection to life threatening sepsis.[7] They are responsible for a variety of infections like urinary tract infection, septicemia, hospital acquired pneumonia, intra-abdominal abscess, brain abscess and device related infections. Keeping in view the importance of ESBLs-producing strains, this study was carried out in Microbiology Department of Fauji Foundation Hospital, Rawalpindi over a period of 2 years (March 2004–April 2006) to find out the prevalence of ESBLs (Extended spectrum β-lactamases) among Enteric Gram-negative rods isolated from different age groups and comparison of prevalence of ESBLs-producing Gram-negative bacilli in male and female patients in various samples.

MATERIALS AND METHODS

Six hundred and nine isolates of Enteric Gram-negative rods from various samples were tested for ESBLs- production. The isolates were sub- cultured, identified and double disc diffusion test/double disc synergy test was performed for detection of ESBLs according to the method of *Jarlier et al.*[8] In 176 ESBLs-producing isolates, source of samples in term of indoor/outdoor was analyzed. In 165 ESBLs-producing isolates, patients' gender and age was known which varied from 3 months to 70 years.

RESULTS

In 176 ESBLs-producing isolates, source of samples in term of indoor/outdoor was analyzed. Out of these 176 isolates, 155 (88.1%) were from indoor patients while 21 (11.9%) were from outdoor patients (Figure-1).

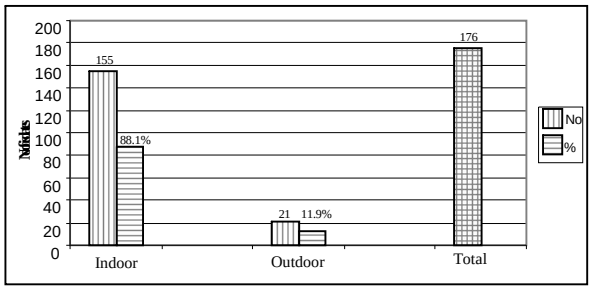

Figure-1: Indoor and outdoor distribution of ESBLs-producing isolates

Out of 155 indoor ESBLs-producing isolates, 72 were from pus samples (46.5%), 63 were from urine (40.6%), 15 were from sputum samples (9.7%) and 5 were from high vaginal swabs (3.2%). Out of 21 outdoor ESBLs-producing isolates, 8 were from urine samples (38.1%), 6 were from pus and high vaginal swabs (28.5% each) and one was from sputum samples (4.8%) (Table-1).

Table-1: Indoor and Outdoor distribution of ESBLs-producing isolates in different samples

	Indoor		Outdoor	
Type of samples	**No**	**%**	**No**	**%**
Pus	72	46.5	6	28.5
Urine	63	40.6	8	38.1
Sputum	15	9.7	1	4.8
HVS	5	3.2	6	28.6
Total	155	100.0	21	100.0

Regarding indoor patients, out of 155 ESBLs-positive isolates, Escherichia coli was found to be most prevalent organism, 79 (51%) followed by Klebsiella pneumoniae, 62 (40%) and Pseudomonas aeruginosa 9 (5.8%). In case of outdoor patients, the most prevalent ESBLs-producing EGNR was Klebsiella pneumoniae 10 out of 21 (47.6 %) followed by Escherichia coli, 8 out of 21 (38.1%), Pseudodomonas aeruginosa, 2 out of 21 (9.52%) and Salmonella spp. 1 out of 21 (4.76%) (Table-2).

Table-2: Distribution of ESBL-producing organisms in indoor and outdoor patients

Organism	Indoor	%	Outdoor	%
E coli	79	51.0	8	38.1
K. pneumoniae	62	40.0	10	47.6
P. aeruginosa	9	5.8	2	9.52
Acinetobacter	1	0.6	-	-
Salmonella spp	-	-	1	4.76
Proteus spp	1	0.6	-	-
Providencia spp	1	0.6	-	-
Aeromonas spp	2	1.4	-	-
Total	155	100.0	21	100.0

In 165 ESBLs-producing isolates, patients gender was known, 106 were females (64.3%) and 59 were males (35.7 %), (Figure-2). Out of 106 ESBLs-producing isolates in females, 44 were from urinary isolates (41.5%), 42 from pyogenic isolates (39.6%), 10 each from vaginal and sputum isolates (9.4% each). Out of 59 ESBLs-producing isolates in males, 32 were from pus isolates (54.2%), 23 from urinary isolates (39%) and 4 were from sputum samples (6.8%), (Table-3).

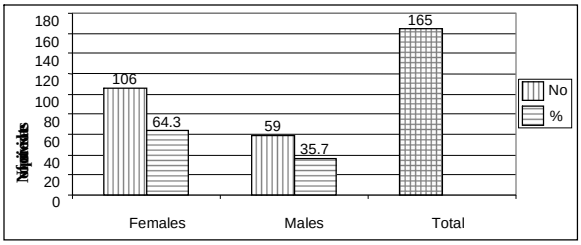

Figure-2: Gender distribution in 165 ESBLs-producing isolates

Table-3: Gender distributions of ESBL-producing isolates in different samples

	Females		Males	
Samples	**No**	**%**	**No**	**%**
Urine	44	41.5	23	39.0
Pus	42	39.6	32	54.2
HVS	10	9.4	-	-
Sputum	10	9.4	4	6.8
Total	**106**	**100**	**59**	**100.0**

In 165 ESBLs-producing isolates, the age of the patients was known which varied from 3 months to 70 years. ESBLs-producing Enteric gram-negative rods (EGNRs) were most frequent in 61–70 years of age group, 46 out of 165 (27.9%), 41–50 years of age group, 33 out of 165 (20.0%) , followed by 51–60 years of age group, 28 out of 165 (16.9%), (Table-4). Trends of prevalence of ESBLs-producing EGNRs at different age groups is shown in Figure-3.

Table-4: Overall Prevalence of ESBLs-producing organisms at different age groups

Age groups (years)	No	%
0-10	9	5.5
11-20	22	13.3
21-30	18	10.8
31-40	9	5.5
41-50	33	20.0
51-60	28	16.9
61-70	46	27.9
Total	165	100.0

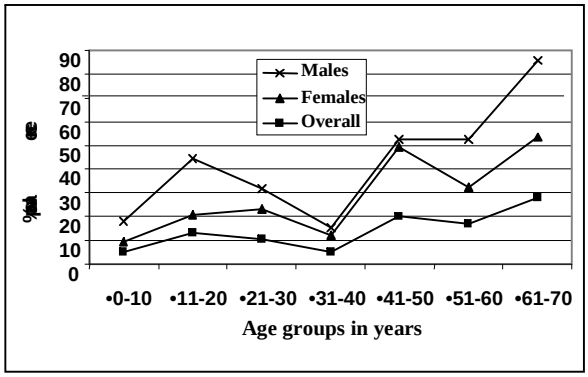

Figure-3: Trends of ESBLs-producing organisms at different age groups in male & female patients

In case of female patients, ESBLs-producing isolates were most frequent at 41–50 years of age group, 31 out of 106 (29.2%) followed by 61–70 years, 27 out of 106 (25.5%), and 51–60 years, 16 out of 106 (15.1%), (Table-5). In case of female patients, in 41–50 years of age group, ESBLs-producing isolates were most frequent in urinary isolates, 14 out of 31 (45.1%) followed by ESBLs-producing isolates from pus samples, 10 out of 31 (32.2%). At the age group of 61–70 years, ESBLs-producing isolates were more frequent in pus isolates, 13 out of 27 (48.1%) followed by urinary isolates, 9 out of 27 (33.3%), (Table-6).

In case of males, the ESBLs-producing organisms were most prevalent in the age group 61–70 years, 19 out of 59 (32.2%), followed by 11-20 years, 14 out of 59 (23.7%) and 51–60 years ,12 out of 59 (20.3%), (Table-7).

In case of males, at the age group 61–70 years, ESBLs-producing organisms were most common in urinary isolates, 13 out of 19 (68.4%) followed by pus isolates, 5 out of 19 (26.3%). At the age group of 11–20 years and 51–60 years, ESBLs-producing organisms were most common in pus isolates, 10 out of 14 (71.4%) and 6 out of 12 (50%), (Table-8).

Table-5: Prevalence of ESBLs-producing organisms at different age groups in 106 female patients

Age Groups (years)	No	%
0-10	4	3.8
11-20	8	7.5
21-30	13	12.3
31-40	7	6.6
41-50	31	29.2
51-60	16	15.1
61-70	27	25.5
Total	106	100.0

Table-6: Distribution of ESBLs-producing Organisms at Different Age Groups in Various Samples in Females

Samples	Age Groups (years)						
	0–10	11–20	21–30	31–40	41–50	51–60	61–70
Urine	1	4	5	2	14	9	9
Pus	2	3	6	3	10	5	13
HVS	-	-	1	2	3	1	3
Sputum	1	1	1	-	4	1	2
Total	4	8	13	7	31	16	27

Table-7: Prevalence of ESBLs-producing organisms at different age groups in males.

Age groups (years)	No	%
0-10	5	8.5
11-20	14	**23.7**
21-30	5	8.5
31-40	2	3.4
41-50	2	3.4
51-60	12	20.3
61-70	19	**32.2**
Total	**59**	**100.0**

Table-8: Distribution of ESBLs producing Organisms at different age groups in different samples in males

Samples	Age Groups (Years)						
	0–10	11–20	21–30	31–40	41–50	51–60	61–70
Pus	2	10	5	2	2	6	5
Urine	3	3	-	-	-	4	**13**
Sputum	-	1	-	-	-	2	1
Total	**5**	**14**	**5**	**2**	**2**	**12**	**19**

DISCUSSION

Over the past decade, ESBL-producing Enterobacteriaceae have emerged as serious nosocomial pathogens throughout Europe.[9] Outbreaks have occurred among the most critically ill patients in intensive care units.[10] Outbreaks were caused by multidrug resistant Klebsiella carrying a TEM-3 gene. Patients with septicaemia due to ESBL-producing organisms had a significantly higher fatality rate than those with non-ESBL isolates (71% *vs* 39%).[12]

Nosocomial infections in patients occur through the administration of extended-spectrum beta-lactam antibiotics or via transmission from other patients through health care workers. ESBL-producing strains can survive in the hospital environment,[13] can be transmitted from patient to patient, through hands of hospital staff[14] and are usually found in those areas of hospitals, where

antibiotic use is heavy and patient's condition is critical.[15]

In the present study the ESBLs-producing isolates were more commonly isolated from indoor patients (88.1%) as compared to outdoor patients (11.9%). A study by Spencer *et al*[16] shows that, more than half of the patients were colonized after 30 days stay in the hospital. Apart from ICUs, ESBL-producing strains have also been isolated from patients in general wards and nursing homes. According to Luzzaro *et al*,[17] the prevalence of ESBL-producers was 7.4% among indoor and 3.5% among outdoor patients.

In the present study, regarding indoor patients, ESBLs-producing E coli was found to be most prevalent organism (51%) followed by Klebsiella pneumoniae (40%) and Pseudomonas aeruginosa (5.8%). While in case of outdoor patients, Klebsiella pneumoniae (47.1%) was the most prevalent ESBLs-producing organism, followed by Escherichia coli (38.1%) and Pseudomonas aeruginosa (9.52%).

According to Calbo *et al*,[18] the prevalence of infection due to community-onset ESBL-producing E coli in urinary tract infections (UTIs) increased from 0.4% in 2000 to 1.7% in 2003. Community-onset ESBL-producing E coli infection shifted from 50% in the first period to 79.5% in 2003 ($p<0.001$). According to Pena *et al*,[19] from Barcelona, Spain, between 1996 and 2002, 68% of the hospitalized patients develop infection, yielding one or more clinical isolates of ESBLs-producing E coli. A significant increase was observed in the incidence of ESBL-producing E coli colonization or infection during the study period. Another study shows that among hospitalized patients, the most prevalent ESBL-producing species was E coli, whereas in his previous study in 1999, K pneumoniae was at the top.[17] A study by Solórzano *et al*[20] shows that ESBL-producing E coli were 16.3% from outpatients. Other study shows that ESBL was rare in community-acquired K pneumoniae infection.[21]

Regarding the gender distribution ESBLs-producing isolates were more common in females, 106 out of 165 (64.3%) as compared to males, 59 out of 165 (35.7%). These findings were incomparable to other studies where the ESBLs-producing isolates were more common in males, as compared to female patients.[22,23] Analysis of ESBLs - producing isolates in females from various samples showed, that they were more commonly isolates from urine (41.5%), followed by pus samples 42 (39.6%), and from vaginal and sputum samples 10 (9.4% each). In case of males ESBLs-producing isolates were more commonly isolated from pus samples 32 (54.2 %) followed by urinary, 23 (39%) and sputum samples, 4 (6.8%). ESBLs-producing EGNRs were most frequent at later part of life where they were most common (27.9%), in older age group (61–70 years) followed by 41–50 years of age group, (20.0%). Another peak was also seen at younger age group, 11–20 years (13.3%). The least prevalence (5.5%) was seen in two age groups (0–10 and 31–40 yrs). The studies of Shah *et al*[23] showed that ESBLs-producing EGNRs were most common at 50–60 years (48%) followed by 40–50 years (29.33%) of age groups while lowest prevalence (6.67%) was seen at 60 years and above which is not comparable to this study. According to Gold and Moellering,[24] in USA in 1996, the prevalence of ESBLs in the patients below and above 50 years age groups, the highest prevalence was noted in the patients belonging to the age group of above 50 years , about 20.53%. While a prevalence of about 10.67% was reported in the age group below 50 years. The ESBLs-producing EGNRs were most frequent in older age group in this study; it can be due to the reason that older patients are immunocompromised and more prone to infections by resistant organisms.

In case of female patients, ESBLs-producing EGNRs were most frequently (29.2%) isolated from middle age group (41–50 years) followed by later age groups (51–60 and 61–70 years, (15.1%, 25.5%). Most common sample from where ESBLs-producing EGNR were isolated at 41–50 years of age group was urine (45.1%) followed by pus samples, (32.2%) whereas at the age group of 61–70 years, ESBLs-producing isolates were more frequently isolated from pus samples (48.1%) followed by urine samples. The best explanation which can given in support of above is that the females are more to UTI at this age group which is the most common sample from which ESBLs producing EGNR were isolated.

In males, the ESBLs-producing organisms showed the highest peak at the age group 61–70 years, (32.2%), followed by a second peak at 11–20 years, (23.7%). In case of male patients at the age group 61–70 years, ESBLs-producing organisms were most common in urinary isolates, (68.4%) followed by pus isolates (26.3%). At the age group of 11–20 years and 51–60 years, ESBLs-producing organisms were most common in pus isolates, (71.4% and 50%). The best comments which can be given in support of above is that the males are most commonly prone to all type of infections especially UTI due to resistant organisms at later age of life.

CONCLUSIONS

Because ESBL-producing strains are resistant to a wide variety of commonly used antimicrobials, their proliferation poses a serious global health concern that has complicated treatment strategies for a growing

number of hospitalized patients. Many ESBL-producing strains of Enterobacteriaceae do not show resistance to newer cephalosporins or aztreonam in routine susceptibility tests so may be missed on routine disc diffusion susceptibility testing. Therefore, a clinical microbiology laboratory must not rely solely on routine susceptibility tests but should also use a more accurate method of detecting ESBLs. Special techniques to identify the presence of strains possessing such enzymes must be developed in the laboratory so that their detection & containment can be realized.

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