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Full Length Research Paper

Prevalence of extended spectrum beta lactamases (ESBL) in clinical isolates from a teaching hospital in Peshawar, Pakistan

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Infections caused by ESBL producing members of the enterobacteriaceae have rapidly increased all over the world. ESBL increase the possibility of failure of empiric antimicrobial regimens. The aim of this study was to determine the prevalence of ESBL in bacterial isolates and to look into the options for treating infections caused by these organisms. A total of 4,150 isolates of enterobacteriaceae were studied. ESBL producer isolates were 371 (8.94%) out of which 281 (75.7%) were recovered from admitted patients while 90 (24.3%) were recovered from outdoor patients. ESBL detection was carried out according to Clinical Laboratory and Standard Institute (CLSI) criteria. Majority of the ESBL producing isolates were obtained from urine 282 (76.0%), followed by swabs 69 (18.6%) fluids 12 (3.2%) blood 06 (1.7%) and sputum 02 (0.5%). The ESBL phenotype was detected in 322 (89.5%) of the isolates of *E. coli*, 20 (5.4%) of *Klebsiella* spp. 14 (3.8%) *Enterobacter* and 05 (1.3%) *Citrobacter* spp. Carbapenems was the drug of choice for serious infection with ESBL – producing organisms in Peshawar. These should not be administered as empiric therapy, because their over use can result in significant resistance in future.

Key words: Extended spectrum beta lactamases (ESBL), cephalosporins, *Escherichia coli*, phenotype screening.

INTRODUCTION

Extended spectrum beta lactamase (ESBL) was first identified in Western Europe in mid 1980. These enzymes are capable of hydrolyzing oxyimino – cephalosporins and are inhibited by β-lactamase inhibitors (Babypadmini and Appalaraju, 2004). These enzymes hydrolyze cephalosporins, penicillin, aztreonam but inactive against cephamycins and carbapenems (Chaudhary and Aggarwal, 2004). Major defense strategy of Gram negative bacteria against ß-lactam antibiotics is the production of ß-lactamases. Bacteria respond to new ß-lactamases with plasmid encoded enzymes called ESBL and Carbapenem - hydrolyzing ß-lactamases (Carbapenemases). They confer resistance to latest ß-lactam antibiotics – with variable success (Jacoby and Price, 2005). ESBL producing isolates frequently show associated resistance to other antibiotic classes such as aminoglycosides, trimethoprim-sulfamethoxazole and quinolones (Lautenbach et al., 2001). These enzymes are produced due to mutation in TEM-I, TEM-2 and SHV-1. β-lactamases are widely distributed in members of enterobacteriaceae (Al-Zahrani and Akhtar, 2005).

Gram negative pathogens harboring ESBL are becoming an increasing therapeutic problem. Uropathogens, such as *Escherichia coli, Klebsiella* spp. and *Citrobacter* spp. have the ability to produce large quantities of ESBL

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(Ullah et al., 2009). These enzymes are encoded on plasmid and lead to multidrug resistance in organisms. They have caused numerous outbreaks of infections (Pena and Pujol, 1998; Chlebicki, 2005). Detection of ESBL producing strains is important because its spread within the hospital may lead to endemic occurrence and repeated outbreaks from time to time. Another important reason of its detection is failure to treat ESBL producing organisms because of limited therapeutic choices (Ullah et al., 2009).

According to Pena and Pujol (1998) an outbreak caused by ESBL producing *Klebsiella pneumonia* was reported in May 1993 – June 1995. Studies suggest that these outbreaks are controlled by restricted use of expanded spectrum cephalosporins along with other strict infection control measures.

Zakaria et al. (2008) have stressed upon specific risk factors involved in the spread of ESBL, which include severity of illness, mechanical ventilation, intubations, invasive procedures, such as arterial catheterization, prolong hospitalization and extensive use of broad spectrum antibiotics including third generation cephalosporins, aminoglycosides and quinolones. ESBL producing strains are more prevalent than currently recognized, because they are often undetected by routine susceptibility testing methods. They have been identified worldwide in a number of different organisms, but unfortunately not detected routinely in many laboratories in developing countries. If not detected, it is highly likely to result in misreporting and treatment failures. Thus, more rapid diagnostic testing of ESBL producing bacteria and possible modifications of guidelines for treating such infections is required (Pitout and Laupland, 2008).

This study was designed to investigate the prevalence of ESBL producing *E. coli* and *K. pneumonia* in a tertiary care hospital and to investigate their susceptibility pattern for different antimicrobial agents so that effective management of infection is achieved.

MATERIALS AND METHODS

A prospective analysis was carried out investigating ESBL detection and susceptibility pattern of clinical isolates of *E. coli*, *Klebsiella* spp., *Enterobacter* and *Citrobacter*, collected during one year, between January - December 2009, in clinical Microbiology Laboratory at Khyber Teaching Hospital Peshawar and Khyber Medical College, Peshawar. Susceptibility pattern of Amikacin (AK) trimethoprim-sulfamethoxazole (SXT) Gentamicin (GEN) Ciprofloxacin (CIP) Doxycycline (DOXY) and Meropenem (MEM) were determined according to CLSI criteria (CLSI, 2006). Samples were collected from in-patients and out-patients attending Khyber Teaching Hospital, Peshawar and microbiology laboratory of Khyber Medical College, Peshawar.

Fresh samples of urine, blood, body fluid, CSF, pus and high vaginal swabs (HVS) swabs were collected aseptically in sterile containers and inoculated onto media within two hours of collection. A total of 4150 recovered pathogens cultured on Blood agar and Mac-Conkey agar were included in this study. Urine samples were cultured on CLED Media. Inoculated media were incubated at 37°C for 24 to 48 h. Recovered organisms were identified by their characteristic appearance on the media, Gram stain reaction and pattern of biochemical reaction using a commercial identification system (API-20-E). In phenotypic ESBL confirmatory test, the test organism was grown on Muller-Hinton agar and Amoxicillin – Clavulanic acid disc (20/10 µg) and a disc of cefotaxime (30 µg) were placed in the centre of the plate having 20 mm center to center distance. The Aztreonam (30 µg), ceftazidim (30 µg) and Cefepime discs were placed at centre to centre distance of 25 mm from Amoxicillin – Clavulanic acid.

Petri plates were examined for enhancement of zone inhibition of Cefotaxime and Cefepime at the side facing Amoxicillin – Clavulanic acid and piperacillin / Tazobactem disc respectively. Organisms, which showed enhanced zone of inhibition, were labeled ESBL- Positive.

Statistical analysis

Statistical analysis was performed using chi-square test to analyze the susceptibility pattern in ESBL producers and non-producers.

RESULTS

A total of 4,150 clinical isolates of enterobacteriaceae were studied. The results revealed that majority of ESBL producer isolates were collected from patients admitted in different departments of the hospital, 281 (75.7%) while 90 isolates (24.2%) were collected from samples obtained from outpatients. ESBL non-producers recovered from outdoor patients were 54.5% and non-producers from the wards were 45.55% (Figure 1). It was found that, majority of these isolates were recovered from urine 282 (76.0%) followed by swabs 69 (18.6%), fluids 12(3.2%), blood 06 (1.6%) and sputum 02 (0.5%) (Table 1).

The ESBL phenotype was detected in 332 (89.5%) of the isolates of *E. coli*, 20 (5.4%), *Klebsiella* spp. 14 (3.8%) *Enterobacter* spp. and 5 (1.3%) *Citrobacter* spp. (Table 2). Susceptibility testing revealed that 355 (95.7%) of the ESBL producing isolates were susceptible to meropenem, it had the best activity against ESBL producing isolates. A total of 76% of the isolates were susceptible to amikacin, 32.6% to gentamicin, only 8% susceptible to trimethoprim-sulfamethoxazole and 7.3% to doxycycline (Table 3).

The age of patients included in this study ranged from 06 months to > 45 years. Patients included in this study were divided into different age groups; group I included patients ≤ 12 years (n=111, 29.2%) Group II included patients having 13 to 45 years age (n=68, 18.3%) and group III included patients having >45 years of age (n=192, 51.6%) (Table 4).

DISCUSSION

Gram negative bacteria are increasingly involved in

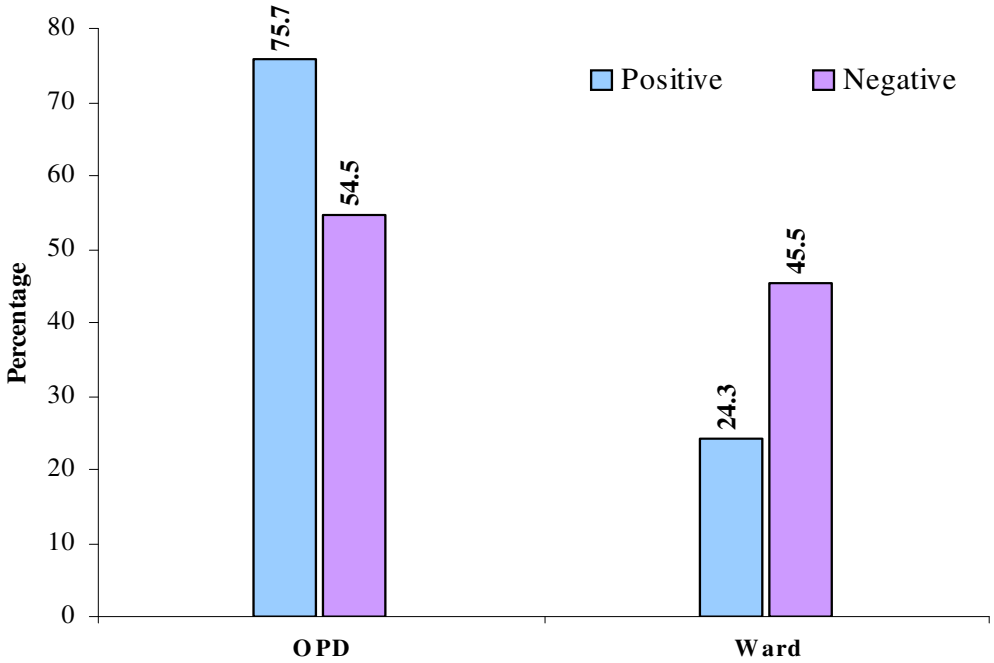

Figure 1. Prevalence of ESBL in isolates recovered from specimens of inpatients and outpatients.

Table 1. Prevalence of ESBL in different specimens collected from indoor and outdoor patients.

Specimen	Positive (%)	Negative (%)	Total (%)
Urine	282 (76.0)	1394 (36.9)	1676 (40.4)
Swabs	69 (18.6)	1200 (31.5)	1269 (30.6)
Blood	06 (1.7)	920 (24.3)	926 (22.3)
Sputum	02 (0.5)	85 (2.2)	87 (2.1)
Fluid	12 (3.2)	180 (4.7)	192 (4.6)

Table 2. Prevalence of ESBL in different bacterial pathogens recovered in this study.

Organism	Positive (%)	Negative (%)	Total
Escherichia coli	332 (89.5)	812 (21.5)	1144 (27.6)
Klebsiella spp.	20 (5.4)	1080 (28.6)	1100 (26.5)
Enterobacter spp.	14 (3.8)	966 (25.5)	980 (23.6)
Citrobacter spp.	05 (1.3)	921 (24.4)	926 (22.3)
Total	371 (100%)	3779 (100)	4150 (100)

nosocomial infections. Due to intrinsic and acquired capabilities to develop resistance to anti-microbial agents, they are difficult to treat. One of the important mechanisms of anti-microbial resistance is the production of extended-spectrum β-lactamases. ESBLs occur worldwide with varying prevalence and rapidly changing overtime. They are produced mostly by nosocomial and nursing home isolates of *E. coli* and *Klebsiella pneumonia*. Unfortunately, these organisms often possess resistance determinants to other important antibiotic groups, such as fluoroqninolones and aminoglycosides. Thus, antibiotic options in the treatment of these organisms are extremely limited. This may lead to prolonged hospital stay and is associated with high mortality (Weinbern and Borthwick, 2005).

A study was conducted by Subha and Ananthan (2002)

Table 3. Antibiotic susceptibility pattern of organisms expressing ESBL.

Antibiotic	Sensitive (%)	Resistant (%)	Not tested (%)
AK	282 (76)	75 (20.2)	14 (3.80)
SXT	31 (8.4)	315 (84.9)	25 (6.70)
GEN	121 (32.6)	223 (60.1)	27 (7.30)
CIP	53 (14.3)	255 (68.7)	63 (17.0)
DOXY	27 (7.3)	298 (80.3)	46 (12.4)
MEM	355 (95.7)	05 (1.30)	11 (3.00)

AK (Amikacin) SXT (Septran) GEN (Gentamicin) CIP (Ciprofloxacin) DOXY (Doxycycline) MEM (Meropenem).

Table 4. Prevalence of ESBL in different age groups of patients.

Age group	Positive (%)	Negative (%)	Total (%)	*P* value	OR
1-12	111 (29.2)	988 (26.1)	1099 (26.5)	0.005	3.04
13-45	68 (18.3)	1843 (48.8)	1911 (46)		
> 45	192 (51.6)	948 (24.9)	1140 (27.5)	0.005	5.49

to show the incidence of multi-drug resistance and ESBL producing *Klebsiella* isolates among children in Chennai, India. According to that study, *K. pneumoniae* accounted for increased ESBL production from 6-17% of nosocomial isolates involved in urinary tract infections. In our study, *E. coli* has shown very high incidence of ESBL production (89.5%). This may be due to the reason that they have not considered *E. coli* in their study.

Observations made by Zakria et al. (2008) indicated that meropenem is active in both ESBL producers and non ESBL producers. In our study, carbapenem has been reported as a drug of choice for serious infections with ESBL producers, the same conclusion has also been made by Romanus (2009).

E. coli shows high prevalence as uropathogen in community as well as in hospital set up. Large numbers of urinary isolates are found to be ESBL producers. In our study, the prevalence of ESBL producing members of enterobacteriaceae is high. Out of 371 positive cases of ESBL producers 332 (89.5%) were *E. coli.* It was the most commonly collected isolate from urine specimen (n=282, 76.0%). Gupta et al. (2002) have also reported similar results. According to that study, *E. coli* predominated amongst the indoor as well as outdoor patients suffering from UTI. Our study, confirmed previous observations that were made among ESBL producing enterobacteriaceae. It predominantly affects specific groups of patients. These are usually at extreme of ages, either elderly or infants.

Most of our patients admitted with symptoms of infection received empiric, broad spectrum antibiotic coverage. In fact, the use of such type of broad spectrum antimicrobial agents lead to the emergence of multi-resistant bacterial strains that ultimately complicate the management of such patients even further. Similar observation was made by Chlebicki and Oh (2004). Further research is required in investigation of ESBL producers and their management practices: because choices of antibiotics for treating infections caused by ESBL-producing enterobacteriaceae are very limited.

Conclusion

Our findings demonstrate that there is increasing incidence of infection with ESBL producing organisms and high rates of antimicrobial resistance among these organisms. Laboratories should be encouraged for regular screening and detection of ESBL and clinicians should be familiar with clinical importance of these enzymes and potential strategies for dealing with them. It is alarming in low-income settings where expensive second line agents are not affordable. Our study supports urgent need for regular screening and surveillance of these organisms in developing countries:

1. Carbapenem is the drug of choice for serious infections with ESBL producers; however, it should not be administered as empiric therapy because its overuse can pose significant problems.
2. Local or institutional anti-biograms should be used to determine the choice of antimicrobial agents.
3. Implementation of infection control measures are of primary importance, as treatment options are limited for

these infections.
4. Awareness of clinicians to clinical significance of these enzymes.

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