

Full Length Research Paper

# The frequency and antimicrobial sensitivity pattern of extended spectrum $\beta$ -lactamase (ESBLs) producing gram negative bacilli isolated from urine in a tertiary care hospital of Pakistan

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Accepted 19 April, 2013

Urinary tract infections (UTIs) are one of the most common bacterial infections in humans, both in the community and the hospital settings. An extensive use of  $\beta$ -lactam antibiotics in hospitals and community has created major resistance problem leading to increased morbidity, mortality and health-care costs. Local knowledge of antimicrobial susceptibilities of these organisms is important for implementation of effective hospital anti-infective policies. The objective of the study was to determine the prevalence of ESBL producers along with their antimicrobial sensitivity pattern in the urinary isolates of Gram negative bacilli. The study was carried out in the Department of Microbiology, Army Medical College, looking after an 1100 bedded tertiary care hospital from December 2009 to November 2010. A total of 826 clinical isolates of Gram negative bacilli were recovered from the routine clinical samples of urine from the inpatient and outpatient departments of the hospital. Three hundred and sixty four (364) ESBL producers were identified from these isolates. *Escherichia coli* was the most frequent ESBL producer in this group followed by *Klebsiella pneumoniae* and *Enterobacter spp.* Carbapenems were found to be the most effective drug followed by Amikacin and Nitrofurantoin. The high prevalence of ESBL producers in the urinary isolates in our study warrants the need for judicious use of antibiotics to control the spread of antibiotic resistance in these bacteria.

**Key words:** Urinary isolates, gram negative bacilli, ESBL, antibiotic resistance.

## INTRODUCTION

The increasing incidence and complexity in the resistance mechanisms of the pathogenic bacteria and their widespread distribution globally has created a worrisome clinical dilemma with the treatment of common ailments becoming more and more of a challenge. Infections caused by resistant microorganisms often fail to respond to conventional treatment, resulting in prolonged illness and increased morbidity and mortality (Mehrgan and

Rahbar, 2008). The alarming number of new cases of multi-drug resistant (MDR) and extremely drug resistant (XDR) tuberculosis, widespread resistance to earlier generation of anti-malarial medicines in malaria endemic regions, a high percentage of hospital-acquired infection caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus species* are all harbingers of the tough times ahead for the

infection treatment and control. The inappropriate and irrational use of antimicrobial medicines is the single most important cause of the alarming increase in the resistance of these organisms, providing favorable conditions for the resistant organisms to emerge, spread and persist (Johann et al., 2005). The inadequate and ineffective treatment of these resistant organisms hampers the control of these infectious diseases as the patient harbors the resistant organism longer, thus further transmitting the resistant organisms to the naïve population.

Urinary tract infections (UTIs) remain the most prevalent and common infection in the community and hospital setting and the economic burden on the global economy due to UTI is enormous (Supriya et al., 2004). The lifetime risk for UTI in females is greater than 50% (Griebing, 2005). This is mainly because of shorter urethra in women; this is why women are more likely to get an infection after sexual activity or when using a diaphragm for birth control. Menopause also increases the risk of a UTI. *Escherichia coli* is the most common causative organism responsible for more than 70% of the cases both in the outpatients and inpatients (Gupta et al., 2001). There have been significant changes in the antimicrobial resistance patterns of the urinary pathogens over the years including resistance due to extended-spectrum beta lactamase (ESBL) producing pathogens (Mohammed et al., 2007). The increasing incidence of infections due to resistant urinary pathogens makes the empirical treatment of these infections difficult. The prevalence of antibiotic resistance varies according to the geographic region and mainly depends on the antibiotic usage tendencies of the population. It is very important to understand the changing trends in the antibiotic resistance of the causative organisms as it has a direct bearing on the treatment of UTI. Also, the detection of ESBL production among uropathogens is an important marker of endemicity and is an epidemiological marker of colonization. The knowledge of the presence of this resistance mechanism indicates the potential of spread to other patients as well as within the community.

Beta-lactam antibiotics are among the safest and most frequently prescribed medication in the world; however, the emergence and worldwide spread of resistance to beta-lactam antibiotics in clinically important bacteria has largely limited their efficacy. Among the resistance mechanisms, extended spectrum beta lactamase production in the Enterobacteriaceae is the most widely encountered with strains being increasingly isolated from all over the world. Since the detection of the ESBL producer for the first time from Germany in 1983 (Knothe et al., 1983), the problem is now endemic in many regions of the world rendering the beta-lactam drugs largely useless in infections caused by these resistant organisms (Nijssen et al., 2004). Infections due to extended spectrum  $\beta$ -lactamases (ESBL) producing Gram negative bacteria in the urine have become an important clinical problem, conferring resistance to all  $\beta$ -lactam antibiotics except

cephamycins and carbapenems. In addition, ESBL-producing organisms frequently show cross-resistance to many other classes of antibiotics; including aminoglycosides and fluoroquinolones, thus treatment of these infections is often a therapeutic challenge. Antibiotic resistance surveillance has a central role among all the strategies to manage the problem of antibiotic resistance. The production of ESBL is frequently plasmid encoded and bears clinical significance. Plasmids responsible for ESBL production frequently carry genes encoding resistance to other drug classes also. Hence ESBL producing organisms commonly show cross-resistance to many other classes of antibiotics (Paterson and Bonomo, 2005). Therefore, treatment options for ESBL producing organisms are extremely limited.

Local knowledge of the antimicrobial susceptibilities of these organisms is important for the introduction of proper treatment guidelines and implementation of effective hospital anti-infective policies. It is important to investigate the prevalence of ESBL positive strains in the hospitals so as to formulate a policy of appropriate empirical therapy in high risk units where infections due to resistant organisms are more likely to be encountered.

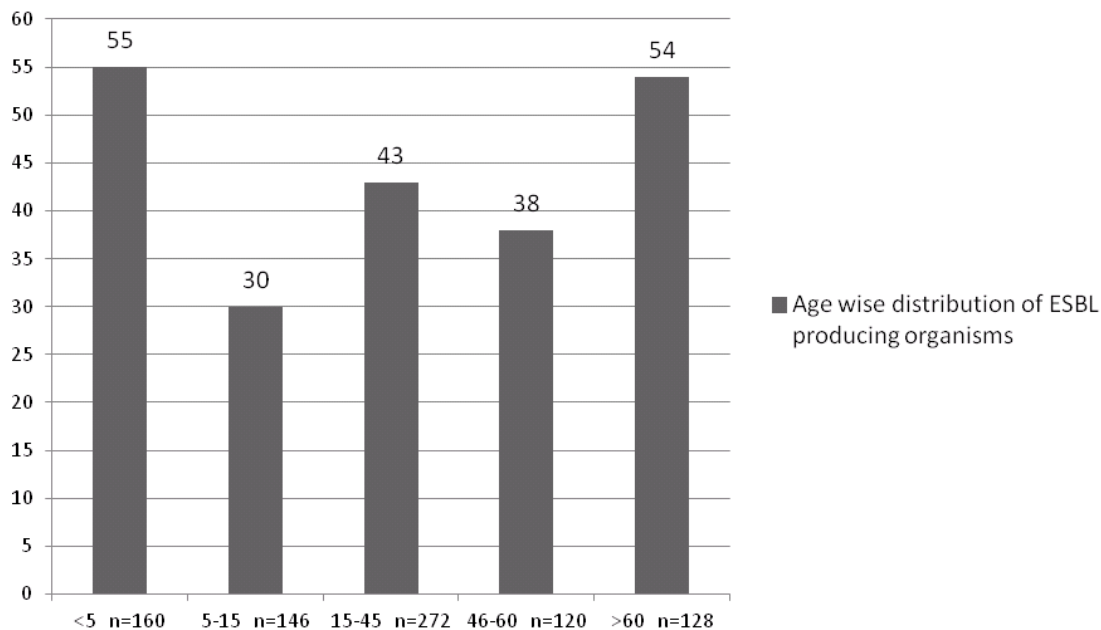
## MATERIALS AND METHODS

### Bacterial isolates

This prospective descriptive study was carried out in the Department of Microbiology, Army Medical College, National University of Sciences and Technology, Rawalpindi, Pakistan from December 2009 to November 2010. The urine samples sent for culture and sensitivity from the Military Hospital, Rawalpindi were studied for the growth of Gram negative Enterobacteriaceae. The samples were inoculated on Cysteine Lactose Electrolyte Deficient (CLED) agar. Duplicate samples from the same patient were discarded. After aerobic incubation at 37°C, the isolates were identified to the species level using standard biochemical tests like indole, triple sugar iron, citrate and urease. Motility was also checked to aid in the proper identification of the organisms. A total of 826 consecutive urine cultures growing Enterobacteriaceae including *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter* spp. isolated during the collection period were included in the study and subjected to testing for the detection of ESBL production and routine antibiotic sensitivity testing.

### ESBL detection

In keeping with the Clinical and Laboratory Standards Institute (CLSI, 2012) recommended guidelines, screening for ESBL production was performed by the double disk synergy method as described by Jarlier et al. (1988). Briefly, a disc of amoxicillin + clavulanic acid (20 + 10  $\mu$ g) was placed in centre of the petri plate already inoculated with the test organism while Aztreonam (30  $\mu$ g), Cefotaxime (30  $\mu$ g) and Ceftriaxone (30  $\mu$ g) were placed at a distance of 20 to 25 mm (centre to centre) from the amoxicillin + clavulanic acid disc. Zones of inhibition around the third generation cephalosporin discs and aztreonam were observed after 18 to 24 h for an enhancement /extension on the side nearest to the amoxicillin + clavulanic acid disc. The organisms showing this synergy were labeled as ESBL producers.



**Figure 1.** Age wise distribution of ESBL producing organisms exhibiting increase frequency of ESBL isolates at extremes of ages. % ESBL isolates represents ESBL positive isolates divided by total number of isolates obtained from a particular age group.

**Table 1.** Susceptibility pattern of the ESBL producers in urine (n = 364).

Drug	Sensitivity (%)
Imipenem/Meropenem	98
Tigecycline	91
Amikacin	84
Piperacillin-tazobactam	79
Nitrofurantoin	71
Cefoperazone-sulbactam	70

Note: Disc diffusion testing was performed to determine the antibiotic susceptibility of the ESBL producing Gram negative organisms isolated from urine in our study and MIC determination was not done so MIC 90/50 and MIC range data is not available. Only antibiotics active against 70% or more of the isolates are shown in this table.

#### Antimicrobial susceptibility testing

The susceptibility of the isolates to a panel of antibiotics was evaluated by the Kirby Bauer Disc Diffusion method (CLSI M02-A11, 2012) using Mueller Hinton agar (Oxoid, England) and the results were interpreted according to the CLSI recommended guidelines (CLSI, 2012). The antibiotic disks were obtained from Oxoid, England. The antibiotics used for the antibiogram determination of the isolates were: ampicillin (AMP), amoxicillin - clavulanic acid (AMC), cefoperazone - sulbactam (SCF), piperacillin - tazobactam (TZP), ceftriaxone (CRO), ceftazidime (CAZ), ciprofloxacin (CIP), levofloxacin (LEV), norfloxacin (NOR), nitrofurantoin (N), meropenem (MEM), imipenem (IMP), amikacin (AMI), gentamicin (CN), co-trimoxazole (SXT) and aztreonam (ATM).

## RESULTS

A total of 826 isolates of Enterobacteriaceae were obtained from the urine samples submitted to the Microbiology Laboratory from both the outpatient and inpatient departments of the Military Hospital, Rawalpindi during the study period. 364 (44%) of these isolates were identified as having ESBL phenotype, of which 258 (71%) were *E. coli*, 69 (19%) were *K. pneumoniae*, 19 (5%) were *K. oxytoca*, 15 (4%) were *Enterobacter* spp. Females constituted 73% of this patient group while 27% were male patients. Mean age of the female patients with ESBL producing organisms was 43 (Range: 1 to 86) and for the male patients it was 46 (Range: 1 to 92). Age wise break up of ESBL positivity rate is shown in Figure 1. Majority of the samples were from the admitted patients (n = 226 to 62%) and 138 (38%) of the samples were from the OPD patients. The OPD patients were mostly from the Medical OPD, General OPD and the Family Clinics.

The organisms were most susceptible to carbapenems (98%) followed by tigecycline (91%), amikacin (84%), piperacillin-tazobactam (79%), nitrofurantoin (71%) and cefoperazone-sulbactam (70%) (Table 1).

## DISCUSSION

ESBL production in Gram negative bacilli is among the fastest and most alarming problem in the arena of infectious diseases. The elaboration of this enzyme create a therapeutic dilemma as the organism frequently

show cross resistance to many of the commonly used antibiotics. The high prevalence of ESBL producers among uropathogens in our study is in line with the studies done elsewhere. Ullah et al. (2009) have documented 58.7% ESBL producers among the uropathogens in their study from Pakistan in 2009 with 71% of the isolates resistant to three or more of the tested antibiotics. They also found carbapenems to be the most effective drug against these organisms. Ava et al. (2010) from Iran found 21% ESBL producers among the urinary isolates in their study in 2010 and nitrofurantoin was the most effective drug. Aggarwal et al. (2009) have reported 36% ESBL producers from the urinary isolates in their study from India in 2009 and found all the isolates uniformly sensitive to carbapenems. Johann et al. (2005) in their study from Canada in 2005 found 26% ESBL producers among the uropathogens and carbapenems and tigecycline were the most effective drugs.

In our study, we found a high resistance among the ESBL producers against fluoroquinolones with only 19% of the isolates sensitive to these drugs. This is compatible with the findings reported by Burgess et al. (2003). Fluoroquinolones are particularly effective for the treatment of UTI because high concentrations can be achieved in the urine and it is orally available. However, the high resistance in urinary pathogens against this drug class is a clinical concern with restricted available options for the treatment of these infections particularly in the outpatient setting. It is interesting to note the high sensitivity of nitrofurantoin against these resistant pathogens in our study with 71% of the ESBL producers sensitive against this drug. Nitrofurantoin is an oral drug with excellent efficacy against the urinary pathogens, including the ESBL producers. Amikacin has also shown good activity against these resistant organisms in our study with 84% sensitivity. The intravenous administration of the drug is a drawback but can be effective for patients who are admitted in the hospital. The high proportion of patients harboring these resistant organisms in the community are a cause of concern with the potential of spread to naïve population a probable hazard and treatment a clinical dilemma with most of the oral drug options not effective against these bacteria. Nitrofurantoin may provide relief in this scenario with its good activity against these isolates in our study.

Antibiotic resistance surveillance has a central role among all strategies to manage the problem of antibiotic resistance. The results of our study show a high prevalence of ESBLs among the urinary isolates. Routine ESBL detection should be made imperative and empirical use of third generation cephalosporins must be discouraged. Strict hygiene protocols and implementation of appropriate infection control measures in the hospital should be encouraged, especially while treating high risk patients in order to contain the problem and limit its spread.

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