

## Nosocomial blood-stream infections from extended-spectrum-beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* from GB Pant Hospital, New Delhi

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### Abstract

**Background:** Nosocomial septicemia due to extended spectrum beta-( $\beta$ )-lactamase (ESBL) producing *Klebsiella pneumoniae* and *Escherichia coli* are a therapeutic challenge due to resistance. Knowledge of disease burden and resistance patterns is required for proper and timely management. We report the prevalence and antimicrobial susceptibility of ESBL producing *E. coli* and *K. pneumoniae* from septicemia at a tertiary care hospital.

**Methodology:** A total of 2,870 blood samples of suspected cases of septicemia were studied between January and December 2009. Antimicrobial susceptibility was determined by Kirby Bauer's disc diffusion method and MICs for imipenem, meropenem, and ertapenem were determined using the E-test. All isolates of *E. coli* and *K. pneumoniae* were tested for ESBL production by E-test method.

**Results:** Forty-one (70.7%) *K. pneumoniae* isolates and ten (41.7%) *E. coli* isolates were ESBL producers. Two (5%) of ESBL producing *K. pneumoniae* isolates, but no *E. coli* isolates, were resistant to carbapenems. *In vitro*, all ESBL producers were sensitive to tigecycline.

**Conclusion:** Our data indicated that the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* strains isolated from blood cultures from hospitalized patients is high. ESBL-producing organisms were found to be more susceptible to meropenem than to imipenem and ertapenem. Tigecycline is active against all the ESBL or multidrug resistant (MDR) *E. coli* and *Klebsiella spp.* isolates.

**Key words:** nosocomial; bacteremia; extended spectrum beta-lactamase

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### Introduction

Extended spectrum beta-( $\beta$ )-lactamase (ESBL) producing strains of *Enterobacteriaceae* have now emerged as a major problem in hospitalized patients. These organisms are responsible for a variety of infections, such as septicemia, urinary tract infection, hospital-acquired pneumonia, intra-abdominal abscess, brain abscess, and device-related infections [1]. Among all types of nosocomial infections, the nosocomial blood-stream infection (BSI) creates a serious health problem in hospitals all over the world. *Escherichia coli* and *Klebsiella pneumoniae* are major nosocomial pathogens causing intra-abdominal infection, urinary tract infection, and primary bacteremia. ESBL producers show resistance to cephalosporins and aztreonam, and often carry resistance to aminoglycosides and other drugs, but they remain susceptible to carbapenems [2]. Unfortunately, there is now an increasing occurrence of carbapenem resistance in *Enterobacteriaceae*. In this context, clinical response to new antibiotics with good *in vitro* activity against ESBL producers, such

as tigecycline, needs to be evaluated. This study investigated the nosocomial BSI in the adult intensive care units and prevalence of extended spectrum  $\beta$ -lactamase producers among *E. coli* and *Klebsiella sp.* It also studied the *in vitro* activity of imipenem (IPM), meropenem (MEM), ertapenem (ETP), and tigecycline (TGC) against ESBL-producing common pathogens isolated from patients in intensive care.

### Material and methods

All adult patients admitted at different intensive care units (ICUs) of GB Pant Hospital (coronary care unit, CCU; cardiothoracic surgery ICU; neurological ICU; general ICU; and neurosurgical ICU) were included in the study during January 2009 to December 2009. Surveillance of these patients was conducted by the laboratory-based ward Liaison method to identify BSI. BSI were defined as a blood-stream infections documented by at least one blood culture positive from a patient with systemic inflammatory response syndrome (*e.g.* fever,

**Table 1.** Susceptibility patterns of ESBL-producing organisms

Antimicrobial Agent	<i>E. coli</i> n = 10 (%)	<i>K. pneumonia</i> n = 41(%)
Imipenem	10 (100)	39 (95.1)
Meropenem	10 (100)	39 (95.1)
Ertapenem	10(100)	39 (95.1)
Amikacin	7 (70)	15 (36.6)
Gentamicin	3 (30)	5 (12.2)
Piperacillin-Tazobactam	6(60)	11(26.8)
Ticarcillin-Clavulanic acid	(0)	(0)
Ofloxacin	3 (30)	9 (21.9)
Cefepime	(0)	(0)
Cefotaxime	0	0
Tigecycline	10 (100)	41 (100)

tachycardia, tachypnea, and leukocytosis) [3]. All the blood samples were processed by the clinical microbiology laboratory in GB Pant Hospital. The pathogens were identified by conventional biochemical procedures [4]. All isolates were screened phenotypically for ESBL production by E-test (AB Biodisk, Solna, Sweden). Minimum inhibitory concentration (MIC) of ESBL-producing strains to cefotaxime was also determined by E-test. ESBL-producing *E. coli* and *K. pneumoniae* were included in the study. Only one non-repeat isolate from each bacteremic episode was included in the analysis. The antibiotic susceptibility of each isolate was determined by the disc diffusion method, employing the criteria of the Clinical and Laboratory Standards Institute (CLSI) guidelines [5]. The antibiotics included in the susceptibility test were cefotaxime (30 µg), cefepime (30 µg), ofloxacin (5 µg), piperacillin-tazobactam (100/10 µg), ticarcillin-clavulanic acid (75/10 µg), amikacin (30 µg), gentamicin (10 µg), imipenem IPM (10 µg) (HiMedia, Mumbai, India), meropenem MEM (10µg) (Becton Dickinson, Sparks, MD, USA), ertapenem ETP (10 µg) (Becton Dickinson, Sparks, MD, USA), and tigecycline TGC (10 µg) (Becton Dickinson, Sparks, MD, USA). Isolates were considered resistant to MEM and IPM if the zone of inhibition was ≤ 13 mm, intermediate when the zone was 14–15 mm, and sensitive when the zone was ≥ 16 mm, per CLSI guidelines [5]. For ETP, isolates were considered resistant when the zone diameter was ≤ 15 mm, intermediate when the zone diameter was 16–18 mm, and sensitive when the zone diameter was ≥ 19 mm. For TGC, isolates were considered resistant when the zone diameter was ≤ 14 mm, intermediate when the

zone diameter was 15–18 mm, and sensitive when the zone diameter was ≥ 19 mm. The MIC of ETP, IPM, and MEM was also recorded for all the isolates by the E-test (AB Biodisk, Solna, Sweden). The MIC breakpoints for IMP, MEM, and ETP were taken per CLSI guidelines [5]. For IPM and MEM, isolates were considered sensitive if the MIC was ≤ 4 µg/mL, intermediate if the MIC was 8 µg/mL, and resistant if the MIC was ≥ 16 µg/mL. Isolates were considered sensitive to ertapenem if the MIC was ≤ 2 µg/mL, intermediate if the MIC was 4 µg/mL and resistant if the MIC was ≥ 8 µg/mL. Two control organisms, *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603, were inoculated in each set of tests for quality control.

## Results

Out of 2,870 blood cultures obtained from suspected cases of septicemia, 244 (8.5%) yielded growth of microorganisms. The total number of pathogenic isolates was 253, of which six were *Candida* spp. and 247 were bacterial isolates. Nine samples showed polymicrobial growth of Gram-negative bacteria. The Gram-negative bacteria most commonly isolated from blood cultures were *K. pneumonia* 64 (26.2 %), *Acinetobacter* spp. 63 (25.8 %), *Escherichia coli* 26 (10.6%), *Citrobacter* spp.15 (6.1%), *Pseudomonas* spp. 24 (9.8 %), *Enterobacter* spp. 9 (3.7 %), and *Proteus mirabilis* 1 (0.4%). The Gram-positive bacteria isolated were *Staphylococcus aureus* 14 (5.7 %), *Enterococcus* spp. 26 (10.6 %), *Streptococcus* spp. 4 (1.6%), and coagulase negative *staphylococcus* 1(0.4%).

All strains of *E. coli* and *K. pneumoniae* were tested for ESBL production. ESBL was detected in

41 (64 %) isolates of *K. pneumoniae* and 10 (38.5%) isolates of *E. coli*. All ESBL isolates had MIC > 8 µg/mL to cefotaxime. The susceptibility data of the ESBL-producing *E. coli* and *K. pneumoniae* are summarized in Table 1. Tigecycline and carbapenems showed sensitivity of 100% and 95%, respectively, by disc diffusion. Two *Klebsiella* spp. isolates were resistant to all the carbapenems tested, with MICs for IPM, MEM, and ETP of 8, 4, and 8 µg/mL respectively. A follow-up of the cases of septicemia due to ESBL-producing bacteria revealed that four out of 51 patients (7.8 %), including two ESBL cases resistant to carbapenems, expired during their hospital stay and the rest were discharged after recovery.

## Discussion

Extended spectrum β-lactamase producing Gram-negative bacteria, particularly *Klebsiella pneumoniae* and *Escherichia coli*, are now widespread nosocomial pathogens causing bacteremia. The most frequent isolates in the present study were *K. pneumoniae* (26.2%) and *Acinetobacter* (25.8%). Similar results are reported by previous studies from India [6,7]. The current study shows prevalence of ESBL-producing *E. coli* and *K. pneumoniae* as 10 (41.7%) and 41 (70.7%), respectively. Other recent studies from India have reported a prevalence of ESBL production ranging from 41.0-63.6% in *E. coli* and 40-66.7% in *K. pneumoniae* [7,8]. Carbapenems are effective alternative antibiotics against ESBL-producing bacteria. The carbapenems tested in the present study showed high activity against all strains of *E. coli* and *K. pneumoniae* with the exception of two *K. pneumoniae* isolates. Resistance in ESBL-producing members of Enterobacteriaceae has been reported as 3-6% to carbapenems [9].

Tigecycline has a bacteriostatic mode of action against a broad spectrum of both aerobic and anaerobic Gram-positive (including methicillin-resistant *Staphylococcus aureus* and vancomycin resistant *enterococci*) organisms and Gram-negative organisms [10]. Regarding ESBL-producing Enterobacteriaceae, *in vitro* susceptibility to tigecycline for *E. coli* and *K. pneumoniae* has been reported as 99.8% and 92.3%, respectively [10,11]. All ESBL producers in the present study showed sensitivity to tigecycline. *E. coli*, and *K. pneumoniae* isolates showed a sensitivity of 75% and 35% to amikacin, respectively. Similar findings observed by Winokur *et al.* [12] showed that 80-87% of ESBL strains were resistant to ofloxacin. Regional studies

have reported the emergence of fluoroquinolone co-resistance in ESBL-producing organisms [13]. All the *E. coli* and *K. pneumoniae* ESBL-producing isolates were resistant to cefotaxime and cefepime

In conclusion, our data indicated the high prevalence of ESBL-producing *E. coli* and *K. pneumoniae* strains isolated from blood cultures from hospitalized patients. ESBL-producing organisms were found to be more susceptible to meropenem than to imipenem and ertapenem; however, the MICs of ertapenem and imipenem were well within sensitive levels. Tigecycline is active against almost all the ESBL or MDR *E. coli* and *Klebsiella* spp. isolates. Acquiring ESBL-producing bacteria can be prevented by adherence to infection control practices in the hospital, such as the removal of medical devices as soon as possible and adherence to sterile techniques when performing procedures. Tigecycline holds promise as an alternative choice of therapy for infections caused by ESBL-producing isolates, keeping in mind the emerging carbapenem resistance.

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**Conflict of interests:** No conflict of interests is declared.