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Detection of resistance pattern, extended-spectrum Beta -lactamase producing clinical isolates *Escherichia coli* and *Klebsiella pneumoniae*

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Abstract
The incidence of carbapenem resistance among gram-negative bacteria is a major cause of concern which threatens to disrupt therapeutic options. This study document the antibiotics susceptibility pattern of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* in clinical isolates. 54 isolates of *E. coli* and 24 isolates of *K. pneumoniae* were screened as ESBL-producers against any one or more of the cefpodoxime, ceftazidime, cefotaxime, ceftriaxone and aztreonam by Kirby-Bauer disc diffusion method. Norfloxacin and nitrofurantoin were tested only against isolates from urine samples. Highest numbers of ESBL-producing *E. coli* were detected by cefpodoxime while ceftriaxone showed the least sensitivity. In some cases the isolates produced ESBL against only one of the antibiotics and in some cases more than one. ESBL-producing *E. coli* isolates showed highest susceptibility to meropenem (94.6%) followed by amikacin (82.6%), and imipenem (90.7%). ESBL-producing *K. pneumoniae* showed highest susceptibility to meropenem (88.5%) followed by gentamicin & piperacillin-tazobactam (81.6%), and amikacin, ciprofloxacin & levofloxacin (78.2%). Susceptibility of both the organisms to other antibiotics was below 75%. The susceptibility of urinary isolates of *E. coli* and *K. pneumoniae* to nitrofurantoin was 81.1% and 96% respectively.

Keywords: ESBL, β-LACTAM, *Escherichia coli, Klebsiella pneumoniae*, antimicrobial resistance

Introduction
Beta-lactam antimicrobial agents are used profusely in the treatment of bacterial infections. Resistance to β-lactam antibiotics among clinical isolates, especially among gram-negative bacteria is most often due to the production of β-lactamases. These enzymes are numerous and they mutate continuously in response to heavy pressure of antibiotic use and have lead to the development of extended spectrum β -lactamases (ESBLs). Many of these ESBLs have evolved from the β -lactamases that are widely distributed among the Enterobacteriaceae [2-3].
ESBLs are enzymes that mediate resistance to extended-spectrum (third generation) cephalosporins (e.g., ceftazidime, cefotaxime, and ceftriaxone) and monobactams (e.g, aztreonam) but do not affect cephamycins (e.g., cefoxitin and cefotetan) or carbapenems (e.g, imipenem or meropenem). These ESBLs are commonly inhibited by β -lactamase-inhibitors such as clavulinic acid, sulbactam and tazobactam [4-7].
ESBLs were first discovered in 1983 [8]. Since that time, these have been identified worldwide and have been found in a number of different organisms, including *Klebsiella pneumoniae, Klebsiella oxytoca, Escherichia coli, Proteus mirabilis, Enterobacter cloacae, Morganella morganii*, *Serratia marcescens*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Capnocytophaga ochracea*, *Citrobacter* species, and Salmonella species [9-12].
Guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) recommend screening all *K. pneumoniae, K. oxytoca*, and *E. coli* for which MICs of cefpodoxime, ceftazidime, cefotaxime, ceftriaxone, and aztreonam are 2 µg/ml [7]. The organism may produce ESBL against anyone or more of the above antibiotics. Various conventional or automated laboratory methods are available to detect this [13].

Among gram-negative bacteria, the emergence of resistance to extended-spectrum cephalosporins and carbapenems is a major cause of concern major concern. Treatment of infections caused by ESBL-producers is complicated not only by resistance to extended-spectrum cephalosporins, but also because many ESBL genes are on large plasmids containing genes which also encode resistance to many other antibiotics including aminoglycosides, chloramphenicol, sulfonamides and tetracycline antibiotics. These infections have a significant impact on patient's mortality and additional financial burden. The present study is aimed to document the existence of ESBL-producing *E. coli* and *K. pneumoniae* in a teaching hospital in Chhattisgarh, India and to know about the status of alternative antibiotics in case the organism is ESBL-producer [14].

Materials and Methods

A retrospective study was conducted to know the susceptibility patterns of clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* that were isolated during the year January 2015 to December 2015 and were identified as ESBL-producers in Clinical Microbiology Laboratory at Chhattisgarh Institute of Medical Sciences, Bilaspur, Chhattisgarh, India. A Microbial work-up was set-up and the isolates were identified by standard procedure. Antimicrobial sensitivity testing was carried out on Mueller Hinton Agar (MHA) plates with commercially available discs (Hi-Media, Mumbai) by the Kirby-Bauer disc diffusion method and interpreted according to NCCLS criteria.

There were 54 isolates of *E. coli* and 24 isolates of *K. pneumoniae* that were detected as ESBL-producer if the MIC of one or more of cefpodoxime, ceftazidime, cefotaxime, ceftriaxone, or aztreonam against these isolates was ≥2 µg/ml. An ESBL-producing strain was considered as resistant to aztreonam, cefotaxime, cefpodoxime, ceftazidime, ceftriaxone and other cephalosporins even if the MICs for these antibiotics were within sensitive range. The susceptibility pattern of penicillins in combination with any of the β-lactamase inhibitors i.e. clavulinic acid, sulbactam or tazobactam was reported as such. Norfloxacin and nitrofurantoin were tested only against isolates from urine samples.

Results

ESBL-producing strains were recovered from various representative samples viz. pus / wound swabs, urine, bronchial aspirates, cervical swabs, catheter tips and blood as shown in Table 1.

Most of the *E. coli* isolates were from urine while majority of *K. pneumoniae* were from pus specimens. There were 54 isolates of *E. coli* and 24 isolates of *K. pneumoniae* that were detected as ESBL-producers. Highest numbers of ESBL-producing *E. coli* were detected by cefpodoxime (n=28) followed by aztreonam (n=24), ceftazidime (n=18), cefotaxime (n=10) and ceftriaxone (n=7). For ESBL-producing *K. pneumoniae* it was cefpodoxime (n=18) followed by cefotaxime (n=13) ceftazidime (n=ll), aztreonam (n=10) and ceftriaxone (n=8) as shown in Table 2.

Variations were observed in ESBL-production by the isolates against antibiotics tested. In some cases the isolates produced ESBL against only one of the antibiotics and in some cases more than one. ESBL-producing *E. coli* isolates showed highest susceptibility to meropenem (94.6%) followed by amikacin (82.6%), and imipenem (90.7%). ESBL-producing *K. pneumoniae* showed highest susceptibility to meropenem (88.5%) followed by gentamicin & piperacillin/ tazobactam (81.6%), and amikacin, ciprofloxacin & levofloxacin (78.2%). Susceptibility of both the organisms to other antibiotics was below 75%. The susceptibility of urinary isolates of *E. coli* and *K. pneumoniae* to nitrofurantoin was 81.1% and 96% respectively.

Table 1: Sources of the ESBL-producing strains.

Specimens	No. of *Escherichia coli* isolated	No. of *Klebsiella pneumoniae* isolated
Urine	23	6
Pus/wound swabs	21	11
Bronchial aspirate	5	2
Cervical swab	3	-
Catheter tips	3	3
Blood	1	2
Total	54	24

Table 2: No. of isolates that produced ESBL against different antibiotics.

Antibiotics	Organisms	
	Escherichia coli	*K. pneumoniae*
Aztreonam	24	10
Cefotaxime	10	13
Cefpodoxime	28	18
Ceftazidime	18	11
Ceftriaxone	7	8

Discussion

The emergence, selective multiplication and dissemination of antimicrobial resistance in bacteria has been well documented as a serious global problem [15]. Among gram-negative bacteria, the emergence of resistance to extended-spectrum cephalosporins is a major cause of concern, owing to its propensity to hybridize with other strains and ability to cause nosocomial outbreaks, and possibility to present great therapeutic challenges [16].

In present study, 54 ESBL-producing *E. coli* and 24 ESBL-producing *K. pneumoniae* isolated from different clinical specimens were studied. Bade Kirby-Bauer disc diffusion method was used in this study for screening the isolates as ESBL-producers. Although the system has variable results but has an acceptable sensitivity [17].

Cefpodoxime showed the highest sensitivity ESBL detection in both *E. coli* and *K. pneumoniae* as has been reported earlier (NCCLS 1996), while ceftriaxone was the least sensitive. More than 90% of ESBL-producing *E. coli* isolates were susceptible to meropenem (94.6%), amikacin (82.6%) and imipenem (90.7%). More than 90% of similar *K. pneumoniae* isolates were susceptible to only meropenem (88.5%). Imipenem was not tested against *K. pneumoniae.* Similar susceptibility has been reported earlier [18].

Organisms that express an ESBL are frequently resistant to other antimicrobial agents, as many of these additional resistance genes are encoded on the ESBL-associated plasmid (NCCLS 1996). In our study, high level of resistance was detected against trimethoprim-sulfamethoxazole. Gentamicin and tobramycin had reduced but variable activity; however the activity of amikacin against these isolated remains high. This better activity of amikacin may be due to its less vulnerability to bacterial enzymes than other aminoglycosides [19].

Also there was a reduced activity of fluoroquinolones including norfloxacin (in urinary isolates). Fluoroquinolone-

resistance is typically encoded chromosomally. This resistance against fluoroquinolones in our study may reflect significant antibiotic pressure in the environment rather than co-carriage of this resistance gene on plasmids.

There was a high level-resistance to β-lactam-β-lactamase inhibitor combination (amoxicillin-clavulinic acid, ampicillin-sulbactam and ticarcillin-clavulanic acid). This is likely to be due to the heavy selection pressure from overuse of these antibiotics and seem to be losing the battle. However, good activity was shown by piperacillin-tazobactam as has been reported earlier [20-21].

Carbapenem are the drug of choice for serious infections with ESBL-producing organisms. Resistance to carbapenems is due to decreased outer membrane permeability, increased efflux system, alteration of penicillin binding proteins, and the production of carbapenem hydrolyzing enzymes-carbapenemases. The carbapenemases belong to class metalo-β-lactamases (MBL).The resistance by MBL can be chromosomally encoded or plasmid mediated [22]. Carbapenem have been rendered ineffective by ESBL producing resistant stains. Paucity of alternative therapeutic option in carbapenem resistant cases compounds the problem in critically ill patients of ICUs [14]. The sharing of data and information on carbapenem and antibiotics resistance will be immense help in devising strategies against bacterial infection of critical care unites. However, these should not be administered as empirical therapy for gram-negative infections that are not life threatening because their overuse can pose a significant problem [21] the situation warrants an immediate implementation of infection control measures, an effective antibiotics policy including antibiotics recycling and stringent antibiotics resistance surveillance measures.

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