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Occurrence of extended spectrum beta lactamases among Escherichia coli and Klebsiella Pneumoniae isolates in a tertiary care teaching hospital

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Abstract
Background: Extended spectrum beta-lactamases (ESBLs) represent a major group of of β-lactamases currently being identified worldwide in large numbers, most commonly produced by *Klebsiella pneumoniae* and *Escherichia coli* but also occur in other gram negative bacteria. The most common cause of bacterial resistance to β-lactam antibiotics is the production of β-lactamase enzymes.
Objective: To determine the prevalence and antibiotic susceptibility pattern of ESBL producers among *Escherichia coli* and *Klebsiella pneumoniae* isolates.
Material and Methods: A total of 400 clinical isolates, 203 *Escherichia coli and* 197 *Klebsiella pneumoniae* were isolated from various clinical specimens and tested for ESBL production. Screening and confirmatory tests for ESBL production were done according to Clinical Laboratory Standard Institute's guidelines.
Results: Out of 400 isolates of *Escherichia coli* and *Klebsiella pneumoniae*, 197 (49.25 %) were found ESBLs producers. Out of 203 *Escherichia coli* isolates 92 (45.32 %) were found ESBLs producers and of the 197 *Klebsiella pneumoniae* isolates 97 (49.23%) were found ESBLs producers. ESBL producing *E. coli* and *K. pneumoniae* strains were most frequently recovered from urine 42.39% and pus 43.29% respectively. ESBL producing both *E. coli* and *K. pneumoniae* strains were most sensitive to imipenem followed by gentamycin.
Conclusion: Routine detection of ESBL-producing microorganisms is required to be done by each laboratory by the standard detection methods so as to control the spread of these infections.

Keywords: Beta-lactam resistance, *Escherichia coli*, Extended-Spectrum Beta-lactamases, *Klebsiella pneumonia*

Introduction
β-lactam antibiotics are one of the most frequently prescribed antimicrobial agents worldwide. The emergence of resistance to these antibiotics in the past few decades has resulted in a major clinical crisis [1, 2]. The most common cause of bacterial resistance to β-lactam antibiotics is the production of β-lactamase enzymes. β-lactamases production by several gram negative and gram positive organisms is perhaps the most important single mechanism of resistance to penicillins and cephalosporins. In the past it was believed that cephalosporins were relatively immune to attack by β-lactamases, but latter on *Klebsiella* spp. was found to be resistant to cephalosporin. The mechanism of this resistance was production of extended spectrum β-lactamases (ESBLs) [3].
Extended spectrum beta-lactamases (ESBLs) represent a major group of of β-lactamases currently being identified worldwide in large numbers, most commonly produced by *Klebsiella pneumoniae* and *Escherichia coli* but also occur in other gram negative bacteria [4-6]. They are plasmid mediated beta lactamases capable of hydrolyzing oxyimino-cephalosporins such as cefotaxime, ceftazidime, ceftriaxone and monobactams such as aztreonam and are inhibited by beta lactamase inhibitors such as clavulanate, sulbactam, and tazobactam [7-9].
As ESBL-positive isolates show false susceptibility to expanded-spectrum cephalosporins in standard disk diffusion test, so it is difficult to reliably detect ESBL production by the routine disk diffusion techniques. Therefore specific detection methods recommended by CLSI have to be adopted. ESBLs are specifically inhibited by β-lactamase inhibitors like clavulanic acid, and this property is commonly utilized for the detection and confirmation of ESBLs in the laboratory [10, 11].
ESBLs-producing strains have emerged as a major problem in hospitalized as well as community based patients. 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 to determine the prevalence and antibiotic susceptibility pattern of ESBL producers among *Escherichia coli* and *Klebsiella pneumoniae* isolates.

Material and Methods
Study Design
This study was carried out in the Department of Microbiology, JLN Medical College Ajmer from August 2016 to November 2016 to detect extended spectrum beta-lactamase producing strains of *Escherichia coli* and *Klebsiella pneumoniae* in various clinical specimens from attached Hospitals. Out of a total of 400 clinical isolates, 203 *Escherichia coli and* 197 *Klebsiella pneumoniae* were isolated from various clinical specimens.

Sample Collection and Methods

The samples were processed for the identification of organisms on the basis of conventional microbiological procedures and were screened for ESBLs. All isolates were cultured on MacConkey Agar and Blood Agar and urinary isolates on CLED media (obtained from Hi-Media, Mumbai, India) also and incubated at 37°C for 24 hrs. They were identified to species level by their characteristic appearances on the media, Gram's stain, Oxidase test, Motility and the pattern of the biochemical reactions.

Antimicrobial susceptibility test

Antimicrobial susceptibility of all the isolates was performed for amikacin, amoxicillin-clavulanic acid, cefotaxime, ceftriaxone, cotrimoxazole, ceftazidime, tetracycline hydrochloride, ciprofloxacin, gentamycin, imipenem, nitrofurantoin and norfloxacin on Mueller Hinton Agar (MHA) using Modified Kirby-Bauer disk diffusion method as per Clinical Laboratory Standards Institute (CLSI) guidelines.[12] The culture media and antibiotic disks were purchased from Hi-Media, India. *E.coli* ATCC 25922 and *K.pneumoniae* ATCC 700603 were used as controls to validate these susceptibility tests.

Initial Screening Tests [10, 13]:

A lawn culture was made from the inoculum on Mueller-Hinton agar medium then Ceftazidime (30µg) and Cefotaxime (30µg) discs were applied with all sterile precautions. The plates were incubated for 18-24 hours at 37°C. According to the CLSI guidelines, isolates showing inhibition zone size of ≤ 22 mm with Ceftazidime (30 µg) and ≤ 27 mm with Cefotaxime (30 µg) were identified as potential ESBL producers and shortlisted for confirmation of ESBL production.

Phenotypic confirmatory test with combination disk [10, 13]:

This test was performed on MHA by disk-diffusion method as recommended by CLSI.[12] In this test, a disk of Ceftazidime (30µg) and a disk of Ceftazidime + Clavulanic acid (30 µg/10 µg) combination with a disk of cefotaxime (30µg) and a disk of cefotaxime+ clavulanic acid (30 µg/10 µg) combination were used. Both the disks in combinations were placed at least 24 mm apart, center to center. When there was an increase of ≥ 5 mm in inhibition zone diameter around combination disk of Ceftazidime + Clavulanic acid versus the inhibition zone diameter around Ceftazidime disk alone, or When there was an increase of ≥ 5 mm in inhibition zone diameter around combination disk of Cefotaxime + Clavulanic acid versus the inhibition zone diameter around Cefotaxime disk alone or when for both kind of disks combinations there was an increase of ≥ 5 mm in inhibition zones the isolate was considered as confirmed ESBL producer.

Results

Out of 400 isolates of *Escherichia coli* and *Klebsiella pneumoniae,* 197 (49.25 %) were found ESBLs producers. Out of 203 *Escherichia coli* isolates 92 (45.32 %) were found ESBLs producers and of the 197 *Klebsiella pneumoniae* isolates 97 (49.23%) were found ESBLs producers.

ESBL producing *E. coli* strains were most frequently recovered from urine 42.39% followed by stool 19.56%, sputum & respiratory tract specimens 15.21% and the least was high vaginal swab1.08%. Similarly ESBL producing *K. pneumoniae* strains were most frequently recovered from pus & other wound discharges 43.29% followed by sputum & respiratory tract specimens 21.64 % (21/97), blood 17.52% and the least was high vaginal swab 1.03% (Table 1)

ESBL producing both *E. coli* and *K. pneumoniae* strains were most sensitive to imipenem followed by gentamycin. Antimicrobial Susceptibility Pattern of ESBL Producing *E.Coli* and *K. Pneumoniae* are depicted in Table 2

Discussion

During past 60 years, bacteria have demonstrated a remarkable ability to resist almost every antibiotic that has been developed [14]. In this study the overall ESBL production was found in 47.25% isolates. Our results are consistent with the study conducted by Wani K A *et al*. [15] who reported 53.4% ESBL producer. The overall prevalence of ESBL producers was found to vary greatly in different geographical areas and in different institutes. Previous studies from India have reported ESBL production varying from 6% to 87%. [16-20] One reason for such variability may be the very low number of samples studied.

We found that *Klebsiella pneumoniae* (49.23%) was the most frequent ESBL producer followed by followed by *Escherichia coli* (45.32%) which is in concordance with Mohanty S *et al*. [21] and Chaurasia E *et al*. [22] However there are studies conducted in India [4, 11, 23, 24] which reported *E.coli* was found to be the most frequent ESBL producer followed by *Klebsiella* spp. whereas *Enterobacter cloacae* (79%) was the most frequent ESBL producer in another study conducted in Pakistan. A higher rate of ESBL production by *Klebsiella pneumoniae* (55%) was observed in Riyadh [25].

In this study we found that ESBL-producing *E. coli* strains were recovered most frequently from urine (42.39%) which is in agreement with the study conducted by Kader AA *et al*. (57.5%) [6], Agarwal P *et al*. (70%) [10], Sasirekha B *et al*. (76%) [26] Xiong Z *et al*. (64.3%) [27] Indicating the need for active screening of urine cultures for ESBL producers. Similarly we found that ESBL-producing *K. pneumoniae* strains were recovered most frequently from pus & other wound discharges (43.29%). Our results are consistent with the study conducted by El Astal ZY *et al*. [8] who also reported *K. pneumoniae* strains were recovered most frequently from pus & other wound discharges (48.4%). However Waiwarawooth J *et al*. (2006) [28] reported higher incidence of ESBL production in sputum (44.69%), followed by urine (21.60%), pus (18.24%) and blood (10.28%).

In this study ESBL producing *E. coli and K. pneumoniae* were found highest susceptible to imipenem 94.56% and 92.78% respectively which is similar to observations made by Kader AA *et al*. (92%) [6]. However there are studies [24, 29-31] who reported 100% susceptible to imipenem. We also found that ESBL producing *E. coli and K. pneumoniae* isolates were highly resistant to IIIrd generation cephalosporin ceftriaxone and only 4.31% and 2.06% ESBL producing *E. coli and K. pneumoniae* respectively were found susceptible to ceftriaxone which is in agreement with the study conducted by El Astal ZY *et al*. [8] where none of the tested isolates were

susceptible to cephalosporins and Wani K A *et al*. [15] who reported 2.5% susceptibility of ceftriaxone.

Conclusion

ESBLs have become a widespread serious problem and several aspects of them are worrying. These enzymes are becoming increasingly expressed by many strains of pathogenic bacteria with a potential for dissemination. Routine detection of ESBL-producing microorganisms is required to be done by each laboratory by the standard detection methods so as to control the spread of these infections and also to institute proper therapeutic strategies

Table 1: Distribution of ESBL Producing *E.Coli* and *K. Pneumoniae* Isolates from Various Clinical Specimens

Sl. No.	Clinical sample	*E. coli* ESBL positive isolate (%)	*K. pneumonia* ESBL positive isolate (%)
1.	Urine	39 (42.39%)	05 (5.15%)
2.	Sputum & Respiratory tract specimens	14 (15.2%)	21 (21.64%)
3.	Pus & other wound discharges	11 (11.95%)	42 (43.29%)
4.	Blood	01 (1.08%)	17 (17.52%)
5.	Body fluids	18 (19.56%)	01 (1.03%)
6.	High vaginal swab	00 (0.00%)	01 (1.03%)
7.	Stool	09(9.78%)	07 (7.21%)
8.	Others	00 (0.00%)	03 (3.09%)
9.	TOTAL	92	97

Table 2: Antimicrobial Susceptibility Pattern of ESBL Producing *E.Coli* and *K. Pneumoniae*

S.N.	Antibiotics	*E. coli* ESBL positive isolate (n=92)	*K. pneumonia* ESBL positive isolate (n=97)
1.	Amikacin	79 (85.86%)	63 (64.94%)
2.	Amoxycillin+Clavulanicacid	08 (8.6%)	06 (6.18%)
3.	Ceftriaxone	04 (4.3%)	02(2.06%)
4.	Cotrimoxazole	06 (6.52%)	07 (7.21%)
5.	Ciprofloxacin	10 (10.86%)	12 (12.37%)
6.	Gentamycin	64 (69.56%)	55 (56.70%)
7.	Imipenem	87 (94.56%)	90 (92.78%)
8.	Nitrofurantoin *	29 (74.35%)	03 (60%)
9.	Norfloxacin*	17(43.58%)	2 (40%)
10.	Tetracycline hydrochloride	33 (35.86%)	42 (43.29%)

* Norfloxacin and nitrofurantoin were tested against urinary isolates only

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