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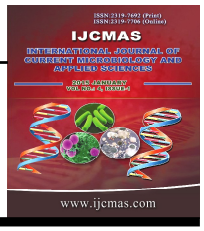

Original Research Article

Prevalence of ESBL Production in *Escherichia coli* and *Klebsiella spp* from Different Clinical Samples – A Study in a Teaching Hospital in Telangana, India

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Keywords

ESBLs, E-test, *E. coli*, *Klebsiella spp*

A B S T R A C T

ESBL producing Gram Negative bacteria have emerged as a major threat worldwide as they produce the enzyme Beta-lactamase which hydrolyse β-lactam antibiotics containing an oxyimino group (Third generation cephalosporin's and aztreonam) and are inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam. A total of 582 of *E. coli* and *Klebsiella spp* from clinical samples like pus/wound swab, sputum, suction tip, pleural fluid and urine obtained from both out and in patients of this hospital over a period of 2 years were included in the study. Organisms were identified by using standard microbiological culture and biochemical reactions and then subjected to antibiotic susceptibility testing by modified Kirby Bauer's disc diffusion method. ESBL production was confirmed by phenotypic confirmatory combination disc diffusion test and E Test. Out of the 582 isolates, 41.4% were *E. coli* and 30.4% were *Klebsiella*. Of these, 56.4% of *E. coli* and 62.3% of *Klebsiella* were ESBL producers. The overall ESBL production among these two organisms was 58.8%. Detection of ESBls should be done and reported regularly along with normal antibiotic sensitivity testing as soon as possible so that a proper antibiotic policy can be formulated to contain and solve the ever prevalent problem of antibiotic resistance.

Introduction

Ever since Penicillin was introduced as an antibiotic, bacteria have acquired a variety of well developed mechanisms to resist their action. This system of defense is so intricate and adaptable that contemporary medicine has been has found it very difficult to maintain an advantage (Louis Rice, 2001). Extended spectrum beta-lactamases (ESBLs) were first described in 1983, and ever since ESBL producing Gram Negative bacteria have emerged as a major threat worldwide as they produce the enzyme Beta-lactamase which hydrolyses β-lactam antibiotics containing an oxyimino group (Third generation cephalosporin and aztreonam) and are inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam Typically, they derive from genes for TEM-1, TEM-2, or SHV-1 by mutations that alter the amino acid configuration around the active site of these β-lactamases. This extends the spectrum of

β-lactam antibiotics susceptible to hydrolysis by these enzymes. An increasing number of ESBLs not of TEM or SHV lineage have recently been described (Paterson and Bonomo, 2005). ESBLs are usually plasmid-mediated β-lactamases, most commonly found in *Klebsiella pneumoniae*, *Escherichia coli* and other Gram-negative bacilli (Ankur Goyal *et al.,* 2009). They have also been found in *Pseudomonas aeruginosa, Proteus mirabilis, Enterobacter, Serratia marcescens, Citrobacter, Morganella morganii, Shigella dysentriae* and *Burkholderia cepacia* (Macrae *et al.,* 2001; Wu et a., 2003; Gupta *et al.,* 2004).

Since its discovery, the number of ESBL variants has been constantly growing with at present more than 300 different ESBL variants known (Louis Rice, 2001). The ESBL producing bacteria are typically associated with multidrug resistance to other drugs like Quinolones, Aminoglycosides because genes with these mechanisms of resistance often reside on the same plasmid as the ESBL gene (Duttaroy, 2005; Araj, 2003).

Multiple outbreaks of ESBL-producing Enterobacteriaceae in intensive care units (ICUs) and increased rates of illness and death, especially in neonatal ICUs, have been reported (Pagani *et al.,* 2002; Singhal *et al.,* 2005). Physical contact is the most likely mode of transmission. The gastrointestinal tract of colonized or infected patients is the most frequent reservoir. Several studies indicate that transient carriage of bacteria on the hands of healthcare workers (HCWs) may lead to transmission to patients (Dichen, 2009). Enterobacteriaceae, especially *Klebsiella spp*.-producing ESBLs such as SHV and TEM types, have been established since the 1980s as a major cause of hospital-acquired infections. However, during the late 1990s, several community-acquired pathogens that commonly cause urinary tract infections and diarrhea have also been found to be ESBL producers. These include *Escherichia coli, Salmonella, Shigella and Vibrio cholera* (Paterson and Bonomo, 2005; Pitout, 2005; Doi, 2007). In order to suppress the emergence and spread of drug-resistant bacteria in our hospital, we feel it is very important to be vigilant of the ESBL producing organisms. Thus, we have conducted this study to demonstrate the prevalence of ESBLs among *Escherichia coli* and *Klebsiella spp* in our geographical area.

Materials and Methods

The present study was carried out in the Department of Microbiology, Mallareddy Institute of Medical Sciences, Hyderabad, Telangana. A total of 582 of *E. coli* and *Klebsiella spp* from Clinical samples like pus/wound swab, sputum, suction tip, pleural fluid and urine obtained from both out and in patients of this hospital over a period of 2 years (September 2012–August 2014) were included in the study. Organisms were identified by using standard microbiological culture and biochemical reactions and then subjected to antibiotic susceptibility testing by modified Kirby Bauer's disc diffusion method on Mueller Hinton agar plates using antibiotic discs Ampicillin (10µg), Amikacin (30µg), Gentamicin (10µg), Ciprofloxacin (5µg), Cefepime (30µg), Cefadroxil(30µg), Ceftadizime (30µg), Cefotaxime(30µg), Imipenem (10µg), Piperacillin-tazobactum (100µg/10µg), Cefoxitin (30µg), Aztreonam (30µg), Cefoperazone – Sulbactum (z75/10µg), Ceftadizime - Clavulanic acid (30/10µg), Meropenem (10µg), Cefuroxime (30µg), Ceftriaxone (30µg), Cefoperazone (75µg), Amoxyclav (20/10µg).

Screening test for identification of potential ESBL producers

Since, the use of more than one of the agents for screening improves the sensitivity of the detection. The screening test was carried out by Kirby-Bauer disc diffusion method on Mueller Hinton Agar, using antibiotic discs such as Ampicillin (10µg), Cefadroxil (30µg), Cefotaxime (30µg), Ceftazidime (30µg), Aztreonam (30µg) and Imipenem (10µg), as per CLSI guidelines.

The results were interpreted using the following zones of inhibition: Ceftazidime<27mm, Aztreonam<15mm, Cefadroxil<14mm, Ampicillin<13mm, and Imipenem>16mm. The isolate was considered a potential ESBL producer, if it was found to be resistant to Cefotaxime, Ceftazidime or Aztreonam. It was then subjected to phenotypic confirmatory tests to ascertain the diagnosis.

Phenotypic confirmatory combination disc diffusion test (PCCDDT)

Cefotaxime (30 µg) and Cefotaxime - Clavulanic acid (30/10 µg) and Ceftazidime (30 µg) and Ceftazidime - Clavulanic acid (30/10 µg) discs were placed Mueller Hinton Agar Plate at a distance of 25mm apart from centre to centre on the lawn culture of the test isolate, and incubated at 35^0C for 18 to 24 hours (Fig. 1). The zone differences in zone diameter, with or without Clavulanic acid, were measured. A positive result was indicated when there was an increase in >=5mm inhibition zone diameter around combination disc of Cefotaxime + Clavulanic acid Ceftazidime + Clavulanic acid versus the inhibition zone diameter around Cefotaxime and Ceftazidime disc alone, which confirmed ESBL production.

E- test

Confirmation was done by E-test procured from HiMedia. The test was performed in according to guidelines of the manufacturer. In this method, a 90mm Mueller Hinton Agar plate was taken, 0.5 Mc Farlands standardized inoculum of the test organism was swabbed and allowed to dry at room temperature. An E-test strip with Ceftazidime at one end, and Ceftazidime + Clavulanic acid at the other end in a concentration gradient, was placed on the lawn culture plate and incubated at 35^0C overnight. After overnight incubation, results were interpreted. The minimum inhibitory concentration (MIC) was interpreted as the point of intersection of the inhibition ellipse of bacterial growth with the MIC gradient on the E-test strip. The lowest concentration gradient which inhibits the bacterial growth is the MIC of the drug. The ratio of the Ceftazidime MIC and Ceftazidime + Clavulanic acid MIC equal to or greater than 8 indicated the presence of ESBL. The presence of Extended-spectrum betalactamases was confirmed by the appearance of phantom zone below, or deformation of Ceftazidime inhibition ellipse or when Clavulanate caused a more than or equal to three doubling concentration decrease (ratio of more than 8) in the MIC values of Ceftazidime.

Results and Discussion

Out of the 582 isolates, 41.4% were *E. coli* and 30.4% were *Klebsiella* (Table 1). 56.4% of *E. coli* were ESBL producers while more than 60 % of *Klebsiella* showed ESBL production showing higher rates of esbl production in *Klebsiella* (Table 2). Overall ESBL production among these two organisms was 58.8%.

Extended-spectrum β-lactamases (ESBLs) are a group of plasmid-mediated, diverse, complex and rapidly evolving enzymes that are posing a major therapeutic challenge today in the treatment of hospitalized and community-based patients. Infections due to ESBL producers range from uncomplicated urinary tract infections to life-threatening sepsis. They share the ability to hydrolyze third-generation cephalosporins and aztreonam and yet are inhibited by clavulanic acid. In addition, ESBL-producing organisms exhibit co-resistance to many other classes of antibiotics, resulting in limitation of therapeutic option (Deepti Rawat and Deepthi Nair, 2010). Carbapenems are now the choice of drugs for such ESBL producers. Although a few studies have reported on the prevalence of ESBL producers in Indian hospitals, ESBL producing bacteria have evolved in several hospitals all over the country. In India, the prevalence rate varies in different institutions from 28 to 84 % (Das *et al.*, 2009).

In our study, we have also reported a prevalence of 58.8% of ESBLs which is of a very high concern. *E. coli* has shown a prevalence of 56.4%, majority of it being in the urine samples. This could be because of the injudicious use of drugs for urinary tract infections. *Klebsiella* has shown a prevalence of 62.3% which is similar to studies conducted by Ankur Goyal *et al.* (2009) showing a prevalence of 66.7% for *Klebsiella*. Other studies from in and around India are shown in table 3. In the global scenario also, there has been a steady increase in the emergence of ESBls since its first report in 1983. In a 1997-1998 survey in Europe, over 74% of *Klebsiella* e possessed ESBLs (11, Paterson and Bonomo, 2005). In Turkey, 58% *Klebsiella* spp. from intensive care units harbored ESBLs (22, Günseren *et al.*, 1999).

Moland and colleagues have shown that ESBL-producing isolates were found in 75% of 24 medical centers in the United States (23 Moland *et al.*, 2002) while in other studies around USA, 4.2–44% of *Klebsiella* spp were found to be ESBL producers (24-26 Saurian's *et al.*, 2000; Mathai *et al.*, 2001; Winokur *et al.*, 2001). Spain has seen a prevalence of 20.8% (27 Romero *et al.*, 2007), Taiwan 28.4% (28 Kuo et al, 2007), Turkey 78.6% (29 Hos¸oglu *et al.*, 2007), Algeria 20% (30 Messai *et al.*, 2008) and China 51% (31 Xiong *et al.*, 2002).

This data only shows Extended spectrum Beta lactamases are very much prevalent throughout the world and this seems to be only increasing as time goes by. Not only are they predominantly present but also vary with different geographical regions. In each of these areas and many more which were reported, the choice for treatment as very limited due to the antibiotic resistance to multiple drugs.

ESBls are not tested on regular basis in many clinical laboratories probably because of lack of awareness of their importance and of efficient methods to detect them. They also lack the resources needed to curb the spread of the resistance mechanisms. This is probably the reason as to why there is a continuous rise of newer bacteria with multidrug resistance and their therapeutic failure to respond to this spread. The consequence of this is avoidable inappropriate and wrong treatment sometimes resulting in fatalities. Therefore, the detection of ESBls should be done and reported regularly along with normal antibiotic sensitivity testing as soon as possible so that a proper Antibiotic Policy can be formulated to contain and solve the ever prevalent problem of Antibiotic resistance.

Table.1 Total number of *E. coli* and *Klebsiella spp*

Isolate	Number	percentage
E. coli	346	41.4%
Klebsiella	236	30.4%

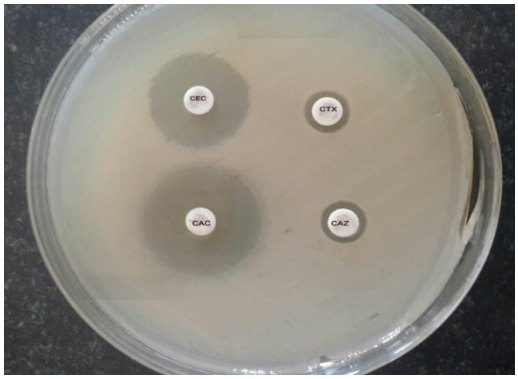
Table.2 No of ESBL and NON ESBL producers

Isolate	No of ESBL producers	%	No of ESBL non producers	%
E. coli	195	56.4%	151	43.6%
Klebsiella	147	62.3%	89	37.7%
Total	342	58.8%	242	41.2%

Table.3 ESBL producers among *E. coli* and *Klebsiella* in other studies

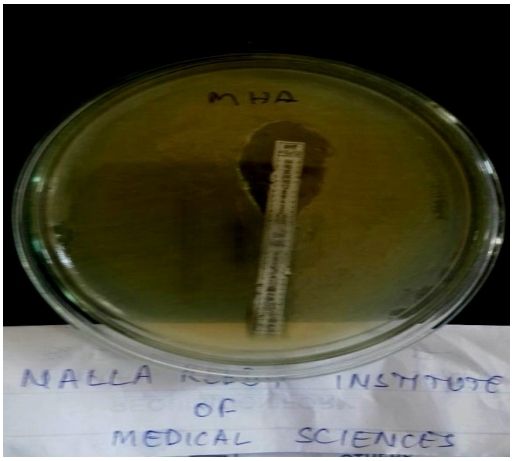
Author	% of ESBLs	
	In *E. coli*	*Klebsiella*
Our study	56.4%	62.3%
Ankur Goyal *et al.* (2009)	63.6%	66.7%
Dichen *et al.* (2009)	26%	57%
Jain *et al.* (2003)	63.6%	86.6%
Gaurav Dalela (2012)	73.5%	58.1%
Sarojamma and Ramakrishna (2011)	-	17%
Anil Chander and Shrestha (2013)	13.51%	16.55%
Sasirekha (2013)	52.8%	45.1%

Figure.1 Phenotypic confirmatory test by combination of disc diffusion test



Phenotypic confirmatory test of an extended spectrum Beta lactamase producing strains using combination disk: Cefotaxime (CTX) (30 µg) and Cefotaxime - Clavulanic acid (CEC) (30/10 µg) and Ceftazidime (CAZ) (30 µg) and Ceftazidime - Clavulanic acid (CAC) (30/10 µg)

Figure.2 Epsilometer test (E-Test)



Confirmatory test of an extended spectrum Beta lactamase producing strains using Epsilometer test (E-test): Ceftazidime (CAZ) and Ceftazidime - Clavulanic acid (CAC)

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