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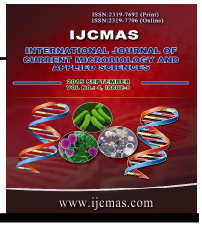

Original Research Article

Prevalence and Anti-Biogram of Extended Spectrum β- Lactamase Producing *Escherichia coli*

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Keywords

Extended-Spectrum β-Lactamase (ESBL), *Escherichia coli* (*E. coli*), Anti-biogram, Resistance

A B S T R A C T

Extended-Spectrum β-Lactamase (ESBL) producing strains of Enterobacteriaceae particular among *Klebsiella pneumoniae* and *Escherichia coli* (*E. coli*) have emerged as a major problem in hospitalized as well as community based patients. ESBL producers most often acquire and exhibit additional resistance to other antimicrobials such as quinolones, cotrimoxazole, trimethoprim, tetracyclines and aminoglycosides, thus making the therapeutic options very limited. This was a retrospective study conducted in a tertiary care hospital in South India. Data was obtained from the microbiology records from June 2011 to March 2013. ESBL screening was done as per Clinical Laboratory Standards Institute (CLSI) guidelines, isolates showing inhibition zone size of ≤22 mm with ceftazidime (30 µg), ≤25 mm with ceftriaxone (30 µg), and ≤27 mm with cefotaxime (30 µg) were processed for confirmatory tests, confirmation using combination disk and double disk synergy test. Antimicrobial susceptibility testing was done by Kirby–Bauer disk diffusion method as recommended by the CLSI guidelines. *E. coli* was isolated commonly from urine, pus and wound samples. Out of 194 *E. coli* isolated, 86(44.3%) were ESBL producers. Highest resistance among ESBL producers was seen with ampicillin and ciprofloxacin and least resistance with imipenem. Resistance to aminoglycosides and nitrofurantoin was relatively low. These findings suggest that antibiotic susceptibility varies region to region and time. The incidence of ESBL producing strains among clinical isolates has been steadily increasing over the past years, resulting in limitations of therapeutic options. We suggest the use of aminoglycosides and nitrofurantoin as the drug of choice for ESBL producers and Imipenem should be reserved for life threatening infections. Resistance to cephalosporin and ciprofloxacin group is a cause of concern. Periodic susceptibility studies will help the physicians in choosing empirical therapy and preserve the higher antimicrobials to life threatening infections.

Introduction

The precise definition of the term Extended-Spectrum β-Lactamase (ESBL) remains unclear but is generally used to refer to any β-lactamase, 'generally acquired rather than inherent to a species, that is either able to confer resistance to oxyimino-cephalosporins (but not carbapenems), or that has an increased ability to do so, as

compared with classic members of its genetic family' (Livermore, 2008). ESBL producing strains of Enterobacteriaceae particular among *Klebsiella pneumoniae* and *Escherichia coli* (*E. coli*) have emerged as a major problem in hospitalized as well as community based patients (Rodriguez-Bano *et al.,* 2004). Infections associated with ESBL producing isolates are difficult to detect and treat, thereby causing increased mortality and morbidity of patients (Krishnakumar *et al.,* 2012).

β-lactamase production is perhaps the single most important mechanism of resistance to penicillins and cephalosporins (Chaudhary *et al.,* 2004). *E. coli* possess a naturally occurring chromosomally mediated plasmid mediated β-lactamases. These enzymes are thought to have evolved from penicillin binding protein. Detection of ESBL producers may be of utmost importance because this represents an epidemiologic marker of colonization and therefore there is potential for transfer of such organisms to other patients (Aggarwal *et al.,* 2009).

ESBL producers most often acquire and exhibit additional resistance to other antimicrobials, thus making the therapeutic options very limited (Chopra *et al.,* 2008; Morosini *et al.,* 2006; Talbot, 2008). There also seems to be a discrepancy between geographical regional resistances and susceptibilities of ESBL organisms (Akram *et al.,* 2007; Hoban *et al.,* 2011).

Knowledge of local prevalence and antibiotic susceptibility pattern will help the physician in choosing empirical therapy and help in formulating hospital antibiotic policy. Hence the present study was done to know the prevalence and antibiogram of ESBL producing *E. coli* from various clinical samples.

Material and Methods

This was a retrospective study conducted in a tertiary care hospital in South India. Data was obtained from the microbiology records from June 2012 to March 2014. Clinical samples including pus, urine, blood, stool, sputum were received in the department of microbiology.

Isolation and identification

Urine samples collected in universal container were inoculated using an inoculating loop of 10 µL volume calibration on cystine lactose electrolyte deficient (CLED) agar plates. Other liquid specimens such as CSF, sputum, stool, and different body fluids collected in sufficient amount were inoculated on the blood agar plates and MacConkey agar plates using an inoculating loop. Identification of the isolated organism was done on the basis of routine biochemical tests and gram staining was performed to confirm *E. coli* using standard protocol (Pamela, 2007).

ESBL screening

Screening of ESBLs was done as per CLSI guidelines (Clinical and Laboratory Standards Institute, 2010), isolates showing inhibition zone size of $\leq$22 mm with ceftazidime (30 µg), $\leq$25 mm with ceftriaxone (30 µg), and $\leq$27 mm with cefotaxime (30 µg) were processed for confirmatory tests.

Confirmatory tests for ESBLs

a) Phenotypic confirmatory test with combination disk

Disk of ceftazidime plus clavulanic acid (30/10 mcg) and cefotaxime plus clavulanic acid (30/10 mcg) discs were also included

along with ceftazidime (30 mcg) and cefotaxime (30 mcg) discs on Muller-Hinton agar. Organism was considered as ESBL producer if there was an increase of ≥5 mm in the zone diameter of ceftazidime/clavulanic acid disc with respect to that of ceftazidime disc alone and or ≤5 mm increase in the zone diameter of cefotaxime/clavulanic acid disc with respect to that of cefotaxime disc alone

b) Double Disc Synergy Test

30 µg antibiotic disks of ceftazidime, ceftriaxone, cefotaxime, and aztreonam are placed on the lawn culture plate of *E. coli*, 30 mm (center to center) from the amoxicillin/clavulanic acid (20/10 µg) disk. This plate was incubated aerobically overnight at 37°C and examined for an extension of the edge of zone of inhibition of antibiotic disks toward the disk containing clavulanate. It is interpreted as synergy, indicating the presence of an ESBL.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done by Kirby–Bauer disk diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2010). Commercially available antibiotic disks (Hi Media India) were used for antimicrobial susceptibility testing. The following antibiotic disks were used, ampicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), piperacillin(100 µg), piperacillin-tazobactam (100/10 µg), ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cefepime (30 µg), aztreonam (30 µg), imipenem (10 µg), amikacin (30 µg), gentamicin (10 µg), ciprofloxacin (30 µg), ofloxacin (5 µg) and nitrofurantoin (300 µg). *E.coli* ATCC 25922 and *E. coli* ATCC 35218 (for β-lactam/β-lactamase inhibitor combination) were used as control strains.

Results and Discussion

A total of 194 isolates of *E. coli* were obtained from various clinical samples as shown in table 1.

E. coli was isolated commonly from urine, pus and wound samples

The demographics of the *E. coli* isolated cases are shown in table 2.

Most cases were from females and most common affected age group was 21–30 years.

Out of 194 *E. coli* isolated, 86(44.3%) were ESBL producers. The antibiogram of ESBL and non-ESBL producers is shown in table 3.

Highest resistance among ESBL producers was seen with ampicillin and ciprofloxacin and least resistance with Imipenem

ESBL producing Gram-negative bacilli especially *E.coli* and K. pneumonia have acquired resistance to commonly used drugs both in hospital and community acquired infections worldwide. Nosocomial infections are mainly caused by gram negative bacteria they are difficult to treat, due to ability to develop resistance (intrinsic and acquired) to most commonly used anti-microbial agents. One of the important mechanisms of antimicrobial resistance is the production of extended spectrum β-lactamases (Shahina *et al.,* 2011). The incidence of ESBL producing strains among clinical isolates has been steadily increasing over the past years, resulting in limitations of therapeutic options (Ananthakrishnan *et al.,* 2000).

In the present study, prevalence of ESBL

producing *E. coli* was 44.3%. Prevalence of ESBL varies across geographical regions as demonstrated by large scale studies like SENTRY, SMART, MYSTIC. As per the SMART study conducted in Asian-Pacific in 2007, the prevalence of ESBL production in Enterobacteriaceae was reported to be highest from India (79%) (Hawser *et al.*, 2009). In the United States, ESBL producing *E.coli* ranges from 0 to 25% with the average being around 3% (National Nosocomial Infections Surveillance System, 2004). In Asia, the percentage of ESBL production in *E. coli* is 4.8, 8.5, and up to 12% in Korea, Taiwan, and Hong Kong, respectively (Pai *et al.*, 1999; Yan *et al.*, 2000; Ho *et al.*, 2000).

ESBL producers showed higher resistance than non-ESBL producers (Table 3). Highest resistance among ESBL producers was seen with ampicillin and ciprofloxacin and least resistance with Imipenem. Resistance to Cephalosporin group ranged from 77% to 83%. Resistance to aminoglycosides and nitrofurantoin was relatively low. This finding is similar to other studies (Shanthi and Sekar, 2010; Al-Zarouni *et al.*, 2008). Other studies have found varying sensitivity pattern, 81.37% to piperacillin-tazobactam, 76.06% for cefoperazone-sulbactam and 45.48% for ticarcillin-clavulanic (Mohanty *et al.*, 2005).

Table.1 *E. coli* isolated from clinical samples

Organism	Frequency	Percentage
Urine	82	42.2
Pus	45	23.1
Wound	24	12.3
Sputum	12	6.1
Blood	18	9.2
Stool	8	4.1
Body fluids	5	2.5
Total	**194**	**100**

Table.2 Demographics of *E. coli* isolated cases (n=194)

Age group (Years)	Male	Female	Total
0-10	4	3	7
11-20	14	15	29
21-30	18	29	47
31-40	19	22	41
41-50	17	23	40
51-60	7	9	16
61-70	6	8	14
Total	**85**	**109**	**194**

Table.3 Antibiogram of *E. coli* (Resistance pattern)

Antibiotic	ESBL producers (n=86) n (%)	Non ESBL producers (n=108) n (%)
Ampicillin	54 (62.7)	59 (54.6)
Amoxicillin/clavulanic acid	22 (25.5)	18 (16.6)
Piperacillin	28 (32.5)	12 (11.1)
Piperacillin-tazobactam	09 (10.4)	02 (1.8)
Ceftazidime	18 (20.9)	04 (3.7)
Cefotaxime	17(19.7)	05 (4.6)
Ceftriaxone	15 (17.7)	04 (3.7)
Cefepime	20 (23.2)	09 (8.3)
Aztreonam	04 (4.6)	0
Imipenem	0	0
Amikacin	10 (11.6)	06 (5.5)
Gentamicin	14 (16.2)	08 (7.4)
Ciprofloxacin	52 (60.4)	45 (41.6)
Ofloxacin	48 (55.8)	39 (36.1)
Nitrofurantoin	12 (13.9)	14 (12.9)

Another study reported 89.7% sensitivity to amikacin and 85.3% to piperacillin-tazobactam (Manoharan *et al.*, 2011). A recent study reported sensitivities to piperacillin-tazobactam 89%, amikacin 22%, gentamicin 56% and tobramycin 78% (Sarma *et al.*, 2011). These findings suggest that antibiotic susceptibility varies region to region and time. We suggest the use of aminoglycosides and nitrofurantoin as the drug of choice for ESBL producers and Imipenem should be reserved for life threatening infections.

Limitations of the study

This was a retrospective study, we did not include other Enterobacteriaceae. Futures studies should be prospective and should include other Enterobacteriaceae like *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

The prevalence of ESBL producing *E. coli* was 44.3%. ESBL producing *E. coli* was resistant to commonly used drugs. Resistance to cephalosporin and ciprofloxacin group is a cause of concern. Periodic susceptibility studies will help the physicians in choosing empirical therapy and preserve the higher antimicrobials to life threatening infections.

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Conflict of interest: None

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