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Original Work

Prevalence and Susceptibility of extended spectrum beta-lactamases in urinary isolates of *Escherichia coli* in a Tertiary Care Hospital, Chennai-South India

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ABSTRACT: Extended spectrum beta – lactamases (ESBLs) are on the rise in hospital settings across the globe. The presence of ESBLs significantly affects the outcome of an infection and poses a challenge to the management of infection worldwide. Therefore, the aim of the present study is to determine the prevalence and susceptibility of extended spectrum beta – lactamase in urinary isolates of Escherichia coli (*E.coli*) in a tertiary care hospital, Chennai-South India. A total of 450 urinary isolates of *E.coli* were collected over a period of six months from April 2008 to September 2008. Antimicrobial susceptibility testing was determined to commonly used antibiotics using the modified Kirby-Bauer's disc diffusion method. ESBL detection was done by the screening method of double disc synergy test and then confirmed by the phenotypic confirmatory test with combination disc as recommended by the Clinical Laboratory Standards Institute (CLSI) and the minimum inhibitory concentration (MIC) method using the E test strips (AB Biodisk,Sweden)- as per manufacturer's instructions. The prevalence of *E.coli* ESBL was 60%. The ESBL producing isolates were significantly resistant ($p < 0.01$) to ampicillin, trimethoprim / sulfamethoxazole, norfloxacin and nalidixic acid as compared to non-ESBL producers. Multidrug resistance was significantly ($p < 0.01$) higher (69%) in ESBL positive isolates than non-ESBL isolates (21%). Knowledge of the prevalence of ESBL and resistance pattern of bacterial isolates in a geographical area will help the clinicians to formulate the guidelines for antibiotic therapy to avoid inappropriate use of extended spectrum cephalosporins.

KEY WORDS: ***ESBL; Escherichia coli; Third-generation cephalosporins***

INTRODUCTION

Resistant bacteria are emerging worldwide as a threat to favorable outcome in the treatment of common infections in community and hospital settings[1]. Among the wide array of antibiotics, β-lactams are the most widely used agents. The most common cause of resistance to β-lactam antibiotics is the production of β-lactamases. Emergence of resistance to β-lactam antibiotics began even before the first β-lactam, penicillin, was developed. The first plasmid-mediated β-lactamase TEM-1 was originally isolated from blood culture of a patient named Temoniera in Greece, in the early 1960s[2]. TEM-1 being plasmid and transposon mediated has facilitated its spread to other species of bacteria. Another common plasmid-mediated β-lactamase SHV-1 (sulfhydryl variable), is chromosomally encoded in the majority of isolates of *K. pneumoniae* but is usually plasmid-mediated in *E. coli*. Over the years, many new β-lactam antibiotics have been developed; however, with each new class of antibiotic, a new β-lactamase emerged that caused resistance to that class of drug. Presumably, the selective pressure imposed by the use and overuse of new antibiotics in the treatment of

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patients has resulted in the emergence of new variants of β-lactamase.

The introduction of third-generation cephalosporins into clinical practice in the early 1980s was heralded as a major breakthrough in the fight against β-lactamase-mediated bacterial resistance to antibiotics. Soon after the introduction, the first report of plasmid-encoded β-lactamase capable of hydrolyzing the extended-spectrum cephalosporins was published in 1983 from Germany[3]. Hence these new β-lactamases were coined as extended-spectrum β-lactamases (ESBLs). In the present scenario the total number of ESBLs characterized exceeds 200.. There is no consensus on the precise definition of ESBLs. A commonly used working definition is that the ESBLs are β-lactamases capable of conferring bacterial resistance to the penicillins; first-, second- and third-generation cephalosporins; and aztreonam (but not the cephamycins and carbapenems) by hydrolysis of these antibiotics and which are inhibited by β-lactamase inhibitors such as clavulanic acid[4].

ESBLs are encoded by transferable conjugative plasmids which often code resistant determinants to other antibiotics. The plasmid-mediated resistance against cephalosporins can spread among related and unrelated gram-negative bacteria. ESBLs are mostly the products of point mutations at the active site of TEM and SHV enzymes[5]. Nosocomial outbreaks of infections caused by ESBL-producing gram-negative bacteria have also been reported, which are mainly the result of extensive and inappropriate use of third-generation cephalosporins. Majority of ESBL-producing organisms are *E. coli* and *K. pneumoniae*. Others include *Enterobacter spp., Salmonella spp., Morganella, Proteus mirabilis, Serratia marcescens,* and *Pseudomonas aeruginosa*. The major risk factors implicated are long-term exposure to antibiotics, prolonged ICU stay, nursing home residency, severe illness, instrumentation, or catheterization[6].

METHODOLOGY

A total of 450 consecutive non-repeat culture isolates of *Escherichia coli* were obtained from urine samples, over a period of six months (April to September 2008). The isolates were identified on the basis of conventional microbiological procedures[7].

Antimicrobial susceptibility was determined by Kirby-Bauer disk diffusion method as per CLSI recommendations[8]. Antimicrobial discs used were Ampicillin (10 µg), Ampicillin/Sulbactam (20/10 µg), Piperacillin-tazobactam (100/10 µg), Cephotaxime (30 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg), Amikacin (30 µg), Norfloxacin (5 µg), Nalidixic acid, Nitrofurantoin, Trimethoprim-sulfamethoxazole (1.25/23.75 µg), and Imipenem (10 µg).

According to the 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 identified as potential ESBL producers and shortlisted for confirmation of ESBL production. *E. coli* ATCC 25922 was used as the control strain.

The following two procedures were carried out in the present study as per CLSI guidelines:

Phenotypic confirmatory test with combination disc: This test requires the use of a third-generation cephalosporin antibiotic disc alone and in combination with clavulanic acid. In this study, a disc of Ceftazidime (30µg) alone and a disc of Ceftazidime + Clavulanic acid (30 µg/10 µg) were used. Both the discs were placed at least 25 mm apart, center to center, on a lawn culture of the test isolate on Mueller Hinton Agar (MHA) plate and incubated overnight at 37°C. Difference in zone diameters with and without clavulanic acid was measured. Interpretation: When there is 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, it confirms ESBL production. ESBL producing Klebsiella pneumoniae ATCC 700603 was used as the control strain.

E-test ESBL strips: Confirmation of ESBL was also done by E-test ESBL strips (AB BIODISK, Solana, Sweden), and the test was performed in accordance with the manufacturer's guidelines. Double ended strips containing gradient of cefotaxime or ceftazidime at one end and cefotaxime or ceftazidime plus clavulanic acid at the other end were tested. The presence of ESBL was confirmed if the ratio of the MIC of cefotaxime or ceftazidime to the MIC of cefotaxime or ceftazidime plus clavulanic acid was ≥ 8.

Statistical analysis

Chi-square test was used with appropriate correction for the observation. Where the cell frequency was less than five, Fisher exact test was applied to see the significance of difference between the resistance levels of various drugs in ESBL producer strains and non-ESBL producer strains using SPSS version15. $P \leq 0.01$ was considered significant.

RESULTS

A total of 450 urinary isolates of *E.coli* were collected over a period of six months from April 2008 to September 2008. By the screening test, 202 of the 450 isolates were short listed as potential ESBL producers.

Of the 202 isolates, 122 isolates were found to be ESBL producers by phenotypic confirmatory test. This was further confirmed by MIC using the E-test strips. Our study revealed 100% agreement of the two methods - phenotypic confirmatory test and MIC E-test strips in detection of ESBL producers.

A significant proportion of the ESBL producing strains were found to be resistant to antimicrobial agents including ampicillin (100%), ampicillin/sulbactam (81.29%), nalidixic acid (70.88%), piperacillin/tazobactam (51.89%), trimethoprim/sulfamethoxazole (78.48%), nitrofurantoin (74.68%), norfloxacin (51.89%) and amikacin (54.43%). Imipenem was found to be the most effective antibiotic against ESBL producers (97.53% of isolates were sensitive); while in non-ESBL producing isolates, resistance was nil. ESBL producing isolates were resistant to more antimicrobial agents than non-ESBL producing isolates. The highest rate of resistance in ESBL negative isolates was seen against ampicillin (81.29%) which was significantly ($p < 0.01$) lower than ESBL producing isolates. This was followed by resistance to ampicillin/sulbactam (78.29%). However, in this case, the difference was not significant ($p > 0.05$) (**Table 1**).

Multidrug resistance was seen in 69.14% ESBL-positive isolates and 21.66% non-ESBL isolates. This difference was highly significant ($p < 0.01$).

Table 1: Antibiotic susceptibility pattern of ESBL* & Non-ESBL

Antibiotics	ESBL (n=202) % resistant	Non-ESBL (n=248) % resistant	Difference (*p*)
Ampicillin	100	81.29	<0.01
Ampicillin/Sulbactam	81.29	78.29	>0.05
Piperacillin /Tazobactam	51.89	27.09	<0.01
Co-trimoxazole	78.48	38.06	<0.01
Nalidixic acid	70.88	27.09	<0.01
Nitrofurantoin	74.68	39.35	<0.01
Norfloxacin	51.89	18.70	<0.01
Amikacin	54.43	34.19	<0.01
Imipenem	2.47	0	>0.05

*ESBL =Extended spectrum beta lacatmase

DISCUSSION

This study demonstrates the presence of ESBL-mediated resistance in urinary isolates of *E.coli* in a tertiary care teaching hospital in Chennai, (capital of Tamil Nadu), India. The prevalence was 60%. 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 28% to 84%[9].

There is considerable geographical difference in ESBLs in European countries. Within countries, hospital-to-hospital marked variability occurs [10]. A large study from more than 100 European intensive care units (ICU) found that the prevalence of ESBLs in *Klebsiella* ranged from as low as 3% in Sweden to as high as 34% in Portugal[11]. In Turkey, a survey of *Klebsiella* sp. from ICUs from eight hospitals showed that 58% of 193 isolate harbored ESBLs[12]. Moland and colleagues have shown that ESBL-producing isolates were found in 75% of 24 medical centers in the United States[13]. ESBLs have also been documented in Israel, Saudi Arabia, and a variety of North African countries[14-16]. From China, the figures of ESBL producers vary between 25-40%[17]. National surveys have indicated the presence of ESBLs in 5-8% of *E. coli* isolates from Japan, Korea, Malaysia and Singapore but 12-24%

of isolates from Thailand, Taiwan, Philippines and Indonesia[4].

ESBLs have emerged due to selective pressure imposed by extensive use of antimicrobials, especially in intensive care units. Since ESBL-positive isolates show false susceptibility to expanded-spectrum cephalosporins in standard disc diffusion test, it is essential to adopt the specific detection methods recommended by CLSI.

The high rate of resistance noted among the isolates in the present study, is of serious concern. 60% of urinary isolates were ESBL producing. In this study, ESBL producing isolates were significantly more resistant to ampicillin ($p < 0.01$), nalidixic acid ($p < 0.01$), cotrimoxazole ($p < 0.01$), nitrofurantoin ($p < 0.01$), norfloxacin ($p < 0.01$) and amikacin ($p < 0.01$) as compared to non-ESBL producing gram-negative isolates.

In our study, resistance to third generation cephalosporin's was found to coexist with resistance to two or more antibiotics like ampicillin, nalidixic acid, cotrimoxazole, nitrofurantoin, norfloxacin, amikacin as also reported by Subha *et al*[18] and Duttaroy *et al*[19] indicating multidrug resistance pattern. Mechanisms of co-resistance are not clear, but one possible mechanism is the co-transmission of ESBL and resistance to other antimicrobials within the same conjugative plasmids[20].

Almost all the ESBL-positive isolates were found to be resistant to Ampicillin and sensitive to Imipenem, which again advocates the usage of carbapenem antibiotics as the therapeutic alternative to β-lactam antibiotics as indicated in many previous studies.

CONCLUSION

The prevalence of ESBL producers at our institute was 60% in accordance to the prevalence reported from other hospitals in India as well as across the globe. Multidrug resistance was significantly ($p < 0.01$) higher (69%) in ESBL positive isolates than non-ESBL isolates (21%). All the ESBL-positive isolates were found to be sensitive to Imipenem, which again advocates the usage of carbapenem antibiotics. The high level of ESBL prevalence in our set up should ensure regular monitoring and judicious usage of extended-spectrum cephalosporins, periodic surveillance of antibiotic resistance patterns, and efforts to decrease empirical antibiotic therapy. This would go a long way in addressing some of the problems associated with ESBLs.

The control measures include judicious use of antibiotics, strict hand-hygiene protocols, and implementation of appropriate infection-control measures in the hospital, especially while treating high-risk patients.

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