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DETECTION OF EXTENDED SPECTRUM BETA LACTAMASES IN *ESCHERICHIA COLI* ISOLATES

SAMEENA K, LAKSHMI K*, CHITRALEKHA S AND AISHWARYA JR

Department of Microbiology, Sree Balaji Medical College and Hospital, (Bharath University), Chennai, India

ABSTRACT

Escherichia coli is known to cause various infections. The continued emergence of ESBLs in *E.coli* presents a great challenge to the practitioners in treating the infections. The aim of the study is to assess the prevalence of Extended-spectrum ß-lactamase (ESBL) producers among the *E.coli* isolates. Hundred isolates of *E.coli* from various samples were included in the study. All isolates were identified by standard conventional methods. Antibiotic sensitivity testing was done using disc diffusion technique by Kirby Bauer method and noted. Isolates with resistance or decreased susceptibility to third generation cephalosporins or aztreonam were selected as ESBL producers. ESBL production was detected by Double disk synergy test (DDST). 37% of the *E.coli* isolates were found to be ESBL producers. Most of the ESBL producing *E.coli* were found to be multidrug resistant. Hence, monitoring of ESBL production and antimicrobial susceptibility testing as a routine is necessary to prevent therapeutic failure and development of multidrug resistance among the bacterial isolates.

KEYWORDS: ***Escherichia coli*, ESBL**

LAKSHMI K
Department of Microbiology, Sree Balaji Medical College and Hospital, (Bharath University), Chennai, India

Corresponding author

INTRODUCTION

E.coli is one of the most common pathogens isolated from various clinical samples in the microbiology laboratory. Antibiotic was discovered first in 1928[1]. Initially antibiotics were used successfully for treating bacterial infections. However, slowly resistant organisms started to emerge which was a threat to the practitioners and the community. Beta-lactam antibiotics were one of the safest drugs which were used most frequently in treating the Gram positive and Gram negative organisms[2]. Bacteria became resistant to these antibiotics by production of beta lactamases. Extended-spectrum ß-lactamases (ESBLs) are a group of enzymes that can hydrolyze a variety of ß-lactams including cephalosporins (e.g. ceftazidime, cefotaxime and ceftriaxone) and monobactam (aztreonam) in addition to penicillins. They are found in a variety of Enterobacteriaceae. ESBL are reported commonly in isolates of *E.coli, Klebsiella* sp., etc. Recent studies on ESBL production in members of Enterobacteriaceae isolated from clinical specimens showed 9-50 per cent ESBL producers[3,4,5]. *E. coli* and *Klebsiella pneumoniae* often carry genes for TEM-1, -2 and SHV-1 ß-lactamases. These TEM- and SHV-derived ESBLs were the commonly observed ESBL types in *E. coli* throughout the 1980s and 1990s[2]. On the other hand, reports on a newer group of ESBLs, coined CTX-M for their preferential hydrolysis of cefotaxime over ceftazidime, started to emerge in E. coli in the late 1990s[6]. Initially ESBL producing *E.coli* were reported mainly from hospital acquired infections. But nowadays, they are found to be more common even in community acquired infections. The majority of community-acquired ESBL-producing *E. coli* infections were presenting as urinary tract infections (UTIs). But now ESBL are being reported in other *E.coli* infections too. Thus, knowledge about the ESBL producing *E.coli* is very essential before treating the infections in order to give appropriate therapy. Empirical antibiotic therapy without proper antibiotic sensitivity pattern analysis may not be helpful in all cases. Hence, we carried out this study to assess the prevalence of ESBL producers among the *E.coli* isolates.

MATERIALS AND METHODS

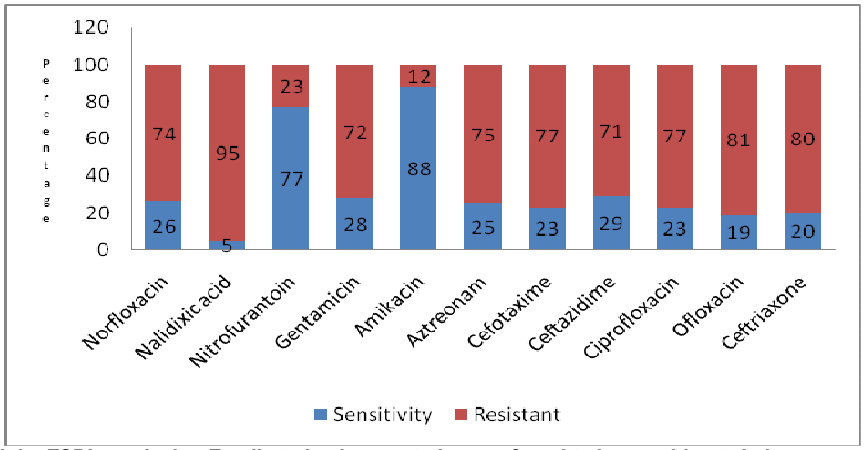
This study was done in a tertiary care hospital from August 2012 to November 2012. Hundred *E.coli* isolates from various clinical samples identified by conventional techniques were included in the study. Antibiogram of the isolates was done by Kirby Bauer's method using antibiotic discs. Antibiotics used were gentamicin (10 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), cephotaxime (30 µg), ceftazidime (30 µg) and amikacin (10 µg). The results were interpreted as per Clinical and Laboratory Standards Institute (CLSI) (formerly known as National Committee on Clinical Laboratory Standards – NCCLS) recommendations.[7] The isolates of *E.coli* with resistance or decreased susceptibility to the third generation cephalosporins were tested for ESBL production by double disc synergy test (DDST). An inhibition zone of ≤ 27 mm for cefotaxime and ≤ 22 mm for ceftazidime is considered that the organism may be an ESBL producer. A lawn culture of test strain on Muller Hinton agar was exposed to disc of cephotaxime (30 µg) and a disc of amoxiclav (augmentin) (20 µg amoxicillin /10 µg clavulanic acid) arranged in pairs. The discs were arranged so that the distance between them was approximately twice the radius of the inhibition zone produced by cephotaxime tested on its own. The test isolate was considered to produce ESBL, if the zone size around the antibiotic disc increased towards the augmentin disc. Test of proportion was used for data analysis.

RESULT

Six hundred and fifty two clinical samples from various infections were included in the study. Out of which, 100 screened isolates of *E.coli* were processed. Among them, 53 were from urine samples, 16 from pus, 4 from vaginal secretion, 8 from stool, 11 from blood samples and 8 from wound swabs. Among the *E.coli*

isolates, 95% showed resistance to nalidixic acid. The antibiogram also revealed that the isolates showed 77% resistance to Ciprofloxacin and cefotaxime, 75% to aztreonam, 72% to gentamicin and 71% resistance to ceftazidime. 88% of the *E.coli* were found to be susceptible to amikacin. By double disc method, the incidence of ESBL among *E.coli* isolates was found to be 37%.

Figure 1
Sensitivity pattern of the E.coli to various drugs:



All the ESBL producing E.coli strains in our study were found to be sensitive to imipenem.

DISCUSSION

ESBLs are now becoming a threat to the treating doctors. The prevalence of ESBLs is now increasing rapidly in *E.coli*. By screening 100 *E.coli* isolates, we found that maximum resistance was observed against nalidixic acid. Nitrofurantoin showed lower resistance (23%) indicating that it can be used for treatment of *E.coli* infections. 88% of the *E.coli* isolates were found to be susceptible to amikacin indicating the possibility of it becoming the drug of choice.77% of the isolates showed resistance to cefotaxime. By confirmatory test using double disc method, 37 % were found to be ESBL producing E.coli. Previous reports showed that the ESBL production in *E.coli* varies from 21 to 34 per cent[4,8,9,10]. Our study reports 37% of *E.coli* to be ESBL producers which is similar to another study by Shoba *et al*[11]. All the ESBL producing *E.coli* isolates were found to be susceptible to imipenem. The production of the beta-lactamases may be of chromosomal or plasmid origin. Plasmid mediated production is often acquired by transfer of genetic information from one organism to another. Such transferable plasmid also codes for resistant determinants to other antimicrobial agents. Hence multidrug resistance is expected to be more common in ESBL producing organisms. In our study also, most of the ESBL producing *E.coli* were found to be multidrug resistant.

CONCLUSION

The recent spread of ESBL producing *E.coli* is becoming explosive. They have already become a part of the community, making its elimination highly impracticable. Our study showed that the prevalence of ESBL production is reasonably high among the *E.coli* isolates. Hence, routine ESBL testing for the pathogens along with conventional antibiogram would be useful for most of the cases of E.*coli*. ESBL should be highly suspected in all patients who do not respond to the first line drugs and appropriate treatment should be given after proper antibiotic sensitivity and ESBL testing. Judicious use of antibiotics can reduce the incidence of ESBL producing organisms.

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