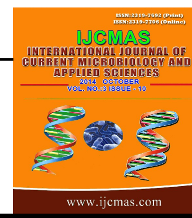

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Original Research Article

Prevalence of Extended Spectrum β-Lactamases In *E.coli* and *Klebsiella* spp. in a Tertiary Care Hospital

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A B S T R A C T

Increasing resistance to 3rd generation Cephalosporins amongst *E.coli* and *Klebsiella* spp. is predominantly due to the production of Extended-spectrum β-lactamases (ESBLs). ESBL producers are associated with increased morbidity and mortality. Accurate laboratory detection is important to avoid inappropriate antimicrobial therapy. *E.coli* and *Klebsiella* spp. isolates obtained from various clinical specimen were identified by conventional microbiological techniques. All these isolates were tested for antimicrobial susceptibility on Muller-Hinton's agar by Kirby-Bauer disk diffusion method as per CLSI guidelines. *E.coli* and *Klebsiella* spp. isolates were screened for production of ESBL and phenotypic confirmation of all potential ESBL producers was done by the double disc synergy test. A total of 417 isolates of *E.coli* (344) and *Klebsiella* spp.(73) were recovered. Of which, 222 (53.23%) were the potential ESBL producers. Out of 222, 15(6.75%) were confirmed as ESBL producers. Out of 181 *E.coli*, 11(6.07%) and out of 41 *Klebsiella* spp. 4(9.75%) were confirmed as ESBL producer. The prevalence of ESBL producers in our study is low as compared to other studies. The ESBL producers pose a major problem for clinical therapeutics. Thus, routine detection of these microorganisms is required.

Keywords

ESBL,
Third generation Cephalosporins,
E.coli,
Klebsiella spp

Introduction

β-lactam antimicrobial agents are the most common treatment for bacterial infections.[10] β-lactamases continue to be the leading cause of resistance to β-lactam antibiotics in gram negative bacteria.[15] In the past it was believed that Cephalosporins were relatively immune to attack by β-lactamases. It was surprising to find Cephalosporin resistant *Klebsiella* spp. among the clinical isolates. The mechanism of this resistance was production of extended spectrum β-lactamases.[12]

Extended Spectrum β-lactamases (ESBLs) are hydrolytic enzymes that mediate resistance to extended-spectrum (3rd generation) Cephalosporins namely Cefotaxime, Ceftazidime, Ceftriaxone and

Monobactams (e.g. Aztreonam) but do not affect Cephamycins (e.g. Cefoxitin and Cefotetan) or Carbapenems (e.g. Meropenem or Imipenem).[4] These are inhibited by Clavulanic acid, Sulbactum, Tazobactum.[8] Because of their greatly extended substrate range, these enzymes were called extended spectrum β-lactamases.

The first ESBL isolates were discovered in Western Europe in mid 1980s. They can be found in a variety of *Enterobateriaceae* spp. However, majority of ESBL producing strains are *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *E.coli*. Other organisms reported to harbor ESBL include *Enterobacter*, *Salmonella*, *Morganella morganii*, *Proteus mirabilis*, *Serratia marcescens* and *Pseudomonas aeruginosa*. However, the frequency of ESBL production in these organisms is low.[4]

Infection due to ESBL producers range from uncomplicated urinary tract infection to life threatening sepsis.[6] ESBL producers are associated with increased mortality and morbidity. Organisms producing ESBLs are clinically relevant and remain an important cause for failure of therapy with Cephalosporins.[2] Being plasmid mediated, these enzymes spread fast among various bacteria and are important by infection control, clinical and therapeutic implications.[15]

E.coli and *Klebsiella* are the most common ESBL producers and there is not enough data on prevalence of ESBL producers in clinical isolates in our area, hence the present study was undertaken to find out the prevalence of ESBL producing *E.coli* and *Klebsiella* isolates.

Materials and Methods

The study was conducted on consecutive isolates of *E.coli* and *Klebsiella* spp. obtained from various clinical specimen over a period of 31months [August 2010-February 2013].

The isolates were identified to the species level by standard microbiological methods like cultural characters, biochemical reactions etc.[5]

Disc diffusion test was carried out with antibiotic discs on Muller-Hinton agar (Hi media laboratory, Mumbai). The results were expressed as susceptible or resistant according to interpretative zone diameters recommended by the Clinical and Laboratory Standards Institute (CLSI).

The following antimicrobials were tested-
Amikacin, Gentamycin, Cefotaxime, Ceftazidime, Ciprofloxacin, Netilmycin, Cefadroxil, Sparfloxacin, Ceftriaxone, Cefoperazone, Lomefloxacin, Ampicillin+ Sulbactum, Ceftazidime. *E.coli* ATCC 25922 was used as control strain.[2]

Screening test

The CLSI has proposed disc diffusion method for screening of ESBL producing *E.coli* and *Klebsiella* species. Cefpodoxime, Ceftazidime, Cefotaxime, Aztreonam, Ceftriaxone discs can be used. Since the affinity of ESBL for different substrates is variable, the use of more than one of these agents for screening improves sensitivity of detection.[6] Hence, both Ceftazidime (best indicator of TEM and SHV derived ESBL) and Cefotaxime (best indicator of CTX-M type) were used.[2]

E.coli and *Klebsiella* isolates with zone of inhibition less than 22mm in diameter for Ceftazidime and less than 27mm in diameter for Cefotaxime were presumed to be potential ESBL producer.[18] Resistance to 3rd generation Cephalosporin in *Klebsiella*

and *E.coli* is not due to ESBL only, other potent β-lactamases such as AmpC and Kl enzymes may be responsible. Hence National Committee for Clinical Laboratory Standards (NCCLS) recommends phenotypic confirmation of ESBL production. Many different techniques exist for confirming ESBL production but those utilizing similar methodology to standard susceptibility test are most convenient. Confirmatory test depends on detecting synergy between Clavulanic acid and indicator Cephalosporins used in primary screening. It distinguishes ESBLs from other β-lactamases.[2]

Isolates presumed to be ESBL producers on the basis of screening test results (as described above) were picked up and emulsified in saline to a 0.5 McFarland turbidity standard. By the use of a sterile cotton swab, the broth was evenly spread on Muller-Hinton agar and allowed to dry.

Discs of Ceftazidime (30mcg), Cefotaxime (30mcg) and Co-amoxyclav (Amoxycillin 20mcg + Clavulanic acid 10mcg) were placed 20mm apart on the plate in a straight line with Co-amoxyclav disc in the middle. The plates were incubated aerobically overnight and the results were read the following day. Isolates which showed an enlargement of the zone of inhibition greater than 5mm on the Co-amoxyclav side of the disc compared to the results seen on the side without Co-amoxyclav were confirmed as ESBL producers.[11] ESBL producing organism *K.pneumoniae* ATCC700603 (positive control) and *E.coli* ATCC25922 (negative control) were used for quality control of ESBL tests.[2]

Results and Discussion

During the study period, a total of 417 isolates of *E.coli* and *Klebsiella* spp. were recovered. Of these 344 were identified as *E.Coli* and 73 as *Klebsiella spp*. Out of 417 isolates, 222 (53.23%) were the potential ESBL producers. Of these 222, 181 were *E.coli* and 41 were *Klebsiella spp*. The potential ESBLs were screened by measuring zone of inhibition ≤22mm for Ceftazidime and ≤27mm for Cefotaxime. Confirmatory tests for ESBL production were performed on these 222 isolates. Out of 222, 15(6.75%) were confirmed as ESBL producers of which 11 (6.07%) were *E.coli*, and 4(9.75%) were *Klebsiella* spp. The confirmation of ESBL producers was done by phenotypic double disc synergy test.

All over the world, ESBL producing strains spread in the hospital. It is necessary to know the prevalence of ESBL producers in the hospital, so as to formulate a policy for empirical therapy.

Equally important is the information of an isolate from a patient to avoid misuse of extended spectrum third generation Cephalosporin which still remain an important component of antimicrobial therapy.[13]

In our study, 222 (53.23%) isolates of *E.coli* and *Klebsiella* species were suspected as ESBL producer on screening test, of which only 15 were confirmed as ESBL producers. Remaining 207 showed resistance to beta lactamase inhibitor (Clavulanic acid) and therefore they were negative by confirmatory test.

This could be due to presence of some derivatives of TEM and SHV, which are not inhibited by Clavulanic acid. These are known as inhibitor resistant TEM and AmpC Beta lactamases. Co-existence of these in ESBL producing strains may give false negative result.[12] Currently, detection of organisms with multiple beta lactamases

that may interfere with the phenotypic confirmatory test can only be accomplished using isoelectric focusing and DNA sequencing, which are not usually available in the clinical laboratories.[12] The prevalence of ESBL producers was found to be low (6.75%) in our study. Results from the SENTRY Asia-Pacific Surveillance Program of 9 countries reported 5.9% *E.coli* and 17.2% *Klebsiella pneumoniae* as the ESBL producers.[9] Also a recent survey from 31 countries reported the prevalence of ESBL producers among *E.coli* and *Klebsiella pneumoniae* as low as 1.5% in Germany and as high as 39-47% in Russia, Poland and Turkey.[7] Studies from India reported varied prevalence of ESBL producers ranging from 6-87%.[1] The prevalence of *E.coli* and *Klebsiella* spp. as ESBL producers in our study is 6.07% and 9.75% respectively. However, it ranges from 14-63% for *E.coli* and 16-80% for *Klebsiella* spp.[14,16]

The isolates which have a positive phenotypic confirmatory test should be reported as resistant to all Penicillins, Cephalosporins except Cephamycins (Cefotetan and Cefoxitin) and Aztreonam, regardless of zone of inhibition diameters.

β-lactam and β-lactamase inhibitor combinations are reported as susceptible, if the diameters of zone of inhibition are within appropriate range.[1]

Current therapy for strains of *Enterobacteriacece* that express ESBL, is limited to Carbapenem.[4] Carbapenem are expensive and have potential side effects.Thus, ESBL producing organisms pose a major problem for clinical therapeutics.[17]

Institutions with high ESBL prevalence need to determine whether there is high rate of Cephalosporin use, especially third generation Cephalosporins. Several studies have shown that by limiting the use of these agents alone or in combination with infection control measures, the frequency of ESBL isolates can be reduced substantially.[4]

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