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A Prevalence Study of Extended Spectrum β-Lactamases in *Escherichia coli* and Klebsiella spp. in a Tertiary Care Hospital in Rajkot City of Gujarat (India)

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Original Research Article

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Abstract: β-Lactam antibiotics are commonly used to treat bacterial infections. Increased use of antibiotics, particularly the third generation of cephalosporins, has been associated with the emergence of ESBL producing bacteria, most commonly in E. coli. And Klebsiella pneumonia. Infection due to ESBL producers range from uncomplicated urinary tract infection to life threatening sepsis. This study was undertaken to assess the prevalence of ESBL producing E.coli and Klebsiella isolates. Patients taking treatment in P.D.U. Hospital and Medical College, Rajkot- a tertiary care hospital in Gujarat, India, were included in study. During the half year period from July 2014 to December 2014**,** a total of 600 E. coli and Klebsiella isolates from various clinical specimens were included in the study. The isolated organisms of E. coli and Klebsiella were characterized for their production of ESBL. Out of 600 E. coli and Klebsiella isolate, E. coli were 224 (37.3% and Klebsiella spp. 376 (62.7%). The overall rate of production of ESBL (by phenotypic confirmatory test with double disc diffusion method) in total isolates of E. coli and Klebsiella spp. is 22.5% (135/600). The rate of production of ESBL in E. coli is 29.9% (67/224) and in Klebsiella spp. is 18.1% (68/376). ESBL producing E. coli and Klebsiella showed high prevalence in present study. Routine laboratory testing for ESBL is needed in order to optimize antibiotic management to reduce ESBL associated morbidity & mortality.
Keywords: Extended spectrum beta-lactamase, E. coli, Klebsiella, prevalence, India.

INTRODUCTION

β-Lactam antibiotics are commonly used to treat bacterial infections. The groups of antibiotics in this category include penicillins, cephalosporins, carbapenems & monobactams. Increased use of antibiotics, particularly the third generation of cephalosporins, has been associated with the emergence of β-Lactamases mediated bacterial resistance, which subsequently led to the development of ESBL producing bacteria. ESBLs are enzymes that mediate resistance to extended spectrum e.g., third generation cephalosporins as well as monobactams such as aztreonam but do not affect cephamycins (e.g. Cefoxitin & Cefotetan) or carbapenems (e.g. Meropenem or Imipenem) [1, 2]. These enzymes are inhibited by Clavulanic acid, Sulbactum and Tazobactum [3].

The first ESBL isolates were discovered in Western Europe in mid 1980s [2]. These enzymes catalyse the hydrolysis of the β-lactam ring of antibiotic, thereby destroying the antimicrobial activity. ESBLs have been reported worldwide in many different genera of enterobactericeae and Pseudomonas aeruginosa [4]. However, these are most common in Klebsiella pneumoniae & E. Coli [5]. ESBL producing organisms are often resistant to several other classes of antibiotics, as the plasmids with the gene encoding ESBLs often carry other resistance determinants. Initially ESBL producing organisms were isolated from nosocomial infections but these organisms are now also being isolated from community [6]. The colonization rate for K. pneumoniae is low in healthy individuals in the general population. But it is increased in hospitalized patients especially with long care facilities, health care manipulations. e.g., use of catheters [7].

Infection due to ESBL producers range from uncomplicated urinary tract infection to life threatening sepsis [8]. 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. Being plasmid mediated, these enzymes spread fast among various bacteria and are important by infection control, clinical and therapeutic implications [9].

ESBLs have been reported from all parts of the world. However, prevalence varies widely even in closely related regions. The true incidence is difficult to determine because of the difficulty in detecting ESBL production & due to inconsistencies in testing & reporting [7]. Prevalence of ESBL in many parts of the world was 10-40% among E. coli and Klebsiella pneumonia [10]. ESBL screening as a routine test has not yet been practiced in many centres in India. ESBL occurs at an alarming rate among enterobactericeae isolates among the hospitalized patients which can result in an outbreak in the community that may be difficult to treat.

E.coli and Klebsiella being the the most common ESBL producers , the present study was undertaken to find out the prevalence of ESBL producing E.coli and Klebsiella isolates.

MATERIALS AND METHODS

The study was undertaken in Department of Microbiology, P.D.U. Medical College, Rajkot (Gujarat, India). The study was conducted on 600 consecutive isolates of E.coli and Klebsiella spp. obtained from various clinical specimen over a period of 6 months [July 2014- Dec 2014].

The isolates were identified to the species level by standard microbiological methods like cultural characters, biochemical reactions etc [11, 12].

Disc diffusion test was carried out with antibiotic discs on Muller-Hinton agar. The results were expressed as susceptible or resistant according to interpretative zone diameters recommended by the Clinical and Laboratory Standards Institute (CLSI) [1].

The following antimicrobials were tested

Ampicillin (10µg), Amoxycillin-clavulanic acid (20/10µg), Piperacillin (100µg), Cephotaxime (30µg), Ceftriaxone (30µg), Ceftazidime (30µg), Gentamicin (10µg), Amikacin (30µg), Netilmicin (30µg), Tetracycline (30µg), Ciprofloxacin (5 µg), Chloramphenicol (30µg), Imipenem (10 µg) and Azythromycin (30 µg).

Screening test for ESBLs

Isolates were screened for ESBL production by using disc Diffusion of cefotaxime (CTX), ceftazidime (CAZ), ceftriaxone (CRX) and Aztreonam (AZM) placed on inoculated plates containing Mueller Hinton agar according to the CLSI recommendations. Isolates showing inhibition zone size of ≤ 22 mm with ceftazidime (30gµ), ≤ 25 mm with ceftriaxone (30µg), ≤ 27 mm with cefotaxime (30µg) ,≤ 27 mm with Aztreonam (30µg) were suspected for ESBL production. 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 [8].

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. Confirmatory test depends on detecting synergy between Clavulanic acid and indicator Cephalosporins used in primary screening. It distinguishes ESBLs from other *β*-lactamases [13].

Confirmatory test for ESBLs

Isolates presumed to be ESBL producers on the basis of screening test were subjected to Phenotypic confirmatory test for ESBL production by double disc diffusion test (DDDT) as per CLSI 2014 guidelines.

In this test a disc of ceftazidime (30µg) alone (‘a’ in fig.1) and a disc of ceftazidime in combination with clavulanic acid (30µg/10µg) (‘b’ in fig.1) were used for each isolates. Both the discs were placed 25 mm apart, centre to center, on a lawn culture of the test isolate on Muller Hinton agar plate and incubated overnight at 37°C. A ≥5 mm increase in zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone was designated as ESBL positive (Figure-1).

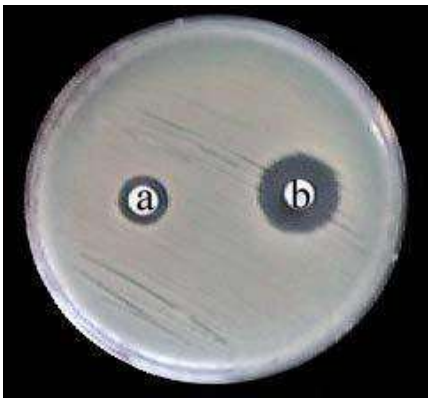

Fig-1: Showing double disc diffusion test

RESULTS

During the study period, a total of 600 isolates of *E.coli* and *Klebsiella* spp. were recovered. Of these 224 (37.3%) were identified as *E.Coli* and 376 (62.7%) as *Klebsiella spp* (Table-1).

Table-1: Shows distribution of E. coli & Klebsiella spp. Isolates

Name of the organisms	Total	%
E. Coli	224	37.3
Klebsiella spp.	376	62.7
Total	600	100.0

Table-2: Detection of ESBL production by DDDT from E. coli and Klebsiella spp. Isolates.

Name of organism	Total	ESBL positive (%)
E. Coli	224	67 (29.9)
Klebsiella spp.	376	68 (18.1)
Total	600	135 (22.5)

Table-2 Shows prevalence of ESBL producing isolates from different clinical specimen by double disc diffusion test.

DISCUSSION

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 [14].

In present study total of 600 E. coli and Klebsiella isolates from various clinical specimens were studied for ESBL production. Of these 224 (37.3%) were identified as *E.Coli* and 376 (62.7%) as *Klebsiella spp*.

The overall rate of production of ESBL in total isolates of E. coli and Klebsiella spp. is 22.5% (135/600). Different studies in India & abroad showed prevalence of ESBL producers from 6.75% to 69.3% (Table-4).

In present study the rate of production of ESBL in E. coli is 29.9% (67/224) and in Klebsiella spp. is 18.1% (68/376). Different studies in India & abroad showed prevalence of ESBL in E. coli 6.07%-73.5% and in Klebsiella spp 9.75%-72% (Table-3).

Table-3: studies in India & abroad showed prevalence of ESBL in E. coli and in Klebsiella spp.

STUDY	ESBL positive E. coli.	ESBL positive Klebsiella spp.	Overall ESBL positive in both.
OUR Study	29.9%	18.1%	22.5%
Puri J *et al.*, [15]	6.07%	9.75%	6.75%
Dugal S *et al.*, [16]	24.4%	38.9%	26.9%
K. Aruna *et al.*, [17]	40.6%	27.6%	37.6%
Trupti B *et al.*, [18]	41.6%	26%	37%
Atit Shah *et al.*, [19]	59.7%	57.14%	59%
Gaurav *et al.*, [20]	73.5%	58.1%	69.3%
Meeta S *et al.*, [21]	57.18%	67.08%	60.4%
Sufia M *et al.*, [22]	56%	72%	62.9%

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 [23].

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 [5].

Current therapy for strains of *Enterobacteriacece* that express ESBL, is limited to Carbapenem [2]. Carbapenem are expensive and have potential side effects. Thus, ESBL producing organisms pose a major problem for clinical therapeutics [24].

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 [2, 15].

CONCLUSION

ESBL producing E. coli and Klebsiella showed major prevalence in our hospital. Phenotypic confirmatory test double disc diffusion method is simple and economical to detect ESBL producers. Routine laboratory testing for ESBL is needed in order to optimize antibiotic management to reduce ESBL associated morbidity & mortality.

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