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ORGINAL ARTICLE

Detection of ESBL prevalence among E.coli and Klebsiella Species; A Comparative Study

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ABSTRACT

This study was undertaken to determine the Extended Spectrum Beta Lactamases (ESBLs) producers among Escherichia coli and Klebsiella species isolated from the clinical specimens. A total of 200 specimens collected from the patients admitted in the tertiary care hospital were included in this study. The specimens like urine, blood and pus were processed according to the standard microbiological methods. The specimens were inoculated onto the Nutrient agar, Blood agar and MacConkey agar and incubated at 37°C for 48 hrs. Bacteria were identified by standard Biochemical reactions and other identification tests. Culture was positive in 150 specimens and those 150 isolates were processed for ESBL by Double Disk Synergy Test (DDST) and Inhibitor Potentiated Disk Diffusion (IPDD) test. Result of the 150 isolates showed that 74% (n=111) isolates of E.coli and 26% (n=39) isolates of Klebsiella showed synergism by DDST. IPDD test showed potentiation in 80% isolates of E.coli (n=120) and 20% in Klebsiella (n=30) isolates. In this study majority of ESBLs were E.coli.

KEY WORDS : ESBL, DDST, IPDDT, Cephalosporins drugs.

Introduction

Beta lactams are the most important class of antibiotics widely used in treating various infections. They inhibit peptidoglycan synthesis and the fundamental change in the substrate of the enzymes [1-2].

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All beta lactams contain an essential four membered Beta lactam ring. The activity of particular beta lactam is influenced by the type of substitution (R group) attached to the basic structure. The Role of Beta-Lactamase in bacterial resistance to beta lactam antibiotics is a continuing problem in treatment of infectious diseases. Beta lactamase can be either chromosomal in origin or plasmid mediated, these enzymes hydrolyze the cyclic amide bond in the beta lactam molecule resulting in the loss of their antibacterial activity [3].

Increasing resistance to 3rd generation cephalosporins has become a cause for concern especially among Enterobacteriaceae family like Escherichia coli and Klebsiella. ESBLs classified under functional classification and molecular classification [4].

The most common beta lactamases among Enterobacteriaceae are the plasmid borne TEM and SHV beta lactamases [5]. The TEM enzymes have a transferable resistance mechanism and are named after the patient Temoniera. The first plasmid-mediated β-lactamase in gram-negative bacteria TEM 1 was described in the early 1960s [6]. SHV is a sulphydryl variable enzyme which shows resistance against Ampicillin, first generation cephalosporins and piperacillin [7]. The ability of ESBL is enhanced by mutation at several positions within their amino acid sequence [8]. Beta lactamases has the ability to attack expanded spectrum of cephalosporins like ceftazidime, cefotaxime, cefpodoxime and monobactams such as aztreonam. ESBLs are inhibited by beta lactamase inhibitors like clavulanic acid. These enzymes transferred between various species of enterobacteriaceae like Escherichia coli and Klebsiella species. The Phenotypic confirmatory methods like DDST and IPDDT are used to identify the Extended spectrum beta lactamase producing pathogens [9].

Materials and Methods

A total of 200 isolates from different clinical specimen (Urine, Blood, and Pus) were obtained from the central laboratory. The samples were screened as per CLSI guidelines for potential ESBL activity.

The specimens were collected and processed for aerobic culture plate method. The aerobic cultures were performed on Basic media like Nutrient agar for the Pigment production, Enriched media like Blood agar for the Haemolysis, Differential media like MacConkey agar and CLED agar for the Lactose fermenting and Non-Lactose fermenting. All the plates were incubated at 37°C; overnight and identification tests of bacteria's were carried out using standard methods. Bacterial identification was done based on colony morphology, Grams staining, Catalase reaction, Oxidase reaction and Biochemical reactions [Figure-1 & 2]. The bacterial isolates were subjected to antimicrobial susceptibility testing using standard Kirby Bauer disc diffusion method

Figure-1

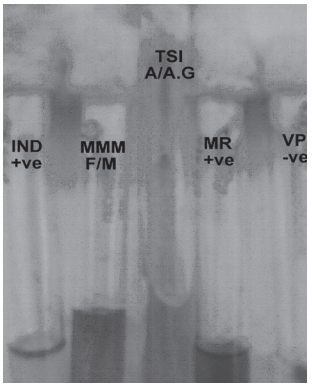
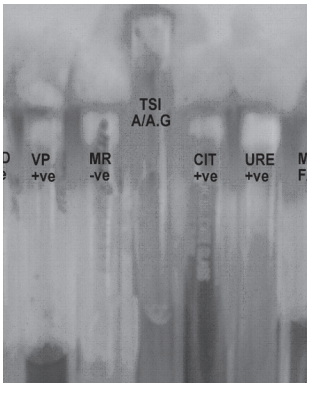


Figure-1



Antibiogram profiles were determined to commonly used antibiotics, those found resistance to any one of the 3rd generation cephalosporins were subjected for confirmation of ESBL production by performing DDST and IPDD test

DOUBLE DISK SYNERGY TEST (DDST)

This test was done for detection ESBL enzymes in E.coli and Klebsiella isolates.

1. A standardised inoculum of the test isolate was swabbed on the surface of a Mueller Hinton agar.

2. Amoxyclav (Amoxicillin/ Clavulanic acid 30/10 µg) is placed at the center of the plate.
3. Disks containing 30 µg of ceftazidime, cefotaxime, ceftriaxone, 10 µg cefopodoxime are placed 20-30mm away from the central disc.

An extension in the zone of inhibition around the peripheral disks towards the centrally placed Amoxyclav disk indicate ESBL production.

INHIBITOR POTENTIATED DISK DIFFUSION TEST [IPDDT]

This method recommended by CLSI was performed as disk diffusion.

1. A standardized inoculum of the test isolates is swabbed on the surface of a Mueller-Hinton agar.
2. Ceftazidime (30 µg) and Ceftazidime/ Clavulanic acid (30/10µg) or Cefotaxime (30 µg) and Cefotaxime/Clavulanic acid (30/10 µg) disks.
3. Placed on the plate and incubated in ambient air for 16-18 hours of incubation at 37°C.
4. Confirmatory testing requires use of both. Cefotaxime and Ceftazidime alone and in combination with Clavulanic acid.

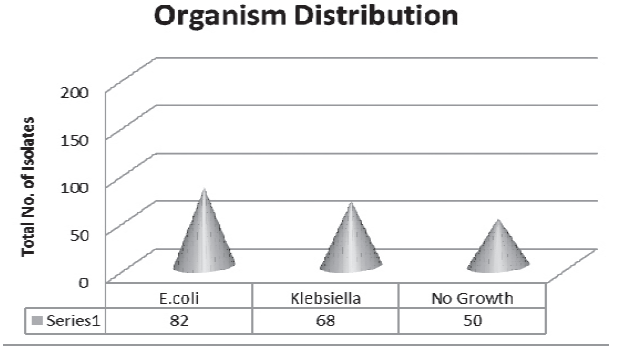
Increase in the zone diameter by ≥ 5 mm around the disks with clavulanic acid over the disks with Cephalosporins alone confirms ESBL production.

Results

Out of 200 specimens 150 specimens were culture positive for growth. Of this 82 (41%) samples showed growth of Escherichia coli, 68 (34%) samples showed growth of Klebsiella and 50 (25%) of the samples revealed no bacterial growth [Table-1]. All the bacteria isolated were recovered from both Blood agar and MacConkey agar. The Antibiogram performed on the Mueller Hinton Agar as per the guidelines issued by the Clinical Laboratory Standards Institute (CLSI) [10] [Table-2].

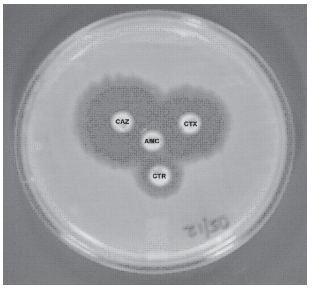
[Table-1]

Table-2 [Antibiotic Resistant Pattern:-] Phenotypic Confirmatory Methods:



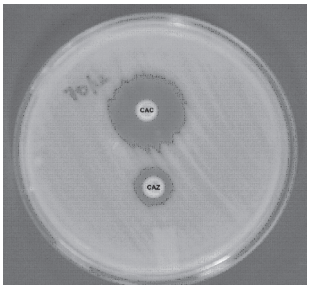
Drugs	E.coli [n=82]	Kleb-siella [n=68]
Ampicillin (10µg)	72	47
Cefopodoxime (30µg)	70	52
Co-trimoxazole (30µg)	60	42
Gentamicin (10µg)	60	52
Nitrofurantoin (300µg)	35	22
Norfloxacine (10µg)	56	55
Amikacin (10µg)	32	20
Cefoperazone/Sulbactum	18	13
Cefepime (30µg)	75	61
Ceftazidime (30µg)	64	56
Ceftazidime/Clavulanic acid	33	23
Oflaxocin (5µg)	69	49

Out of the 150 isolates screened 74% (n=111) isolates of E.coli and 26% (n=39) isolates of Klebsiella showed synergism by DDST [Figure-3].



ESBL Detection by DDST

IPDD test showed potentiation in 80% isolates of E.coli (n=120) and 20% in Klebsiella (n=30) isolates [Figure-4].



ESBL Detection by IPDDT

Discussion

There are reports on the predominant distribution of E.coli and Klebsiella in the environment, hospital acquired infection and frequently encountering organisms [11]. E.coli causes the Pyogenic, UTI and Haemolytic disease. Klebsiella causes the Pyogenic and UTI infections mainly.

A total of 150 Escherichia coli [n=82] and Klebsiella spp [n=68] isolates were resistant to third generation cephalosporins were subjected to screening for the presence of ESBLs. A high degree of resistance was observed in E.coli isolated from urine and pus specimens to the beta lactam drugs, namely Ampicillin, Cephalothin, Cefuroxine and Cefepime. This explains that the ESBLs are capable of hydrolyzing the second generation (Cephalothin, Cefuroxime) and fourth generation (Cefepime) Cephalosporins.

Most of the isolates from the above said specimens were sensitive to the beta lactam and beta lactam + beta lactamase inhibitor drug Piperacillin + Tazobactam and Imipenem [12].

Among the two methods used, in the present study, IPDDT is a phenotypic confirmatory test, was found to be more sensitive procedure for detection of ESBL than the DDST. 53% of the 150 ESBL producing strains were detected by DDST while IPDDT detected 75% of isolates.

It shows that the resistance rates to cephalosporins among Enterobacteriaceae (E.coli and Klebsiella) from clinical samples are very high. Increasing resistance to other classes of antibiotics will make the treatment options difficult. Rational antibiotic use and cycling of antibiotic use may help to revert this situation.

Conclusion

The 150 isolates were tested for DDST and IPDD test to detect the ESBLs in E.coli and Klebsiella species. Result shows that 74% (n=111) isolates of E.coli and 26% (n=39) isolates of Klebsiella showed synergism by Double Disk Synergy Test (DDST). Inhibitor Potentiated Disk Diffusion (IPDD) test showed potentiation in 80% isolates of E.coli (n=120) and 20% in Klebsiella (n=30) isolates. This study indicates that the majority of ESBLs were expressed in E.coli.

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