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A study of extended spectrum beta-lactamases among clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*

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Abstract

Aim: To identify the prevalence of these strains in hospitals and to characterize their epidemiology to control the spread of these strains and to determine suitable prevention and treatment policies.

Materials and Methods: A total of 450 isolates of *E. coli* and *K. pneumoniae* were isolated from different clinical specimens from patients admitted under different clinical disciplines,

Results: Out of 69 putative ESBL producing isolates of *K.pneumoniae* 91.31% were resistant to ceftazidime, while 92.76%, 91.03%, 100% and 92.76% were resistant to cefotaxime, ceftriaxone, cefpodoxime and aztreonam. It is to be noted that 86.96% isolates were resistant to ceftazidime. All the isolates i e, 100% were susceptible to imipenem and mipenem + cilastin.

Co-resistance was observed with ciprofloxacin 81.92%, gentamicin 41% and nalidixic acid 100%. All the isolates i e, 100% were multidrug resistant.

Conclusion: A knowledge of resistance pattern of bacterial strains in a geographical area will help to guide appropriate and judicious antibiotic use. ESBL's occurrence and spread need to be controlled. Appropriate antimicrobial selection, surveillance system and effective infection control procedures are the key partners in their control"

Keywords: Beta-Lactamases, *Escherichia Coli*, *Klebsiella Pneumoniae*

Introduction

Infectious diseases are not the main cause of mortality and morbidity in this era of chemotherapeutic and antibiotic agents. Unfortunately this is not true in all infections as incidence of some infections is on the increase and is causing concern especially because of development of drug resistance by some of the microbiological agents^[1]. Emergence of drug resistance among human pathogens is not only important to microbiologist but also to clinician's who face a major therapeutic problem. This problem is mainly due to irrational and inadequate therapeutic doses of antibiotics and chemotherapeutic agents. Extended Spectrum BetaLactamases (ESBL's) are a rapidly evolving group of beta-lactamases which share the ability to hydrolyze third generation cephalosporins and aztreonam yet are inhibited by clavulanic acid. Typically they derive from genes for TEM-1, TEM-2 or SHV-1 by mutations that alter the amino acid configuration around the active site of these beta-lactamases. This extends the spectrum of betalactam antibiotics susceptible to hydrolysis by these enzymes^[2]. ESBL's are encoded by transferable conjugative plasmids which often code resistance determinants to other antimicrobial agents such as aminoglycosides. These conjugative plasmids are responsible for the dissemination of resistance to other members of gram negative bacteria in hospitals and community^[3]. Incidence of these organisms is being continuously increasing throughout the world with limited treatment alternatives. ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* pose a major problem for clinical therapeutics. It is necessary to identify the prevalence of these strains in hospitals and to characterize their epidemiology to control the spread of these strains and to determine suitable prevention and treatment policies. Bearing in mind this massive problem of ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* the present study was carried out.

Material and Method

A total of 450 isolates of *E. coli* and *K. pneumoniae* were isolated from different clinical specimens from patients admitted under different clinical disciplines of Krishna Hospital and Medical Research Centre, Karad during 01.09.2008 to 31.03.2010 out of which 123 were included in the present study. These included *E.coli* isolates and *K. pneumoniae* isolates.

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All these isolates were isolated from various clinical specimens like urine, pus, sputum, blood, bone marrow and body fluids. These strains were subjected to Antibiotic susceptibility testing.

Determination of MIC of ceftriaxone, ceftazidime and cefotaxime. Phenotypic confirmatory tests.

Resistance transfer of experiment using E.coli J53AZR as recipient strain.

All these specimens were processed and clinical isolates isolated using standard procedure [4].

a) Pus, sputum and body fluids:-Specimens were inoculated on blood agar, chocolate agar and

MacConkeys agar. Precaution was taken to see that specimens were collected before administration of antibiotics. Lactose fermenting colonies were selected for morphological and biochemical identification. Biochemical tests were performed as per standard procedures [5], namely MR, Citrate, Urease, Indole, Sugar fermentation, TSI, and OF. Motility testing was also done.

Blood/ Bone Marrow culture:- 5-10 ml blood was collected aseptically in two culture bottles containing 50ml of tryptose phosphate broth and bile broth with SPS in the final concentration of 0.05%. Precaution was taken to see that the blood was collected before administration of antibiotics; subculture was done on 5% sheep blood agar and MacConkeys agar after 24hours and 10days of incubation at 37 °C. Lactose fermenting colonies were selected for morphological and biochemical identification. These were further identified by using biochemical tests as above.

b) Urine Specimen collection was done by sampling the midstream flow by clean catch technique.

Male – the glans was retracted and the urethral meatus cleaned immediately before voiding.

Female – the patient was asked to remove underclothing completely and sit comfortably on the toilet seat, swinging one knee to the side as far as possible. The periurethral area and perineum were first cleaned with soap water followed by a rinse with water. The labia were held apart during voiding and the first few millimeters of urine passed into a bedpan or toilet bowl to flush out bacteria from urethra. The midstream portion of urine was then collected in a sterile wide mouthed Container [6].

Urine sample was obtained from an indwelling catheter using a number 28 gauge needles and syringe. The area from where the urine has to be aspirated is disinfected and then the needle is inserted through the soft rubber connector between the catheter and the collecting tubing [7].

The specimens thus obtained were cultured on Blood agar and MacConkeys agar using standard loop method (Semi-Quantitative culture) [8].

A colony count of 10³ CFU/ml of a single potential pathogen in symptomatic males or catheterized urine were processed further [8]. Lactose fermenting colonies were selected for morphological and biochemical identification. These were further identified by using biochemical tests as above.

Control

The standard E.coli (ATCC 25922) was employed as a check on activity of discs and on reproducibility of the test.

The donor was grown un aerated to late log phase at 37 °C in nutrient broth approximately 2x10⁸ bacteria per ml while the recipient was grown aerated, by shaking to late log phase at 37 °C approximately 5x10⁸ bacteria per ml diluted with equal quantity of fresh warmed broth.

One part of donor to a 9 parts of recipient were mixed together and the mixture was incubated without shaking overnight at 37 °C, similarly another set of the same was mixed with the recipient in 1:10 ratio 0.2ml donor 2.02ml recipient strain and the mixture incubated without shaking overnight at room temperature. 0.1ml volumes of both sets of mixture was then spread evenly over one half of the selection plates and streaked thinly over the other half, so as to obtain single colonies to know whether the frequency of transfer was high or low. Donor and recipient strains were also inoculated individually on MacConkeys agar (without bile salts) to serve as controls. Interpretation of results. If growth occurred on the selection plate containing antibiotics as well as on the control MacConkey plate then it was concluded that antibiotic resistance of donor was transferred to the recipient. If the growth was obtained only on the control MacConkey agar plate and not on the selection plate till 48hours, it was concluded that antibiotic resistance of donor strain was not transferred to the recipient.

A single colony of transconjugants was subcultured on MacConkeys agar plate and the antibiotic sensitivity pattern was again checked by Kirby-Bauer method to confirm the resistance transfer. MIC of trans conjugants for Cefotaxime, Ceftriaxone and Ceftazidime were again determined by agar dilution method Antibiotic susceptibility test, and phenotypic confirmatory test were also done.

Statistical Analysis

The analyzed results were expressed as percentage and proportion for the description of the distribution of specimens. Z-test for Standard error of difference between two proportions was used to compare the proportion between DDST and PCT of two groups. If probability (p) value is less than 0.05, the association or the difference was said to be significant.

Results

Out of the 54 putative ESBL producing isolates of E. coli 90.98% were resistant to ceftazidime, while 94.45%, 81.04%, 100% and 88.89% were resistant to cefotaxime, ceftriaxone, cefpodoxime and aztreonam. It is to be noted that 90.70% isolates were resistant to cefoxitin. All the isolates i e, 100% were susceptible to imipenem and imipenem + cilastin. Co-resistance was observed with ciprofloxacin 72.23%, gentamicin 58.71% and nalidixic acid 100%. All the isolates were that is 100% were multidrug resistant.

Out of 69 putative ESBL producing isolates of *K.pneumoniae* 91.31% were resistant to ceftazidime, while 92.76%, 91.03%, 100% and 92.76% were resistant to cefotaxime, ceftriaxone, cefpodoxime and aztreonam. It is to be noted that 86.96% isolates were resistant to cefoxitin. All the isolates i e, 100% were susceptible to imipenem and imipenem + cilastin.

Co-resistance was observed with ciprofloxacin 81.92%, gentamicin 41% and nalidixic acid 100%. All the isolates i e, 100% were multidrug resistant.



Fig 1: *K. Pneumoniae*

Discussion

The clinical samples included in the present study were urine, pus, sputum, blood/bone marrow and body fluids. Out of the 37 ESBL producing isolates of *E.coli* 23/37(62.16%) were from urine, 9/37(24.32%) from pus, 2/37(05.40%) from sputum and 3/37(08.10%) from body fluids.

Out of the 40 ESBL producing isolates of *K.pneumoniae* 16/40(40.00%) were from urine, 7/40(17.05%) from pus, 8/40(20.00%) from sputum, 4/40(10.00%) from blood/bone marrow and 5/40(12.05%) from body fluids.

In the present study majority of the ESBL isolates were from urine this compares well with the studies carried out in India and abroad. Resistant bacteria are emerging worldwide as a threat to favorable outcome in the treatment of common infections in community and hospital settings. Among the wide assay of antibiotics, beta-lactams are the most widely used agents. The most common cause of resistance to beta-lactam antibiotics is the production of beta-lactamases. Emergence of resistance to beta-lactam antibiotics began even before the first betalactam, penicillin was developed. The first plasmid mediated betalactamase TEM-1 was originally isolated from blood culture of a patient named Temoniera in Greece, in the early 1960s. TEM-1 being plasmid and transposon mediated has facilitated its spread to other species of bacteria. Another common plasmid mediated beta-lactamase is SHV-1 (sulfhydryl variable), which is chromosomally encoded in the majority of the isolates of *K. pneumoniae* but is unusually plasmid mediated in *E. coli* [9].

In the present study the prevalence of ESBL producers was found to be 18.22% (37/203) among *E. coli* isolates and 16.19% (40/247) among *K. pneumoniae* isolates. The overall prevalence of ESBL producers was found to vary greatly in different geographical areas and in different institutes.

In the present study the prevalence of ESBL producers was 18.22% (37 out of 203) among *E.coli* isolates and 16.19% (40 out of 247) among *K. pneumoniae* isolates. This compares well with the study have reported 18.05% of *E. coli* isolates to be ESBL producers 16.00% of *K. pneumoniae* isolates to be ESBL producers.

In comparison to other studies conducted in India the percentage of ESBL producers is less in the present study

In the present study all ESBL producing isolates were multidrug resistant. All were resistant to cefpodoxime, 94.45% were resistant to cefotaxime, 90.98% resistant to ceftazidime, 81.04% resistant to ceftriaxone and 88.89% to aztreonam. It is important to note all the ESBL producing strains were sensitive to imipenem and imipenem + Cilastin.

Results of our study compare well with that of Agarwal P *et al.*, who found that multidrug resistance was significantly higher among ESBL producers than non ESBL producers. However all the isolates were sensitive to imipenem.

Conclusion

Thus ESBL have become a widespread serious problem and several aspects of them are worrying. ESBL's compromise the activity of wide spectrum antibiotics creating major therapeutic difficulties with a significant impact on the outcome of patients. In hospital environment plasmids could be transferred easily between patients through health care workers due to hand carriage and selection pressure. The continued emergence of ESBL producing isolates of *E. coli* and *K. pneumoniae* presents diagnostic challenge to the clinical microbiology laboratories, which need to be more aware of the need for their detection. A knowledge of resistance pattern of bacterial strains in a geographical area will help to guide appropriate and judicious antibiotic use. ESBL's occurrence and spread need to be controlled. Appropriate antimicrobial selection, surveillance system and effective infection control procedures are the key partners in their control"

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