Detection of extended spectrum β-Lactamase production in clinical isolates of E.coli recovered from patients of D.G.H. Hospital

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ABSTRACT

Background: Resistance to various antibiotics is a big problem faced in clinical therapeutics since the initiation of use of Penicillin as an antibiotic. It has created a state of crisis in chemotherapy with antibacterial agents in case of bacterial infection.

Aim: To determine the incidence of ESBL producing isolates amongst the clinical isolates of Dhiraj General Hospital and determine the susceptibility pattern of the ESBL producer as well as ESBL non producer isolates for other antimicrobial agents and to find out treatment alternative for ESBL producer isolates

Methods: All samples received from D.G.H. at the microbiology laboratory, and found to be E.coli positive were selected for processing. Retrospectively the clinical histories were reviewed in terms of diagnosis, treatment and further follow up of the patients. One hundred samples were susceptible to be ESBL producer were screened by phenotypic disc synergy test (PDCT) by using Cefotaxime(30)/ Cefotamixe+ Clavulinicacid (30/10), and Ceftazidime (30)/ Ceftazidime + Clavulinic acid(30/10). Further confirmation was done by E. Test.

Results: One hundred E. coli isolates were obtained from different wards. Majority were from Medicine and Surgery wards. Prevalence was found to be high of ESBL production in this hospital. Highest isolates were obtained from urine samples. All isolates were sensitive for Imipenem.

Conclusions: Specific test to detect ESBL production should be done routinely and an empirical therapy policy should be adopted by the high risk units.

Key Words: ESBL, e.coli, enterobacteriaceae, DDST, PDCT

INTRODUCTION

Resistance to a range of antibiotics is issue of immense crisis faced in clinical therapeutics since the initiation of use of Penicillin as an antibiotic.[1,2] It has created a state of disarray in chemotherapy with antibacterial agents in case of bacterial infection, an area which has received wide coverage in academic as well as popular press over the last few years.[1] Enzyme β-lactamase which confers resistance to Penicillins and early Cephalosporins, is produced by wide variety of bacteria that is from aerobic grampositive to gram negative also in anaerobes.[2] To overcome the resistance to penicillin and early Cephalosporins newer Cephalosporins were developed which were resistant to β-lactamase, these includes Cephamycins, Oxyiminocephalosporins, Carbapenems, Monobactams.[3] Overuse of newly developed β-lactams ultimately resulted in birth of more potent plasmid mediated enzyme known as Extended Spectrum β-lactamase (ESBL), which are having wide range of hydrolysis than the previous β-lactamase.[2] Transfer of resistance among different bacteria by various natural gene transfer mechanism has resulted in global spread of resistance at high rate and has created a worldwide problem.[2,4]

Failure to detect the ESBL in clinical setups has increased morbidity, mortality and outbreaks of highly resistant pathogens.[4,5] Till date so many phenotypic methods for ESBL detection have been tried by different workers, but inhibitor based method guided by Clinical Laboratory Standards Institute (CLSI) is most widely accepted and extensively used in treatment with the inhibitor combination.[2,4]

MATERIALS AND METHODS

Study comprises of total 100 clinical isolates of E.coli that have been isolated from different samples received at Diagnostic Microbiology Section of Microbiology Department, D.G.H. Hospital & S.B.K.S M.I.& R.C.. These isolates were subjected to study their colony morphology and various biochemical reactions. Confirmation was done using ESBL screening by phenotypic confirmatory disc diffusion method.

As per guideline provided by CLSI discs of cefotaxime (30 mgm), ceftazidime (30 mgm) , cefotaxime (30 mgm) + clavulanic acid (10 mgm), ceftazidime (30 mgm) + clavulanic acid (10 mgm) are kept on Muller Hilten agar plate. Ceftazidime and Cefotaxime discs are obtained from Himedia laboratory, Mumbai. Discs of ceftazidime + clavulanic acid and cefotaxime + clavulanic acid were obtained from Becton Dickenson Microbiology System USA.

If there is increase of 5 mm or more in discs containing Clavulanic acid in comparison of the respective antibiotic disc; then the isolate is confirmed as ESBL producer while in cases of no increase in the zone diameter it is presumed to be ESBL non producer.

MIC Reduction Test

The MIC of the isolates in the presence of 4 gm/ml Clavulanic acid was determined and matched against the MIC of antibiotic without Clavulanic acid combination. Epsilometer test (E-test) was obtained from Bio Merieux, Delhi. Any isolates showing reduction of 3 fold or more than that, double dilution range in MIC with the clavulanic acid combination than the antibiotic alone then it is ESBL producer on the basis of MIC reduction test. After 24 hours of incubation, an elliptical zone of inhibition is produced and the point at which the ellipse meets, the strip gives a reading for the minimum inhibitory concentration of the drug.

RESULTS

One hundred E.coli isolates were obtained from various wards of the hospital. Majority of the isolates obtained were from Medicine and Surgery wards. The lowest numbers of isolates were obtained from Obstetrics and Gynecology wards; it may be due to less frequency of samples being received. In the present study out of total 100 E.coli isolates tested, 68 isolates found ESBL producer and 32 were ESBL non producer.

Table 1 : Ward wise Prevalence of ESBI producer isolates from Total ESBL Producer E.coli isolates

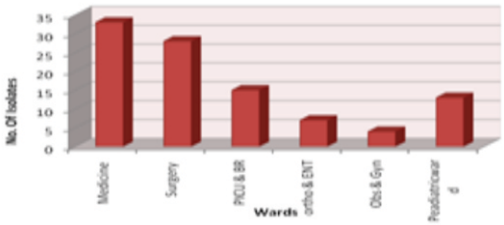


Fig.1: Isolate Showing Negative Phenotypic Disc Diffusion Confirmatory Test

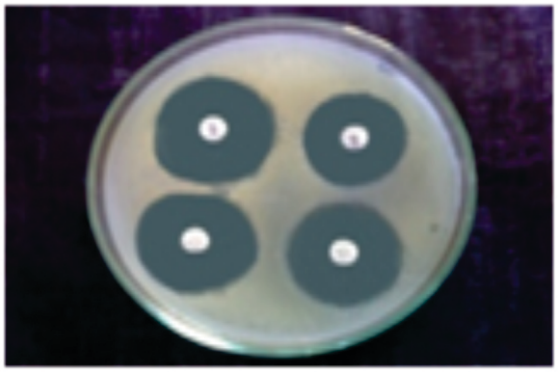
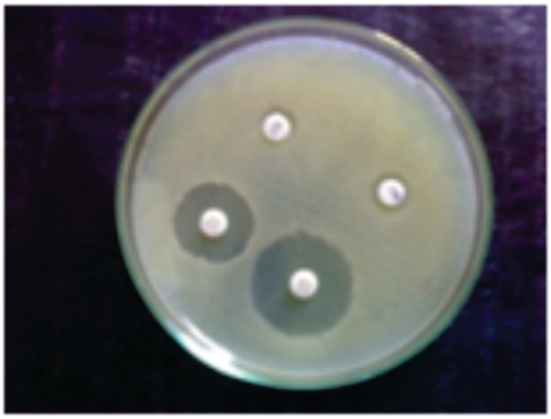


Fig.2: Isolate Showing Positive Phenotypic Disc Diffusion Confirmatory Test



DISCUSSION

In the last fifteen years ESBLs have gone from being an interesting scientific observation to a reality of great medical importance. Since the discovery of β-lactamase, till date the variety has expanded a lot and it does not shows any sign of slowing down. Most likely it is caused by overuse of Extended Spectrum Cephalosporins as broad spectrum empirical therapy. Numerous methods have been proposed for the detection of ESBL in different setup but it is imperative to note that none of the methods based on phenotypic expression of ESBL detects each and every ESBL producing isolates.

The routine resistance being observed in the routine sensitivity work was higher to the β-lactamase as compared to other compounds. E.coli is one of the most common members found to be pathogenic in Enterobactriaceae family and the occurrence of ESBL in E.coli isolates of our setup was not worked out before.

Several factors govern the ESBL production and multiple drug resistance in clinical isolates; like age, immune status, duration of hospital stay, duration of empirical therapy with broad spectrum antibiotics are to name a few.

CONCLUSION

ESBL producing isolates shows multiple drug resistance compared to ESBL non producer isolates. Isolates were comparatively more resistant to Cefotaxime, suggestive of probable presence of CTX-M type ESBL as major class of total prevalence.

Data suggest that the isolate on addition of Clavulanic acid reduces MIC up to significant level. In case of the isolates showing higher resistance to Ceftazidime and Cefotaxime there is dramatic reduction in MIC on addition of 4 mg/ml of Clavulanic acid which is presumptive of higher expression of ESBL enzyme which is inhibited well by Clavulanic acid. The alternate treatments for ESBL producing isolate are Carbapenems and Quinolone, as quoted by Clinical Microbiology Reviews, Oct, 2005; institutional discretion for its application is recommended for clinical evaluation.

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