Introduction

Gram negative bacteria can cause serious infection in hospitalized patients. Treatment of these infections is often lengthy and get complicated because of the increasing bacterial resistance mediated by varying degrees of beta lactamase enzymes. Extended-spectrum β-lactamases (ESBLs) are Ambler class A penicillinases, which confer resistance to and hydrolyze the expanded spectrum cephalosporins like ceftazidime, cefotaxime, monobactam-aztneronam and related oxyimino β-lactams as well as older penicillins and cephalosporins. They arise from mutations in the genes for common plasmid-mediated β-lactamases, especially Temoniera (TEM) and sulfhydryl variable (SHV) enzymes, which alter the configuration of the enzyme near its active site to increase the affinity and hydrolytic ability of the β-lactamase for oxyimino compounds while simultaneously weakening the overall enzyme efficiency. Widespread use of third generation cephalosporins and aztreonam is the major cause of the mutations leading to emergence of ESBLs. ESBL occur predominantly in Klebsiella spp. and Escherichia coli but have also been increasingly reported in other genera of the family Enterobacteriaceae. ESBLs are encoded by transferable conjugative plasmids which often code resistant determinants to other antibiotics. The plasmid-mediated resistance against cephalosporins can spread among related and unrelated gram-negative bacteria. ESBLs are mostly the products of point
mutations at the active site of TEM and SHV enzymes. Nosocomial outbreaks of infections caused by ESBL-producing gram-negative bacteria have also been reported, which are mainly the result of extensive and inappropriate use of third-generation cephalosporins. The major risk factors implicated are long-term exposure to antibiotics, prolonged ICU stay, nursing home residency, severe illness, instrumentation, or catheterization.

The aim of this study is to evaluate the utility of ceftazidime+clavulanic acid as a better method for detection of extended spectrum beta lactamases (ESBL) in the family Enterobacteriaceae.

Materials and Methods

The present study was conducted in the Department of Microbiology, Pt. B.D. Sharma PGIMS, Rohtak over a period of one year (February 2014 to January 2015). A total of 500 isolates of family Enterobacteriaceae were obtained from various clinical samples collected from patients, irrespective of age and sex. The samples included were urine, pus, blood, body fluids, sputum, CSF, high vaginal swabs (HVS), stool, and throat swabs. Organism identification was done according to the standard microbiological protocol. ESBL detection was done by combined disc method. A disc of ceftazidime (30µg) alone and ceftazidime + clavulanic acid (30µg/10µg) and cefotaxime (30µg) alone and cefotaxime + clavulanic acid (30µg/10µg) were placed at a distance of 25 mm, centre to centre on Muller Hinton agar plate inoculated with a bacterial suspension of 0.5 MacFarland turbidity standard and incubated overnight at 37°C (Fig 1). An increase in inhibition zone diameter of ≥ 5 mm for the combination disc versus ceftazidime or cefotaxime alone confirmed ESBL production.

Data collection and Statistical Analysis

At the end of the study, results were collected and analysed by using Chi-square test and p value tests was calculated by SPS software version 20.0 The prevalence of ESBL was calculated by the following formula.

Results and Discussion

A total of 500 isolates of Enterobacteriaceae were included in the study. Maximum rate of isolation of members of Enterobacteriaceae was from urine samples (39%), followed by blood (25%), pus samples (11.2%), stool samples (8.4%), sputum samples (6%), body fluids samples (4.8%), throat swab samples (2.4%), HVS (2%) and CSF samples (1%). The majority of patients from which members of the family Enterobacteriaceae were isolated belonged to age group of 21-30 years in both sexes, followed by 31-40 years and 11-20 years of age group in both sexes. Majority of isolates recovered were E. coli (41.6%), followed by K. pneumonia (32%), C. freundii (8.4%), C. koseri (6.4%), E. aerogenes (6.2%), E. cloacae (2.4%), P. vulgaris (1.8%), and P. mirabilis (1.6%). Of the 500 isolates tested, ESBL was detected in 226 (45.2%) of isolates by combined disc method. The use of ceftazidime (30µg) and ceftazidime+ clavulanic acid (30µg/10µg) detected 226 (45.2%) isolates as ESBL positive as compared to cefotaxime (30µg)
alone and cefotaxime + clavulanic acid (30µg/10µg) which detected 180 (36 %) isolates as ESBL positive and the results were statically significant (p<0.05).

<table>
<thead>
<tr>
<th>Isolates</th>
<th>ESBL +ve</th>
<th>Ceftazidime± Clavulanic acid</th>
<th>Cefotaxime± Clavulanic acid</th>
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<tr>
<td>500</td>
<td>226(45.2%)</td>
<td>226 (45.2%)</td>
<td>180(36%)</td>
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Fig.1 Showing Zone Enhancement (> 5 mm) by the use of Cefazidime+ Clavulanic acid (CAC) in comparison to Ceftazidime (CAZ) alone Similarly by using Cefotaxime + Clavulanic acid (CEC) in Comparison to Cefotaxime (CTX) alone

Antibiotic resistance surveillance has a central role among all strategies to manage the problem of antibiotic resistance. Since their first description in the mid 1970s, ESBLs have been isolated worldwide and form a major contributor of drug resistance in many genera of Enterobacteriaceae. The present study reported 45.2% prevalence of ESBL by combined disc method. The use of ceftazidime± clavulanic acid detected more ESBL as compared to cefotaxime ± clavulanic acid indicated the former to be a more sensitive indicator. Zali et al also reported higher sensitivity of ceftazidime ± clavulanic acid as compared to cefotaxime ± clavulanic acid. The result of present study were in accordance with the study conducted by Shoorasheety et al. who reported 41 % ESBL producing strains. Similarly Mita et al. reported 43 %ESBL producing strains. Jaishree et al. reported 53.2 %ESBL producing strains. Similarly Vishwanath et al. reported 57.5 %isolates as producing ESBL. Giriyapur et al. reported 63.89 %of ESBL positive strains. However, Rawat et al. demonstrated 18.6%of ESBL strains . This difference could be attributed to the fact that all gram negative bacilli were included.

References

1. Upadhyay S, Sen MR, Battacharjee A. Presence of different beta- lactamase
classes among clinical isolates of Pseudomonas aeruginosa expressing AmpC beta-lactamase enzyme. J Infect Dev Ctries 2010;4:239-42


