Original Article

Detection of ESBL and Co-production of AmpC in various samples by DDST, PDCT, MDDST method at a tertiary care hospital in Kanpur.

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ABSTRACT

BACKGROUND : The increase prevalence of Enterobacteriaceae producing ESBLs creates a great need for identifying these organisms for infection control and epidemiological surveillance. The aim of this study was to find a better technique for detection of ESBLand its co–producers with AmpC. **MATERIALS AND METHOD :**A total of 100 isolates of GNB were isolates from various sample over a period of 6 month from November 2017 to April 2018 were included in the study. The isolates were simultaneously screened for ESBL and AmpC production .ESBL production was confirmed by original double disk synergy test ,phenotypic disc confirmatory test (PDCT) and modified double disk synergy test (MDDST), and the results were compared. **RESULT:** All the100 isolates were screened from both ESBL and AmpC β-lactamases production among these 65 were screened positive for ESBL and 45 screened positive for AMPc. The most common ESBL produces were isolates. Escherichia coli (65),Klebsiellaspp (18),proteusspp(2),Citrobacterspp(2),Pseudomonas spp(8),and Acinetobacter(5). Out of 65 isolates were screened for ESBL 40 were confirmed positive by confirmatory test used DDST or MDDST. DDST detect ESBL in only 15. While 25 were ESBL positive isolated detected by MDDST. Out of 45 AmpC production was detected only in 20 by Disk impreganated method . All the 10 isolates which were additional detected ESBL producers by MDDST shows positive Disk impregnation method i.e AmpC co-producers by DDST method of ESBL detection of the isolates were AmpC positive . In MDDST cefepime was the best cephalosporin in detecting

ESBL in presence of AmpC production. **CONCLUSION :** From this study we conclude that ,Using only one disk combination might fail to detect ESBL production .The MDDST using cefepime and piperccillin-tazobactum gives the highest sensitivity followed by MDDST using cefepime and amoxicillin –clavulanate.

KEYWORDS: ESBL, AmpC , PCDDT, DDST ,MDDS,Phenotypic method ,Resistance.

Introduction

The increase prevalence of Enterobacteriaceae producing ESBLs creates a great need for identifying these organisms for infection control and epidemiological surveillance. The aim fo this stuhy was to find a better technique for detection of ESBLand its co-producers with AmpC [1]. Extended spectrum β-lactamases (ESBLs) and AmpC β-lactamases are of growing concern to microbiologists for accurate detection and to clinicians for conferring resistance to several antibiotics resulting in treatment failure. They are most commonly produced by *Klebsiella* spp. and *Escherichia coli* but may also occur in other gram negative bacteria. Extended spectrum β-lactamases (ESBLs) are typically plasmid-mediated enzymes that hydrolyze penicillins, third generation cephalosporins and aztreonam [2].

Material and Methods

A total of 100 isolates of GNB were isolates from various sample over a period of 6 month from November 2017 to April 2018 were included in the study. The isolates were simultaneously screened for ESBL and AmpC production .ESBL production was confirmed by original double disk synergy test ,phenotypic disc confirmatory test (PDCT) and modified double disk synergy test (MDDST), and the results were compared. AST was done to according CLSI guideline[3].

Result

All the 100 isolates were screened from both ESBL and AmpC β-lactamases production among these 59 were screened positive for ESBL and 41 screened positive for AmpC. The most common ESBL producing isolates were. Escherichia coli(69), Klebsiella spp (19), Proteus spp(2) ,Citrobacterspp(2), Pseudomonas spp(8). Out of 65 isolates were screened for ESBL 40 were

confirmatory test used DDST or MDDST detected ESBL in only 15. While 25 were ESBL positive isolated detected by MDDST. Out of 45 AmpC production was detected only in 20 by Disk impregnated method. All the 10 isolates which were additionally detected as ESBL producers by MDDST shows positive disk impregnation method i.e. AmpC co-producers. By DDST method of ESBL detection of the isolates were AmpC positive. In MDDST cefepime was the best cephalosporin in detecting ESBL in presence of AmpC production.

Fig:1 –Percentage of isolates positive for ESBL and AmpC

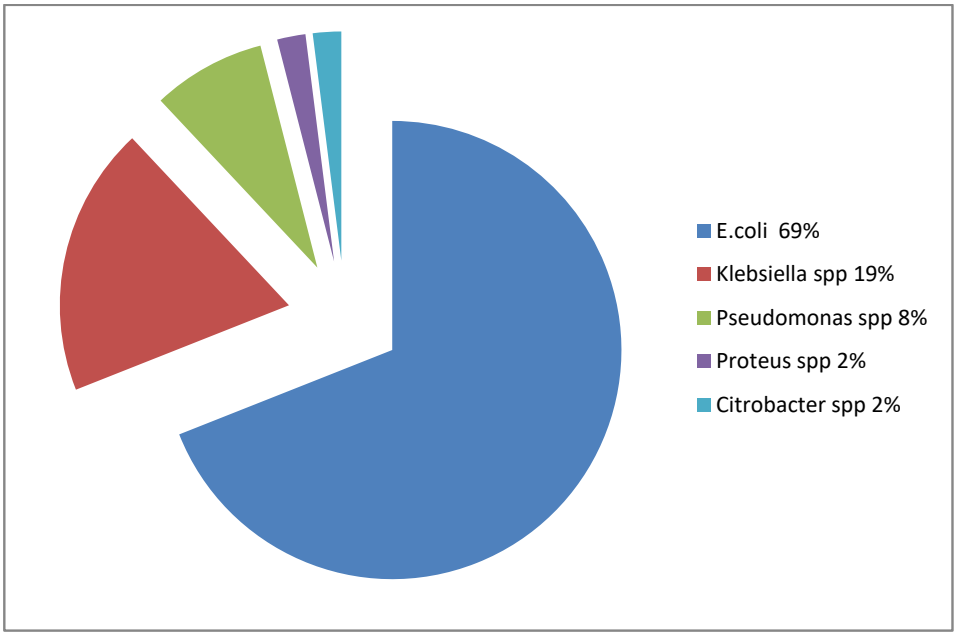

Fig :2-Most common isolates among ESBL producers

Discussion

ESBL producing organisms are of ever increasing concern since their first description more than 20 years ago. Prevalence rate of 35%-85% has been demonstrated in various Indian studies . A study from Uganda revealed ESBL production in 62% isolates [4] . In Latin America upto 32% of *Escherichia coli* and upto 58% of *Klebsiella pneumoniae* isolates are ESBL positive [5] .

Systemic infections due to ESBL-producing Enterobacteriaceae were associated with severe adverse clinical outcomes. Primarily characterized in limited bacteria such as *Escherichia coli* and *Klebsiella* spp, ESBLs have been spreading and reaching other genera, principally *Enterobacter* and *Proteus* spp.

AmpC- producing organisms can act as hidden reservoirs for ESBLs. So it is important for clinical microbiology laboratories to be able to detect ESBL production in these organisms on a routine basis for epidemiological and therapeutic purposes. AmpC β-lactamases are resistant to β-lactamase inhibitors like clavulanic acid and hence the augmentation in zone diameter in DDST by ESBL producers can be completely masked by AmpC enzymes. In order to detect ESBLs in isolates that co-produce AmpC β-lactamase, modification of DDST by using combination of cefepime and piperacillin-tazobactam or incorporation of inhibitors of AmpC enzyme like boronic acid compounds, cloxacillin and novel inhibitors such as Syn2190 have been recommended [6,7]. In our study In MDDST cefepime was the best cephalosporin in detecting ESBL in presence of AmpC production.

In the study conducted by K. Jaspal etal., [8] Cefepime was the best cephalosporin in detecting ESBL in presence of AmpC as it is less affected by AmpC β-lactamases. Cefotaxime and ceftazidime were not able to show any potentiation of zones in presence of β lactamase inhibitor in AmpC co-producers as the AmpC β-lactamase gave a resistant zone to the cephalosporin which was similar to our study.

Although there are no CLSI guidelines for phenotypic methods to screen and detect AmpC, several methods have been developed for detection of AmpC. Reduced susceptibility to cefoxitin is taken as indicator of AmpC production However, reduced susceptibility to cefoxitin can also be due to reduced outer membrane permeability.

Conclusion

The frequency of ESBLs can easily be underestimated in clinical isolates using the current CLSI recommended methods i.e. DDST and PDCT since these organisms often produce multiple β-lactamases. In such situations, where AmpC β-lactamases can interfere with clavulanate synergy, the modification of double disc synergy tests that combine piperacillin-tazobactum with cefepime may increase the possibility of ESBL detection.

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