Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883 (Online)**
Sch. Acad. J. Biosci., 2014; 2(4): 268-271 **ISSN 2347-9515 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com **DOI:** 10.36347/sajb.2014.v02i04.005

Research Article

Prevalence of Extended Spectrum Beta Lactamase Producing Strains of *Klebsiella pneumoniae* and *Escherichia coli* Isolated from Clinical Samples, at VIMS, Bellary

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Abstract: Infections caused by Extended Spectrum Beta Lactamase (ESBL) producers are on the rise worldwide, which increase the pressure to the use of carbapenems or cephamycins and betalactamase inhibitors as a combination therapy, depending on sensitivity result and also cause increase in hospital stay ,delay in appropriate therapy, increase in health care costs and higher morbidity and mortality, as they are resistant to penicillins, third generation cephalosporins and aztreonam. These infections are difficult to control, as they are usually associated with aminoglycoside and multidrug resistance. Hence detection of prevalence helps in management of the disease as well as containment of further spread of these organisms. The objective of present study is to determine the prevalence of ESBL producers among the *klebsiella pneumoniae* and *Escherichia coli* isolates from various clinical samples. A total of 261 strains of *klebsiella pneumoniae* (161) and *Escherichia coli* (100) isolated from various clinical samples between January – June 2012 were included in the study. They were studied for ESBL production by Phenotypic Confirmatory Disk Diffusion Test (PCDDT) and by Double Disk Synergy Test (DDST). It is found that ESBL producers detected by PCDDT were 48% (50% in *klebsiella pneumoniae* and 46% in *Escherichia coli*) and 45% by DDST (48% in *klebsiella pneumoniae* and 42% in *Escherichia coli*). The results indicate a high prevalence rate of ESBL producers and that detection by PCDDT is better than DDST. Routine antibiotic susceptibility test may fail to detect ESBL producers. PCDDT is simple and cost effective for the detection of ESBL production and hence should be routinely employed in diagnostic laboratories.
Keywords: ESBL Producers, Double Disk Synergy Test, Phenotypic Confirmatory Disk Diffusion Test, *E.coli, Klebsiella pneumoniae* .

INTRODUCTION

Infections by Extended Spectrum Beta Lactamase (ESBL) producers are on the rise worldwide, leading to serious opportunistic and nosocomial infections [1, 2]. ESBLs are a super family of active site serine proteases capable of conferring bacterial resistance to penicillins, Cephalosporins (I , II , and III generation) and Aztreonam [3]. ESBLs arise by point mutations in genes for common plasmid-mediated β-lactamases TEM-1/-2 and SHV-1 [1,4]. In recent years, non-TEM and non-SHV plasmid-mediated ESBLs are increasingly being reported, mainly the CTX-M enzymes [1]. They are inhibited by beta lactamase inhibitors such as Clavulanate [2]. Since ESBL-positive isolates show false susceptibility to extended-spectrum Cephalosporins in standard disk diffusion test, it is difficult to reliably detect ESBL production by the routine disk diffusion techniques [4].

Hence the present study was undertaken to know the prevalence of ESBLs among the *E. coli* and *K. pneumoniae* isolates and to compare the antibiotic susceptibility pattern of ESBL producers with that of non-ESBL producers.

MATERIALS AND METHODS

The study was conducted from January – June 2012.A total of 261 strains [*klebsiella pneumoniae* (161) and *Escherichia coli* (100)] isolated from various clinical samples obtained from VIMS, Bellary were studied. Confirmation of the strains was done by their colony morphology on MacConkey agar and Chocolate agar & by standard biochemical tests [5,6].

Antibiotic Susceptibility Testing [1,5,6].

Kirby-Bauer disk diffusion method was done and the antibiotics tested included: Ampicillin (10 µg), Amoxyclav (20/10 µg), Cefepime (30 µg), Piperacillin – Tazobactam (100/10 µg), Gentamicin (10 µg), Levofloxacin (5 µg), Amikacin (30 µg), and Imipenem (10 µg). III generation (3 GC) Cephalosporins –Cefotaxime and Ceftazidime each 30 µg was used. They were then studied for ESBL production.

Double Disk Synergy Test (DDST) [2].

Done to determine synergy between a disc of augmentin and each of III generation Cephalosporin drugs. The III generation Cephalosporin drugs were placed 15 mm center to center from augmentin disks. Increase in inhibition zone towards augmentin disk or if neither discs were inhibitory alone but inhibited growth where they diffuse was considered as ESBL producers.

Phenotypic Confirmatory Disk Diffusion Test (PCDDT) [7].

Cefotaxime and Ceftazidime 30 µg discs alone and in combination with clavulanic acid 10 µg were used. An increase in zone > 5 mm diameter for either drug in combination with clavulanic acid as compared to the drug used alone was considered as ESBL producer.

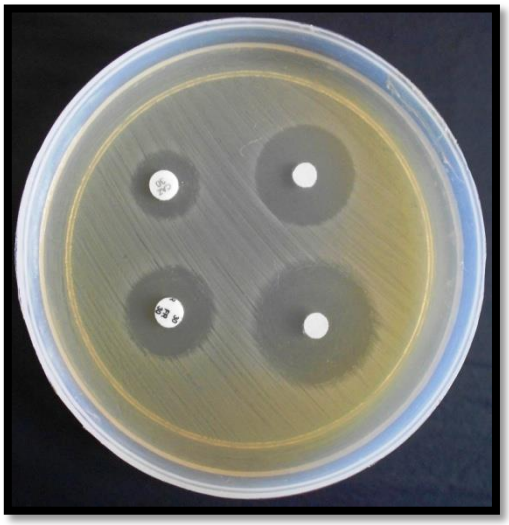


Fig-1:Phenotypic Confirmatory Disk Diffusion Test – PCDDT

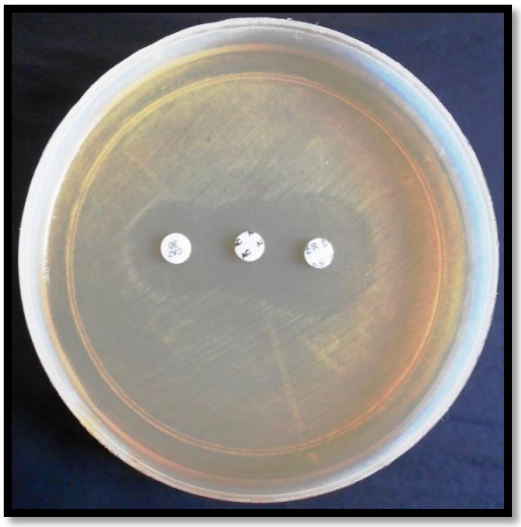
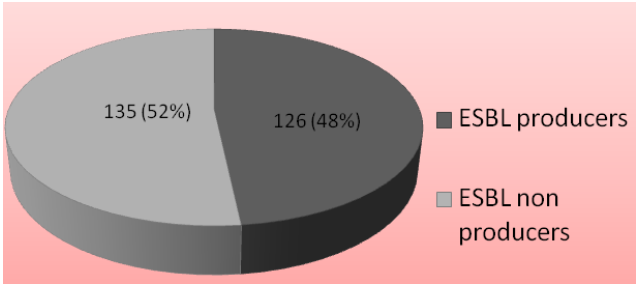


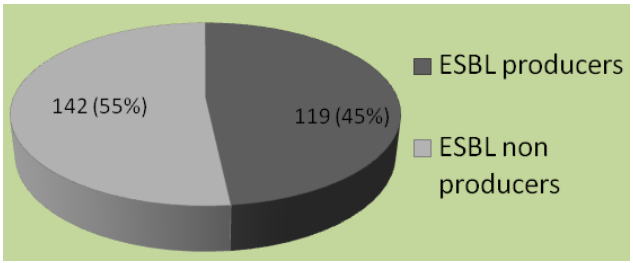
Fig-2:Double Disk Synergy Test (DDST)

RESULTS

In the present study out of the total 261 isolates studied, the number of ESBL producers as detected by PCDDT were 126 (48%) and by DDST it was 119 (45%), as shown in graphs 1 and 2. Among the *K. pneumoniae* isolates, 80 (50%) and 77 (48%) were ESBL producers as detected by PCDDT and DDST respectively. Among the *E. coli*isolates, 46 (46%) and 42 (42%) were ESBL producers as detected by PCDDT and DDST respectively, as shown in tables 1 and 2. The antibiotic susceptibility patterns of the isolates (ESBL non producers and ESBL producers) were analyzed and shown in graphs 3 and 4. It is noted that Non ESBL producers show lower resistance patterns to the antibiotics as compared to the ESBL producers.



Graph 1: ESBL Producers As Detected By PCDDT



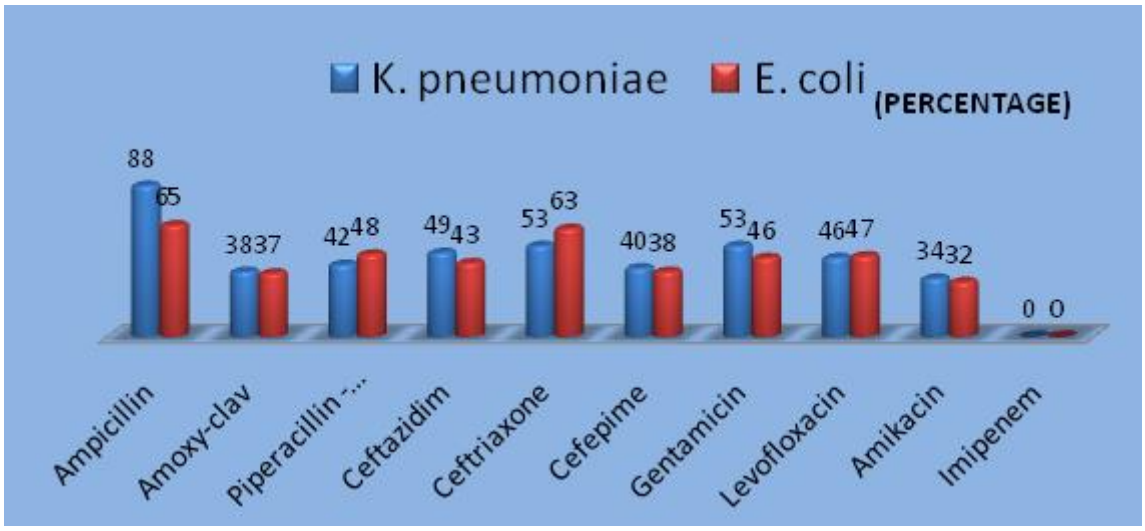
Graph 2: ESBL Producers As Detected By DDST

Table 1: Phenotypic Confirmatory Disk Diffusion Test

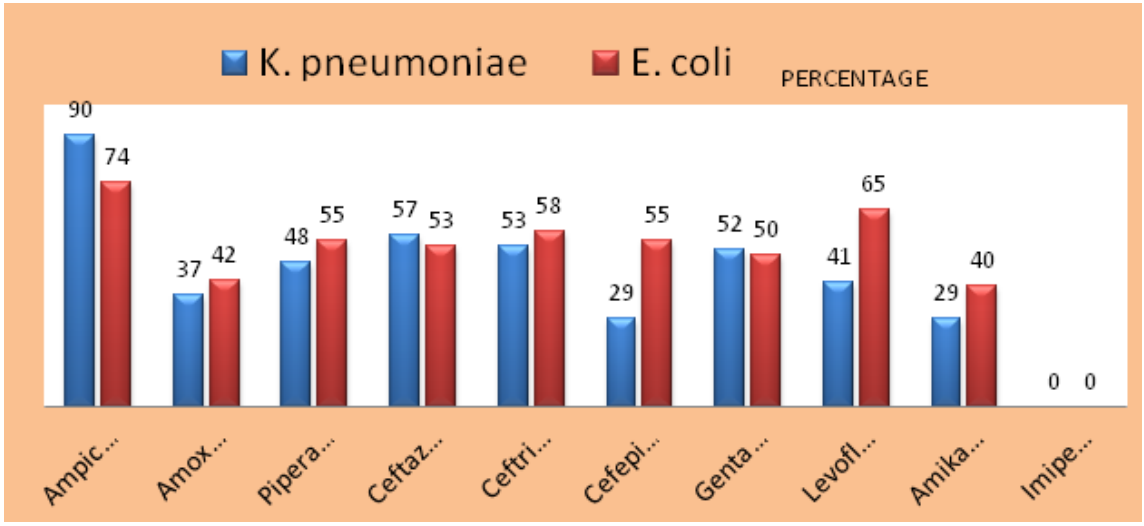
ORGANISMS	ESBL Producers	ESBL Non Producers	TOTAL
E. coli	46 (46%)	54 (54%)	100
K. pneumoniae	80 (50%)	81 (50%)	161
TOTAL	126 (48%)	135 (52%)	261

Table 2: **Double Disk Synergy Test (DDST)**

ORGANISMS	ESBL Producers	ESBL Non Producers	TOTAL
E. coli	42 (42%)	58 (58%)	100
K. pneumoniae	77 (48%)	84 (52%)	161
TOTAL	119 (45%)	142 (55%)	261



Graph 3: Antibiotic Resistant Patterns of Non-ESBL producers



Graph 4: antibiotic resistant patterns of ESBL producers

DISCUSSION

Extended spectrum β-lactams are commonly included in the empirical antibiotic regimens for treatment of gram negative sepsis and other serious infections [8]. Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* have spread rapidly worldwide and pose a serious threat in healthcare-associated infections, leading to serious opportunistic infections and also treatment failures [1, 9]. These infections are difficult to control as they are associated with aminoglycoside and multidrug resistance [10]. In the present study a high prevalence of ESBL producers (48%) are detected. The ESBL producing strains are also highly resistant to other first and second line antibiotics that are routinely used. The resistance patterns of ESBL producers are higher compared to that of non ESBL producers, with only few options available like Imepenem and Amikacin. The isolates which have a positive phenotypic confirmatory test for ESBL production should be reported as resistant to all cephalosporins (except cephamycins, cefoxitin, and cefotetan) and aztreonam, regardless of the MIC of that particular cephalosporin [8]. Penicillins are reported as resistant and β-lactam/β-lactamase inhibitor combinations are reported as susceptible if the zone diameters are within the appropriate range [11].

CONCLUSION

The results indicate a high prevalence rate of ESBL producers. Detection of ESBL producers by PCDDT is better than DDST. PCDDT is simple and cost effective for the detection of ESBL production and hence should be routinely employed in diagnostic laboratories. Routine surveillance is essential for containment of further spread of multidrug resistant strains.

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