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Detection of Extended-Spectrum Beta-Lactamase (ESBL) Producing Entrobacteriaceae from Pus Samples and Antibiotic Co-Resistance in a Tertiary Care Hospital, Uttarakhand

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ABSTRACT

Background: The present study was designed to detect ESBL producing Enterobacteriaceae and determine their antibiotic susceptibility pattern in pus samples received in the Microbiology laboratory of our tertiary care hospital. Extended spectrum beta-lactamase (ESBL) producing Enterobacteriaceae are responsible for causing serious health-care associated infections worldwide. They pose a major therapeutic challenge because they tend to be multidrug resistant resulting in limited options for treatment of infections caused by them. **Methods:** A total of 1334 pus samples were received in the Microbiology laboratory of our tertiary care hospital from October 2013 to September 2014. ESBL Enterobacteriaceae organisms were identified by conventional methods and antimicrobial susceptibility testing was done by Kirby Bauer's disc diffusion method and the results were interpreted according to Clinical Laboratory Standards Institute-2012 guidelines. **Results:** Out of 1334 pus samples processed, out of which 378 (86.69%) were obtained Enterobacteriaceae isolates. A total of 123 (32.53%) ESBL producing Enterobacteriaceae obtained [Escherichia coli (47.15%), Klebsiella pneumonia (29.26%), Klebsiella oxytoca (2.43%), Proteus mirabilis (12.19%), Proteus vulgaris (4.06%), Citrobacter freundii (2.43%), Enterobacter aerogenes (1.62%) and Providencia rettgerii (0.81%)]. A high degree of resistance to co-trimoxazole (96.74%) followed by gentamicin (78.04%) and Ciprofloxacin (34.95%) was found. The susceptibility of these strains ware found 100% to Imipenem followed by Amikacin (75.61%). **Conclusion:** In the present study, Family Enterobacteriaceae isolates which producing ESBL enzyme found in large number. Most of the ESBL producing Enterobacteriaceae members showed resistant to different groups of antibiotics.

Keywords: Aminoglycosides, Escherichia coli, Multi drug resistance, Nosocomial infection.

INTRODUCTION

Resistant bacteria are emerging worldwide which is threat to the community and hospital settings. The outbreaks of infection in various hospitals globally have been supplanted by endemicity of ESBL producers. This may lead to increased patient mortality when antibiotics inactive against ESBL producers are used.- Many bacteria belongs to Enterobacteriaceae family carry beta-lactamases enzymes which are responsible for resistance to the beta-lactam group of antibiotics such as penicillins, cephalosporins, cephamycins, and carbapenems. These enzymes hydrolyze of the amide bond of four-membered beta-lactam ring and render the antibiotic inactive against its original cellular target, the cell wall transpeptidase.[2] Pyogenic infections are one of the common hospital infections caused by members of Enterobacteriaceae. The main risk factors are long term antibiotic exposure, hospital stay, severe illness and instrumentation.[3] The use of the third-generation cephalosporin in clinical practice early 1980s was heralded as a major breakthrough in the fight against β-lactamase mediated bacterial resistance to antibiotics.[4] Knowing resistance pattern of such multidrug resistant pathogens will help in the appropriate usage of antimicrobial agents and prevent further emergence of resistant strains. ESBLs are derived from genes for the narrow-spectrum beta-lactamases (TEM, SHV and CTX-M) by mutations that alter the amino acid configuration around the enzyme active site. They are typically encoded by plasmids that can be exchanged readily between bacterial species.[5] These enzymes are most commonly produced by the members of the Enterobacteriaceae. Now days, more than 350 different natural ESBL verities based on their amino acid sequence have been classified into nine distinct families comparises such as TEM, SHV, CTX-M, PER, VEB, GES, BES, TLA, and OXA.[5-8] ESBL-producing Enterobacteriaceae have been responsible for numerous outbreaks of infection throughout the world and pose challenging infection control issues. Data based on clinical outcome indicate that ESBLs are clinically significant, and hence it is essential to know the prevalence of ESBL producing strains in a

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geographical area because it can help in judicious use of antibiotics and can guide the empirical therapy in high risk units.

MATERIALS AND METHODS

The descriptive study was carried out in Government medical college and Hospital, Haldwani in between November 2013 to September 2015. A total of 1334 pus sample collected from OPD and ward patients as abscess, post operative wound, sepsis, Cellulites, Ulcer, Injuries, and Burns patients. Identification of Enterobacteriaceae organisms were identified by conventional biochemical methods and antimicrobial susceptibility testing was done by Kirby Bauer's disc diffusion method.[9,10] Antibiotics penal were includes Levofloxacin (5µg), Ciprofloxacin (5µg), Ceftriaxone (30µg), Cefotaxime (30µg), Ceftazidime (30µg), Amoxyclav (30µg), Aztreonam (30µg), Gentamicin (10µg), Imipenem (10µg). All Antibiotic discs were obtained from Hi-Media, Mumbai, India.

Screening test for ESBL producing Enterobacteriaceae: All isolates of family Enterobacteriaceae detected from pus were tested for resistance to Aztreonam (30µg) and the third generation cephalosporins namely Cefotaxime (30µg), Ceftriaxone (30µg) and Ceftazidime (30µg) by the Kirby-Bauer disc diffusion method. This was a part of the routine antimicrobial susceptibility testing. Resistance to at least one of the antibiotics was considered as positive in the screening test for possible ESBL production as per 2012 CLSI guidelines.[11]

Confirmatory tests:

1. The double disc synergy test (DDST):An Amoxyclav disc (amoxicillin/clavulanic acid 30/10µg) was placed at the centre of the plate. Cephlosporins discs containing 30µg were placed 20 mm away from the central disc. Distances were measured from the centre of one disc to that of the other disc. Plates were incubated overnight at 370C. Enhancement zone cephalosporins discs and Amoxyclav disc was considered as a positive result.
2. Phenotypic confirmatory disc diffusion test (PCDDT):The ceftazidime (30µg) discs alone and in combination with Clavulanic acid (ceftazidime + Clavulanic acid, 30/10µg disc) were applied onto a plate of Mueller Hinton Agar (MHA).After overnight incubation at 370C.an increase of ≥5mm in the zone of inhibition of the combination disc in comparison to the ceftazidime disc alone was considered to be a marker for ESBL production.
3. E-test:The MICs were determined by E-test ESBL strips (Hi-Media Laboratories Pvt. Limited, Mumbai). The E-test ESBL strips were impregnated with Cefotaxime (CTX) at one end and Cefotaxime + Clavulanic acid (CTX+) at another end. The MIC was interpreted as the value at the intersection of the growth ellipse with the strip. The isolate was confirmed to be an ESBL producer when the ratio of the MIC value of Cefotaxime to the MIC value of cefotaxime in combination with clavulanic acid was more than 8 and no zone was obtained for cefotaxime but zone was observed in cefotaxime and clavulanic acid combination.

Escherichia coli ATCC 25922 was used as the negative control and ESBL-producing Klebsiella pneumonia ATCC 700603 was used as the positive control for all phenotypic methods.

Data entry and Static analysis: It is a descriptive study. All data among the ESBL producing Enterobacteriaceae will be done and quantified in number, Percentage and tabulated form.

RESULTS

A total of 1150 isolates were obtained out of 1334 pus sample. Of these, 436 isolates were gram negative bacilli (GNB). Out of 436 GNB isolates, 378 isolate were belongs to Enterobacteriaceae family, in which 123; 32.53% [Table 1] were ESBL producers accounting E. coli (47.15%), K. pneumonia (29.26%), K. oxytoca (2.43%),Proteus mirabilis (12.19%), Proteus vulgaris(4.06%), Citrobacterfreundii (2.43%), Enterobacteraerogenes (1.62%), Providencia spp. (0.81%), ESBL producing organisms are depicted in [Table 2]. Among ESBL positive strains, more drug resistance was seen to Co-trimoxazole (96.74%) followed Gentamicin (78.04%) and Ciprofloxacin (34.95%). All these ESBL Enterobacteriaceae strains ware sensitive to Amikacin (75.61%) and Imipenem (100%). Antibiotic Resistance pattern of ESBLs producing organisms is depicted in [Table 3].

Table 1: Distribution of ESBL and Non ESBL Enterobacteriaceae

Gram negative organisms	Total Enterobacteriaceae isolates N=378 (86.69%)		Other organisms
	ESBL Enterobacteriaceae	Non ESBL Enterobacteriaceae	
436	No=123 (32.53%)	No=255 (67.47%)	58

ESBL: Extended spectrum beta lactamase

Table 2: Distribution of ESBL producing organisms (n=123)

S.No	ESBLs producing Enterobacteriaceae	Numbers=123 (%)
1	Escherichia coli	58 (47.15 %)
2	Klebsiellapneumoniae	36 (29.26%)
3	Klebsiellaoxytoca	3 (2.43%)
4	Proteus mirabilis	15 (12.19%)
5	Proteus vulgaris	5 (4.06%)

6	Citrobacterfreundii	3 (2.43%)
7	Enterobacteraerogenes	2 (1.62%)
8	Providencia spp.	1 (0.81%)

ESBL: Extended spectrum beta lactamase

Table 3: Antibiotic Resistance pattern in ESBLs producing Enterobacteriaceae & Non ESBLs producing Enterobacteriaceae

S. No	Antibiotics	ESBL producing Enterobacteriaceae (n=123)	Non ESBL producing Enterobacteriaceae (n =255)
		Number (%)	Number (%)
1	Gentamicin	96 (78.04 %)	110 (43.13 %)
2	Amikacin	30 (24.39 %)	63 (24.70%)
4	Levofloxacin	24 (19.51 %)	60 (23.52%)
5	Ciprofloxacin	43 (34.95 %)	82 (32.15 %)
6	Co – trimoxazole	119 (96.74 %)	208 (81.56 %)
11	Imipenem	0 (0%)	0 (0%)

ESBL: Extended spectrum beta lactamase

Table 4: Prevalence of ESBL producing Enterobacteriaceae from different parts of India

S. N	Author	Year	Place of study	Percentage of ESBL Enterobacteriaceae
1	Hansotia et al.[15]	1997	Nagpur	25.8%
1	Kurana et al.[16]	2002	Chandigarh	38.5%
2	Neelumtaneja et al.[17]	2008	Chandigarh	40.2%
3	Vinod kumar et al.[18]	2004	Gulburga	13.5%
4	Present study	2017	Haldwani	32.53%

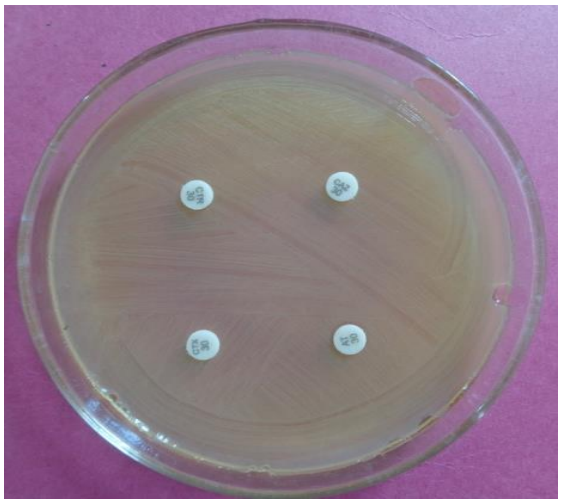

Figure 1: A photograph of an isolate showing resistance to all the four antibiotics in screening test for ESBL production

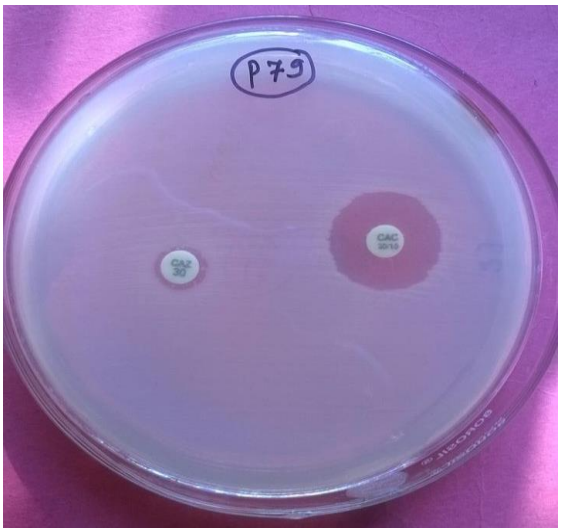

Figure 3: A photograph showing production of ESBL by Phenotypic confirmatory disc diffusion test

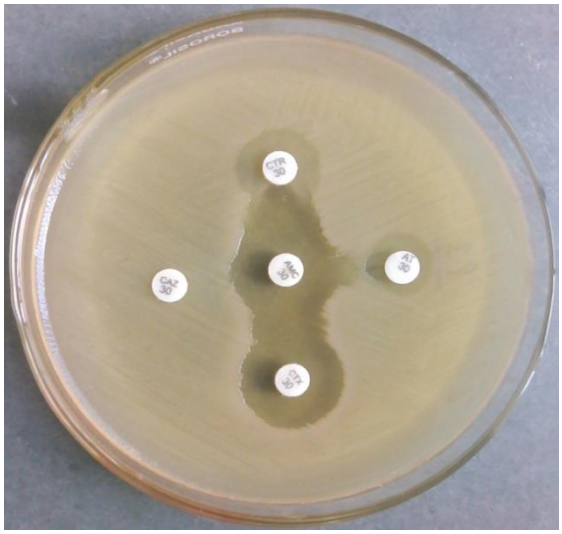

Figure 2: A photograph of an isolate showing ESBL production by Double disc synergy test

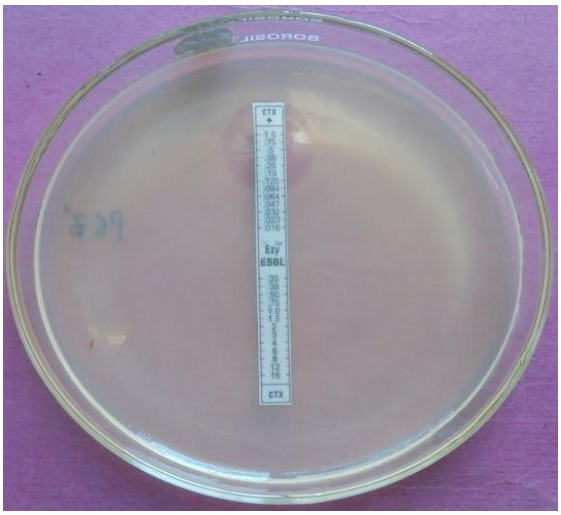

Figure 4: A photograph showing production of ESBL by E-test

DISCUSSION

Pyogenic infections are common conditions, for which patients seek medical attention. The emergence of multidrug resistance in bacteria has posed challenges in the management and control of wound infections, reducing treatment options and adding to the overall patient morbidity and mortality.[12] In the current era, ESBLs producing Enterobacteriaceae are a major problem in the hospital and community setting due to the increasing use of broad-spectrum antimicrobial agents e.g. beta-lactams and cephalosporins antibiotics.

In this study indicated that high prevalence of ESBL producing Enterobacteriaceae in pus sample was detected 32.53% as compared to the prevalence recorded by Padmaja et al.[13] and Shashwati et al.[14] both were showed a very high prevalence of ESBL producing Enterobacteriaceae 76% and 45.02% respectively. The high prevalence of ESBLsamong Enterobacteriaceae in India varies, [Table 4] probably due to the instrumentation, prolonged hospital stay and multiple courses or prolonged exposure to antibiotics may be a contributing factors. We have noticed high ESBL production in E. coli (47.15%) followed by K. pneumoniae (29.26%) where as Gupta et al.[19] detect ESBL Production in 44.1% of E. coli and 29.4 % in klebsiella sp. which is concordance to our study.

In our study, resistance shown to co-trimoxazole is maximum 96.74% and 81.56% in ESBL producing Enterobacteriaceae isolates and non ESBL producing Enterobacteriaceae respectively because commonly used in community as well as hospital settings in past. Gentamicin and Ciprofloxacin both are significantly resistance in ESBL producing Enterobacteriaceae because of commonly use in hospital. No resistance was found to Imipenem which is similar to the study conducted by N. Padmaja et al.[13] Because of reflects their less use for treatment of community acquired infections. We conclude that E. coli and Kleibsella spp. are most common ESBL producers and theses strains are shown a significant level of resistance. The PCDDT performed comparably well with the E-test. Clinicians be aware with ESBL producing Enterobacteriaceae member and should consider the probability of treatment failures with beta-lactam group of antibiotics.

CONCLUSION

Phenotypic methods are widely applied for the detection of ESBL producing organisms, particularly in rural and resource-constrained settings where the feasibility for application of molecular methods is not possible on routine basis. Majority of the Enterobacteriaceae isolates producing ESBLs also have showed high degree of resistance for various other antibiotic groups, which were routinely being used in our hospital setting. The multi drug resistant attribute being shown by these isolates is a cause of concern from clinician as well as patient's perspective, as they impose substantial pharmacotherapeutic and pharmacoeconomic burden. We realize that data extrapolated from our study may not be representative of the whole Uttarakhand State's scenario and must be interpreted cautiously. However, the findings of our study can be used as a template to optimize hospital antimicrobial policy and antimicrobial prescribing guidelines.

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