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ESBL Production: Resistance Pattern in *Escherichia coli* and *Klebsiella pneumoniae*, A Study by NCCLS method

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ABSTRACT

Various factors are involved in resistance to third generation cephalosporins by E. coli and K. pneumoniae. The objectives of present study were to study the resistance pattern due to ESBL's. The study comprised a total of 200 clinical specimens which included urine, pus, sputum, blood, throat swabs, suction tips and vaginal swabs. E. coli and K. pneumoniae isolates were studied for ESBL production by NCCLS method. Culture of 200 samples yielded 117 E. coli and 83 K. pneumoniae isolates. Among them, 98 (49%) were screen positive for ESBL. Among them 53 E. coli and 26 K. pneumoniae were found to produce ESBL. In that, 52 isolates of E. coli and 26 isolates K. pneumoniae were positive by NCCLS-PCT method. The present study observed ESBL production in 39.5% of cases. All the ESBL isolates were susceptible to imipenem, however 98.1% cases were resistant to cefoxitin. The present study observed higher prevalence of ESBL producing E. coli and K. pneumoniae. We suggest that routine screening of ESBL should be performed on all isolates which are showing decreased susceptibility to third generation cephalosporins.

Keywords: ESBL; NCCLS; PCT; cephalosporins; multidrug resistance

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INTRODUCTION

Penicillins and Cephalosporins remained the first line of defense against microbes for over 20 years until resistance due to β-lactamase produced by gram negative bacilli became a serious problem [1]. Many species of gram negative bacteria possess naturally occurring chromosomally mediated β-lactamases. Within a few years after its isolation, the TEM-1 β-lactamase spread was worldwide and is now found in different species of the members of family enterobacteriaceae, pseudomonas aeruginosa, hemophilus influenzae and neisseria gonorrhoeae [2]. Over the last 20 years, many new β-lactam antibiotics have been developed that were specifically developed to resistant for hydrolytic action of β-lactamases. However, with each new class that has been used to treat patients, new β-lactamases emerged that caused resistance to that class of drug [2]. Extensive use of newer generation cephalosporins has been the strong factor for evolution of newer β-lactamases such as ESBLs.

Major outbreaks involving ESBL producing strains have been reported from all over the world, thus making them emerging pathogens [3]. There have been sporadic reports of ESBL from major hospitals from India and some of them have recorded the incidence to be as high as 60-68% [4,5]. The ESBL are result of various mutations of TEM-1, TEM-2 and SHV-1, all of which are β-lactamase enzymes found in enterobacteriaceae family. Normally, TEM-1, TEM-2 and SHV-1 enzymes confer high level resistance to early penicillins and low level resistance to first generation cephalosporins [6,7]. The widespread use of third generation cephalosporins and aztreonam are believed to be the major cause of mutation in these enzymes that have led to the emergence of ESBLs [7-11].

In 1999, National Committee for Clinical Laboratory Standards (NCCLS) has published methods for screening and confirmatory methods for ESBL detection for K. pneumoniae, K. oxytoca and E. coli [12]. It was opined that the identification of prevalence of ESBL strains in a hospital is important to control their spread [3]. There are various factors involved in the resistance of E. coli and K. pneumonia to third generation cephalosporins. In the present study, the objectives were to study the resistance pattern due to ESBL's by NCCLS method.

MATERIALS AND METHODS

The present investigation comprised a total of 200 samples including 95 urine, 45 pus, 32 sputum, 11 blood, 9 throat swabs, 6 suction tips and 2 vaginal swabs. All the samples were processed immediately within two hours of collection. On MacConkey's media lactose fermenting colonies (pink flat or mucoid colonies) were taken for identification (Fig. 1). The E. coli and K. pneumoniae isolates were screened for possible ESBL production using ceftazidime, cefotaxime, aztreonam, ceftriaxone and cefpodoxime disks.

NCCLS phenotypic confirmatory disc diffusion test (PCT)

E. coli and K. pneumoniae isolates were subjected for phenotypic confirmatory test using ceftazidime 30μg and ceftazidime – clavulanic acid (30μg + 10μg). The isolated organisms

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were inoculated on to peptone water and incubated at 37^0C for 4-6 hours. The turbidity of the growth was adjusted to 0.5 Macfarland's standard. This suspension was inoculated onto Muller-Hinton agar plate by lawn culture. Ceftazidime (30μg) and ceftazidime- clavulanic acid (30μg + 10μg) discs were placed. Additionally ceftiaxone (30μg) and ceftiaxone–sulbactum (30μg + 10μg) were also placed on the same plate such a way that none of these zones overlapped or interfered with each other. Plates were incubated at 37^0c for overnight. An increase in zone diameter of ≥ 5mm for ceftazidime – clavulanic acid disc when compared to ceftazidime disc alone was considered as ESBL producer (Fig. 2). The same criterion was applied to ceftriaxone and ceftriaxone – sulbactum.

RESULTS

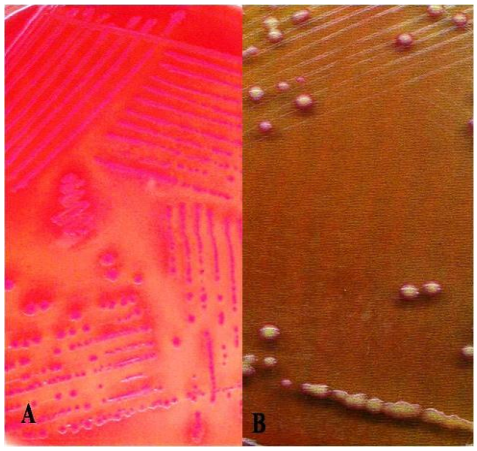

Fig 1. Mac Conkey's agar showing lactose fermentors (1A. flat colonies and 1B. mucoid colonies)

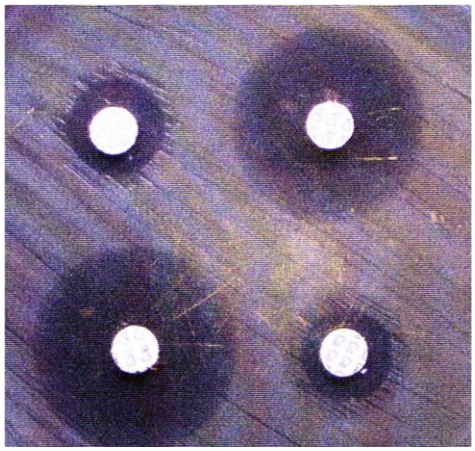

Fig 2. NCCLS – Phenotypic confirmatory test (PCT) with ceftazidime (Ca) / ceftazidime – clavulanic acid (Cac) disks along with ceftriaxone (Ci) / ceftriaxone – sulbactum (Cis).

The cultures of specimens yielded 117 E. coli and 83 K. pneumoniae isolates. In them, 98 (49%) were screen positive for ESBL. Among them 53 E. coli and 26 K. pneumoniae were found to produce ESBL. In that 52 (98.1%) isolates of E. coli and 26 (100%) isolates K. pneumoniae

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were positive by NCCLS-PCT method. The present study observed ESBL production in 39.5% of cases. All the ESBL isolates were susceptible to imipenem, however 98.1% cases were resistant to cefoxitin. It was also observed that all but one E. coli isolate that were confirmed ESBL positive by NCCLS-PCT method were also positive by ceftriaxone (Ci) / ceftriaxone with sulbactum (Cis) method. However in 7 screen positive but ESBL negative E. coli isolates and 1 K. pneumoniae isolate, ceftriaxone (Ci) / ceftriaxone with sulbactum (Cis) method gave positive result (Table 1).

Table 1 showing the role of ceftriaxone (Ci) / ceftriaxone -sulbactum (Cis) in ESBL detection

		E. coli	K. pneumoniae	Total
Total tested		117	83	200
Screen positive but ESBL negative isolates		7	1	8
In ESBL positive isolates	NCCLS-PCT positive	52	24	66
	NCCLS-PCT negative	1	0	1

NCCLS-PCT: national committee for clinical laboratory standards phenotypic confirmatory test.

DISCUSSION

When penicillin was introduced in 1944, 5% of S. aureus isolates were β-lactamase producers but this proportion had risen to 50% within 5 years and now stands at more than 90% [13]. β-lactamase production by several gram negative and gram positive organism is perhaps the most important mechanism of resistance to penicillins and cephalosporins [1]. With the widespread use of oxyimino cephalosporins, resistance to extended spectrum β-lactam antibiotics has emerged. Because of increased spectrum of activity, especially against expanded spectrum cephalosporins, these enzymes were called extended spectrum β-lactamases (ESBL) [2]. ESBLs are the derivatives of common β-lactamases such as TEM and SHV that have undergone one or more amino acid substitutions near the active site of enzyme, thus increasing the affinity and hydrolytic activity against third generation cephalosporins and monobactams [14]. These enzymes are the result of various mutations of TEM-1, TEM-2 and SHV-1, all of which are β-lactamase enzymes found in enterobacteriaceae family. Normally, TEM-1, TEM-2 and SHV-1 enzymes confer high level resistance to early penicillins and low level resistance to first generation cephalosporins [6,7]. The presence of ESBL could be suspected if an isolate of K. pneumoniae or E. coli demonstrate resistance to cefotaxime and cefpodoxime, but might show susceptibility to other third-generation cephalosporins. Unfortunately, some strains appear susceptible even to ceftazidime [15].

The increased prevalence of enterobacteriaceae producing ESBLs create a great need for necessary testing methods that will accurately identify the presence of these enzymes in clinical

isolates. Detection of ESBL expression has proved to be difficult for many laboratories because in vitro testing may not reveal intermediate susceptibility or resistance to cefotaxime or ceftriaxone at the NCCLS interpretative breakpoint for susceptibility [16]. The incidence of ESBL producing strains among clinical isolates has been steadily increasing over the past few years resulting in limitations of therapeutic options [3]. They were initially associated with nosocomial outbreaks caused by a single enzyme producing the strain. So it is necessary to identify the prevalence of these ESBL strains in hospital and to characterize their epidemiology to control their spread [3]. ESBL's confer resistance to all third generation cephalosporins and since these antibiotics are the predominantly used ones in several hospitals, such a resistance can lead to treatment failures. Most often, isolates possessing these enzymes also exhibit resistance to fluoroquinolones, aminoglycosides, sulphonamides and tetracycline [17-19].

So the detection of isolates expressing ESBLs is crucial in patient health care as well as limiting the spread of ESBL producing isolates. Though ESBLs might be produced by several members of enterobacteriaceae, the present study was restricted only to detect their presence in clinically significant E. coli and K. pneumoniae isolates that were obtained from samples submitted by patients. Various methods have been described to detect and confirm the ESBL production which include DDST, NCCLS, inhibitor potentiated test, three dimensional test, E-test, Vitek system. It was reported that all these tests have their own advantages and limitations. NCCLS recommends the use of phenotypic confirmatory test using ceftazidime (Ca) alone with ceftazidime (Ca) and clavulanic acid combination. The difference of 5mm or more of zone inhibition around ceftazidime (Ca) and that of ceftazidime – clavulanic acid (Cac) indicates positive test. Babypadmini et al. [20] studied ESBL production by NCCLS method by using ceftazidime (Ca) and ceftazidime - clavulanic acid (Cac). Nath et al. [21] performed NCCLS phenotypic confirmatory method by using ceftazidime (Ca), cefotaxime (Ce), ceftriaxone (Ci) and cefotaxime-clavulanic acid (Cec) disks. In the present study NCCLS phenotypic confirmatory method was done by using ceftazidime (Ca) and ceftazidime–clavulanic acid (Cac). We observed that the present study correlates with Babypadmini et al. [20] study.

We suggest that proper infection control practices and barriers are essential to prevent spreading and outbreaks of ESBL producing bacteria. Limiting the use of cephalosporins, especially third generation cephalosporins in an effective way reduces the frequency of ESBLs. Encouraging the use of β-lactam and β lactamase inhibitor combination as an alternative to third generation cephalosporins is another alternative [7]. There are some precautionary methods to be taken to prevent the spread of hospital infections and limit the spread of ESBL harboring bacteria. The health care workers should be advised to wash their hands regularly. Environmental contamination should be avoided while disposing the hospital wastes. Clean and disinfected equipments to be used among the patients and some equipment must be dedicated for a single patient use. The housekeeping practices should be intensified. Other programmes include clinical and bacteriological surveillance of patients admitted to intensive care units and antibiotic cycling as well as policies of restriction of empirical use of broad-spectrum antimicrobial agents such as third and fourth generation cephalosporins and carbapenems [6]. It is recommended by NCCLS (2002) that for all confirmed ESBL producing strains, the test interpretation should be reported as resistant to all penicillins, cephalosporins and aztreonam.

Among all the available β-lactam drugs, carbapenems (imipenem and meropenem) are more effective and reliable antibiotics against ESBL producing isolates. Carbapenems are highly resistant to the hydrolytic activity of all ESBL enzymes, due to the trans-6 hydroxyl ethyl group [7,22]. Among the available carbapenems, meropenem is the most active agent against ESBL producing organism in vitro [7]. Effect of a β-lactam and β-lactamase inhibitor combination varies depending on the subtype of ESBL present [7].

CONCLUSION

Present study observed that ESBL positive isolates exhibited high levels of multidrug resistance and the prevalence of ESBL producing E. coli and K. pneumoniae was found to be high. NCCLS-PCT test was more efficient in detecting the ESBL production. We suggest that routine screening of ESBL should be performed on all isolates showing decreased susceptibility to one or more of third generation cephalosporins. Resistance strains must be confirmed for ESBL production by NCCLS-PCT phenotypic confirmatory test.

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