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COMPARITIVE STUDY OF ESBL MEDIATED DRUG RESISTANCE AMONG CLINICAL AND COMMUNITY ISOLATES OF ESCHERICHIA COLI

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ABSTRACT

The emergence and spread of resistance to antibiotics among common pathogenic bacteria is an important health care concern. Today the magnitude of the problem has become a threat to reverse the scientific progress made so far. The aim of the study is to identify various strains of *Escherichia coli* isolated from hospital and community infections, to study their production of ESBL, and their antibiogram so as to guide the clinician to plan proper antibiotic policy, thereby reducing the mortality and morbidity due to bacterial infections. Antibiotic sensitivity test is done by using Kirby– Bauer's method of disc diffusion susceptibility test and it is confirmed by Double disc synergy test, Phenotypic confirmatory disc diffusion test. : Among 100 isolates of *Escherichia coli* 25 isolates from hospital, 8 isolates from community are resistant to 3rd generation cephalosporins. Among these, 12 isolates from hospital, and 4 isolates from community are ESBL producers. The present study highlights that there is a significant difference in drug resistance pattern between hospital to community strains.

KEYWORDS: Extended spectrum beta -lactamases, 3rd Generation cephalosporins, Minimum inhibitory concentration, Double disc synergy test, Phenotypic confirmatory double disc test.

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INTRODUCTION

The introduction of third-generation cephalosporins into clinical practice in the early 1980s was heralded as a major breakthrough in the fight against β-lactamase-mediated bacterial resistance to antibiotics. Soon after the introduction, the first report of plasmid-encoded β-lactamase capable of hydrolyzing the extended-spectrum cephalosporin was published in 1983 from Germany. Hence these new β-lactamases were coined as extended spectrum β-lactamases (ESBLs).[1] B Lactamases continue to be the leading cause of resistance to β-lactam antibiotics among gram-negative bacteria. In recent years there has been an increased incidence and prevalence of extended-spectrum β-lactamases (ESBLs), The extended spectrum _-Lactamase (ESBL) enzymes are plasmid-mediated enzymes capable of hydrolyzing and inactivating a wide variety of _-Lactams, including third generation cephalosporins, penicillins andaztreonam [2]. The majority of ESBLs are derived from the widespread broad-spectrum β-lactamases TEM-1 and SHV-1[3].

The ESBL producing bacteria are increasingly causing urinary tract infections both in hospitalized and outpatients. This is making therapy of UTI difficult and promoting greater use of expensive broad spectrum antibiotics, such as carbapenems. Detection of ESBLs using conventional antimicrobial susceptibility methods and delay in the detection and reporting of ESBL production by Gram-negative bacilli are associated with prolonged hospital stay, increase morbidity, mortality and health care costs. [4] The incidence of ESBL-producing strains among clinical isolates has been steadily increasing over the past years resulting in limitations of therapeutic option. Over the last fifteen years numerous outbreaks of infection with organisms producing extended spectrum β-Lactamases have been observed worldwide.[5] Extended Spectrum BetaLactamases (ESBL) hydrolyses expanded spectrum cephalosporins, which are used in the treatment of UTI. They arise by mutations in genes for common plasmid-mediated beta lactamases that alter the configuration of the enzyme near its active site to increase the affinity and hydrolytic ability of the beta lactamases for oxyimino compounds while simultaneously weakening the overall enzyme efficiency. [6] Acute urinary tract infections are common, occurring in 10% to 20% of otherwise healthy women during their lifetimes. These infections are usually limited to the bladder and urethra and are most commonly caused by Escherichia coli. [7]

The emergence of ESBL-producers along with multiple resistant isolates poses a serious problem in the hospital setting. The widespread use of antibiotics coupled with the transmissibility of resistance determinants mediated by plasmids, transposons, and gene cassettes in integrons are factors that contribute to the increase in antibiotic resistance in bacterial pathogens. [8] Despite the widespread availability of antibiotics, urinary tract infection (UTI) remains the most common bacterial infection in the human population. Antibiotics are usually given empirically before the laboratory results of urine culture are available. To ensure appropriate therapy, current knowledge of the organisms that cause UTI and their antibiotic susceptibility is mandatory. [9] Urinary tract infection is the second most common infectious presentation in community practice. Worldwide, about 150 million people are diagnosed with UTI each year, costing the global economy in excess of 6 billion US dollars .There has been significant changes in the antimicrobial resistance patterns of uropathogens over the years including resistance due to extended spectrum beta lactamase (ESBL)-producing pathogens. The increasing prevalence of infections caused by antibiotic-resistant bacteria makes empirical treatment of these infections difficult. [10]

MATERIALS AND METHODS

The material for study consists of 100 clinical isolates of *Escherichia coli* from 375 clinical samples of urine are taken for study. Among them 50 are from hospital 50 are from community. All samples were inoculated in nutrient agar, blood agar and CLED and

incubated at 37ºC for overnight. Identification is done based on cultural characteristics, biochemical reactions.

Antimicrobial susceptibility testing

The antibiogram of all the isolates were studied with a set of 9 antibiotic disc containing amoxicillin(20µg),gentamicin(10µg), amikacin(30µg), ciprofloxacin(30µg), Ceftazidime(30µg), cefotaxime(30µg), ceftriaxone(30µg), imipenam(10µg), azithromycin(10µg). Kirby-Bauer' method of disc diffusion susceptibility testing was done as per the NCCLS guide lines. The isolates were categorized in to 2 groups based on this susceptibility or resistance to 3rd generation cephalosporins (3GC). Group –I consisted of sensitive strain which have shown a zone of diameter of more than 17mm, Group- II are resistant strains which have shown a zone of diameter less than 17mm for all 3GC.

ESBL confirmatory test

1. Double disc synergy test (DDST)

The isolated colonies were inoculated in peptone water at 37ºC for 2-6 hours. The turbidity was adjusted to 0.5 Mc forlands standard and lawn culture was made on Mueller Hinton agar using sterile swab. Augmentin disc (amoxicillin 20µg/clavulinic acid10µg) was placed in the centre of the plate. On both sides of augmentin disc, disc of cefotaxime (30µg) and ceftazidime (30µg) were placed with centre to centre distance of 15mm to centrally placed disc. The plates were incubated at 37ºC overnight. ESBL production was interpreted as widening of the zone of inhibition around a 3GC of more than 3mm towards the Augmentin disc is considered as ESBL producer by DDST[6].

2. Phenotypic confirmatory disc diffusion test (PCDDT)

In this procedure a zone of diameter more than 5mm around a ceftazidime + clavulinic acid disc discs when compared to ceftazidime disc alone is considered as ESBL producer.

RESULTS

Out of 100 isolates of Escherichia coli, tested for their antibiogram, 67 (67%) isolates have shown sensitivity to 3rd generation cephalosporins and 33 (33%) have shown resistance.

Table 1
Antibiogram for 3rd generation cephalosporins

Total No. of isolates	Sensitive 3 GCS		Resistant to 3 GCS	
	No	Percentage	No	Percentage
100	67	67%	33	33%

Among 50 hospital isolates 25 (50%) and out of 50 communities isolates 8(16%) are resistant to 3GC. Out of these 25 hospital resistant isolates 12 (24%) are found to be ESBL producers, among 8 community resistant isolates 4(8%) are ESBL producers. Definitions of hospital and community acquired infection: A hospital-acquired infection is usually one that first appears three days after a patient is admitted to a hospital or other health care facility. Infections acquired in a hospital are also called nosocomial infections. An infection contracted outside of a health care setting or an infection present on admission is community acquired infection.

Table 2
Table showing Extended spectrum beta-lactamase production among hospital and community patients

Group	Total No. of isolates	Sensitive to 3 GCS		Resistant to 3 GCS=33			
		No	Percentage	ESBL +Ve		Non ESBL	
Hospital	50	25	25%	12	24%	13	26%
Community	50	42	84%	4	8%	4	16%
Total	100	67	67%	16	16%	17	17%

So there is a significant difference in drug resistance pattern between hospital and community isolates.

DISCUSSION

In hospitals, intensive care units are often epicentre for ESBL production. In the hospital the environmental sources of ESBL producing organisms are ultra sonography coupling gel, bronchoscopes, and glass thermometers. Transit carriage on hands of health care workers is an important means of transfer from patient to patient [11]. It is important to recognise that many patients may have asymptomatic colonization with ESBL producing organisms without signs of overt infection. They act as reservoir in some hyper endemic intensive care units and transplant units. Nosocomial infections caused by ESBL producing pathogens are associated with risk factors such as elderly age, prolonged hospitalization, previous antibiotic use, and presence of invasive devices[9][12]. Infections caused by ESBL-producing Enterobacteriaceae are serious concerns in the current environment. Many ESBLs represent enzymes that have evolved from class A ß -lactamases—namely, TEM-1, TEM-2,and SHV-1, which are frequently expressed in gram negative bacteria and which confer resistance to ampicillin, amoxicillin, and other penicillins, as well as to early- but not later-generation cephalosporins. The first plasmid mediated ß -lactamase in Gram negatives, TEM-1, was reported in 1965 from an Escherichia Coli isolate belonging to a patient in Athens, Greece, named Temnoniera (hence the designation TEM.). Another common plasmid mediated β - lactamase found in Klebsiella pneumoniae and Escherichia coli is SHV-1 (named after the sulfhydryl “variable”active site) [11]. ESBLs arose when mutations of the genes encoding TEM-1, TEM-2, or SHV-1 gave rise to new lactamases that became able to hydrolyze third-generation cephalosporins and aztreonam [12, 13]. ESBL-containing plasmids often carry resistance genes for other antibiotics thus; aminogycosides and fluoroquinolones may be ineffective[14]. ESBL producing organisms pose a major problem for clinical therapeutics. Initially they are restricted to hospital acquired infections, gradually they have also been isolated from infections in out patients[13].

As many as 33 isolates of Escherichia coli among 100 test strains are resistant to 3 GCS (Table 1) in the present study. Among the 50 nosocomial isolates in the present study, 25 isloates are resistant to 3 CGS (50%) and among them 12 showed ESBL production (24%) as per (Table I1). A similar study have been recorded by Md. Akram et al[15] – 34% ESBL positive strain of Escherichia Coli. The results of present study correlate well with that of Shukla et al, 2004[16]. The present study indicates the importance of implementation of routine detection of ESBL producing microorganisms in each laboratory by the standard detection methods, so as to control the spread of infections. Phenotypic confirmatory disc diffusion test is simple, sensitive and cost effective for the detection of ESBL production. Monitoring and judicious usage of extended cephalosporins should be emphasized. Periodic surveillance of antibiotic resistance

pattern, and efforts to decrease empirical antibiotic therapy would go a long way in addressing some of the problems associated with ESBLs.

CONCLUSION

In conclusion, the findings in this study document the emerging threat of ESBL pathogens in our setting with the occurrence of these strains as aetiological agents of infection in the hospital and community. While the findings shed light on *E. coli*, which are the predominant ESBL producers, we recommend further work on evaluating the ESBL types in these isolates as well as the prevalence of other ESBL-producing Gram negative bacteria which are emerging as pathogens of concern in the clinical setting. We advocate increased surveillance as well comprehensive multicenter/ multinational studies to address this emerging problem of ESBL-associated infections.

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