Original Research Article

Comparative study of ESBL producing *Escherichia coli* in OPD and IPD patients of urinary tract infections

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A B S T R A C T

K e y w o r d s	Urinary tract infection is one of the most common infections diagnosed in outpatient and in hospitalizes patients. It is caused by gram positive and gram negative organisms. Most prevalent amongst them is *E. coli*. Current knowledge on antimicrobial susceptibility pattern is mandatory for appropriate treatment. Many of them produce enzymes capable of inactivating drugs having beta-lactam rings. These are called as beta-lactamases. Since these enzymes extended their spectrum over a period of time and started acting upon second and third generation of cephalosporins, they are now called ESBLs i.e. extended spectrum beta-lactamases. The aim of this study is to determine the prevalence and antibiotic susceptibility pattern of *E. coli* strains isolated from OPD and IPD patients, producing beta-lactamases. Antibiotic susceptibility testing and detection of beta-lactamases are carried out as per standard guidelines. Study shows a significant number of ESBL producers in our hospital and their sensitivity pattern shows high degree of resistance against most of the drugs.
ESBL; beta-lactamases; carbapenems; *Escherichia coli;* OPD; IPD.	

Introduction

Urinary tract infections are one of the most common bacterial infections in community and hospital admitted patients. They cause serious health problems affecting millions of people every year. Some people are more prone to getting infections than others. Any pathology of the urinary tract that obstructs the flow of urine e. g. kidney stone increases the chances of infection. A common source of infection is catheter placed in urethra and bladder. People with diabetes have higher risk of UTI because of poor response to immune system.

Despite wide spread availability of antibiotics, urinary tract infections remain the most common bacterial infection in human population. Antibiotics are usually given empirically before the laboratory results of the cultures are available. UTIs

are most commonly caused by *E. coli* in both OPD and IPD patients. Infections are usually treated by broad spectrum antibiotics like cephalosporins, fluoroquinolones and aminoglycosides. Cephalosporins inhibit cell wall synthesis and are used mainly for treating gram negative bacterial infections. These include mainly cefotaxime, cefaclor, cefuroxime and ceftazidime etc. Among members of enterobacteriaceae family resistance to beta-lactamases have been reported due to presence of ESBL (Kader and Kumar, 2005).

ESBLs are defined as beta-lactamases capable of hydrolyzing oxi-imino beta-lactams like cefotaxime, ceftriaxone, ceftazidime and monobactums and are inhibited by clavulanic acid. ESBLs have no effect on cephamycins and most strains are susceptible to cefoxitin and cefotetan (Philippon et al., 1989). However ESBL producing strains can become resistant to cephamycins due to loss of outer membrane proteins (porins). ESBL producing *E. coli* in this part of world has been observed by several workers and its prevalence was variously reported from 28-67% (Akram, 2007; Babtpadmini, 2004; Hammer,2007; Jabeen, 2003; Mehrgan, 2008).

Detection of ESBL is very important and of great concern as spread of these ESBL producing organisms in the hospital may lead to the outbreak of epidemic. Another reason of concern is treatment failure as the therapeutic choices are very limited.

Materials and Methods

Present study was conducted on patients, visited Mohan Dai Cancer hospital between Jan 2010 to May 2011. A total of 271 urine samples were collected in sterile disposable containers. Samples were inoculated on MacConkey agar and blood agar plates. After overnight incubation at $37^{o}C$, plates were observed for growth of gram negative and gram positive organisms. The organisms were identified on the basis of their morphology and biochemical reactions.

Antibiotic susceptibility testing

All the isolates were subjected to antibiotic susceptibility testing by disc diffusion Kirby–Bauer method using Mueller-Hinton agar plates as described by the clinical laboratory standard institute (CLSI 2006). Following discs were used for sensitivity testing.

Augmentin (AG), cefotaxime (CF), cefoperazone (CP), cefuroxime (CB), cefaclor (KF), ceftazidime (FG), ceftriaxone (RP), cefadroxil(CU), cefixime (SF), cefoperazone+sulbactum (MG) cefotaxime+sulbactum (CG), cefpodoxime (CE), gentamicin (GM), amikacin (AK), norflox (NX), cefipime+amikacin (CPT), ofloxacin (ZN), sparfloxacin (DC), moxifloxacin (MO), imipenem (IM), meropenem (MR) and piperacillin+tazobactum (TZP).

Detection of extended spectrum beta-lactamases

The test organisms were grown on MHA and discs of ceftazidime (30 ug) and disc of ceftazidime in combination of clavulanic acid (10ug) were placed on the sensitivity plate at a distance of 20 mm. Difference of 5 mm or more between zones of inhibition of the two discs was considered as ESBL positive isolate (Paterson and Bonomo, 2005).

Result and Discussion

Results are based on the study in which a total of 271 urine samples were processed. 188 samples were from OPD patients while 83 were from IPD. Rate of infection is more in IPD patients (59%) than in OPD patients (50%) which are depicted in Table 1. Various gram negative organisms were isolated. Out of these *E. coli* colonies were identified as lactose fermenting, flat and irregular margin, which were further confirmed by biochemical tests. Results in Table no. 2 shows the number of *E. coli* isolates among total positive isolates. It is also showing the numbers of *E. coli* that are producing ESBLs.

Results in Table no.3 shows sensitivity pattern of ESBL positive and negative *E.coli* against conventional and newer antibiotics. This is very much clear from this table that ESBL positive *E. coli* is mostly resistant to a large no. of antibiotics.

Urinary tract infections are one of the most common infections diagnosed in outpatient and hospitalized patients. Current knowledge on antimicrobial susceptibility pattern is mandatory for appropriate therapy. Extended spectrum beta-lactamases (ESBL) hydrolyse all generations of cephalosporins which are used in the treatment of UTI. Treatment of UTI without detection of ESBL may lead to inappropriate use of antibiotic leading to treatment failure. The production of beta-lactamases may be chromosomal or plasmid mediated(Philippon,1989). Plasmid mediated production is often acquired by transfer of genetic information from one organism to other. High degree of resistance can be explained by the fact that such transferable plasmids also carry resistant determinants to other antimicrobial agents making them resistant to ESBL producing organisms. In the study 56 organisms were ESBL producing and were resistant to more than 4 to 5 drugs whereas multidrug resistance was much less in non ESBL producing organisms.

Many factors are responsible for higher incidence of infections with ESBL producing organisms. These include prior hospitalization, prior stay in ICU, sex, diabetes, catheterization, patient on parenteral nutrition, age more than 65 yrs, patient already on antibiotics, lower immunity due to anti cancer drugs (NNISS, 2004; NIPHE, 2008). Resistance of most of the organisms to antibiotics has increased due to ESBL production (Rodriguez and Pascual, 2008). In most European countries the prevalence of *E. coli* resistant to fluroquinolones, third generation cephalosporins and aminoglycosides is on the increase every year (Calbo, 2006). We have also noticed the similar kind of observation in our study. The current situation on antibiotic resistance has reached a serious level. Antibiotics that can be used for the treatment of multidrug resistant bacteria in urinary tract infections are limited (Ortega, 2009).

In our study excluding carbapenems, amikacin and gatifloxacin, there are few antibiotics showing sensitivity over 50 % to ESBL producing organisms. In addition, infections with these organisms increase mortality, prolong hospital stay and also increases relative treatment cost (Azap,

Table.1 Various gram negative organisms

Patients	No of cases	Positive cases	%age positivity
OPD	188	106	56.3
IPD	83	55	66.2

Table.2 The number of *E. coli* isolates among total positive isolates.

Patients	Total isolates	*E. coli*	ESBL positive	%age positivity
OPD	106	39	24	63
IPD	55	20	15	76

Table.3 Sensitivity pattern (%) of ESBL positive and negative *E. coli* in OPD and IPD patients

OPD	AG	CF	CP	CB	KF	FG	RP	CU	SF	MG	CG
ESBL POSITIVE	2.68	7.8	13.2	8	5	5	11	5	2.5	79	47
ESBL NEGATIVE	32	82	86	82	68	90	82	73	77	99	97
	GM	AK	NX	CPT	ZN	DC	GF	MX	IM	TZP	AZK
ESBL POSITIVE	39	84	2.6	72	8	5	89	87	88	82	37
ESBL NEGATIVE	68	98	62	98.5	55	73	98	92	90	96	73
IPD	AG	CF	CP	CB	KF	FG	RP	CU	SF	MG	CG
ESBL POSITIVE	5	5	5	2	2	11	5	2	2	58	16
ESBL NEGATIVE	17	50	53	54	49	90	47	55	55	98	75
	GM	AK	NX	CPT	ZN	DC	GF	MX	IM	TZP	AZK
ESBL POSITIVE	9	63	2	47	5	6	79	80	67	53	20
ESBL NEGATIVE	80	99	30	97	52	56	96	97	97.5	98.2	65

Therefore it is very important to assess the risk factors for the emergence of multidrug resistant bacteria and provide means to prevent emergence of resistance. Recent studies showed that emergence of ESBL producing bacteria are more frequent in patients with history of hospitalization, having antibiotics and urogenital surgery (Austin, 1999; Platt, 1986). In our study also more isolates from indoor female patients have been found to be associated with ESBL production. Since ours is a cancer hospital and most patients are immunologically compromised and terminally ill, require catheterization which is one of the most important risk factors for the emergence of ESBL producing bacteria.

In our country, second generation cephalosporins like cefaclor and cefuroxime are extensively used by general practitioners, unregistered medical *practitioners* and chemists and that too in inadequate doses and duration to treat not only urinary tract infections but all kinds of infections. This may be the most important reason of high prevalence of ESBL producing organisms. Therefore to prevent emergence of resistance, efforts should be made to follow the indications for the administration of antibiotics and to use them appropriately for optimum period. Preferably antibiotics should be given on the basis of culture and sensitivity report.

Foley catheterization is well known to be a risk factor for urinary tract infections. About 20% of urinary tract infections are associated with Foley catheterization due to faulty techniques during insertion of catheter (Bukhari, 1993). Infection may occur due to contamination of the collecting specimen through the lumen of the catheter (Tenke, 2008). Other cases

have been reported to occur through the biofilm formed between catheter and urethral mucosa (Babini, 2000). Therefore unnecessary Foley catheterization should be avoided in patients with urinary tract infections to prevent emergence of ESBL producing bacteria.

Among the beta-lactams, the carbapenems have the widest spectrum of activity. Imipenem was the most active antimicrobial agent having 95-98 % activity. Imipenem was followed by meropenem with 85 to 95 % activity. Aminoglycosides have good activity against gram negative and gram positive isolates. Among non beta-lactams both gentamicin and amikacin are showing good sensitivity. Gentamicin is showing poor sensitivity against beta-lactamase producing organisms while amikacin is showing very good sensitivity. This is because of extensive use of gentamicin by the general practitioners and unregistered medical practitioners. Antibiotic resistance problem is not a new problem in India or even in the world. What is alarming is the apparent failure of newly introduced and potent agents in settings where resistance is widespread. Generally resistance rate is low when a new antibacterial agent is introduced. Resistance then appears and increases while it reaches a steady state level. We have seen in our study that even third generation of cephalosporins, combination formulations and carbapenems are showing high degree of resistance and this is simply because of injudicious use of antibiotics and not having any policy regarding usage of antibiotics in most of the hospitals.

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