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Full Length Research Paper

Antibiotic susceptibility pattern and ESBL prevalence in nosocomial *Escherichia coli* from urinary tract infections in Pakistan

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Urinary tract infections (UTI) are the most prevalent infections worldwide, mostly caused by *Escherichia coli*. These bacteria also produce enzymes called extended spectrum β-lactamases (ESBL) which render penicillins and cephalosporins inactive. The present study included 116 *E. coli*, isolated from urine of in-patients suffering from UTI. Antibiotic susceptibility testing and ESBL detection were carried out according to Clinical Laboratory and Standards Institute (CLSI) criteria. Fifteen antibiotics were tested in this study. A total of 66 (56.9%) isolates were found to be ESBL producers. A total of 83% isolates were resistant to 4 or more antibiotics. Imipenem and meropenem showed high potency with 98 and 97% isolates being susceptible, respectively. Susceptibility to gentamicin was 48%, kanamycin 43%, both ofloxacin and enoxacin 38%, ciprofloxacin 35%, doxycycline 18% and to co-trimoxazole only 17%. This study reveals that *E. coli* isolated from UTI in this region are multi-drug resistant and produce ESBL in large proportions.

Key words: Antibiotic resistance, *Escherichia coli*, Extended Spectrum β-Lactamase, Urinary Tract Infections.

INTRODUCTION

Urinary Tract Infections (UTIs) are one of the most common bacterial infections in humans, both in the com-munity and the hospital settings (Cox, 1988; Gonzalez and Schaeffer, 1999). UTIs are amongst the most prevalent infectious diseases affecting approximately 150 million people worldwide annually which results in more than 6 billion US dollars loss to the global economy (Gonzalez and Schaeffer, 1999; Stamm and Norrby, 2001). The lifetime risk for UTI in females is greater than 50% (Griebling, 2005) . In the United States, about 8 million physician visits and more than 100,000 hospital admissions per year are due to UTIs (Warren et al., 1999).

UTIs are mostly caused by *Escherichia coli* accounting for more than 70% of uncomplicated cases both in out-patients and inpatients (Gupta et al., 2001). Other Gram negative bacteria include *Klebsiella* spp., *Enterobacter* spp., *Pseudomonas aeruginosa, Proteus* spp. Gram-positive bacteria account for 5 to 15% of UTIs and include *Enterococcus* spp., *Staphylococci,* and *Strep-tococci* (Hryniewicz et al., 2001; Akram et al., 2007).

UTIs are usually treated with broad-spectrum cephalo-sporins, flouroquinolones and aminoglycosides. Cephalo-sporins are cell wall inhibitors and are used commonly for treating infections caused by gram negative organisms. These include cephradine, cefaclor, cefotaxime, ceftazidime etc. Fluoroquinolones are antibiotics which act by inhibiting the activity of DNA gyrase and topoisomerase, enzymes essential for bacterial DNA replication and include ciprofloxacin, ofloxacin, enoxacin, sparfloxacin etc. The aminoglycoside antibiotics include gentamcin, kanamycin, amikacin etc. These act by inhibiting bacterial protein synthesis (Trevor et al., 2001).

The *E. coli*, worldwide, have developed resistance to antimicrobial agents and the phenomenon is increasing both in outpatients and hospitalized patients (Akram et al., 2007; Garcia et al., 2007). Among members of the *Enterobacteriaceae* family, resistance to β-lactams has been reported to be associated with ESBL (Babini and

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Livermore, 2000), which hydrolyze oxyimino β- lactams like cefotaxime, ceftriaxone, ceftazidime and mono-bactams but have no effect on cephamycins, carba-penems and related compounds (Philippon et al., 1989). ESBL producing *E. coli* in this part of the world has been observed by several workers; its prevalence was variously reported from 28 to 67% (Jabeen et al., 2003; Babypadmini and Appalaraju, 2004; Tankhiwale et al., 2004; Akram et al., 2007; Hammer et al., 2007; Mehrgan and Rahbar, 2008).

Production of ESBL is frequently plasmid encoded and bears clinical significance. Plasmids responsible for ESBL production frequently carry genes encoding resis-tance to other drug classes also. Therefore, antibiotic options in the treatment of ESBL producing organisms are extremely limited (Paterson and Bonomo, 2005). Detection of ESBL production is important. One major concern is the spread of ESBL positive bacteria within hospitals, which may lead to outbreaks or to endemic occurrence (Meyer et al., 1993; Pena et al., 1998; Lucet et al., 1999; Quale et al., 2002). Another concern is failure to treat infections caused by ESBL positive organisms, as therapeutic choices are limited (Paterson and Bonomo, 2005) . It is necessary to investigate the prevalence of ESBL positive strains in hospitals so as to formulate a policy of empirical therapy in high risk units where infections due to resistant organisms are much higher (Mathur et al., 2002). There is not much information available on the susceptibility pattern of urinary isolates and ESBL prevalence in Pakistan. The aim of this study was to determine the frequency of the uropathogens, their susceptibility pattern and identifi-cation of the ESBL production for effective management of UTIs.

MATERIALS AND METHODS

Bacterial isolates

Between April 2005 and February 2006, 116 strains of *Escherichia coli* were isolated from urine of admitted patients in Khyber Teaching Hospital, Peshawar, in the North West of Pakistan. Fresh mid-stream urine (n = 342) was collected aseptically in sterilized bottles or disposable sterile plastic bags and submitted to clinical microbiology laboratory. Only one isolate per patient was included in the study. The samples received were inoculated onto blood agar and Cysteine Lactose Electrolyte Deficient (CLED) agar. After 24 h aerobic incubation at 37°C, isolates were identified to the species level using biochemical tests. The plates showing significant growth as per Kass count (single specie count of more than 10^5 organisms per ml of urine) were processed further (Kass, 1956).

Antimicrobial agents susceptibility testing

Susceptibility to antimicrobial agents was determined both by Disc Diffusion method of Kirby Bauer and Minimum Inhibitory Concen-tration (MIC) method on Muller-Hinton agar (Oxoid, England) as described by the Clinical Laboratory Standard Institute (CLSI) (CLSI, 2006). The antibiotic discs were obtained from Oxoid, England while the powder antibiotic was obtained from Sigma, Germany.

The antibiotics used for antibiogram determination of the collected strains were: ampicillin (AMP), cephradine (CE), cefaclor (CEC), cefotaxime (CTX), ceftazidime (CAZ), doxycycline (DOX), ciprofloxacin (CIP), ofloxacin (OFL), enoxacin (ENX), nalidixic acid (NA), meropenem (MEM), imipenem (IPM), sulphamethoxazole + trimethoprim (SXT), gentamicin (CN) and kanamycin (K). *E. coli* NCTC 10418 was used as control for susceptibility testing.

Detection of Extended Spectrum β-Lactamases (ESBL)

The initial screening and phenotypic confirmatory tests recom-mended by the CLSI for ESBL detection were carried to assess the prevalence of ESBL (CLSI, 2006). In the initial screening test a disc of amoxicillin + clavulanic acid (20 + 10 μ g) was placed in centre of the Petri plate already inoculated with the test organism while aztreonam (30 μg) cefotaxime (30 μg) ceftazidime (30 μg) cefpodoxime (30 μg) and ceftriaxone (30 μg) discs were placed at a distance of 20 to 25 mm (centre to centre) from the amoxicillin + clavulanic acid disc on the same plate. Zones of inhibition around the third generation cephalosporin discs and aztreonam were observed after 18 h incubation at 37°C. If the zone of inhibition around one or more cephalosporin discs and aztreonam was extended on the side nearest to the amoxicillin + clavulanic acid, the organism showing this synergy was labelled as ESBL positive. In the phenotypic confirmatory test, the test organisms were grown on Muller-Hinton agar and discs of cefotaxime (30 μg) and ceftazidime (30 μg) separately and each of these in combination with clavulanic acid (10 μg) were placed on the surface of the lawn of bacteria. A difference of 5 mm between the zone of inhibition of a single disc and in combination with clavulanic acid was considered as ESBL positive isolate. *E. coli* NCTC 10418 was used as ESBL negative control and *Klebsiella pneumoniae* ATCC 700603 was used as ESBL positive control strain.

Statistical analysis

Statistical analysis was performed by the chi-square test and *P* values of 0.05 were considered significant.

RESULTS

A total of 342 urine samples were collected and processed for culture and sensitivity assays. However, *E. coli* growth was seen only in 116 (33.9%) specimens. Age range of patients was between 3 days to 75 years with a mean of 28 years. More isolates were recovered from females as compared to males the ratio being 3:1. Middle aged patients, that is, 22 - 45 years accounted for 54.3% of infections. While the second predominant group was children (24% UTI recorded). Patients from gynae-cology wards contributed maximum number of isolates (42.2%) followed by medical (31.9%), children (21.6%) surgical (3.4%) and nephrology wards (0.9%).

Among β-lactams the most effective antibiotic was imipenem with 98.3% of the isolates susceptible to this agent, followed by meropenem with 97.4% of the isolates being susceptible. Among cephalosporins, 62% resis-tance was recorded to cefotaxime, 65% to cefaclor and

Table 1. Susceptibility pattern and MIC of antimicrobial agents tested against *E. coli* (n = 116).

Antibiotic	Sensitive no. (%)	Resistant no. (%)	Intermediate no. (%)	MIC_{50}	MIC_{90}
AMP	13 (11.2%)	103 (88.8%)	0 (00.0%)	>128	>128
CEC	35 (30.2%)	76 (65.5%)	5 (04.3%)	>128	>128
CAZ	40 (34.5%)	76 (65.5%)	0 (00.0%)	32	>128
CTX	44 (38.0%)	72 (62.0%)	0 (00.0%)	>128	>128
CE	14 (12.1%)	84 (72.4%)	18 (15.5%)	>128	>128
DOX	21 (18.1%)	92 (79.3%)	3 (02.6%)	32	128
CIP	41 (35.3%)	72 (62.1%)	3 (02.6%)	64	>128
OFL	44 (38.0%)	72 (62.0%)	0 (00.0%)	16	128
ENX	44 (38.0%)	71 (61.2%)	1 (00.8%)	128	>128
NA	23 (19.8%)	89 (76.7%)	4 (03.5%)	>128	>128
CN	56 (48.3%)	60 (51.7%)	0 (00.0%)	4	>128
K	50 (43.1%)	66 (56.9%)	0 (00.0%)	6	>128
MEM	113 (97.4%)	3 (2.60%)	0 (00.0%)	DD	DD
IPM	114 (98.3%)	2 (1.70%)	0 (00.0%)	DD	DD
SXT	20 (17.3%)	94 (81.0%)	2 (01.7%)	DD	DD

DD - Disc Diffusion data only.

Table 2. Comparison of susceptibility to antibiotics between ESBL positive and ESBL negative *E. coli*.

	ESBL +ve (n = 66)		ESBL –ve (n = 50)	
Antibiotic	Sensitive	Resistant	Sensitive	Resistant
DOX	10 (15.2%)	56 (84.8%)	11 (22%)	39 (78%)
CIP	13 (19.7%)	53 (80.3%)	28 (56%)	22 (44%)
NA	2 (3.0%)	64 (97%)	21 (42%)	29 (58%)
CN	22 (33.3%)	44 (66.7%)	34 (68%)	16 (32%)
SXT	9 (13.6%)	57 (86.4%)	11 (22%)	39 (78%)

ceftazidime both and 72% to cephradine while the highest resistance was recorded to penicillin group (ampicillin) being 89%. The antimicrobial susceptibility for the 15 antibiotics used in this study is mentioned in Table 1. Among aminoglycosides, 48% isolates were susceptible to gentamicin and 43% to amikacin. Activities of different fluoroquinolones (ciprofloxacin, ofloxacin and enoxacin) were almost similar, 38%; with MIC_{50} for ofloxacin as 16 mg/l. Only 17.3% isolates were susceptible to sulphamethoxazole + trimethoprim (SXT) while 18% were susceptible to doxycycline (Table 1).

A total of 0.8% isolates were susceptible to all the antibiotics while 29.3% were resistant to all the antibiotics tested except carbapenems. A statistically significant difference was found in the susceptibilities of flouro-quinolones and aminoglycosides for ESBL positive and ESBL negative isolates (p-value <0.05) (Table 2). Resis-tance to doxycycline and sulphamethoxazole + trimethoprim was slightly high in ESBL positive isolates as compared to ESBL negative ones but it was not statistically significant (p-value >0.05). In total 83% isolates were multi drug resistant (MDR), that is, resistant to at least 4 or more drug classes and all of these were ESBL positive. The most prevalent MDR pattern was resistance to β-lactams, doxycycline, flouroquinolones and co-trimoxazole. There were also slight differences in the susceptibilities of the antibiotics among different wards. Significant difference in susceptibility pattern was recorded for SXT between children and medical ward where its susceptibility was 8 and 29% in these two units respectively. No statistically significant differences were found in the susceptibilities of *E. coli* to antibiotics isolated from males and females (p-value >0.05) (Table 3).

In this study, 66 (56.9%) *E. coli* were found to be ESBL producers, 47 (71.2%) isolated from females and 19 (28.8%) from male patients. Prevalence of ESBL was almost similar in medical and gynaecology wards being 58 and 58.3% respectively while it was 52% in children ward.

DISCUSSION

This study reveals the antibiotic susceptibility pattern and

Table 3. Comparison of susceptibility to antibiotics of *E. coli* isolated from males and females.

Antibiotic	*E. coli* isolated from females (n = 87)			*E. coli* isolated from males (n = 29)		
	Sensitive no. (%)	Resistant no. (%)	Intermediate no. (%)	Sensitive no. (%)	Resistant no. (%)	Intermediate no. (%)
AMP	9 (10.3%)	78 (89.7%)	0 (00.0%)	4 (13.8%)	25 (86.2%)	0 (00.0%)
CEC	28 (32.2%)	56 (64.4%)	3 (03.4%)	7 (24.1%)	20 (69.0%)	2 (06.9%)
CAZ	31 (35.6%)	56 (64.4%)	0 (00.0%)	9 (31.0%)	20 (69.0%)	0 (00.0%)
CTX	34 (39.1%)	53 (60.1%)	0 (00.0%)	10 (34.5%)	19 (65.5%)	0 (00.0%)
CE	11 (12.7%)	63 (72.4%)	13 (14.9%)	3 (10.3%)	21 (72.4%)	5 (17.3%)
DOX	15 (17.3%)	69 (79.3%)	3 (03.4%)	6 (20.7%)	23 (79.3%)	0 (00.0%)
CIP	32 (36.8%)	52 (59.8%)	3 (03.4%)	9 (31.0%)	20 (69.0%)	0 (00.0%)
OFL	35 (40.2%)	52 (59.8%)	0 (00.0%)	9 (31.0%)	20 (69.0%)	0 (00.0%)
ENX	34 (39.1%)	52 (59.8%)	1 (01.1%)	10 (34.5%)	19 (65.5%)	0 (00.0%)
NA	18 (20.7%)	65 (74.7%)	4 (04.6%)	5 (17.2%)	24 (82.8%)	0 (00.0%)
CN	39 (44.8%)	48 (55.2%)	0 (00.0%)	17 (58.6%)	12 (41.4%)	0 (00.0%)
K	38 (43.7%)	49 (56.3%)	0 (00.0%)	12 (41.4%)	17 (58.6%)	0 (00.0%)
MEM	84 (96.6%)	03 (03.4%)	0 (00.0%)	29 (100%)	0 (00.0%)	0 (00.0%)
IPM	85 (97.7%)	02 (02.3%)	0 (00.0%)	29 (100%)	0 (00.0%)	0 (00.0%)
SXT	13 (14.9%)	72 (82.8%)	2 (02.3%)	7 (24.1%)	22 (75.9%)	0 (00.0%)

ESBL prevalence in *E. coli* isolated from patients suffering from UTIs in Peshawar, Pakistan. Majority of *E. coli* were isolated from female patients, (75%); an observation similar to other reports (El Astal, 2005; Hasan et al., 2007). Patients from adult age group (22 - 45 years) contributed 54.3% isolates, which is again similar to that reported by others (Akram et al., 2007).

Among the β-lactams tested, the carbapenems have the widest spectrum of activity. imipenem was the most active antimicrobial agent having 98.3% activity. However, Muhammad et al. (2007) from India have reported 100% activity for imipenem against *E. coli* (Akram et al., 2007). Imipenem was followed by meropenem with 97.4% activity. Again Hryniewicz et al. (2001), have reported 100% activity in Poland (Hryniewicz et al., 2001). The third generation cephalosporins, ceftazidime and cefotaxime had 34.5 and 38% activity respectively, which is comparable to that reported from India (Tankhiwale et al., 2004). Resistance to ampicillin in *E. coli* is high in Pakistan (Rahman et al., 2002; Zaidi et al., 2005). In the present study, 88.8% resistance was recorded to ampicillin which is similar to a study from Jordan (Shehabi et al., 2004). While Noor et al. (2004) have reported 100% resistance to ampicillin in *E. coli* from Pakistan.

Aminoglycosides have good activity against clinically important gram negative bacilli (Gonzalez and Spencer, 1998). Among the non-β-lactams, gentamicin showed good activity with 48% isolates found susceptible in this study, which is more than recorded in Israel (29%) and India (36%) (Tankhiwale et al., 2004; Colodner et al., 2007). This may be due to increased use of gentamicin in India and Israel as compared to Pakistan. According to Miller et al. (1997), pattern of resistance to aminoglycosides is affected by selective pressure in different regions. Resistance to Kanamycin was recorded in 57% isolates. This is lower than reported by Nadia et al. (2004) from Karachi, Pakistan. They have recorded 50% resistance (Gul et al., 2004).

Ciprofloxacin has been recommended as first line therapy in UTI (Paterson, 2000). But resistance to flouroquinolones is increasing throughout the world (Matute et al., 2004; El Astal, 2005; Karlowsky et al., 2006). The observed resistance in *E. coli* to ciprofloxacin, ofloxacin was 62% and to enoxacin was 61% in this study. This is higher than reported in other studies from Palestine, Canada, USA and Turkey (Mazzulli et al., 2001; Sahm et al., 2001; El Astal, 2005; Yuksel et al., 2006).

Generally, pathogens in hospitals are resistant to multiple antibiotics due to increased selection pressure of antibiotics. Surveillance studies have been conducted to monitor antibiotic susceptibility pattern in pathogenic bacteria to help clinicians when using empirical treatment for infections. Studies from USA, Europe and most other countries have shown better susceptibility pattern for pathogens isolated from UTI against SXT (Szczypa et al., 2001; Mazzulli et al., 2001; Sahm et al., 2001; Gordon and Jones, 2003; Bonsu et al., 2006). But in this region of the world SXT has shown poor activity (Tankhiwale et al., 2004; Akram et al., 2007). A reason for this lack of sensitivity may be that in the past, SXT has been extensively used in this region. Among the 116 *E. coli*, 94 (81%) strains were resistant to SXT. Hence, SXT cannot be recommended as an empiric therapy for the treatment of UTI in Pakistan.

Extended spectrum β -lactamases producing strains are mostly associated with UTI (Jamal et al., 2005; Melzer and Petersen, 2007). The mortality rate, length of hospital

stay and cost of hospitalization due to infections caused by ESBL producing *E. coli* isolates is significantly higher than caused by ESBL non-producing *E. coli* (Schwaber et al., 2006; Melzer and Petersen, 2007). ESBL prevalence varies in different countries. ESBL prevalence of 67, 42 and 43% has been reported in *E. coli* from Iran, India and Bangladesh respectively (Rahman et al., 2004; Mehrgan and Rahbar, 2008; Taneja et al., 2008). While less than 1% of *E. coli* isolates produce ESBL in the Scandinavian countries (Stobberingh et al., 1999; Kjerulf et al., 2008). In this study, 56.9% isolates were ESBL producers. This is higher than reported by others from Pakistan (Shah et al., 2003; Ali et al., 2004; Jabeen et al., 2005). Carbapenems have better activity against ESBL producers (Paterson, 2000) . This is in agreement with our study as all of our ESBL producing isolates were susceptible to carbapenems.

Multi-drug resistance is a major problem in the management of uropathogens (Tankhiwale et al., 2004; Akram et al., 2007; Hasan et al., 2007). This MDR may be due to plasmids harboring several resistance genes which are transferred from one bacterium to another (Ram et al., 2000) . Mathai et al. (2004), have linked such resistance pattern to the presence of integrons (Mathai et al., 2004). We recorded 83% isolates as MDR. The prevalent MDR pattern was resistance to ampicillin, co-trimoxazole, doxycycline and quinolones. To investigate MDR, ESBL from other parts of Pakistan, further studies using more isolates are required. Studies of molecular epidemiology of these resistance genes can also be used for comparison with genes already isolated from other parts of the world.

In conclusion this study shows that larger number of *E. coli* recovered from UTI in this region produce ESBL. Thus, they are resistant to penicillins and cephalosporins, which are important drugs in UTI treatment. Such isolates are also resistant to flouroquinolones, aminoglycosides, tetracyclines and co-trimoxazole. Carbapenems are the drugs of choice against UTI caused by *E. coli*. The higher MDR in this region is a cause for concern. Further molecular studies may have to be conducted to establish the basis of this MDR.

Strict antibiotic policy should be adopted in hospitals to estimate the impact of higher resistance in bacteria and to take steps for reducing this resistance. There are several possible methods for overcoming resistance including reduced use of antibiotics, use of synergistic combinations, addition of an anti-resistance factor, attacking the underlying disease, improving the hygienic measures and regular surveillance studies (Hankook, 1998; Huovinen, 1998).

Competing interests

We (FU, SAM and JA) declare that we have no competing interests.

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