Original Article

DISTRIBUTION AND SUSCEPTIBILITY PATTERN OF URINARY TRACT BACTERIAL PATHOGENS IN AN OUTPATIENT SETTING. A LABORATORY BASED STUDY–FAISALABAD

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ABSTRACT

A retrospective study was conducted in the setting of a diagnostic laboratory (Meezan Lab) catering to tertiary care hospitals and out patient population of Faisalabad district. A total of 93 urine samples collected during a time period of 6 months from July 2011 to December 2011 and fulfilling the inclusion criteria were included in the study. The objective was to assess the distribution of urinary tract bacterial pathogens with respect to age and gender and to determine the antibiotic susceptibility profile of the isolates in our setting. Single positive cultures with a colony count of $>10^5$ CFU on CLED medium were identified and their antibiotic sensitivity and resistance profile was depicted in the form of an antibiogram. 78.4% of the total isolates were identified as *E. coli*, followed by *Enterobacter spp.* 6.45%, *Staphylococcus aureus* 6.45%, *Pseudomonas aeruginosa* 5.37%, *Enterococcus fecalis* 2.15% and *Klebsiella pneumonia* 1.07%. It was observed that 95.12% of total tested isolates were sensitive to Cefoperazone/sulbactam followed by Piperacillin/Tazobactam 94.4 %, Amikacin 90.5%, Cefipime 63.73%, Imipenem 62.65%, Gentamicin 52.5%, Ceftazidime 52.4%, Levofloxacin 45%, Sparfloxacin 37.5%, Ceftriaxone 34.6%, Enoxacin 26.6%, Ciprofloxacin 16.6%, Cotrimoxazole 13.95%. This is an alarmingly low sensitivity to Ciprofloxacin and Cotrimoxazole both of which are routinely used as empirical therapy for uncomplicated community acquired urinary tract infections and reinforces the need for proper sensitivity testing to guide the management of UTIs.

Keywords: CFU=Colony Forming Units, CLED=Cysteine Lactose Electrolyte Deficient agar, CA-UTI=Community Acquired Urinary Tract Infection, uropathogens=urinary tract pathogens, IDSA=Infectious Diseases Society of America, spp=species

INTRODUCTION

UTIs are one of the most common bacterial infections worldwide with an estimate of 150 million cases per year and result in a cost of more than 6 billion dollars.[1,2] UTIs are estimated to account for over 7 million outpatient visits, 1 million visits to the emergency room, and approximately 100,000 hospital admissions per year in the USA.[3]

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Conclusive data regarding morbidly burden of UTIs in Pakistan is lacking. Uncomplicated community acquired UTI (CA-UTI) is most frequently caused by *E. coli.*[1,2,3,4] Other pathogens implicated are *Klebsiella spp*, *Proteus spp*, *Pseudomonas spp*, *Enterobacter spp* and *Staphylococcus saprophyticus* amongst others.[3]

In routine practice it is seen that uncomplicated CA-UTIs are treated empirically mostly with Cotrimoxazole, Fluoroquinolones or Nitrofurantoin. In the setting of Pakistan unprescribed antibiotic use is common with patients taking antibiotics from over the counter as self-therapy. Such injudicious use

of antibiotics is one factor amongst others that leads to the rapid development and spread of resistance in uropathogens. According to the IDSA (Infectious Diseases Society of America) uncomplicated CA-UTI in non-pregnant females can be treated with Cotrimoxazole empirically with Fluorquinolones, Nitrofurantoin and Fosfomycin as alternatives in areas with a documented high resistance to Cotrimoxazole.[2,4] These guidelines may not apply to our setting in Pakistan as there is insufficient epidemiological data regarding the common uropathogens and their susceptibility patterns. Regional studies of distribution of uropathogens and their susceptibility profiles can help to develop such necessary data.

MATERIALS AND METHODS

Design and setting

This was a retrospective study conducted on urine samples obtained in the time duration between July 2011 and December 2011. The setting was Meezan lab which serves as a private diagnostic and reference laboratory to a tertiary care hospital and general outpatient population of Faisalabad. It also caters to suburban areas via its four satellite collection centres.

Sample size and Inclusion criteria

Only urine samples from outpatients with significant pus cells on light microscopy were included in the study. A total of 93 isolates from single positive cultures with a colony count of $>10^5$ CFU were included.[1,3,5,6,7,8]

Specimen processing

Sample were received in laboratory in sterile containers, samples consisted of freshly voided mid-stream urine from symptomatic outpatients. The samples were processed upon arrival. After urinalysis samples with significant pus cells were cultured on Cysteine Lactose Electrolyte Deficient (CLED) medium. Inoculation of CLED medium was done using a calibrated loop of 0.001 ml as per standard protocol.[3,5,7] Plates were incubated at 37°C for 24 hours in aerobic environment. Single positive culture isolates of CFU $>10^5$ were identified and further processed.

Microbiological identification

Organisms were identified on the basis of colony morphology, cultural characteristics, gram stain, morphology, and biochemical testing as per standard protocols.[7,8] Biochemical tests included oxidase, TSI, citrate, urease, indole and motility for gram negative organisms and catalase, coagulase, bile esculin, novobiocin and bacitracin sensitivity for gram positive organisms.[7,8] These tests were performed as per standard protocols.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was performed upon confirmed isolates. This was executed according to CLSI guidelines by Kirby–Bauer Disk Diffusion method on Muller Hinton Agar.[3,5,7,9,10] Antibacterial discs (Oxoid) used included representatives from different antibiotic groups which are routinely used in management of UTIs. Antibiotics tested were Amikacin (AK30 mcg), Gentamicin (CN 10 mcg), Ampicillin (AM 10mcg), Amoxicillin/clavulanic acid (AMC 20/10 mcg), Piperacillin/ Tazobactam (TZP 36mcg), Imipenem (IMP 10mcg), Cefixime (CFM 5mcg), Cephradine (CE 30mcg), Ceftazidime (CAZ 30mcg), Cefipime (FEP 30mcg), Ceftriaxone (CRO 30mcg), Cefoperazone/Sulbactam (SCF 10/5mcg), Ciprofloxacin (CIP 5mcg), Enoxacin (EN 10mcg), Levofloxacin (LEV 5mcg), Sparfloxacin (SPX 5mcg), Cotrimoxazole (SXT 1.25/23.75mcg). In addition Vancomycin (VA 30mcg), Linezolid (LZD 30mcg) and Erythromycin (E 15mcg) were also tested in case of gram positive isolates (See Table 1 and Figure 1).

RESULTS

Of the 93 isolates included in the study 78.4% were *E. coli*, followed by *Enterobacter spp.* 6.45%, *Staphylococcus aureus* 6.45%, *Pseudomonas aeruginosa* 5.37%, *Enterococcus fecalis* 2.15% and *Klebsiella pneumonia* 1.07%.

Distribution of the isolates

The distribution of these pathogens with respect to age group and gender was analyzed. The data was organized from 9 distinct age groups, 0–10, 11–20, 21–30, 31–40, 41–50, 51–60, 61–70, 71–80 and 81–90 years. Total number of isolates, the relative frequencies of individual organisms as

percentages and their frequencies in %age in males and females were organized and are depicted in Table 2.

In age group 1–10: 100% isolates were *E. coli* in both males and females, in 11–20 year age group: 77.7% isolates were *E. coli* followed by *Staph aureus* and *Enterobacter spp*., in males 66.6% isolates were *E. coli* followed by *Staph aureus* and in females 50% were *E coli* followed by *Enterobacter spp*. In the 21–30 year group 77.7% isolates were *E. coli* followed by *Staph aureus* 11.11% and *Klebsiella pneumoniae* 11.11%. The 31-40 years age group comprised *E. coli* 90% and *Staph aureus* 10%. In 41–50 years age group maximum isolates were *E. coli* 68.75% followed by *Pseudomonas aeruginosa* 12.5%, *Staph aureus* 12.5% and *Enterobacter spp.* 6.25%. In the age group 51–60 years the relative frequencies of bacterial pathogens was *E. coli* 88.2%, *Pseudomonas aeruginosa* 14.2% and *Enterobacter sp.* 14.2%. In the age group 61–70 years the frequencies were *E. coli* 70%, *Enterococcus fecalis* 11.76%, *Enterobacter spp*. 5.8%, *Pseudomonas aeruginosa* 5.8% and *Staph aureus* 5.8%. In the age group 71–80 years the relative percentages of isolates were *E. coli* 75%, *Enterobacter spp*. 25% and in the age group 81–90 years 71.4% of isolates *E. coli* followed by *Enterobacter spp.* 14.2% and *Pseudomonas aeruginosa* 14.2%. It was observed that in males and females of all age groups relative percentage of *E. coli* was highest without exception.

Susceptibility pattern of the isolates

It was observed that 95.12% of total tested isolates were sensitive to Cefoperazone/ sulbactam followed by Piperacillin/Tazobactam 94.4 %, Amikacin 90.5%, Cefipime 63.73%, Imipenem 62.65%, Gentamicin 52.5%, Ceftazidime 52.4%, Levofloxacin 45%, Sparfloxacin 37.5%, Ceftriaxone 34.6%, Enoxacin 26.6%, Ciprofloxacin 16.6%, Cotrimoxazole 13.95%. Gram positive organisms showed 100% sensitivity to Vancomycin and Linezolid. It was observed that there was an alarmingly low sensitivity to both Ciprofloxacin and Cotrimoxazole both of which are routinely used as empirical therapy for uncomplicated community acquired urinary tract infections (CA-UTI).

Susceptibility patterns of all organisms were observed and documented. The data was organized into two antibiograms, one for gram negative isolates and the other for gram positive organisms in Tables 1 and 3.

Table 1 shows the susceptibly pattern of gram negative isolates to representative antibiotics from routinely used antibiotic groups. *E. coli* isolates displayed 100% resistance to Ampicillin, Augmentin, and Cephradine, closely followed by 88.8% resistance to Cotrimoxazole, 88.2% to Cefixime and 85.9% to Ciprofloxacin. In comparison lesser resistance was observed to levofloxacin 58.8% and least to Imipenem 4.1% followed by 5.9% to Amikacin indicating that these may be better treatment options compared to the conventional empiric therapy. Sensitivity and resistance of *Enterobacter sp.*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* are also described in Table 3.

The tested *Staph aureus* isolates displayed 100% sensitivity to Amikacin, Augmentin, Cefaclor, Vancomycin and Linezolid, whereas there was 100% resistance to Erythromycin, 66.6% to ceftazidine and 33.3% to levofloxacin. The tested *Enterococcus fecalis* isolates showed maximum resistance to Amikacin, Ampicillin, most of the cephalosporins, Gentamicin, Imipenem, and cotrimoxazole. They displayed good sensitivity to Cefaclor, Piperacillin/Tazobactam, Cefoperazone/Sulbactam, Vancomycin and Linezolid.

Systematic review

A systemic review of the distribution of pathogens responsible for CA-UTI in various studies conducted in Pakistan in comparison with the distribution of pathogens in the present study has been depicted in the following Table 4.

DISCUSSION

The most commonly isolated organism in the present study was *E. coli* 78.49%. This is in concurrence with other studies conducted in Pakistan with frequency of *E. coli* being 70% in one Lahore based study[5] and 37% in another. In Islamabad based studies *E coli* was most commonly isolated with a frequency of 46.98%[6] and 66%, in four separate studies

Table 1. Antibiogram showing susceptibility pattern of gram negative organisms isolated

Antibiotic tested	***E. Coli*** **(number of isolates=73)**			***Enterobacter sp.*** **(number of isolates=6)**			***Pseudomonas aeuroginosa*** **(number of isolates=5)**			***Klebsiella pneumoniae*** **(number of isolates=1)**		
	% of total	% S	% R	% of total	% S	% R	% of total	% S	% R	% of total	% S	% R
AMINOGLYCOSIDES												
Amikacin	91.7	94	5.9	83.3	80	20	100	80	20	100	0	100
Gentamicin	90.4	60.6	39.4	100	6.66	3.33	100	60	40	100	0	100
PENICILIINS												
Ampicillin	1.3	0	100	33.3	0	100	20	0	20	NT	NT	NT
Amoxicillin + clavulanic acid	1.3	0	100	NT	NT	NT	NT	NT	NT	NT	NT	NT
Piperacillin + Tazobactam	74	92.6	7.4	66.6	100	0	60	100	0	100	100	0
CARBAPENEMS												
Imipenem	98.6	95.8	4.1	100	100	0	100	40	60	100	100	0
CEPHALOSPORINS												
Cefixime	69.86	9.8	88.2	66.6	50	50	60	0	100	100	0	100
Cephradine	6.8	0	100	NT	NT	NT	20	0	100	NT	NT	NT
Ceftazidime	89	52.3	49.2	100	83.3	16.6	60	66.6	33.3	100	0	100
Cefipime	97.2	66.2	33.8	100	83.3	16.6	100	40	60	100	0	100
Ceftriaxone	91.7	32.8	67.2	83.3	40	60	80	50	50	100	100	0
Cefoperazone + Sulbactam	70.9	92.7	7.2	83.3	100	0	100	100	0	100	100	0
QUINOLONES												
Ciprofloxacin	97.2	14	85.9	100	50	50	100	40	60	100	0	100
Enoxacin	97.2	19.7	80.2	100	50	50	100	40	60	100	0	100
Levofloxacin	93.1	41.2	58.8	100	66.6	33.3	100	40	60	100	0	100
Sparfloxacin	75.3	29	71	66.66	33.3	66.66	80	25	75	100	0	100
SULFONAMIDES												
Trimethoprim + Sulfamethoxazole	98.6	11.1	88.8	100	33.3	66.6	100	20	80	100	100	0

Number of isolates: Total number of clinical isolates obtained of a specific gram negative organism, **% of total:** Percentage of total isolates obtained that were tested for antibiotic susceptibility, **% S:** Percentage of tested isolates that were sensitive to the antibiotic, **% R:** Percentage of tested isolates that were resistant to the antibiotic, **NT:** Not tested

Table 2. Age group and gender-wise distribution of organisms isolated

Age Group (years)	Total No. of isolates	Individual isolates percentage of total	Percentage in Males	Percentage in Females
1 – 10	8	*E. coli* 100%	*E. coli* 100%	*E. coli* 100%
11 – 20	5	*E. coli* 60% *Staph aureus* 20% *Enterobacter spp*.20%	*E. coli* 66.6% *Staph aureus* 33.3%	*E. coli* 50% *Enterobacter spp.* 50%
21 – 30	9	*E. coli* 77.7% *Staph aureus* 11.11% *Klebsiella pneumoniae* 11.11%	*E. coli* 66.6% *Klebsiella pneumoniae* 33.3%	*E. coli* 83.3% *Staph aureus* 16.6%
31 – 40	10	*E. coli* 90% *Staph aureus* 10%	*E. coli* 100%	*E. coli* 83.3% *Staph aureus* 16.6%
41 – 50	16	*E. coli* 68.75% *Pseudomonas aeruginosa* 12.5% *Staph aureus* 12.5% *Enterobacter spp*. 6.25%	*E. coli* 83.3% *Pseudomonas aeruginosa* 16.6%	*E. coli* 60% *Staph aureus* 20% *Pseudomonas aeruginosa* 10% *Enterobacter spp* 10%
51 – 60	17	*E. coli* 88.2% *Pseudomonas aeruginosa* 14.2% *Enterobacter spp.* 14.2%	*E. coli* 100%	*E. coli* 83.8% *Pseudomonas aeruginosa* 8.3% *Enterobacter spp*. 8.3%
61 – 70	17	*E. coli* 70% *Enterococcus fecalis* 11.76% *Enterobacter spp*. 5.8% *Pseudomonas aeruginosa* 5.8% *Staph aureus* 5.8%	*E. coli* 71.4% *Enterococcus fecalis* 14.2% *Enterobacter spp.* 14.2%	*E. coli* 70% *Enterococcus fecalis* 10% *Pseudomonas aeruginosa* 10% *Staph aureus* 10%
71 – 80	4	*E. coli* 75% *Enterobacter spp.* 25%	*E. coli* 66.6% *Enterobacter spp*. 33.3%	*E. coli* 100%
81 – 90	7	*E. coli* 71.4% *Enterobacter spp*. 14.2% *Pseudomonas aeruginosa* 14.2%	*E. coli* 33.3% *Enterobacter spp*. 33.3% *Pseudomonas aeruginosa* 33.3%	*E. coli* 100%

Total number of isolates: Total number of isolates of all organisms from the specific age group, **Individual isolates percentage of total:** Breakdown of all isolates into specific organisms obtained in the specified age group with their relative percentages, **Percentage in males:** Breakdown of all isolates into specific organisms obtained from males in the specified age group with their relative percentages, **Percentage in females:** Breakdown of all isolates into specific organisms obtained from females in the specified age group with their relative percentages.

Table 3. Antibiogram showing susceptibility pattern of gram positive organisms isolated

Antibiotic tested	*Staphylococcus aureus* (No. of isolates = 6)			*Enterococcus fecalis* (No. of isolates = 2)		
	% of total	% S	% R	% of total	% S	% R
AMINOGLYCOSIDES						
Amikacin	66.6	100	0	100	0	100
Gentamicin	NT	NT	NT	50	0	100
MACROLIDES						
Erythromycin	33.3	0	100	NT	NT	NT
PENICILLINS						
Ampicillin	NT	NT	NT	50	0	100
Amoxicillin + clavulanic acid	83.3	100	0	NT	NT	NT
Methicillin	100	83.3	16.66	50	0	100
Piperaciliin + Tazobactam	83.3	100	0	50	100	0
CARBAPENEMS						
Imipenem	NT	NT	NT	50	0	100
GLYCOPEPTIDE						
Vancomycin	100	100	0	50	100	0
OXAZOLIDINONES						
Linezolid	100	100	0	50	100	0
CEPHALOSPONRINS						
Cefaclor	66.6	100	0	50	100	0
Cefixime	NT	NT	NT	50	0	100
Cephradine	100	50	50	50	0	100
Cefipime	100	83.3	16.6	100	50	50
Ceftazidime	100	33.3	66.6	100	50	50
Cefotaxime	100	100	0	50	100	0
Cefoperazone + Sulbactam	NT	NT	NT	50	100	0
QUINOLONES						
Ciprofloxacin	NT	NT	NT	50	0	100
Enoxacin	83.3	80	20	100	50	50
Levofloxacin	100	66.66	33.3	100	50	50
Sparfloxacin	100	83.3	16.6	100	50	50
SULFONAMIDES						
Trimethoprim + Sulfamethoxazole	NT	NT	NT	100	0	100

Number of isolates: Total number of clinical isolates obtained of a specific gram negative organism, **% of total:** Percentage of total isolates obtained that were tested for antibiotic susceptibility, **% S:** Percentage of tested isolates that were sensitive to the antibiotic, **% R:** Percentage of tested isolates that were resistant to the antibiotic, **NT:** Not tested

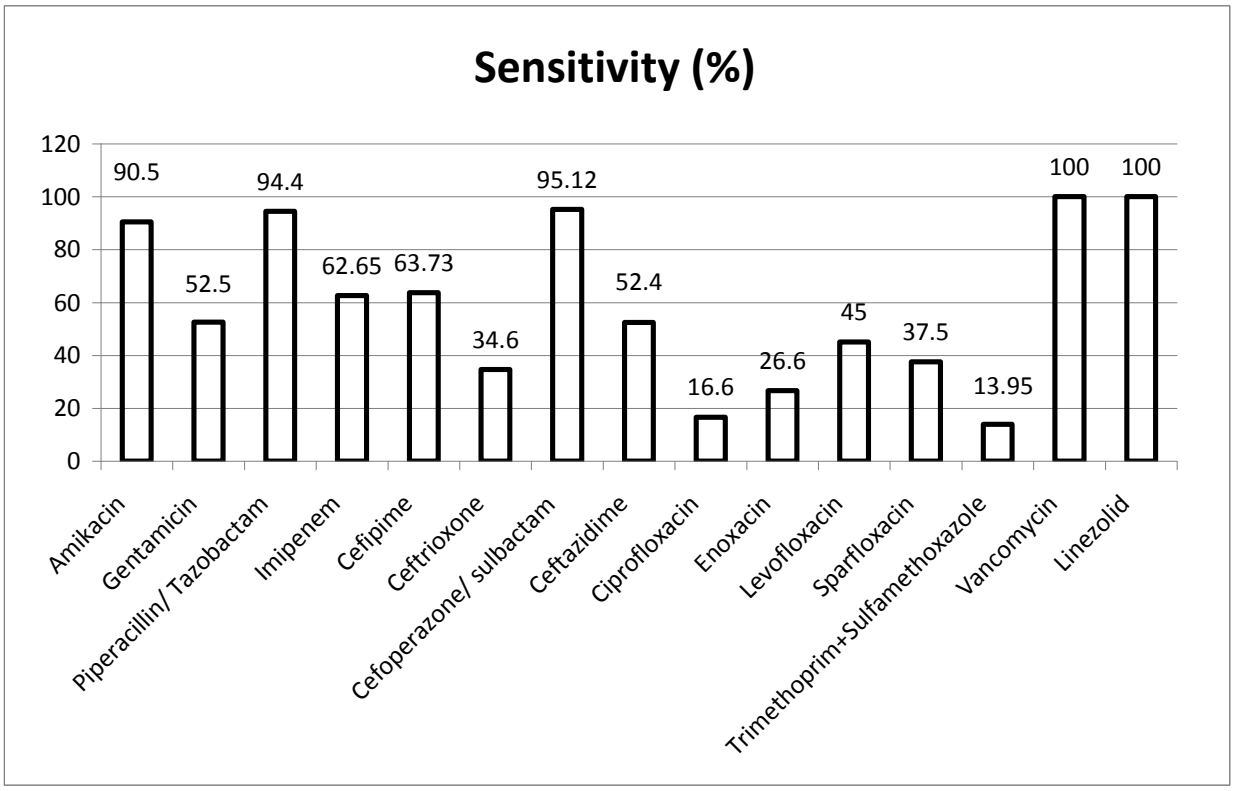

Figure 1. Depicting cumulative antibiotic sensitivities of the majority of isolates tested

Table 4. Systematic Review: year wise and area wise distribution of urinary tract organisms isolated in studies conducted in Pakistan

Common Organisms %	Present study	Humayun *et al.* (5)	Bano *et al.* (6)	Naeem *et al.* (11)	Zafar *et al.* (12)	Ahmad *et al.* (13)	Shaikh *et al.* (14)	Gul *et al.* (15)	Noor *et al.* (16)	Khan *et al.* (17)	Farooqui *et al.* (10)
	Faisalabad 2012	Lahore 2012	Islamabad 2012	Islamabad 2010	Lahore 2009	Gilgit 2008	Karachi 2005	Karachi 2004	Karachi 2004	Abbottabad 2000	Karachi 1989
E. coli	78.49	70	46.98	66	37	48.67	52	47.6	73	76.6	40
Enterobacterspp	6.45		1.2	3				4.6			
Pseudomonasspp	5.37	1.4	2.43	7	23	7.5	7			38	16
Klebsiellaspp	1.07	14	18.07	13	37		22	7.6	16	14.3	11
Proteus spp		1.4	2.43		1	8.86	4	4.6	11	5.2	13
Acinetobacter		4.2			1						
Serratia spp							1	9.2			
Staph. spp	6.45	2.8	16.87	10	1	32.76	0.7	9			
Enterococcusspp	2.15	5.7	3.61					3			
Streptococcusspp			2.43					4.6			
Candida spp		1.4	4.81								

conducted in Karachi *E. coli* was most common being 52%[14], 47.6%[4], 73% (9) and 40%[10] of the total isolates. Similarly in a Gilgit based study *E coli* was commonest with 48.98%[13] and in Abbottabad with 76.6%.[17]

The susceptibility profile of urinary tract pathogens is constantly changing with a trend towards resistance to the more commonly used antimicrobials. In 2000 a study conducted in Karachi over a period of 8 years was published which showed an increasing trend towards resistance to Gentamicin, Amikacin, Oflaxacin, Cefotaxime and Ceftazidime.[18] This is also suggested by a temporal comparison of the results of individual studies conducted in Pakistan. In a study published in 2000 gram negative uropathogens showed 72.8% sensitivity to Ciprofloxacin, 69.2% to norfloxacin, 34.9% to Cotrimoxalzole, 56.1% to gentamicin, and 75.4% to ceftriaxone.[17] In 2004 a study reported 48% sensitivity of gram negative uropathogens to ciprofloxacin, 40.3% to norfloxacin, 55.7% to cotrimoxazole and 69.2% to gentamicin.[15] A 2005 study described 43% sensitivity of *E. coli* to ciprofloaxacin and 13% to cotrimoxazole.[14] In 2008 Ahmed *et al.* reported 45.45% resistance of *E. coli* isolates to Ciprofloxacin and 72.73% resistance to ceftazidime.[13]

Naeem *et al.* documented 63% sensitivity of *E. coli* isolates to ciprofloxacin, 95% to piperacillin/tazobactam, 93% to cefoperazone/ sulbactam, 95% to imipenem, and 88% to amikacin in 2010.[11] The greater sensitivity to piperacillin/tazobactam, cefoperazone/sulbactam and amikacinare comparable to the results of the present study. A recent study Bano *et al.* published in 2012 reported 56% sensitivity of *E. coli* to Amikacin which was the most sensitive in the study and a lowest sensitivity of 5% to Ciprofloxacin. Sensitivity to Cotrimoxazole was 12%.[6] Another recently published study conducted in Lahore reported Amikacin to be the most sensitive drug for *E coli* causing UTI with a sensitivity of 97.61%, sensitivity to Cotrimoxazole was reported as 81% and 37.14% for Levofloxacin.[5]

It is becoming imperative to identify nationwide trends in antibiotic susceptibilities of uropathogens and to develop a continuous surveillance for changes in susceptibility trends.

CONCLUSION

E. coli is the leading cause of CA-UTI in our setting and mirrors the same trend in other studies conducted in Pakistan. Sensitivity of cefperazone/sulbactam, Piperacillin/tazobactam and Amikacin is good across the board whereas Vancomycin and Linezolid are effective for gram positive urinary tract pathogens. A trend towards increased resistance of urinary tract pathogens to Cotrimoxazole, Ciprofloxacin and other Quinolones has been identified and is repeatedly seen across different studies in Pakistan. This warrants the need for nationwide research initiatives to determine and continuously monitor the antibiotic susceptibility profiles of urinary tract pathogens so that local guidelines for empiric therapy can be developed. It is recommended that proper sensitivity testing of UTI causing organisms should be undertaken to guide the management of UTIs until the development of conclusive local guidelines regarding empiric therapy.

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