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Research Article

TWO YEARS RETROSPECTIVE STUDY OF ANTIBIOTIC RESISTANCE PATTERN OF UROPATHOGENS ESPECIALLY *Escherichia coli* IN NORTH INDIA

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ABSTRACT
Urinary tract infection (UTI) is a common health problem. Antibiotic resistance pattern of uropathogens varies from place to place. Moreover, bacteria have developed more resistance to antibiotics due to their extensive and irrational use. The aim behind the study was to report the current antibiotic resistance pattern of uropathogens in North India. Retrospective study was done on patients of Medicine department in Punjab Institute of Medical Sciences, Jalandhar. These patients were symptomatic of UTI. Pathogens isolated on their urine cultures were identified by standard methods and the antimicrobial susceptibility was performed by Kirby-Bauer disk diffusion method. Prevalence of UTI was 27.1% (365/1347); 40% male and 60% female patients. *Escherichia coli* were 68.7% followed by *Candida spp* 8.1%, *Klebsiella spp* 7.3%, *Pseudomonas aerugenosa* 4.8%, *Staphylococcus aureus* 3.2%, *Enterococcus faecalis* 2.7% among common isolates. All the bacteria were mulitdrug resistant and most of these were resistant to quinolones, 3rd generation cephalosporins and meropenem. *Escherichia coli* resistance to norfloxacin was 90.6%, ciprofloxacin 89.4%, cefotaxime 87.1%, ceftriaxone 84.7%, meropenem 62.7% and gentamicin 59.6%. The effective drugs for *E. coli* were nitrofurantoin, amikacin, piperacillin/tazobactam and imipenem. Uropathogens have got high antibiotic resistance. *Escherichia coli* resistance to quinolones, 3rd generation cephalosporins and even meropenem is very alarming. It has become difficult to treat UTI with few effective antibiotics. Irrational and extensive use of antibiotics must be abandoned. Urine culture must be done wherever possible before starting treatment of UTI. Routine surveillance of antimicrobial susceptibility pattern can play a great role in reducing antimicrobial resistance.

Keywords: *Antimicrobial Resistance, Escherichia Coli, Urinary Tract Infection, Uropathogens*

INTRODUCTION
Urinary tract infection is amongst the common infections encountered in clinical practice (Gaterman, 2007). It remains a major public health problem in terms of morbidity and financial cost with an estimated 150 million cases per annum worldwide, costing global economy in excess of 6 billion US dollars (Gonzalez *et al.,* 1999). UTI is a common reason for admission to internal medicine departments. UTI does represent an important cause of morbidity and has the potential for serious and life-threatening sequelae if left untreated or undertreated. *Escherichia coli* is the principal pathogen of UTI, both in the community as well as in the hospital (Karlowsky *et al.,* 2001; Gorbach *et al.,* 2004). Although UTI occurs in all age groups and both genders, its prevalence is higher in women. An estimated 50% of women experience at least one episode of UTI at some point of their lifetime and between 20% and 40% of women can have recurrent episodes (Rock *et al.,* 2007; Vasquez *et al.,* 2004). Approximately 20% of all UTIs occur in men (Giriebling *et al.,* 2007) and about 35% of all UTIs are those of nosocomial origin (Drekonja and Johnson, 2008).

There is increasing trends of antibiotic resistance of uropathogens worldwide, especially to commonly used drugs including quinolones (Karlowsky *et al.,* 2002; Kahlmeter, 2003; Arslan *et al.,* 2005). Moreover, UTI is now becoming increasingly tough to control because of emergence of bacteria harboring extended-spectrum beta-lactamases (ESBL) (Paterson *et al.,* 2005). Resistance pattern of

uropathogens also varies from place to place. The present study was done to appraise the current antibiotic resistance pattern in the uropathogens isolated in a tertiary care hospital in North India.

MATERIALS AND METHODS

Study Design and Criteria

A retrospective study of two years (June, 2011 to May 2013) was done on the patients symptomatic of urinary tract infection who either visited medical outdoor or were admitted in medical wards of Punjab Institute of Medical Sciences (PIMS), Jalandhar during that period. All the patients symptomatic of UTI were included irrespective of their co-morbidities.

Sample Collection and Analysis

Urine was collected by midstream "clean catch" method and from catheters of the catheterized patients. Urine samples were processed within 1 hour of collection. Microscopic examination of urine samples was done. The samples were inoculated on MacConkey and blood agar by semi-quantitative method with caliberated loop (internal diameter 3.26 mm) and incubated at 37 °C for 18-24hours and significant bacteriuria was looked for. Growth of Gram-negative bacteria > 10^5 cfu/ml, while for gram-positive bacteria, growth of $10^3 – 10^5$ cfu/ml was considered significant. The obsereved growth was identified by colony characters, Gram's staining and biochemical reactions by standard methods (CLSI, 2008).

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing was carried out by Kirby Bauer's Disc diffusion method (Bauer AW *et al.,* 1966). Antimicrobial susceptibility testing was performed on all isolates according to Clinical and Laboratory Standard Institute guidelines (CLSI, 2010). The antibiotic discs used and their concentrations were: Nitrofurantoin (NIT, 300µg), Norfloxacin (NOR, 10µg), Ciprofloxacin (CIP, 5µg), Amikacin (AMK, 30µg), Gentamicin(GEN, 10µg), Azithromycin (AZM, 15µg), Erythromycin (ERY, 15µg), Piperacillin-Tazobactam (TZP, 100µg/10µg), Cefotaxime (CTX, 30µg), Ceftriaxone (CRO, 30µg), Meropenem (MEM, 10µg), Imipenem (IPM, 10µg), Oxacillin (OXA, 1µg), Clindamycin (CLI, 2µg), Linezolid (LZD, 30µg) and Vancomycin (VAN, 30µg).

Data Collection and Statistical Analysis

The variables recorded in the data were patient's age, sex and status of the patient whether outdoor or indoor, pathogen grown on urine culture and its antimicrobial susceptibility. The data was analyzed statistically using SPSS statistical software package (version 19).

RESULTS

Demographic Characteristics

The age range of 1,347 patients studied was 15 to 95 years with the mean (±SD) of 49.6 (±17.7) years. Urine culture was positive for 365 (27.1%) patients, 146 (40%) male and 219 (60%) female; male to female ratio 2:3. Patients with UTI were 46.8% outpatients and 53.2% inpatients. Maximum patients (27.7%) with UTI belonged to 55 – 64 year age group and minimum (4.7%) to 15 – 24 year age group. Among patients aged ≥ 75 years, male were more infected than female whereas in all other groups, female were more infected than male. Difference between distrubion of male and female patients of UTI was statistically significant ($x^2 = 18.649$, $p = 0.005$) (Table 1).

Uro-Pathogens Isolated

A total of 371 pathogens were isolated in 365 urine samples. Among these pathogens, Gram-negative bacteria were 86% (319), Gram-positive bacteria were 5.9% (22) and Candida spp 8.1% (30). The frequency distribution of the uro-pathogens is shown in Table 2. The most frequently isolated Gram-negative bacilli were *Escherichia coli* (68.7%), *Klebsiella spp* (7.3%) and *Pseudomonas aerugenosa* (4.8%). Gram-positive bacteria were *Staphylococcus aureus* (3.2%) and *Enterococcus faecalis* (2.7%).

Table 1: Demographic characteristics of patients of UTI

Age (years)	Total (%)	Male (%)	Female (%)	Chi-square (x^2)	*p*-value
15 - 24	17(4.7)	2(11.8)	15(88.2)		
25 - 34	26(7.1)	6(23.1)	20(76.9)		
35 - 44	33(9.0)	9(27.3)	24(72.7)		
45 - 54	78(21.4)	38(48.7)	40(51.3)	18.649	0.005
55 - 64	101(27.7)	40(39.6)	61(60.4)		
65 - 74	66(18.1)	26(39.4)	40(60.6)		
≥ 75	44(12.1)	25(56.8)	19(43.2)		
Total	**365(100)**	**146(40)**	**219(60)**		
Patient status					
Outdoor	171(46.8)	65(38)	106(62)	0.53	0.467
Indoor	194(53.2)	81(41.8)	113(58.2)		

Table 2: Frequency of the uropathogens isolated

Pathogen	Frequency	Percentage
Gram-negative bacteria	**319**	**86**
Escherichia coli	255	68.7
Klebsiella spp	27	7.3
Pseudomonas aerugenosa	18	4.8
Proterus spp	8	2.1
Acinetobacter spp	6	1.6
Citrobacter spp	2	0.6
Enterobacter spp	2	0.6
Edwardsiella spp	1	0.3
Gram-positive bacteria	**22**	**5.9**
Staphylococcus aureus	12	3.2
Enterococcus faecalis	10	2.7
Candida spp	**30**	**8.1**
Total isolates	**371**	**100**

Antibiotic Resistance Pattern of Bacteria

All the bacteria were multidrug resistant having resistance against 2 or more antimicrobials. *Eschericihia coli* showed very high resistance to norfloxacin (90.6%), ciprofloxacin (89.4%), cefotaxime (87.1%) and ceftriaxone (84.7%). Resistance of these isolates against gentamicin and meropenem was also high, 59.6% and 62.7% respectively. The antibiotic with lowest resistance to these isolates was imipenem. *Klebsiella spp* were 88.9% resistant to ceftriaxone, 81.5% resistant to cefotaxime, norfloxacin and ciprofloxacin, 77.8% resistant to nitrofurantoin, gentamicin and meropenem, and 51.8% to piperacillin/tazobactam. *Pseudomonas aerugenosa* showed 100% resistance to nitrofurnatoin and very high resistance to all other antimicrobials except imipenem. *Proteus spp* showed high resistance to nitrofurantoin (87.5%) and ceftriaxone (62.5%). *Acinetobacter spp* were 100% resistant to nitrofurantoin, norfloxacin and ciprofloxacin, 83.3% resistant to amikacin and cefotaxime and 66.7% resistant to gentamicin, ceftriaxone and meropenem. *Citrobacter spp* showed 0% resistance to piperacillin/tazobactam and imipenm, 50% resistance to nitrofurantoin and 100% resistance to all other antimicrobials. *Enterobacter spp* were 100% resistant to all the antimicrobials. *Edwardsiella spp* showed 100% resistance to cephalosporins and quinolones and 0% resistance to all other antibiotics.

Among Gram-positive bacteria, *Staphylococcus aureus* were 67% resistant to oxacillin. These isolates showed high resistance to quinolones and 3rd generation cephalosporins; 91.7% resistance to norfloxacin and cefotaxime and 83.3% resistance to ciprofloxacin and ceftriaxone. Meropenem and imipenem resistance was 91.7%, azithromycin 83%, erythromycin 75% and gentamicin 58.3% among these isolates.

These isolates showed less resistance to vancomycin, linezolid, nitrofurantoin, amikacin and piperacillin/tazobactam, 0%, 16.7%, 25%, 41.7% and 50% respectively. *Enterococcus faecalis* were 100% resistant to quinolones, 3rd generation cephalosporins and carbapenems and 90% resistant to piperacillin/tazobactam and aminoglycosieds and 70% resistant to nitrofurantoin. These isolates were less resistant to vancomycin (10%) and linezolid (0%). Antibiotic resistance pattern of commonly isolated bacteria is shown in Table 3.

Table 3: Antibiotic resistance pattern (in percentage) of commonly isolated bacteria

	All Gram-negative bacilli (n = 319)	Escherichia coli (n = 255)	Klebsiella spp (n = 27)	Pseudomonas spp (n = 18)	Proteus spp (n = 8)	Acinetobacter spp (n = 6)	Staphylococcus auureus (n = 12)	Enterococcus faecalis (n = 10)
Nitrofurantoin	36.9	24.7	77.8	100	87.5	100	25	70
Norfloxacin	88.4	90.6	81.5	83.3	37.5	100	91.7	100
Ciprofloxacin	86.2	89.4	81.5	66.7	25	100	83.3	100
Amikacin	23.2	15.3	48.1	66.7	12.5	83.3	41.7	90
Gentamicin	61.4	59.6	77.8	61.1	50	66.7	58.3	90
Cefotaxime	85.6	87.1	81.5	83.3	50	83.3	91.7	100
Ceftriaxone	84.3	84.7	88.9	83.3	62.5	66.7	83.3	100
Piperacillin/Tazobactam	21.3	14.5	51.8	66.7	12.5	33.3	50	90
Meropenem	65.2	62.7	77.8	88.9	37.5	66.7	91.7	100
Imipenem	15.7	12.2	25.9	44.4	0	33.3	91.7	100
Azithromycin	-	-	-	-	-	-	83.3	-
Erythromycin	-	-	-	-	-	-	75	-
Oxacillin	-	-	-	-	-	-	66.7	-
Clindamycin	-	-	-	-	-	-	75	-
Linezolid	-	-	-	-	-	-	16.7	0
Vancomycin	-	-	-	-	-	-	0	10

" - " means antibiotic not tested

The comparative antibiotic resistance pattern of *E. coli* and all Gram-negative bacilli is shown in Figure I. Against norfloxacin, ciprofloxacin, cefotaxime and ceftriaxone, *Escherichia coli* showed higher resistance than all Gram-negative bacilli whereas against all other antibiotics, *E. coli* resistance was less than all Gram-negative bacilli.

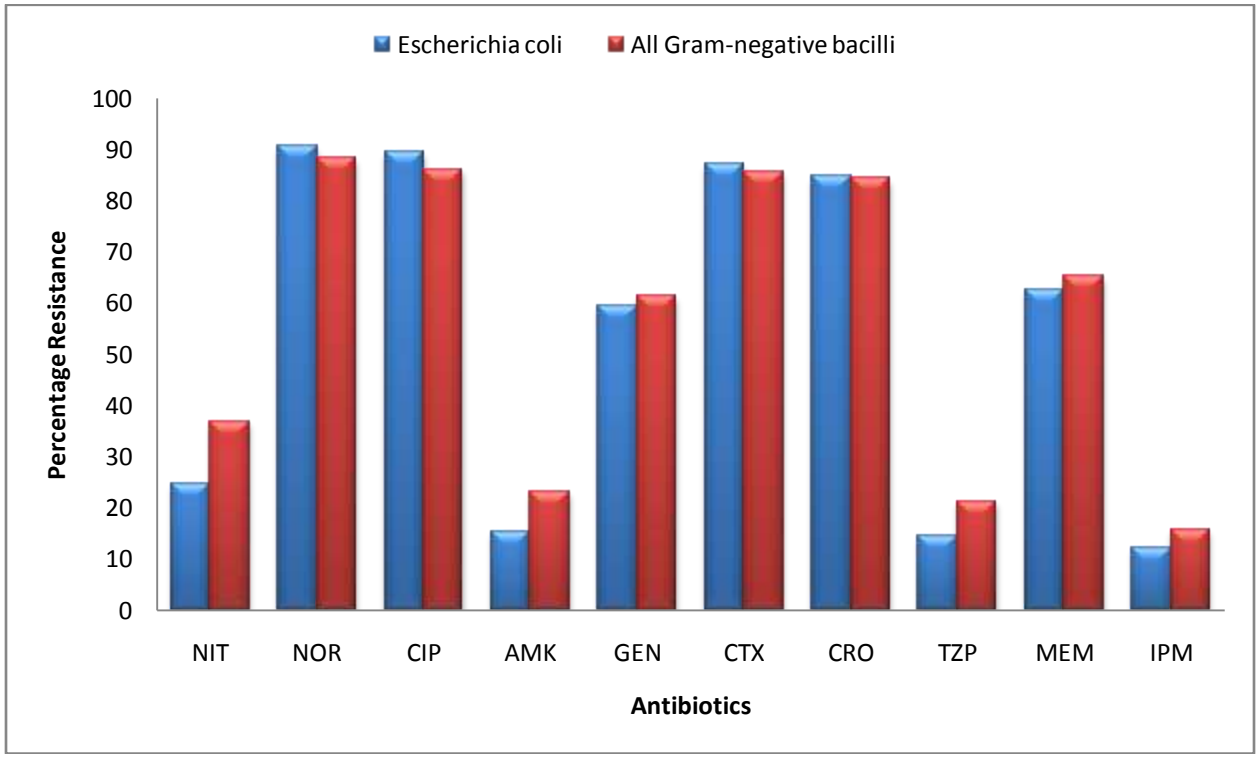

Figure I Antibiotic resistance of *Escherichia coli* and all Gram-negative bacilli
NIT, Nitrofurantoin; NOR, Norfloxacin; CIP, Ciprofloxacin; AMK, Amikacin; GEN, Gentamicin; CTX, Cefotaxime; CRO, Cefttriaxone; TZP, Piperacillin/tazobactam; MEM, Meropenem; IPM, Imipenm

DISCUSSION

In this study, the overall incidence of UTI was 27.1%, comparable to 26.01% reported in Puducherry (Mandal *et al.*, 2010) but lower than the reports of Odisha, 34.5% (Dash *et al.*, 2013), Meerut, 53.8% (Prakash and Saxena, 2013) and Assam, 65% (Sharma and Paul, 2012). It was higher than that reported in Aligarh, 10.8% (Akram *et al.*, 2007). This variation in incidence of UTI might be due to different geographical locations differing in environment, social habits of the community, the standard of personal hygiene and education.

Maximum patients of UTI in our study were aged 55 – 64 years, like the finding of Prakash *et al.*, (2013), while on the contrary, other studies (Dash *et al.*, 2013; Akram *et al.*, 2007; Razak and Gurushantappa, 2012) show maximum incidence of UTI among younger patients. The cause of middle aged patients having more UTI in our study might be underlying risk factors like diabetes mellitus, nephrolithiasis or prostatic enlargement among these patients and different age composition of the studied subjects.

In this study, Gram-negative bacilli constituted 86% and Gram-positive bacteria 5.9% and Candida 8.1% of the isolates. Razak *et al.*, (2012) have reported these results as 82.3%, 10% and 7.7% respectively. Akram *et al.*, (2007) have reported Gram-negative rods 92% and Gram-positive cocci 8% of the isolates, whereas Kothari *et al.*, (2008) have reported these microorganisms 95.7% and 4.3% respectively. This variation might depend upon the different conditions of the patient like urinary catheterization.

Escherichia coli was the predominant (68.7%) isolate followed by *Klebsiella spp* (7.3%). This finding correlates with the results of other Indian studies (Mandal *et al.*, 2010; Akram *et al.*, 2007; Kothari and Sagar, 2008; Shifali *et al.*, 2012). More than 80% of all Gram-negative bacilli were resistant to norfloxacin, ciprofloxacin, cefotaxime and ceftriaxone.

Escherichia coli resistance to norfloxacin was 90.6% and to ciprofloxacin 89.4%. Razak *et al.*, (2012) have found the same results, whereas other studies (Akram *et al.*, 2007; Sood and Gupta, 2012; Banerjee

and Padmashri, 2011) have reported norfloxacin resistance of 69%, 66.7%, and 77.8% respectively. Dash *et al.*, (2013) have reported ciprofloxacin resistance 53.4% and Mandal *et al.*, (2010) 73%.

Escherichia coli showed high resistance to cefotaxime (87.1%) and ceftriaxone (84.7%). Akram *et al.*, (2007) have reported lower resistance to these antibiotics; 56% and 55% respectively. Mandal *et al.*, (2010) have reported 60.5% resistance to ceftriaxone. Hasan *et al.*, (2007) in his study at a tertiary hospital of New Delhi, have reported cefotaxime resistance of 71.4%. Sood *et al.*, (2012) have reported 70% cefotaxime resistance and 69.1% ceftriaxone resistance. Prakash *et al.*, (2013) have reported cefotaxime and ceftriaxone resistance of 87.8% and 53% respectively. Among aminoglycosides, *E. coli* isolates showed higher resistance to gentamicin (55.7%) than amikacin (11%) and results from the different Indian studies (Mandal *et al.*, 2010; Dash *et al.*, 2013; Prakash and Saxena, 2013; Akram *et al.*, 2007; Sood and Gupta, 2012; Hasan *et al.*, 2007) support this finding. Akram *et al.*, (2007) reported 64% resistance to gentamicin and 51% resistance to amikacin in 2008 from Aligarh. Dash *et al.*, (2013) have reported 15.9% resistance to gentamicin and 5.8% resistance to amikacin in these isolates. Resistance of *E. coli* to Piperacillin-tazobactam was 14.5%, higher than other reports; 0% by Kumar *et al.*, (2013) and 6.2% by Sood and Gupta (2012). In our study, nitrofurantoin has shown better activity against *E. coli* with resistance of 15.3% which is significantly lower than 80.9% reported by Hasan *et al.*, (2007), 80% shown by Akram *et al.*, (2007), and also lower than of other studies (Mandal *et al.*, 2010; Prakash and Saxena, 2013). Some studies (Dash *et al.*, 2013; Sood and Gupta, 2012; Kumar *et al.*, 2013) have reported nitrofurantoin resistance lower than that observed in our study. Among carbapenems, *E. coli* showed high resistance to meropenem (57.2%) higher than 9.8% stated by Mandal *et al.*, (2010) and 4.5% described by Prakash and Saxena (2013). *E. coli* showed the lowest resistance to imipenem (11.8%) among all antibiotics tested. Prakash and Saxena (2013) and Akram *et al.*, (2007) also have demonstrated lowest imipenem resistance (0%) in their studies. In our study, resistance of *Escherichia coli* to norfloxacin, ciprofloxacin, ceftriaxone and meropenem is maximum as compared to other previous Indian studies which is an alarming finding.

Resistance of Gram-negative bacilli against quinolones and 3rd generations cephalosporins is increased. Quinolones are quite often taken by the patients as self prescription for this ailment. Third generation cephalosporins are used routinely to treat UTI in indoor patients and now carbaenems are also used commonly. The extensive use of all these antibiotics has led to increased resistane of uropathogens against these drugs. Many of these antibiotics are easily available to the patients over the counter (Kotwani and Holloway, 2011).

The first ever meeting of medical societies in India on the issue of antibiotic resistance has been held in Chennai (Chennai Declaration) on the eve of 2nd annual conference of the Clinical Infectious Disease Society (CIDSCON 2012) on 24th August, 2012. National antibiotic policy has been one of the various recommendations of this declaration to tackle the antibiotic resistance (Ghafur *et al.*, 2013).

Recently, World Health Organization (WHO) reports antimicrobial resistance (AMR) is an increasingly serious threat to global public health. The problem is so serious that it threatens the achievements of modern medicine. A post-antibiotic era— in which common infections and minor injuries can kill—is a very real possibility for the 21st century. Very high rates of resistance have been observed in all WHO regions in common bacteria (for example, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*) that cause common health-care associated and community-acquired infections (urinary tract infections, wound infections, bloodstream infections and pneumonia). Resistance to one of the most widely used antimicrobials for the treatment of urinary tract infection caused by *E. coli*–fluoroquinolones–is very widespread. In the 1980s, when these drugs were first introduced, resistance was virtually zero. Today, there are countries in many parts of the world where this treatment is now ineffective in more than half of patients. The results are cause for high concern, documenting resistance to antibiotics, especially "last resort" antibiotics, in all regions of the world (WHO, 2014).

Conclusion

This study reveals the current antibiotic susceptibility pattern of uropathogens in North India as patients being treated at our institute come from different places of this region of our country. Most of the

uropathogenic bacteria are multi-resistant, showing resistance to more than 2 antibiotics, this is an alarming situation. *Escherichia coli*, the most common uropathogen has shown resistance to quinolones, 3rd generation cephalosporins, gentamicin and even meropenem. This leaves the treating physician with few choices of antimicrobial agents to treat UTI. This increased antimicrobial resistance which is the result of irrational and uncontrolled use of the antimicrobials is threat to the public health. This practice must be abandoned and the treatment of UTI must be urine culture based in a hospital setting. A constant surveillance of antibacterial resistance is required to know its current status.

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