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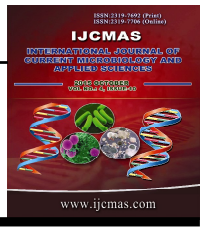


Original Research Article

Antibiotic Susceptibility Pattern of Bacterial Uropathogens Isolated from Patients at a Tertiary Care Hospital in Western Uttar Pradesh of India

Amit A. Rangari[1]*, Sachin Sharma[1], Nidhi Tyagi[2], Paramjit Singh[1], Garima Singh[1] and Rameshwari Thakur[1]

[1]Department of Microbiology, Muzaffarnagar Medical College and Hospital, Muzaffarnagar (U.P.) India
[2]Department of Biotechnology, School of Science, Noida International University, Greater Noida (U.P.) India
**Corresponding author*

Keywords

Uropathogens, Urinary Tract Infection, Antibiotic sensitivity, Antibiotic resistance

A B S T R A C T

Urinary tract infection (UTI) being common bacterial infection in humans is a major cause of morbidity. Aetiology of UTI and their antibiotic sensitivity patterns vary from time to time and across different areas. Antibiotic sensitivity pattern of uropathogens from 300/510 patients with significant bacteriuria was studied at a tertiary care hospital in western Uttar Pradesh India. UTI was frequently encountered in females and adult patients. 15% of the isolates were from in-patients. Most common etiological organisms of UTI isolated were *Escherichia coli* (60%), *Enterococcus* (20%) *Klebsiella spp* (8%), *Pseudomonas aeruginosa* (3%) and *Staphylococcus aureus* (2%). *Pseudomonas aeruginosa* was found only among hospital isolates. Antibiotic susceptibility pattern revealed that >80% of the isolates were sensitive to amikacin, nitrofurantoin and imipenem, while > 60% were sensitive to piperacillin-tazobactum and tobramycin. *E. coli* showed high sensitivity to imipenem 98.88% (178), nitrofurantoin 97.22% (175) and amikacin 92.77% (167). *Enterococcus spp* showed high sensitivity to linezolid 93.33% (56) and nitrofurantoin 91.66% (55). Very high rate of resistance was seen against ceftazidime, carbenicillin, co-trimoxazole, ciprofloxacin, norfloxacin, gentamicin and tetracycline. High rate of multi-drug resistance was recorded among all isolates. In view of the increasing rate of resistance of uropathogens to commonly used antibiotics, rational prescription and use of antibiotics is advocated.

Introduction

Urinary tract infection (UTI) is the most common and a serious health affecting problem both in the community and hospital settings each year worldwide (Bano *et al.*, 2012). It is the second most common infection after respiratory tract infection. It is the most important cause of morbidity in the world affecting all age groups across the life span and in both genders and usually requires medical treatment (Omoregie *et al.*, 2008). About 150 million people are diagnosed with UTI each year, costing the

global economy in excess of 6 billion dollars (Hari P Kattel *et al.,* 2012). One woman in five develops a UTI during her life time. UTI in men are less common, nearly 20 % of women who have a UTI will have another and 30% of those will have yet another episode of UTI. Aetiology of UTI and their antibiotic sensitivity patterns vary from time to time and across different areas (Gruneberg, 1980; Gales *et al.,* 2000; Saffar. *et al.,* 2008). Despite the fact that antibiotics have revolutionized the management of many clinical syndromes caused by infections, their increasing use in many ways such as, indiscriminate prescribing, inappropriate dosing and duration of treatment, over the counter availability of antibiotics to the general public, use in animal husbandry, and use to control infections in horticulture has contributed to the rise of antibiotic resistance among various common pathogens (Kerr, 2005). No systematic national surveillance of antibiotic resistance is available. The emergence of antibiotic resistance in the management of UTI is a serious public health issue, particularly in the developing world where apart from high level of poverty, ignorance and poor hygienic practices, there is also a high prevalence of fake and spurious drugs of questionable quality in circulation (Abubakar, 2009). In patients with suspected UTI, antibiotic treatment is usually started empirically, before urine culture results are available. To ensure appropriate treatment, knowledge of the organisms that cause UTI and their antibiotic susceptibility is mandatory (Ashkenazi *et al.,* 1991). Hence the changing spectrum of microorganisms involved in UTI and emergence of resistance across institutions and geographical areas have made imperative the conduct of antibiotic susceptibility pattern study of UTI pathogens in various regions from time to time (Oluremi *et al.,* 2011). The aim of current study was to (A) Isolate uropathogenic bacteria from urine of patients with Urinary tract infection and (B) To study the antibiotic susceptibility pattern of isolated uropathogens from in-patients and out-patients departments of a tertiary care hospital in western Uttar Pradesh-India. Detection of UTI causing pathogens and resistance of these pathogens to commonly prescribed antibiotics in clinical setups is essential and helpful in improving the efficacy of empirical treatment (Ko *et al.,* 2008).

Material and Methods

This observational and prospective study was conducted at a tertiary care hospital in western Uttar Pradesh-India, from 1st February 2015 to 31st July 2015. A total of 510 mid-stream urine specimens of in-patients (IPD) and out-patients (OPD) suspected to be having urinary tract infection were cultured and further analyzed. Exclusion criteria were patients already on antibiotic therapy. At 37°C for 24 hours of incubation, predominant growth (on blood agar, MacConkey's agar) of single bacteria was seen in 300 (58.82 %) urine samples. Out of the 510 urine culture reports, 300 bacterial isolates from urine of patients which conferred to "Kass concept of significant bacteriuria" were further identified and evaluated for their antibiotic sensitivity using Kirby-Bauer method (Collee *et al.,* 1996; Baur *et al.,* 1996). 'Kass concept of significant bacteriuria' being growth of single bacterial isolate, count of which being $\geq 10^5$ cfu/ml of urine (Collee *et al.,* 1996). The bacterial isolates were identified and confirmed using standard microbiological method which included Gram staining, colonial morphology on media, and growth on selective media, lactose fermentation, catalase, oxidase, coagulase, indole, citrate

utilization, urease tests and hanging drop preparation for motility (Collee *et al.*, 1996).

Antibiotic sensitivity testing: Antibiotic susceptibility testing was performed by the disc diffusion assay on Muller Hinton Agar by Kirby-Bauer method (Collee *et al.*, 1996) using the following antibiotics disc: ampicillin 10 mcg, ampicillin-sulbactum 10 mcg, ciprofloxacin 10 mcg, co-trimoxazole 25 mcg, ceftazidime 10 mcg, ceftazidime/clavulanic30/10 mcg, cephalothin 30 mcg, carbenicillin 100 mcg, clindamycin 2mcg, erythromycin15 mcg, gentamicin 30 mcg, imipenem10 mcg, kanamycin 30 mcg, norfloxacin 10 mcg, nitrofurantoin 300 mcg, nalidixic acid 30 mcg, linezolid15 mcg, piperacillin /tazobactum100/10mcg, tobramycin10 mcg and tetracycline 30 mcg. Interpretation of diameter of growth inhibition zone was done by using the standard interpretative chart provided by disc manufacturer. At 37°C, after 24 hours of incubation, organisms were scored as sensitive or resistant to corresponding antibiotic on the basis of zone of inhibition following the criteria of Clinical and Laboratory Standards Institute. Multiple drug resistance is described as resistance to three or more than three different antibiotic classes tested while Extensive drug resistance is described as resistance to at least one member of all but two antibiotic classes (Sumera Sabir *et al.*, 2014).

Results and Discussion

Out of 510 urine samples examined in our study, 300(58.82%) showed significant bacteriuria; 137(45.66%) were male and 163 (54.34%) were female patients. In our study prevalence of UTI was ranged in patients between 10–75 years old (Table 1). Of the 300 isolates obtained, Gram negative bacteria had a higher frequency of occurrence than Gram positive. Most common isolated uropathogens in Gram negative bacilli and Gram positive cocci were *E. coli*(60%) and *Enterococcus* (20%) respectively (Table 2).

45(15%) patients were from IPD and 255(85%) patients were from OPD (Table 3).

The isolated bacteria showed wide differences in their susceptibility to the tested antimicrobial antibiotics (Table 4 and 5). Most of the bacteria recovered from UTI showed the highest degree of resistance to ceftazidime, kanamycin, ampicillin, tetracycline, co-trimaxazole, ceftazidime-clavulanic, norfloxacin, gentamycin, ciprofloxacin and erythromycin.

UTI are caused by microbial invasion and subsequent multiplication in urinary tract (Boye *et al.*, 2012). In community and hospital settings the etiology of UTIs and the antimicrobial susceptibility of UTI causing bacteria's have been changing over the years (New, 1996; Jones, 1996). In our study, 255(85%) culture positive urine sample belonged to Out-door Patient Department (O.P.D) while 45(15%) were from In-door Patient Department (I.P.D). In the United states, UTIs account for seven million office visits and 1,00,000 hospitalizations annually, making them the most common bacterial infections in outpatient settings (Schappert, 1999).

Approximately 1 in 3 women will require antimicrobial treatment for a UTI before age 24, and 40 % to 50 % of women will have a UTI during their life time (Foxman, 2003). The estimated annual costs of UTI are $1.6 billion for evaluation and treatment. Despite advances in antimicrobial therapy, UTIs remain a significant cause of morbidity (Foxman, 2002; Warren *et al.*, 1999). The sex distribution of patients in our study is consistent with other reported studies. In our

study, the incidence of UTI was high among the females (54.34%) than males (45.66 %). Factors such as short urethra and its closeness to the anus as well as sexual activity have been reported to influence the higher prevalence of UTI in females (Adedeji *et al.,* 2009). The female to male ratio among age groups of less than 20 years and age group of more than 50 years was equal and higher for age group of 20 to 40 years. This may be due to increase sexual activity at this age range which predisposes the female to the possibility of contracting UTI. This result is in agreement with other reports which showed that UTI were more common in females than males during adolescence and adulthood (Adedeji *et al.,* 2009; Gales *et al.,* 2000; Kebira *et al.,* 2009; Kolawole *et al.,* 2009; Orret *et al.,* 1998; Tambekar *et al.,* 2006; Theodore, 2007).

The uropathogens identified in our study are similar to those of many other studies conducted in different countries either in the region or internationally (Astal *et al.,* 2002), however different results have been reported. The similarities and differences in the type and distribution of uropathogens may result from different environmental conditions and host factors, practices such as health care and education programmes, socioeconomic standards and hygiene practices in each country (Mansour Amin *et al.,* 2009). In our study, the Gram negative bacilli constituted 75% of the bacterial isolates implicated in UTI cases while Gram positive cocci constituted 25%. Out of the 6 *Staphylococcus aureus* isolated, 5(1.66%) were found to be methicillin resistant *Staphylococcus*. Apart from gram positive organisms isolated in urine, the other isolates are inhabitants of the large bowel. The Enterobacteriaceae family were the most common microorganisms isolated of urinary tract infection in present study accounting 71% of total isolated bacteria and amongst them *E. coli* (60 %) was the most predominant bacteria. This result is consistent with reports from other studies (Abubakar, 2009; Daza *et al.,* 2001; Dimitrov *et al.,* 2004; Gruneberg, 1980; Orret *et al.,* 1998; Omigie *et al.,* 2009) but differs from the reports of Ehinmidu (Ehinmidu, 2003) and Aboderin *et al.* (2009) which recorded *P. aeruginosa* and *Klebsiella* spp, respectively as the predominant bacteria. *P. aeruginosa* was also reported as the second most common bacterial isolate in UTI from studies in India by Tambekar *et al.* (2006) and Lafia, Nigeria by Kolawole *et al.* (2009). However, *Klebsiella* spp was reported as the second most frequently implicated organism in UTI in some other studies (Aboderin *et al.,* 2009; Abubakar 2009; Gales *et al.,* 2000; Haghi-Ashteiani *et al.,* 2007; Uwaezuoke *et al.,* 2006). The incidence of community acquired UTI due to *E. coli* is more in this study (52%) than hospital acquired infections (8%). *P. aeruginosa* was mostly responsible for UTI cases among in patients where it accounted for 3% of the infections. This is possibly due to the opportunistic nature of the organism and its versatility in causing nosocomial infections in hospitalized patients especially those fitted with catheters. It has been previously reported that *P.aeruginosa* is being increasingly isolated from both community and hospital acquired UTIs (Kolawole *et al.,* 2009; Omigie *et al.,* 2009; Orret *et al.,* 1998; Tambekar *et al.,* 2006).

In present study, Gram positive cocci were sensitive to linozolid and nitrofurantoin. *Enterococuss spp* showed resistance to ciprofloxacin, gentamycin, erythromycin, clindamycin, and norfloxacin. Except for ciprofloxacin, *coagulase negative staphylococcus* and *Staphylococcus aureus* showed good sensitivity to all antibiotics used for Gram positive bacteria. Gram

negative bacilli isolated from UTI were sensitive to nitrofurantoin, imipenem, amikacin, tobramycin and piperacillin-tazobactum. Imipenem and piperacillin-tazobactum was more effective against *Pseudomonas spp.* and nitrofurantoin and imipenem were effective against *Acinetobacter spp.* isolated in present study. *E.coli* was sensitive to imipenem, nitrofurantoin, amikacin, tobramycin and piperacillin-tazobactum. In present study, a high level of bacterial resistance was seen to ampicillin and ceftazidime.

The high prevalence of resistance to the community used antibiotics such as ampicillin, ciprofloxacin and tetracycline has caused considerable alarm (Janet, 2006; Nurullaev, 2004; Orrett *et al.*, 2006).

The most effective antimicrobial agents in our study were imipenem, nitrofurantoin, amikacin, tobramycin and piperacillin-tazobactum for Gram negative bacilli. However, isolated Gram positive cocci were fully sensitive to linozolid and nitrofurantoin. Based on the results of this study, the efficacy of amikacin was comparable to other reports (Kothari *et al.*, 2008). In a recent study done by Devanand Prakash and Ramchandra Sahai Saxena in adjoining Meerut city, their bacterial isolates were comparable to our study, nalidixic acid was found to be the most resistant drug followed by ceftazidime. In their study most sensitive drug against all uropathogens was meropenem followed by imipenem (Devanand Prakash and Ramchandra Sahai Saxena, 2013).

Table.1 UTI in male and female patients of various age groups

Age groups (Years)	Male No(%)	Female No(%)
10-19	8(2.66%)	5(1.66%)
20-29	39 (13%)	46 (15.33%)
30-39	35 (11.66%)	49 (16.33%)
40-49	24 (8%)	26 (8.66%)
50-59	22 (7.33%)	25 (8.33%)
60-75	9 (3%)	12 (4%)
Total UTI case=300	**137(45.66%)**	**163(54.33%)**

Table.2 Isolated uropathogens in male and female patients

ISOLATED BACTERIA	No(%)	Male(%)	Female(%)
Gram negative bacteria			
Escherichia coli	180(60%)	35%	65%
Klebsiella spp	24(8%)	40%	60%
Pseudomonas aeruginosa	9(3%)	55%	45%
Proteus spp.	6(2%)	49%	51%
Acinetobacter spp.	3(1%)	70%	30%
Citrobacter spp.	3(1%)	75%	25%
Gram positive bacteria			
Enterococcus spp.	60(20%)	32%	68%
Coagulase negative staphylococcus	9(3%)	33%	67%
Coagulase positive staphylococcus aureus	6(2%)	22%	78%

Table.3 Isolated uropathogens in Inpatients and Outpatients

ISOLATED BACTERIA(Total no =300)	Inpatients No(%)	Outpatients No(%)
Escherichia coli	24(8%)	156(52%)
Klebsiella spp.	1(0.33%)	23(7.66%)
Pseudomonas aeruginosa	9(3%)	0(0%)
Proteus spp.	2(0.66%)	4(1.33%)
Acinetobacter spp.	0(0%)	3(1%)
Citrobacter spp.	0(0%)	3(1%)
Enterococcus spp.	6(2%)	54(18%)
Coagulase negative staphylococcus	2(0.66%)	7(2.33%)
Coagulase positive staphylococcus aureus	1(0.33%)	5(1.66%)

Table.4 In-vitro antibiotic sensitivity in isolated Gram positive uropathogens.

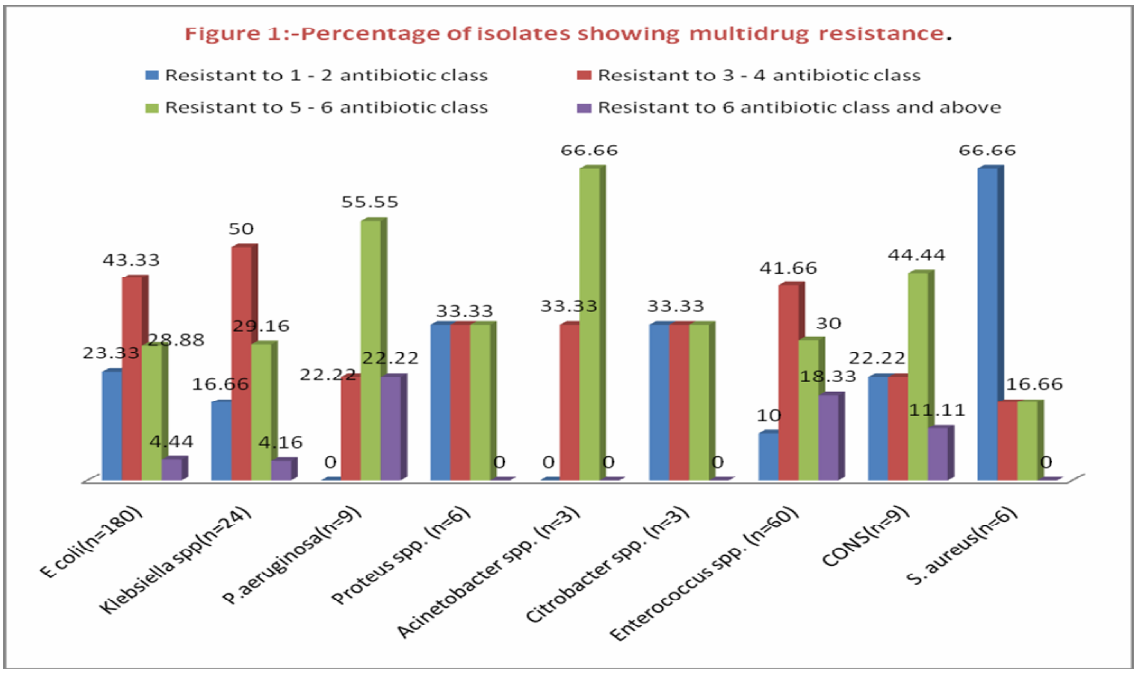
ANTIBIOTICS	GRAM POSITIVE ORGANISM ISOLATED (n=75)					
	Enterococcus spp		***Coagulase Negative Staphylococcus***		***Staphylococcus aureus***	
	S	**R**	**S**	**R**	**S**	**R**
Clindamycin	**11**	**49**	**7**	**2**	**5**	**1**
Ciprofloxacin	**4**	**56**	**1**	**8**	**2**	**4**
Erythromycin	**13**	**47**	**6**	**3**	**4**	**2**
Gentamicin	**7**	**53**	**7**	**2**	**5**	**1**
Linezolid	**56**	**4**	**7**	**2**	**6**	**0**
Nitrofurantoin	**55**	**5**	**9**	**0**	**6**	**0**
Norfloxacin	**15**	**45**	**6**	**3**	**5**	**1**
Kanamycin	**12**	**48**	**4**	**5**	**5**	**1**
Tobramycin	**52**	**8**	**8**	**1**	**5**	**1**

S→ Sensitive, R→ Resistant

Table.5 *In-vitro* antibiotic sensitivity in isolated Gram negative uropathogens.

ANITIBIOTIC	GRAM NEGATIVE ORGANISM ISOLATED(n=225)											
	E.coli		*Klebsiella spp*		*Proteus spp*		*Pseudomonas aeruginosa*		*Acnieto--bacter spp*		*Citrobacter spp*	
	S	R	S	R	S	R	S	R	S	R	S	R
Ampicillin	5	175	12	12	0	6	0	9	0	3	0	3
Ampicillin-sulbactum	30	150	12	12	6	0	0	9	1	2	1	2
Amikacin	167	13	24	0	6	0	3	6	0	3	3	0
Ciprofloxacin	67	113	11	13	2	4	0	9	0	3	1	2
Cotrimoxazole	24	156	18	6	6	0	0	9	0	3	0	3
Ceftazidime	0	180	0	24	0	6	0	9	1	2	0	3
Ceftazidime-clavulanic	30	150	6	18	0	6	0	9	0	3	3	0
Gentamicin	60	120	4	2 0	6	0	0	9	0	3	1	2
Norfloxacin	40	140	12	12	6	0	0	9	0	3	0	3
Nitrofurantoin	175	5	19	5	4	2	0	9	3	0	1	2
Nalidixic acid	35	145	11	13	3	3	0	9	0	3	1	2
Tobramycin	124	56	3	21	1	5	0	9	1	2	1	2
Kanamycin	2	178	8	16	2	4	1	8	1	2	2	1
Cephalothin	81	99	5	19	0	6	2	7	0	3	2	1
Tetracycline	24	156	24	0	1	5	0	9	1	2	1	2
Imipenem	178	2	24	0	2	4	7	2	3	0	3	0
Carbenicillin	0	180	4	2 0	2	4	2	7	1	3	2	1
Piperacillin-Tazobactum	113	67	16	8	6	0	8	1	2	1	2	1

S→ Sensitive, R→ Resistant



E. coli, the predominant etiologic organism of UTI in this study showed moderate to high susceptibility to the fluoroquinolones (ciprofloxacin) and varying degree of susceptibility to other commonly used antibiotics (Table 2 & 5). Earlier studies conducted in Nigeria by Ehinmidu (2003), in Kuwait by Al Sweih *et al.* (2005), in India by Tambekar *et al.* (2006) have reported good susceptibility of the bacteria to fluoroquinolones. However, resistance to fluoroquinolones is on the increase in the locality of our study.

The poor susceptibility of *E. coli* isolates in this report to nalidixic acid (19.44%), norfloxacin (22.22%) and gentamicin (33.33%) is also showing a decreasing trend when compared to higher values recorded in previous works in India by Tambekar *et al.* (2006), in Iran by Saffar *et al.* (2008) and in Nigeria by Uwaezuoke *et al.* (2006)·The *P. aeruginosa* isolates showed reduced susceptibility to the fluoroquinolones (100% resistance for ciprofloxacin), 100% resistance to gentamicin and to majority of other antibiotics tested. These results present a worrying pattern of resistance comparable to what obtained in other similar studies by Daza *et al.* (2001) and by Haghi-Asteiani *et al.* (2007).

The antibiotic susceptibility profile for all the UTI bacterial isolates in this study was ciprofloxacin (29.33%), nitrofurantoin (90.66%), gentamicin (30%), nalidixic acid (22.22%), co-trimoxazole (21.33%), tetracycline (22.66%), and ampicillin (7.55%) (Table 4 & 5). Nitrofurantoin was the most active drug in this study showing the highest activity against the Enterobacteriaceae and S. *aureus* isolates in both community and hospital acquired UTI.

However, our study result showed general increase in resistance of UTI bacterial isolates to fluoroquinolones with higher resistance to ciprofloxacin. Increase in widespread use of fluoroquinolones in medical centres is a possible cause of high-level resistance to fluoroquinolones in UTI patients as reported by in a study by Nakhjavani *et al.* (Nakhjavani *et al.*, 2007). All the isolates were highly resistant to older drugs (nalidixic acid, co-trimoxazole, and tetracycline). Activity of gentamicin against all the isolates may be due to its widespread use in the hospital environment as a broad-spectrum antibiotic. Use of aminoglycosides, especially when only one type is employed, may lead to an increased level of resistance as reported in his study by Swartz (Swartz M.N., 1997). Resistance of UTI pathogens to commonly use antibiotics may be due to their frequent prescription in the hospital, their easy availability in the community without prescription and their low cost which make them subject to abuse (Abubakar, 2009). The near total or complete resistance (100%) of all the isolates in this study to ceftazidime is worrisome as they may have lost their value in the treatment of UTI in this area of study.

Rate of multi-drug resistance was recorded in both community and hospital isolates (Figure 1). High rate of multidrug resistance among the isolates in this study is very alarming and should be of great concern. Hryniewicz *et al.* (2001) in a study conducted in Poland reported that multidrug resistance was usually related to production of Extended Spectrum Beta Lactamases (ESBL) among the enterobacteriaceae in both community and hospital isolates (Hryniewcz *et al.*, 2001). Multi-drug resistant *P. aeruginosa*, *Klebsiella spp*, *S. aureus* and *Enterococcus* spp strains have been widely reported in some studies (Abubakar, 2009; Gales *et al.*, 2000). High prevalence of multiple antibiotic resistance strains is a possible indication that very

large population of bacterial isolates has been exposed to several antibiotics.

In conclusion, the data obtained from this study suggest that while UTI causing pathogens are still susceptible to the fluoroquinolones such as ciprofloxacin, resistance to these antibiotics is on the increase. Other commonly prescribed antibiotics in UTI such as nalidixic acid, co-trimoxazole, tetracycline, ampicillin are rather ineffective and may have lost their value in the chemotherapy of UTI. It is concluded that Gram negative bacilli (enterobacteracae) were responsible for majority of urinary tract infections and most of the strains were multidrug resistant. The most common isolated bacteria from urinary tract infection was *E. coli*, most effective antimicrobial agents against Gram negative bacilli were nitrofurantoin, amikacin, tobramycin and imipenem while most effective antibiotics against Gram positive cocci were lineozolid, nitrofurantoin and tobramycin. Sensitivity of a uropathogen to a particular antibiotic vary from time to time and across different areas. To reduce the incidence of resistance, empirical antibiotic selection in treatment of UTI must be based on the knowledge of local prevalence of causative uropathogens and their respective antimicrobial sensitivities rather than on universal guidelines. Indiscriminate prescription and use of antibiotics must be discouraged in both community and hospital settings by continuous public awareness and education on rational antibiotic use as well as adoption of strict national antibiotic policy to regulate the prescription, sale and use of antibiotics.

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