

Original Research Article

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Antimicrobial Resistance among Uropathogenic Bacteria in Rural Kerala, India

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ABSTRACT

Urinary tract infections caused by multidrug resistant bacteria are increasingly becoming prevalent even in rural areas. Objective of the present study was to find out the distribution and antibiotic resistance pattern of urinary isolates over a period of one year in a tertiary care centre of Central Kerala which has got a rural location. We analyzed the types and antibiotic resistance pattern of all significant urinary isolates in the microbiology department from May 2016 - April 2017. Of the 3772 urine samples processed, 1265(33.5%) showed significant bacteriuria. The predominant isolate was *E. coli* (48.9%) followed by *Klebsiella sp.* (14%), *Enterococcus sp.* (14%), *Staphylococcus sp.* (7%), *Pseudomonas* and other non-fermenters (11%). Among Enterobacteriaceae, 64.7% was ESBL producers and 15.75% were carbapenem resistant. Around 63% of *E.coli* and *Klebsiella sp.* were found to be resistant to fluoroquinolones like norfloxacin and ciprofloxacin. Ampicillin resistance was observed in 35% of *Enterococcus* isolates and vancomycin resistance in 0.65%. Of the *Staphylococcal* isolates, 79.5% was methicillin resistant. From our study, we could realize that the problem of antibiotic resistance is increasing in an alarming rate even in rural areas. Multidrug resistance, since being a universal problem, we all have to be vigilant against this menace and the need for antibiotic stewardship should be reiterated.

Keywords

Urinary isolates,
Antibiotic
resistance, Rural
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Introduction

Urinary tract infection (UTI) is the most common of infections in the clinical practice. Among the bacterial agents of UTI, *E. coli* continues to be the commonest in both community acquired and hospital acquired cases (Gupta *et al.*, 2002). Other pathogens include *Klebsiella*, *Pseudomonas* and other non-fermenters, *Enterococcus*, *Staphylococcus*, *Enterobacter*, *Citrobacter*, *Proteus*, etc. Over the last decade there has been an alarming increase in antimicrobial resistance (AMR) among the common

bacterial agents of UTI in developing and developed countries. In this context, the empirical antibiotics used earlier in UTI such as ampicillin/amoxicillin, co-trimoxazole, norfloxacin, ciprofloxacin, etc. can no longer be used confidently. In the recent years antibiotic resistance is becoming a major problem not only in hospital acquired complicated UTIs but also in uncomplicated community acquired cases (Kavya *et al.*, 2016) which makes the treatment of UTI more complex. On reviewing the literature it

could be easily understood that AMR is not distributed uniformly among different countries and in different parts of the same country. Hence it is mandatory to establish routine mechanisms to assess local resistance rates among UTI pathogens. The standard regimen for empiric therapy should be reassessed periodically in light of changing susceptibility patterns. In the present study, we aim to find out the antibiotic resistance pattern of urinary isolates from a tertiary care centre in central Kerala against the commonly used drugs. An attempt is also made to find out the difference in resistance pattern among hospitalized and outpatient cases.

Materials and Methods

We conducted a prospective analysis of all significant urinary isolates in the microbiology department over a one year period from May, 2016 – April, 2017. Samples from clinically suspected cases of UTI, both hospitalized and out patients, irrespective of their age and sex from PK Das Institute of Medical Sciences, Palakkad, Kerala, India are included in the study. Most of the samples are clean catch mid-stream samples and the rest, catheter samples and supra pubic aspirates. All the samples are processed within 2 hrs of collection. Microscopic examination and culture on MacConkey agar and 5% sheep blood agar are done. Significance of the isolates is assessed by following the recommendations of Kass, microscopic findings as well as the clinical features. Colony counts $<10^5$ also are considered significant on an individual basis by correlating the microscopic findings, type of specimen and the clinical history.

Identification of the isolates is done by standard bacteriological methods (Collee *et al.*, 2006). In addition, Hi Media, Hi Chrome UTI Agar is used for confirming the identification of Enterobacteriaceae and

Pseudomonas. Antibiotic susceptibility testing of the isolates is performed by Kirby-Bauer disc diffusion method on Mueller Hinton Agar as per CLSI guidelines (CLSI, 2016) using Hi Media, India antibiotic discs. Antibiotic discs used are ampicillin (10 μ g), norfloxacin (10 μ g), ciprofloxacin (5 μ g), nitrofurantoin (300 μ g), co-trimoxazole (1.25/23.75 μ g), cephalothin (30 μ g), cefuroxime (30 μ g), cefotaxime (30 μ g), ceftazidime (30 μ g), gentamicin (10 μ g), piperacillin (100 μ g), amikacin(30 μ g), amp-sulbactam (10/10 μ g), piperacillin-tazobactam (100/10 μ g), imipenem (10 μ g), meropenem (10 μ g), high level gentamicin (120 μ g), tetracycline (30 μ g), ceftiofur (30 μ g), vancomycin (30 μ g), linezolid (30 μ g).

Using CLSI phenotypic confirmatory test, ESBL production is checked with cefotaxime clavulanate disc among the members of Enterobacteriaceae. Quality control is performed using standard strains, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923.

Considering the differences in the susceptibility pattern of bacterial agents of UTI among community acquired and hospital acquired cases, characterization of the isolates and their antibiotic resistance pattern based on the hospital status of the patients is also done.

Results and Discussion

Among the 3772 urine samples processed during the one year study period 1265(33.5%) samples gave significant bacterial growth.

The predominant isolate was *E. coli* (48.9%) followed by *Klebsiella sp.* (14%), *Enterococcus sp.* (14%), *Staphylococcus sp.* (7%), *Pseudomonas* and other non-fermenters (11%) (Fig. 1). 61.7% of total isolates in the present study is from inpatients and the rest is

from out patients. In both the groups, *E. coli* stands as the commonest isolate, which constitutes 57.7% of the outpatient and 43.5% of inpatient isolates respectively. Members of enterobacteriaceae constitute 81.6% of the total isolations in outpatients, whereas in inpatients, it is only 59.8% (Fig. 2).

Among the members of Enterobacteriaceae isolated, 64.7% are ESBL producers and 15.75% were carbapenem resistant (Table 1).

Ampicillin resistance is observed in 35% of *Enterococcus* isolates and vancomycin resistance in 0.65%. Of the *Staphylococcal* isolates, 79.5% is methicillin resistant (Table 2).

58.9% of *E. coli* isolates from outpatients and 73.5% from admitted patients are tested positive for ESBL production. For all common organisms from both outpatients and hospitalized patients, nitrofurantoin and amikacin are found to be least resistant followed by piperacillin- tazobactam and carbapenems. For *E. coli*, nitrofurantoin resistance is 14.6% and 20.4% respectively, in outpatients and inpatients and for amikacin it is 2.9% and 11.8% (Table 3).

There are an estimated 150 million cases of UTIs per annum worldwide (Stamm *et al.*, 2001) which along with the consistently increasing antibiotic resistance among the common causative agents of UTI makes the regular surveillance of the changing trends in antibiotic resistance pattern mandatory.

Even though our most common isolate is *E. coli* (49%), the rate is less compared to western studies where the corresponding rate is about 85% (George *et al.*, 2000 and Richard Colgan *et al.*, 2008). Data from other studies from India also show a similar reduced rate of 50-60% (Mohammed Akram *et al.*, 2007 and Dnyaneshwari *et al.*, 2016)

which is an important point to be considered. Another significant finding noticed in the present study, similar to studies from other parts of India (Mohammed Akram *et al.*, 2007), is the rate of isolation of *Klebsiella*(14%), which is more in this part of the world compared to US and Europe where the rate comes only to 3.8 % (George *et al.*, 2000). Along with *Klebsiella*, we have noted the emergence of *Enterococcus* also as a significant pathogen (14%) which agrees with the findings of another recent study from Pune, India (Dnyaneshwari *et al.*, 2016). However, in a study from Mysore, India the rate of isolation of *Enterococcus* was only 5.97% (Kavya *et al.*, 2016). Rate of *Pseudomonas* and other NFGNBs is also increasing in this region and the same has also been reported by Dnyaneshwari *et al.*, (2016). From the above observations we can infer that there is a region wise difference in the bacterial agents of UTIs.

Among the hospitalized patients, predominance of *Enterococci*, *Pseudomonas*, other NFGNB and *Staphylococci* is noted more than outpatients which corresponds with other studies (George *et al.*, 2000 and Jharna Mandal *et al.*, 2012). Co-trimoxazole, ampicillin, ciprofloxacin, early cephalosporins and nitrofurantoin were considered as the front line agents of treatment for UTIs all over the world. But recent regional and nationwide studies in US have reported that resistance to co-trimoxazole among urinary isolates of *E. coli* is approaching or exceeded 20% (James *et al.*, 2006 and Gupta *et al.*, 2001). In the present study, 58.8% of *E. coli* isolates are found to be resistant to co-trimoxazole and the mean resistance rate of all bacteria to co-trimoxazole is 57.1 %. The corresponding rate reported by Karlowsky *et al.*, is 66.5% (James *et al.*, 2006). In a study from Aligarh, India and according to Gupta *et al.*, the rates are 60.4% and 80% respectively (Mohammed

Akram *et al.*, 2007 and Gupta *et al.*, 2002). According to US Infectious Disease Society of America, co-trimoxazole cannot be considered as the empirical therapy for UTI where the resistance rate is $\geq 20\%$ (James *et al.*, 2006). Hence co-trimoxazole, no longer holds good as the first line agent of treatment in our area.

We could demonstrate a resistance rate of 91.1 % for *E. coli* against ampicillin and a mean resistance rate of 84.2% for all the isolates together. *Klebsiella sp.* shows a mean resistance of 99.4 % which could be explained by the inherent resistance of *Klebsiella* to ampicillin. Many other reports from India also show a high resistance of about 80- 98% against *Klebsiella* and *E. coli*, (Dnyaneshwari *et al.*, 2016 and Jharna Mandal *et al.*, 2012). In most parts of the world, the rate of ampicillin resistance among urinary isolates is increasing, which according

to a study from North America in 2006 is 79.8% (James *et al.*, 2006) and this shows that ampicillin has no bearing in the empirical therapy of UTIs.

Considering the high rate of resistance to co-trimoxazole and ampicillin, in many parts of US and Europe, quinolones have been used as the main drugs for UTI (George *et al.*, 2000 and James *et al.*, 2006). However, following its widespread use, studies from various parts of US and Spain report that fluoroquinolones demonstrate a stepwise, threefold increase in resistance from 1995(0.7%) to 2001(2.5%) (James *et al.*, 2002). In the present study, mean resistance of all isolates to ciprofloxacin is 51% in comparison to 18% from a Spanish study (Gobernado *et al.*, 2007). Other Indian studies also have reported a similar high level resistance (Jharna Mandal *et al.*, 2012 and Mohammed Akram *et al.*, 2007).

Fig.1 Distribution of the isolates

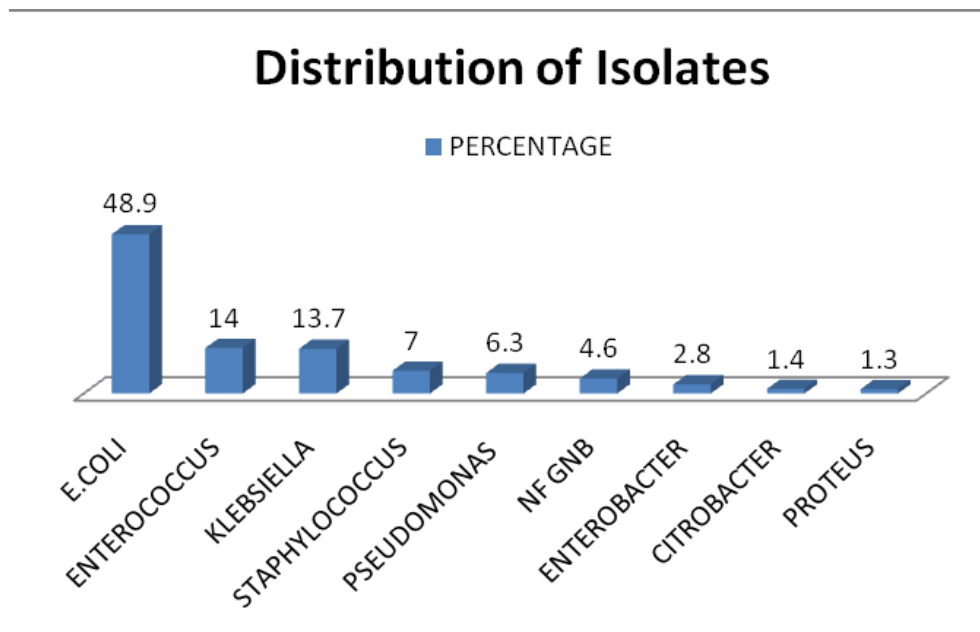


Fig.2 Distribution of isolates based on hospital status of the patients

Distribution of isolates based on hospital status of the patients

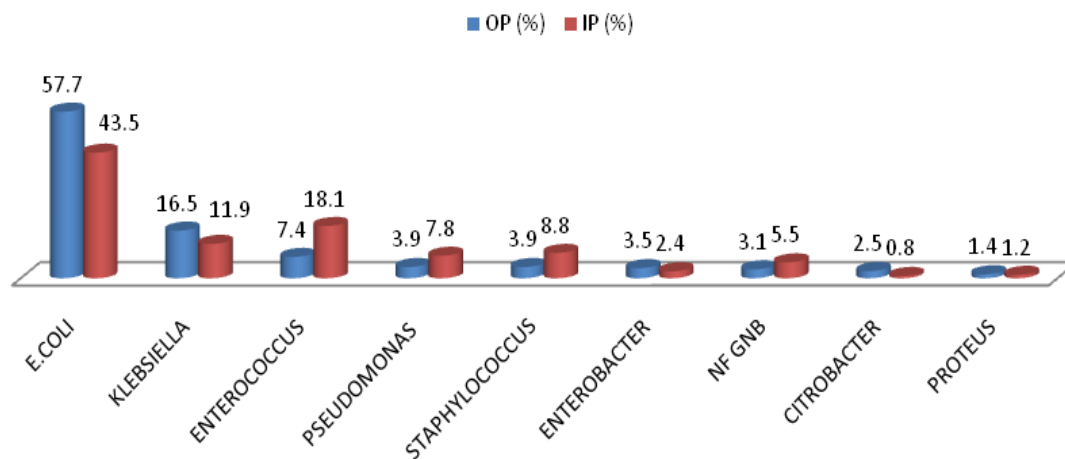


Table.1 Antibiotic resistance pattern of the Gram negative isolates

	<i>E.coli</i> (n=619) %	<i>Klebsiella</i> <i>sp</i> (n=173) %	<i>Proteus</i> <i>sp</i> (n=16) %	<i>Citrobacter</i> <i>sp</i> (n=18) %	<i>Pseudomonas</i> <i>sp</i> (n=80) %	Other NFGNB (n=58) %	<i>Enterobacter</i> <i>sp</i> (n=36) %
AMP	91.1	99.4	56.1	100	80	100	100
CEP	83.7	82.1	56.1	27.8	78.8	100	97.2
CXM	72.7	70.5	50	22.2	78.8	100	75
CTX	70.1	67.1	12.5	22.2	52.5	87.9	63.9
CAZ	70.3	67.1	12.5	22.2	52.5	74.1	63.9
COT	58.8	64.2	25	16.7	100	46.1	58.3
CIP	66.4	59.5	12.5	16.7	52.5	60.3	38.9
NX	66.7	59.5	18.8	16.7	53.8	60.3	36.1
NIT	17.8	75.1	ND	61.1	77.5	93.1	88.9
AK	7.8	26	0	16.7	40	63.8	33.3
GEN	35.1	49.1	0	16.7	47.5	62.1	52.8
PI	37.4	82.1	18.8	27.8	40	75.9	72.2
PIT	25.5	45.1	0	16.7	33.8	48.3	47.2
A/S	75	83.2	25	50	80	55.2	88.8
IPM	7.9	17.3	6.3	5.6	25	50	22.2
MRP	20.4	27.7	0	5.6	46.3	41.4	22.2
ESBL	66.9	67.1	6.3	22.2	ND	ND	63.9

Amp-ampicillin, Cep-cephalothin, Cxm-cefuroxime, Ctx-cefotaxime, Caz-ceftazidime, Cip-ciprofloxacin, Gent-gentamicin, Ak-amikacin, Cot-co-trimoxazole, Nx-norfloxacin, Nit-nitrofurantoin, A/s-amp- sulbactam, PI – piperacillin, PIT-piperacillin-tazobactam, Ipm- imipenem, Mrp-meropenem, ND-not done

Table.2 Antibiotic resistance pattern of the Gram positive isolates

	<i>Enterococcus sp</i> (n=177) %	<i>Staphylococcus sp</i> (n=88) %
AMP	35	96.6
COT	80.2	44.3
CIP	78	73.9
NX	77.4	73.9
NIT	18.1	0
AK	59.3	71.6
GEN	55.9	55.7
PI	35	90.9
PIT	35	81.8
A/S	52	83
VA	0.6	0
LZ	0	0
CX	ND	79.5

Amp-ampicillin, Cip-ciprofloxacin, Gen-gentamicin, Ak-amikacin, Cot-co-trimoxazole, Nx-norfloxacin, Nit-nitrofurantoin, A/s-amp- sulbactam, PI – piperacillin, Pit-piperacillin-tazobactam, Cx - cefoxitin, Va-vancomycin, LZ-linezolid, ND-not done.

Table.3 Distribution of antibiotic resistance based on patients hospital status

		AMP	CEP	CXM	CAZ	COT	CIP	NX	NIT	AK	GEN	PI	PIT	A/S	IPM	MRP
<i>E.coli</i>	IP	91.2	91.7	78.2	76.7	62.2	69.9	70.5	20.4	11.8	42.2	81.4	32.2	77.3	10.9	24.2
	OP	91.1	73.9	66.1	62.5	54.6	62.1	62.1	14.6	2.9	26.4	72.5	17.5	72.1	4.3	15.7
<i>Klebsiella sp</i>	IP	100	83.9	73.1	69.9	66.7	63.4	63.4	78.5	31.2	57	84.9	53.8	88.2	22.6	33.3
	OP	98.8	80	67.5	63.8	61.3	55	55	71.3	20	40	78.8	35	77.5	11.3	21
<i>Pseudomonas sp</i>	IP	80.3	78.7	78.7	55.7	100	54.1	54.1	77	39.3	62.3	41	36.1	80.3	26.2	47
	OP	78.9	78.9	78.9	42.1	100	47.4	52.6	78.9	42.1	0	36.8	26.3	78.9	21.1	42.1
<i>Enterococcus sp</i>	IP	41.8	ND	ND	ND	85.8	86.5	86.5	19.9	65.2	63.8	15.6	15.6	58.9	ND	ND
	OP	8.3	ND	ND	ND	58.3	44.4	41.7	11.1	36.1	0	27.8	27.8	25	ND	ND
<i>Staphylococcus sp</i>	IP	97.1	89.9	89.9	88.4	49.3	82.6	81.2	0	89.9	68.1	92.8	89.9	91.3	ND	ND
	OP	94.7	52.6	52.6	47.4	26.3	42.1	47.4	0	5.3	10.5	84.2	52.6	52.6	ND	ND

Nitrofurantoin which still remains as an important treatment option for UTI in western countries (George *et al.*, 2000) has a mean resistance of 59 % against the urinary pathogens of our study. The resistance rate varies among different pathogens, for *E. coli* it is 17.8 %, *Klebsiella* 75.1 %, for *Enterococcus* and *Staphylococcus* 18.1% and 0 % respectively. In various studies from US, the mean rate of nitrofurantoin resistance for urinary isolates is around 4.5% (James *et al.*, 2006 and George *et al.*, 2000) compared to 34.3% from a multicenter study in Delhi (Atul

et al., 2008). Regardless of the wide spread use for a long period, this drug maintains a low level of resistance which could be explained by its exclusive pharmacodynamic properties (McOsker *et al.*, 1994). Since adequate concentration of this drug cannot be obtained in blood, nitrofurantoin can be used only in lower UTIs.

All the isolates from the present study show a substantially high level of resistance to most of the second line antibiotics; amikacin, extended spectrum cephalosporins, inhibitor

combinations like ampicillin- sulbactam and piperacillin-tazobactam. In a study from Goa, India, 73.5% of the etiological agents of hospital acquired UTI were resistant to all the antibiotics tested (Umesh S Kamat *et al.*, 2009). In another study from Aligarh, India, resistance of *E. coli* to third generation cephalosporins was 55-85% (Mohammed Akram *et al.*, 2007). Similarly in the present study 64.7% of Enterobacteriaceae are ESBL producers and 37% of the isolates are resistant to piperacillin tazobactam. We could demonstrate a mean resistance of 35.4 % to aminoglycoside, amikacin and 41.7% for gentamicin. Mean resistance to imipenem in Gram negative isolates is 19.2% and the same for meropenem is 23.4%. This slight increase in resistance against meropenem compared to imipenem noted among our isolates could be due to the increased use of the former in clinical practice. In a study among community acquired UTI cases by Atul *et al.*, meropenem resistance for *E.coli* was 0% whereas it is 15.7% in the present study (Atul *et al.*, 2008). In another study from Gujarat, carbapenem showed only 16% sensitivity in nosocomial UTI and 41.3% sensitivity in community-acquired UTI and colistin had a total sensitivity of 45.3% only (Raval *et al.*, 2015). However no colistin resistance is detected among our isolates.

Enterococcus sp. is a significant isolate in this study which constitutes 14% of total isolates. These strains exhibit a mean resistance of 35% to ampicillin and the rate of vancomycin resistant *Enterococcus* comes to 0.6%. No case of vancomycin resistant *Staphylococcus* is isolated and the rate of methicillin resistance is 79.5% in the present study. *Pseudomonas* isolates are highly resistant to all the antimicrobials tested except imipenem (25%) and piperacillin tazobactam (33%). Other NFGNB exhibit a reasonably high resistance to all the antibiotics except colistin. Many recent studies also have reported

increasing rates of carbapenem resistant *Acinetobacter* isolates (Li-Yang Hsu *et al.*, 2017 and Paul G. Higgins *et al.*, 2010).

Among the outpatients, 39.9% of *E.coli* isolates are ESBL producers whereas it is almost double in admitted patients. This shows the increased rate of AMR in hospitalized patients compared to community acquired cases as reported in similar studies from India (Kavya *et al.*, 2016 and Atul *et al.*, 2008). 87% of Staphylococcal strains from admitted and 52.6% from outpatients are methicillin resistant which shows the increased prevalence of community acquired methicillin resistance in our locality. In our study, ciprofloxacin sensitivity to *E.coli* among outpatients is 37.9% whereas it was only 28% in a study from Delhi (Atul *et al.*, 2008).

Nitrofurantoin and amikacin are found to be least resistant among the common organisms in both outpatients and hospitalized patients. Even though aminoglycosides have been around for a long while, resistance has not developed as rapidly as to others. The chief reason for this appears to be that these drugs being administered parenterally have not been over-used. In *E. coli*, nitrofurantoin resistance is 14.6% and 20.4% respectively in outpatients and admitted patients which makes this as the first line of treatment in all cases of UTI.

Multidrug resistance (MDR) is very common among uropathogens as reported by Akram *et al.*, where almost all isolates were MDR (Mohammed Akram *et al.*, 2007). In a large scale study on 38,835 urinary isolates of *E. coli*, only 7.1% were MDR (Daniel *et al.*, 2001) whereas 65.9 % of our *E. coli* isolates are resistant to three or more groups of the first line drugs which is incomparably higher than western experience. Indian isolates show higher resistance than the isolates from USA

and Europe. The possible explanation is the uncontrolled antibiotic prescribing practices of our region. Another reason for high rate of resistance in the present study is that the isolates which are tested in a laboratory based study may be mostly from previous antibiotic treatment failed or from patients with other underlying risk factors. Hence this study, like any other laboratory based study, may bias towards an over reporting of resistance in patients with UTI and necessitates more patient based studies in this area.

All over the world, the problem of antibiotic resistance is increasing in a fast pace. Various studies on antibiotic resistance among uropathogens around 2000 show a low rate of resistance to ampicillin, co-trimoxazole, nitrofurantoin and quinolones in US and European countries (James *et al.*, 2002, Daniel *et al.*, 2001). In 2007, a large scale study conducted in Spain showed a drastic increase in the resistance to ampicillin, co-trimoxazole and quinolones, 52.1%, 26% and 18% respectively (Gobernado *et al.*, 2007). There are several factors which contribute to this high level of antibiotic resistance in bacteria. One of the main reasons for *E. coli* resistance to quinolones in Spain has been ascribed to the over use of these drugs (Gobernado *et al.*, 2007). Other factors like use of quinolones in agriculture and in animals, for growth promotion are also contributing (Garau *et al.*, 1999). The above mentioned reasons must have contributed to the high rates of resistance among our isolates as well. More problem oriented, institutional, regional and nationwide studies are required in our country to understand the changing trends of antibiotic resistance among urinary isolates. The implementation of hospital antibiotic policies also will help to check the uncontrolled increase in bacterial drug resistance to a certain extent.

Being a tertiary care centre, most of our patients are referred cases and must have

received various antibiotics from the peripheral practitioners which in turn must have helped in selecting out the resistant mutants. Use the appropriate antibiotic in correct dosage and duration, only in indicated cases. This should be our policy to tackle this growing menace.

Considering many studies from US, Europe and India, it is clear that antimicrobial resistance is markedly high in countries like India. Hence international treatment guidelines for empirical therapy in UTI will not stand true in Indian setting. This makes routine culture and sensitivity mandatory in all cases which is quite impractical also, to prevent treatment failure and development of further drug resistance. Therefore it is important to have periodic region wise analysis of changing trends of drug resistance among urinary isolates to formulate local guidelines for treating UTI. We hope that this study would help the clinicians in deciding the antibiotic therapy for UTIs and also would help in formulating an antibiotic policy in the hospitals.

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