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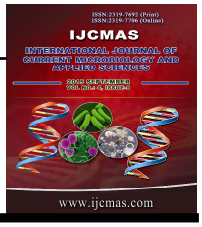

Original Research Article

Clinico-Microbiological Profile of Urinary Tract Infection in Tertiary Care Hospital in Ahmedabad, Gujarat, India

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Keywords

ESBL, Amp C betalactamase, Carbapanemase, CA-Catheter associated UTI (CA-UTI), GNB – Gram negative bacilli

A B S T R A C T

Urinary tract infection is one of commonest infectious disease seen in community worldwide. In a country like India where most of patients are treated empirically, it is important to know prevalent causative agents and their susceptibility pattern in that particular geographical area in context to latest trends in sensitivity pattern. The present study was carried out at a tertiary care hospital in Ahmedabad where total of 950 patients having signs & symptoms of urinary tract infection were subjected to a culture & sensitivity study. *E. coli* (51.88%) is still the commonest uropathogens followed by *Klebsiella* and other enteric gram negative bacilli. There is an alarming increase of cases of UTI by gram positive organisms (16%) mainly associated with instrumentation or catheterisation. Total number of strains showing resistance to betalactam group of antibiotics is increasing steadily over time. 56.17 % of strains of GNB were resistant to betalactam by production of ESBL whereas 16.67% & 10.8% strains showed production of Amp C and carbapanemase respectively. Methicillin resistant was noted in 11.43% of strains of staphylococci. These resistant strains were also resistant to many of other non-betalactam group of antibiotics. Nitrofurantoin (83.88 %) and Amikacin (81.11 %) are still the best effective drugs available.

Introduction

Urinary tract infection (UTI) is one of the most common infectious diseases seen in the community. Empirical antibiotic therapy is usually employed and for this, knowledge of the common uropathogens and their susceptibility to commonly used antibiotics is needed. Treatment becomes even more challenging in the presence of risk factors such as higher age, comorbidity, and immunosupression. Many times, physicians resort to prescribing broad-spectrum antibiotics over specific antibiotics in the view of resistance of the causative organism to the antibiotic. Poor patient compliance and incomplete course of antibiotic therapy have resulted in the evolution of resistance to many of these antibiotics.

Various studies done worldwide have shown changing patterns in the etiology of UTIs. However, studies on UTI and the pattern of antibiotic resistance in context to newer mechanism of resistance in India are few. The present trends of the uropathogens and their susceptibility to various antibiotics are essential to formulate guidelines for the empirical treatment of UTIs while awaiting the culture sensitivity.

The aim of the present study was to record the common clinical presentation and risk factors for UTI. The distribution of bacterial

strains isolated from complicated and uncomplicated UTIs occurring in the community and their resistance pattern against commonly used antibiotics were also studied.

Materials and Methods

The study was done in L.G. Hospital, Maninagar Ahmedabad, India, from January to June, 2014. The study included all the patients who were admitted or visited the out-patient department in the hospital with symptoms of UTI during the study period and had UTI confirmed by positive urine culture reports. Only one sample from each subject was considered. Data were collected using a questionnaire regarding demographic and clinical data.

Isolation and identification of uropathogens

A clean-catch midstream specimen was collected in a sterile wide-mouth leak-proof container (Sterile uricol by Hi Media, India). Using a calibrated loop method of a loop diameter of 4 mm, 10 µl of the uncentrifuged specimen was transferred onto a plate of Blood agar & CLED agar each. Streaking was done using the modified Mayo's technique without flaming the loop for isolation and incubated at 35–37°C for 24 h. A specimen was considered positive for UTI if a single organism was cultured at a concentration of $>10^5$ colony-forming units/ml. Isolated gram-positive and gram-negative organisms were further identified by standard set of biochemical tests up to genus/species levels wherever possible.

Antibiotic sensitivity testing

In the presence of any potential growth, antibiotic sensitivity testing was done by the modified Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotics tested were: imipenem, meropenem, aztreonam, ciprofloxacin, ofloxacin, norfloxacin, nalidixic acid, amikacin, gentamicin, nitrofurantoin, cotrimoxazole, various cephalosporins, tigecycline and colistin. Antibiotic discs used were from Hi Media – Doceda UTI-IV (DE011) & Doceda G-XII minus (DE046). Muller Hinton Agar used for keeping antibiotic disc diffusion test was also obtained from Hi Media (M173). A 15 cm diameter glass petri plates were used for keeping antibiotic sensitivity testing.

Extended spectrum beta lactamase detection

The screening for extended spectrum beta lactamase (ESBL) was done using cefpodoxime (≤17 mm), ceftazidime (≤22 mm), aztreonam (≤27 mm), cefotaxime (≤27 mm), and ceftriaxone (≤25 mm). If the organisms showed a zone of inhibition lower than the minimum for any antibiotic disc, ESBL positivity was suspected. The phenotypic confirmation was done by testing the strain against ceftazidime (Ca) and ceftazidime/clavulanic acid. A >5-mm diameter of the zone of inhibition for ceftazidime/clavulanic acid in comparison to ceftazidime was considered indicative of ESBL production. *Escherichia coli* ATCC 25922 was used as an ESBL-negative and *Klebsiella pneumoniae* 700603 was used as an ESBL-positive reference strain.

AmpC beta lactamase detection

All the isolates were screened for Amp C- β lactamase by Kirby-Bauer disc diffusion method using cefoxitin (30µg) disk. An isolate demonstrating reduced susceptibility (< 18 mm zone of inhibition) to cefoxitin was taken as screen positive. Screen positive were further confirmed by Amp C sterile disk test. *E. coli* ATCC 25922 was used as a control.

Carbapanemase detection

CLSI (2013) recommendation for zone diameter of ertapenem (10 µg) and meropenem (10µg) were used as a screening test. A zone diameter of < 19 mm for ertapenem and < 16 mm for meropenem were considered as indicative of carbapanemase production. Screen positives were confirmed by Modified Hodge test where clover leaf like indentation of standard *E. coli* ATCC 25922 strain growing along the test organism growth streak within disk diffusion zone was considered positive.

Mettalo-betalactamase detection

Screen positives isolates for carbapanemase were also subjected to a double disk synergy test using imipenem and imipenem/ethylene diamine tetraceteic acid disc. Difference in zone diameter of > 5 mm between imipenem disc alone and imipenem- EDTA disc respectively is considered as positive.

Boronic acid test for detection of KPC – *Klebsiella pneumoniae* carbapanemase detection

Infections caused by bacteria producing *K. pneumoniae* carbapanemase are becoming increasing significant problem worldwide since the first detection of these enzymes more than a decade ago. Although KPCs do not represent the first or the sole mechanism of carbapenem resistance, they are remarkable because they are often not detected by routine susceptibility screening and possess exceptional potential for dissemination. Screen positives isolates for carbapanemase were subjected to a double disk synergy test using a betalactam along with betalactam/boronic acid combination. The test is considered positive when the diameter of growth inhibitory zone around a betalactam disk with boronic acid is > 5 mm large than that around a disk containing betalactam alone.

Results and Discussion

Pathogen isolated from 399 (42%) of the 950 patients' urine samples (Table 1). *Escherichia coli* was the most frequently isolated pathogen accounting for 207 (51.88%) of the total isolates & it is followed by *Klebsiella* 78 (19.55%), *Enterococci* 30(7.52%), *Coagulase negative Staphylococci* 22(5.51%), *Pseudomonas* 15(3.76%), *Candida* 12(3.01%), *Staphylococcus aureus* 11(2.76%), *Citrobacter diversus* 6 (1.5%), *Enterobacter aerogens* 5 (1.25%) and *Citrobacter freundii* 3(0.75%). Some cases of mixed infection include *Klebsiella* and *Pseudomonas* 4 (1 %), *E. coli* and *Enterococci* 4 (1%), and *Klebsiella* and coagulase negative *Staphylococci* 2(0.5%). Amongst gram negative bacilli, 83.64 % of strains show resistance by one or another mechanism to betalactam group of antibiotics (Table 2). Out of various mechanism of resistance, ESBL production was commonest mechanism (56.17 %) followed by Amp C production (16.67%) and carbapanemase (10.8%). ESBL production was observed in 59.72% of *Escherichia coli* strain and 57.14% of *Klebsiella* strain. All gram positive cocci were positive for beta lactamase production. However, methicillin resistance was noted only in 11.43 % strains of *Staphylococci* (Table 3). Amongst non-betalactam group of antibiotics, nitrofurantoin and amikacin were most effective drugs showing 83.88 % and 81.11 % sensitivity, respectively (Table 4).

Although UTI ranks among the most common infection in developing countries, in the present study, only 399 of the 950 suspected cases (42%) were proved by culture. This indicates that urine culture is

essential for a definitive diagnosis of UTI. For either short- or long-term catheters, the infection rate is about 5% per day. Infection spreads by biofilm formation on both internal (intraluminal route) and external (periurethral route) catheter surface. Despite precautions, the majority of patients catheterized for >2 weeks eventually develop bacteriuria. Asymptomatic bacteriuria is the most common presentation of catheter-associated UTI. Current recommendations are not to treat asymptomatic catheter-associated UTI as it leads to the emergence of drug-resistant organisms. To prevent infection, intermittent catheterization by either a nurse or by the patient is advised.

Previous studies showed lower rate of ESBL production. Kader and Angamuthu (2005) reported 8.9% ESBL-positive cases in a hospital-based study in Saudi Arabia. Bean *et al.* (2008) reported a community-based ESBL prevalence to be 5.7% in London. In the present study, 56.17% of the isolates were ESBL-positive uropathogens. Previous studies in India have reported an ESBL positivity rate between 26.9% and 48.3%. ESBL producers do not respond to the usually prescribed empirical therapy. Also, there is an increased risk of associated morbidity and mortality, and cost of therapy when these patients are put on the standard empirical therapy.

Presently, alternative antimicrobial therapy to treat ESBL-positive UTI on outpatient basis is limited. Carbapenems are the most effective in this situation but need to be administered intra-venously or -muscularly. So the option remains is Betalactam-betalactamase inhibitor combination drugs which were effective till now.

But with spread of Amp C betalactamase & Carbapanemase production, their effectivity in empirical treatment of UTI remains in question. The experimental use of fosfomycin in treating ESBL-positive UTI has shown promising results in the recent past. All this and the high rate of ESBL positivity in the present study warrant a change in the empirical therapy for UTI to prevent the complications.

The antibiotic susceptibility pattern in the present study is similar to other recent studies. Quinolones were the least active drug against uropathogens in the present study. The resistance rate for ciprofloxacin has been increasing over decades and this is the highest resistance rate reported to date (84.63%). Akram *et al.* (2007) reported ciprofloxacin resistance rates ranging from 47% to 69% among the Gram-negative organisms in their study in India. Though the bacterial spectrum causing community-acquired UTI (CA-UTI) remained the same over time, the antibiotic susceptibility has changed. Prais *et al.* (2003) studied bacterial susceptibility to oral antibiotics in CA-UTI in 1991 and 1999. They reported that the pathogens recovered in the two groups were similar but there was a generalized decrease in bacterial susceptibility to common antibiotics in the two groups. Although, quinolones were considered as one of the drugs of choice for the treatment of UTI, the increasing resistance rate necessitates a change in the empirical treatment against CA-UTI.

The uropathogens showed highest sensitivity to carbapenems. The next best alternatives were aminoglycosides. But again, nearly one-fourth and more than half of the uropathogens showed resistance against amikacin and gentamicin, respectively. Also, the carbapenem-resistant organisms, although few (10.8%) in the present study, raise a concern over the available options to treat complicated and drug-resistant cases.

Table.1 Organisms isolated

Sr. No.	Organism	Numbers	Percentage
1	*E. coli*	207	51.88
2	*Klebsiella pneumoniae*	78	19.55
3	*Enterococci*	30	7.52
4	Coagulase negative *Staphylococci*	22	5.51
5	*Pseudomonas aeruginosa*	15	3.76
6	*Candida* species	12	3.01
7	*Staphylococcus aureus*	11	2.76
8	*Citrobacter diversus*	6	1.50
9	*Enterobacter aerogens*	5	1.25
10	*Citrobacter frundi*	3	0.75
11	*Klebsiella + Pseudomonas*	4	1.00
12	*E. coli + Enterococci*	4	1.00
13	*Klebsiella* +CONS	2	0.50
	Total	399	100.00

Table.2 Resistance pattern among Gram negative bacilli

		ESBL		**Amp C**		**Carbapanemase/MBL**		**Total resistant strains**	
Causative organism	Total	Numbers	%	Numbers	%	Numbers	%	Numbers	%
E.coli	211	126	59.72	34	16.11	17	8.06	177	83.89
Klebsiella pneumoniae	84	48	57.14	16	19.05	11	13.10	75	89.29
Pseudomonas aeruginosa	15	1	6.67	2	13.33	6	40.00	9	60.00
Citrobacter diversus	6	2	33.33	1	16.67	0	0.00	3	50.00
Enterobacter aerogens	5	3	60.00	1	20.00	1	20.00	5	100.00
Citrobacter frundi	3	2	66.67	0	0.00	0	0.00	2	66.67
Overall	324	182	56.17	54	16.67	35	10.80	271	83.64

Fig.1 Showing ESBL production

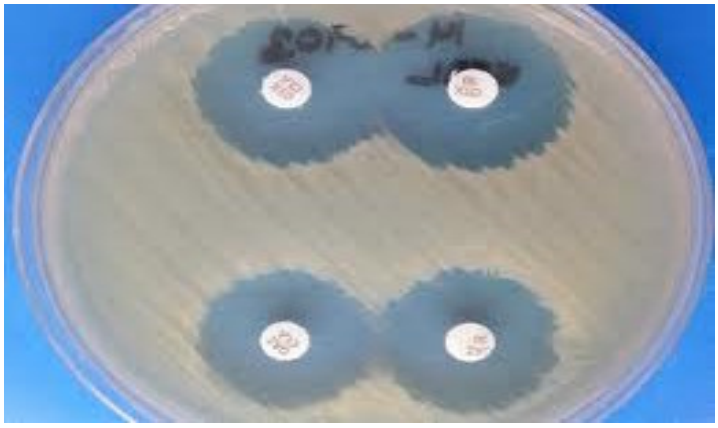
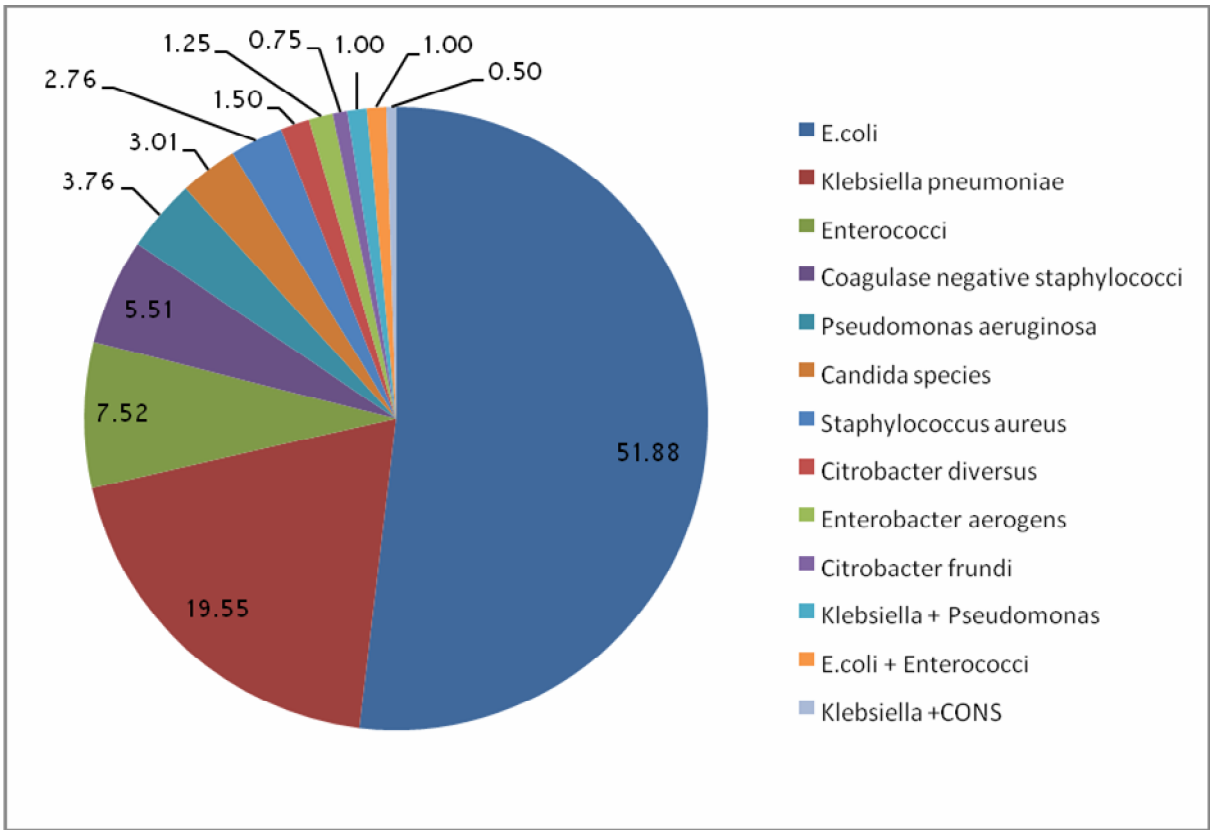


Fig.2 Type of organism isolated



Until recently, carbapenems were almost uniformly active against resistant Gram-negative organisms, but some strains have now developed very effective ways to deal with the carbapenems. There are various mechanisms by which these organisms achieve such feat, by producing beta lactamases which destroy the antibiotics, by blocking the entry of these antibiotics, or by efflux pumps which actively pump out these antibiotics. Furthermore, some of these mechanisms are not antibiotic or class specific, and can also be easily transferred from one organism to another. The situation is worsening everyday as no new antibiotics against these multidrug-resistant organisms are in advanced stages of clinical development.

With limited options and all the above-mentioned growing concerns, it would not be late where we will find ourselves in epidemics with multidrug-resistant organisms. We now have to find alternative and economical options to fend off an otherwise catastrophe. Micek *et al.* (2010) reported an improved outcome in sepsis when patients were put on empiric combination antibiotic therapy rather than the conventional monotherapy. A similar trial in treating UTI is yet to be evaluated. Kristensen and group evaluated a Decision Support Group in a small Danish County in deciding the empirical treatment of bacteraemic urinary tract infection and found that a decision theoretic approach showed promise of improving empirical antibiotic treatment, and may be a measure to support an antibiotic policy. Such feat on a larger scale could help in establishing standardized empiric therapy. But care should be taken to include the prevalent organism and antibiotic susceptibility pattern of the region as it varies over larger geographic areas due to various reasons.

Clinical presentation plays a minor role in establishing diagnosis in UTI. *E. coli* is still the most widely prevalent organism causing UTI in the community, only that the alarmingly high rate of resistant ESBL species should draw our attention. The resistance pattern, though not that different from the rest of the world, is ever increasing due to uncontrolled abuse of the available antibiotics. A strong decision has to be established regarding the antibiotic policies for UTI and stringent measures have to be taken to ensure the effectiveness of the same. Failing to do so, the time is not far where we would have to stand helplessly against these organisms.

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