Original Research Article

A Study on Bacterial Profile and Antibiotic Susceptibility Pattern in Complicated Urinary Tract Infection in a Tertiary Care Centre

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A B S T R A C T

Complicated urinary tract infections (cuti) refer to those infections that occur in the presence of anatomic or functional abnormalities in the kidney. Urine from 120 complicated UTI patients was collected and subjected to gram staining, culture and sensitivity by routine methods and ESBL resistance detected by screening and phenotypic methods. Males contributed to maximum number of isolates with 67.5%. Maximum number of isolates was obtained in the 51-60 yrs age group. The gram negative bacilli constituted 90.83% of isolates and gram positive cocci constituted 9.17% of isolates. *E. coli* was the commonest isolate with 40.83% and *Klebsiella spp* with 33.33%. The other isolates were *Pseudomonas spp*, *Acinetobacter spp*, *S. aureus, Citrobacter spp* and *Proteus vulgaris*. In GNB isolates, Amikacin, Nitrofurantoin and Imipenem had higher sensitivity. The gram positive cocci isolated were *Enterococci spp* and *S. aureus* which were 7 and 4 isolates respectively. The ESBL production in *E. coli* was higher than *Klebsiella spp*. The high rate of resistance demands an effective drug control policy to be formulated by the knowledge obtained in this study for the treatment of these patients.

Introduction

Complicated urinary tract infections (c UTI) refer to those infections that occur in the presence of anatomic or functional abnormalities in the kidney, bladder or collecting systems. A complicated infection is associated with factors that increase the chance of acquiring bacteria and decrease the efficacy of therapy. The host is compromised and / or the bacteria have increased virulence [or] antimicrobial resistance.

Upto 40% to 50% of the female population will experience asymptomatic UTI at some time during their life and about 3% of all women in the United States visit a physician each year for a UTI (Foxman *et al.,* 2002).

Men with bacteriuria frequently have anatomic abnormalities of the urinary tract. The uropathogens causing cUTI and their antibiotic sensitivity patterns varies considerably not only from that in acute uncomplicated UTI but also with time. The ascending route by way of the urethra is the most usual way by which micro-organisms enter the urinary tract.

E coli was the most common organism causing c UTI in a study done by Peterson *et al.,* (2007) in the United states. Strains of *E. coli* that infect patients with complicating factors [or] invasive procedures on the urinary tract, may lack specialized virulence factors.

Antimicrobial resistance rates of these strains vary and are highest among nosocomial strains under intense selection pressure in the hospital environment. ESBL producing organism poses a major problem for clinical therapeutics. The incidence of ESBL producing strains among clinical isolates have been on steady increase posing a problem in therapeutic options.

The knowledge of drug resistance pattern in a geographical area and the formulation of an appropriate hospital antibiotic policy will go a long way in the control of these infections. Therefore it is necessary to know the antibiotic susceptibility pattern of pathogens to select the correct antibiotic(s) for proper treatment of cUTI.

Knowledge of the microbial organisms and antibiotic susceptibility pattern of c UTI is essential for defining empirical treatment.

The main aim of this study to determine the Bacteriological profile in patients with Complicated Urinary Tract Infection and determine the Antibiotic susceptibility pattern using Kirby Bauer Disc diffusion method. Also identify the prevalence of ESBL in *E. coli* and *Klebsiella* spp by screening and phenotypic confirmatory methods. Finally, analyse the drug resistant pattern in uropathogens in cUTI.

Materials and Methods

Sample collection

Urine collection from women by clean catch midstream technique requires personal supervision for best results. The periurethral area and perineum are first cleansed with two or three gauze pads saturated with soapy water, using a forward – to – back motion, followed by a rinse with sterile saline or water.

The labia should be held apart during voiding, and the first few milliliters of urine passed into a bedpan or toilet bowl to flush out bacteria from the urethra. The midstream portion of urine is then collected in a sterile, wide-mouthed container that can be covered with a tightly fitted lid. The soapy water preparation is usually not required for men; rather, simple cleansing of the urethral meatus immediately after retraction of prepuce before voiding and then collection of the midstream sample is usually sufficient.

Specimens should be processed within 2 hours after collection to achieve accurate colony counts.

Urine culturing and bacterial identification

One ml of diluted (1/1000) urine specimen was used for culturing on CLED, Blood, and Mac Conkey agar. Specimen was inoculated using a calibrated loop. The plate was incubated at 35^0C for 24 hrs-48 hrs.

Specimens with more than 10^5 colony forming unit [CFU]/ ml were considered as positive samples. The isolates were identified based on colony morphology on culture plates, gram's staining and by standard biochemical tests. Antibiotic susceptibility testing was done by standard methods according to CLSI guidelines.

Antimicrobial susceptibility testing

Antibiotic susceptibility testing was performed by the Kirbey bauer disc diffusion method on MHA (Mueller hinton agar) according to CLSI protocols. The diameters of zone of inhibition were interpreted according to CLSI standards for each organism. Media and disks were tested for quality control with standard strains.

Detection of ESBL production in GNB

Screening method

Isolates of gram negative bacilli showing the following resistance pattern were considered to be possible ESBL producing strains.

CLSI phenotypic confirmation method

With a sterile bacterial loop 3 – 5 identical colonies were picked from a fresh overnight grown pure culture and inoculated into 5 ml of nutrient broth. The broth was incubated at 35^0 C for 2 -4 hrs and the turbidity matched with 0.5 Mc Farlands standard. Lawn culture of the test organism was made on to MHA plate [Hi media]. Antibiotic discs Ceftazidime [CAZ 30microgram] and Ceftazidime/ Clavulanic acid [CAZ/CA 30micro gram/10micro gram] [Himedia, Mumbai] were placed onto the plate and incubated at 35^0 C overnight. A ≥ 5mm increase in zone diameter for Ceftazidime tested in combination with clavulanic acid versus its zone when tested alone confirmed an ESBL producing organisms (Hussam *et al.,* 2009).

Double disk diffusion synergy test

In this test disks of cefotaxime, ceftazidime and Amoxycillin-clavulanic acid [20 microgram /10 micro gram] were kept 25 - 30mm apart from center to center on an inoculated MHA.

A clear extension of the edge of inhibition zone of Cephalosporin towards Amoxycillin-clavulanic acid disc was interpreted as positive for ESBL production (Chaudhary *et al.,* 2004).

Results and Discussion

The study undertaken at the Institute of Microbiology, Madras Medical College,

Chennai among samples received from patients of Rajiv Gandhi Government General Hospital, Chennai with cUTI showed the following results.120 Patients were included in this study.

Complicated urinary tract infection is a condition where there are structural and functional abnormalities of the urinary tract. The uropathogens isolated in c UTI varies considerably from that in uncomplicated UTI (Mahesh *et al.,* 2010).

The amount of drug resistance in these isolates is very high. Hence this study was conducted to determine the bacterial profile and the drug resistance pattern in these isolates.

The study was conducted at Institute of Microbiology, Madras Medical College and a total of 120 patients were included in this study.

The maximum number of patients were present in the age group 51yrs -60yrs [25%], followed by 41yrs – 50yrs group [17%] [Table 2]. In the study conducted by Mahesh *et al.,* (2010) the maximum patients were seen in 60-69yrs group with 22.5% followed by 50-59yrs group with 21%. This was in comparison with the present study (Mahesh *et al.,* 2010).

Males contributed the maximum number of patients with 67.5% and females constituting 32.5%. The female to male prevalence ratio was 1: 2.1 [Table 1]. This was in comparison with the study conducted by Mahesh *et al.,* where the males were the maximum numbers, with female to male prevalence ratio of 1:1.63 (Mahesh *et al.,* 2010).

Of the total 120 cases of cUTI, Diabetes mellitus [34.17%] was the most common risk factor contributing to the infection. This was

followed by renal calculi [15%], BPH [10%], post renal transplant [9.17%], bladder tumour and nephrotic syndrome each constituting 5.83% and the other cases were HUN, Stricture urethra each constituting 4.17% and 5% respectively [Table 5]. This was similar to the study conducted by Mahesh *et al.,* (2010) where the Diabetes mellitus was the most common risk factor with 42.6%. This was in comparison with this study. However the renal calculi were found in only 2% of the cases.

The majority of the patients presented with dysuria and was found in 37.5% patients. The next presentation was with fever in30.8%; followed by pain abdomen, frequency of micturition, hematuria, oliguria in the decreasing order of presentation [Table 4]. These symptoms were comparable with the study by Mahesh *et al.,* (2010)

In this study *E. coli* [40.83%] topped the list of organisms causing c UTI [Table 6]. The results from world-wide review are comparable to this study. Peterson *et al.,* (Peterson *et al.,* 2007) in their study done in USA have reported *E. coli* as the most common organism causing cUTI. A review by Lindsay Nicolle *et al.,* (1997) on c UTI reported *E. coli* as the most common

uropathogen with a worldwide prevalence rate of 21-54%. The second most common organism is *Klebsiella* species with 33.33%followed by *Pseudomonas* species 7.5%, *Acinetobacter* species and *Enterococcus faecalis* each with 5.84%, *Citrobacter freundii* 2.5%, *Staphylococcus aureus* 3.33% and finally single isolate of *Proteus vulgaris* with 0.83% were isolated in this study.

65.6% of *E. coli* and 62.3% of *Klebsiella spp* were ESBL positive [Table 9]. This was in comparison with the study conducted by Mahesh *et al.,* in a tertiary care centre in Bangalore where the ESBL positive *E. coli* and *Klebsiella* spp were 66.77% and 60.27% respectively (Mahesh *et al.,* 2010).

The *E. coli* isolated in these patients were 88% sensitive to Amikacin,77% sensitive to Nitrofurantoin, and were 26% sensitive to Cephalosporin, and 10% sensitive to Ofloxacin. They were 100% sensitive to Imipenem [Table 7]. In a similar study conducted by Mahesh *et al.,* (2010) only 20% *E. coli* were sensitive to ofloxacin. The resistant rate of the isolates to Amikacin, Gentamicin and Nitrofurantoin were also higher. The high resistance noted is similar to the present study.

Detection of ESBL production in GNB

Antibiotics	Zone diameter for possible ESBL Producing strain
Ceftazidime 30micro gram	≤ 22mm
Cefotaxime 30 micro gram	≤ 27 mm

Table.1 Gender distribution of patients with c UTI (n-120)

Gender	No of patients	Percentage
Male	81	67.5%
Female	39	32.5%
Total	120	100%

Males contributed to maximum number of isolates with 67.5%.

Table.2 Age and sex distribution of patients with c UTI (n=120)

Age group	Male[n=81] No %	Female [n=39] No %	Total [n=120]	No. %
12-20	1(1%)	6 (15%)	7	5.5%
21-30	18 (22%)	4 (10%)	22	18%
31-40	7 (8.5%)	11 (28%)	18	15%
41-50	13 (16%)	7 (17.5%)	20	17%
51-60	22 (27%)	8 (20.5%)	30	25%
61-70	12 (15%)	3 (8%)	15	12.5%
71-80	6 (7%)	-	6	5%
81-90	2 (2.5%)	-	2	2%
Total	81 (100%)	39 (100%)	120	100%

Maximum number of isolates were obtained in the 51-60 yrs age group.

Table.3 Uropathogens isolated in cUTI (n = 120)

Microorganisms	No of patients	Percentage
GNB	109	90.83%
GPC	11	9.17%
Total	120	100%

The Gram Negative Bacilli constituted 90.83% of isolates and Gram Positive Cocci constituted 9.17% of isolates.

Table.4 Symptoms in cUTI patients (n = 120)

Symptoms	No of patients	percentage
Dysuria	45 (37.5%)	
Fever	37 (30.8 %)	
Pain abdomen	23 (19.16 %)	
Frequency of micturition	15 (12.5 %)	
Hematuria	6 (5 %)	
Oliguria	3 (2. 5%)	

Dysuria and fever were the predominant symptoms in these patients.

Table.5 Distribution of diseases associated with c UTI patients [n =120]

Name of the Disease	No of patients	Percentage
Diabetes mellitus	41	34.17%
Renal Calculi	12	10%
BPH	12	10%
Bladder tumour	7	5.83%
Nephrotic syndrome	7	5.83%
HUN	5	4.17%
Stricture urethra	6	5%
Vesical calculi	3	2.5%
Ureteric calculi	3	2.5%
Nephritic syndrome	2	1.67%
PKD	2	1.67%
Phimosis	2	1.67%
Post Renal Transplant	11	9.17%
Carcinoma penis	1	0.83%
Testicular epididymo orchitis	1	0.83%
Renal abscess	1	0.83%
ARF	1	0.83%
Meatal stenosis	1	0.83%
Perinephric abscess	1	0.83%
Carcinoma prostate	1	0.83%
Acute prostatitis	1	0.83%
Cushing's syndrome	1	0.83%

Diabetes mellitus was the commonest associated illness with 34.17% followed by Renal Calculi with 15% of the patients (including vesical and ureteric calculi).

Table.6 Uropathogens isolated in cUTI

Organisms	No of patients	Percentage
E.coli	49	40.83%
K.pneumoniae & K. Oxytoca	40	33.33%
Pseudomonas aeruginosa	9	7.5%
Acinetobacter spp	7	5.84%
Enterococcus faecalis	7	5.84%
Staph. aureus	4	3.33%
Citrobacter freundii	3	2.50%
Proteus vulgaris	1	0.83%
Total	120	100%

E.coli was the commonest isolate with 40.83% and *Klebsiella spp* with 33.33%. The other isolates were *Pseudomonas spp*, *Acinetobacter spp*, *S. aureus, citrobacter spp &Proteus vulgaris*.

Table.7 Antimicrobial sensitivity pattern in gram negative bacilli [n=109]

Name of The Antibiotics	*E. coli* n=49		*K. pneumo niae* n= 27		*K. oxytoca* n=13		*P. vulgaris* n=1		*Pseudo monas aeruginosa* n=9		*Citrobacter freundi* n=3		*Acineto bacter spp* n=7	
	No %		No	%	No	%	No	%	No	%	No	%	No	%
Amikacin	43 (88%)		12 (44%)		8 (61.5%)		1 (100%)		6 (66%)		1 (33%)		6 (86%)	
Nitrofurantoin	38 (77%)		8(30%)		4 (31%)		-		-		1 (33%)		2 (28%)	
Gentamicin	15 (32%)		6(22%)		3 (23%)		1 (100%)		5 (55%)		-		5 (71%)	
Cotrimaxazole	7 (15%)		1(3%)		3 (23%)		-		2 (22%)		-		2 (28%)	
Ceftazidime	12 (26%)		5 (19%)		5 (38.5%)		1 (100%)		6 (66%)		-		4 (57%)	
Norfloxacin	4 (9%)		6 (22%)		3 (23%)		1 (100%)		5 (55%)		1 (33%)		4 (57%)	
Ciprofloxacin	2 (4%)		5 (19%)		1 (7%)		1 (100%)		3 (33%)		-		1 (14%)	
Ofloxacin	5 (10%)		5 (19%)		1 (7%)		1 (100%)		2 (22%)		-		1 (14%)	
Imipenem	49 (100%)		27 (100%)		13 (100%)		1 (100%)		9 (100%)		3 (100%)		7 (100%)	

In GNB isolates, Amikacin, Nitrofurantoin and Imipenem had higher sensitivity.

Table.8 Anti-microbial sensitivity pattern in gram positive cocci [n=11]

Antibiotics	*Enterococcus faecalis* n=7		*Staph. aureus* n=4	
	No	%	No	%
Amikacin	1	14%	3	75%
Nitrofurantoin	4	57%	-	-
Cotrimoxazole	-	-	1	25%
Ciprofloxacin	1	14%	1	25%
Erythromycin	1	14%	1	25%
Amoxycillin	2	26%	1	25%
Amoxycillin Clavulanic Acid	4	57%	2	50%
Cephalexin	3	42%	2	50%
Oxacillin			1	25 %

The Gram positive cocci isolated were *Enterococci spp* and *S. aureus* which were 7 and 4 isolates respectively.

Table.9 List of ESBL producing organisms [n=67]

Organism	Screening test with Cefotaxime	PCDDT No %		DDST N0 %	
E.coli n=49	37	32	65.6%	32	65.6%
Klebsiella spp n=40	30	25	62.5%	25	62.5%

The ESBL production in *E.coli* were higher than *Klebsiella spp*.

The *Klebsiella pnemoniae* isolated in these patients were 44% sensitive to Amikacin, 30% sensitive to Nitrofurantoin and19% sensitive to cephalosporin and Fluoroquinolones [Table 7].

The *Klebsiella oxytoca* isolated in these patients were 61.5% sensitive to Amikacin, 31% sensitive to Nitrofurantoin,38.5% sensitive to Cephalosporin and 7% sensitive to Fluoroquinolones [Table 7]. Both the *Klebsiella spp* were 100% sensitive to Imipenem. In a similar study conducted by Mahesh *et al.,* the sensitive rate to Fluoroquinolones among *Klebsiella spp* was 40%.

The resistance -rate was higher for Amikacin, Garamycin, Nitrofurantoin in these isolates (Mahesh *et al.,* 2010). The high resistance noted is similar to the present study. The *Pseudomonas aeruginosa* was the third commonest isolate in these patients. They were 66% sensitive to Amikacin and Cephalosporin, 33% sensitive to Ciprofloxacin and 22% sensitive to Ofloxacin [Table 7]. In a similar study by Mahesh *et al.,* 74.5% resistance rate to Fluoroquinolones and 64.7% resistance to Amikacin and Gentamicin were noted for *Pseudomonas spp*. This was in comparison to the present study.

In the present study, *Pseudomonas spp* were 100% sensitive to Imipenem whereas 70% of the isolates were sensitive to Carbapenems in the study by Mahesh *et al.,* This is not coherent with the present study. The *Acinetobacter spp* isolated in this study were

86% sensitive to Amikacin, 57% sensitive to Cephalosporin and Norfloxacin; 14% sensitive to Fluoroquinolones and 28% sensitive to Nitrofurantoin and Cotrimoxazole and 100% sensitive to Imipenem [Table 7].

The *Citrobacter freundii* constituted 3 isolates in this study. They were 33% sensitive to Amikacin, Nitrofurantoin and Norfloxacin. They were 100% sensitive to Imepenem [Table 7].

The *Proteus vulgaris* was the single isolate in this study. It was 100% sensitive to Amikacin, Gentamicin, Cephalosporins, Fluoro-quinolones and Imipenem [Table 7].

The *Enterococcus faecalis* constituted 7 isolates in this study. They were 14% sensitive to Amikacin, and Ciprofloxacin. They were 57% sensitive to Nitrofurantoin and 42% sensitive to Cephalexin. They were 100% resistant to Cotrimoxazole [Table 8]. In a similar study by Mahesh *et al.,* 28.5% resistance to Fluoroquinolones and 85% resistant to Cotrimoxazole was noted. This was in comparison to the present study (Mahesh *et al.,* 2010).

The *S. aureus* constituted 4 isolates in this study. They were 75% sensitive to Amikacin. They were 50% sensitive to Amoxicillin Clavulanic acid and cephalexin.3 isolates were oxacillin resistant. The isolates were 100% sensitive to Carbapenems. The highly sensitive rate to Carbapenems, could be due to it usage pattern in our institution. It is an expensive drug, used in critically ill inpatients

where the administration of approximate doses for the full duration is ensured. The high rate of resistance in Cephalosporins and Fluoroquinolones are multifactorial including lack of protocol for c UTI and low compliance of patients in completing the antibiotic course. The increasing rate of multidrug resistant uropathogens and ESBL producing organism pose a serious threat in treating cUTI.

The removal of the stasis factor in these patients with complicated UTI will contribute much towards treating these patients. An effective Antibiotic Policy incorporating these results will help in Treatment and improved Prognosis of these patients.

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