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Bacteriological Profile, Antibiotic Sensitivity Pattern and Detection of ESBL Production in the Isolates of UTI in Tertiary Care Hospital, Davangere, India

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Abstract: ***Background: Urinary tract infection (UTI) is a very commondiseasethat can affect anyone at any age where the infection rate is higher in women than men. Objective:The aim and objectives of this study were to determine the etiological bacterial pathogens of the UTI and to determine the antibiotic sensitivity pattern of pathogens isolated as well as identify Extended-spectrum β-lactamases (ESBL) producers. Methodology:This was a hospital based cross sectional study in which 120 midstream urine samples were collected from April 2015 to June 2015 from clinically suspected UTI patients of various departments. Urine culture was done, using conventional microbiological techniques. Biochemical testing was used to identify the organisms and antibiotic sensitivity was done by the Kirby Bauer disc diffusion method according to standard CLSI guideines . Further ESBLs was detected by double disc synergy and combined disc diffusion test. Results:Out of 120 tested samples, 48 showed growth of pathogens among which the most prevalent were E.coli (29.17%) followed by Klebsiella (22.92%). The majority of the isolates were from female (77.08%). ESBL production was observed in 35.71% of E. coli strain and 27.27% of Klebsiella strains. High rates of resistance was found with Ceftazidime(81.58%), Ceftazidime + Clavulanic acid (76.32%,Erythromycin(53.33%), Cefotaxime (53.19%),Ciprofloxacin(45.83%), Norfloxacin (43.33%) among the isolates but Nitrofurantoin(37.93), Gentamycin (22.97%) and Amikacin (18.75%) are comparatively sensitive. Conclusion: The study revealed that E. coli was the predominant bacterial pathogen of UTIs. An increasing trend in production of ESBLs among UTI pathogens were noted. Proper knowledge of susceptibility pattern of uropathogens is crucial in order to discourage the indiscriminate use of antibiotics as well as in formulating effective empiric therapy.***

Keywords: Urinary tract infection, Antibiotic sensitivity, Extended spectrum β-lactmase.

1. Introduction

Urinary tract infections (UTI) are one of the most common human bacterial infections both in community and hospital settings.(1,2) An estimate of patients suffering from UTI is around 150 million per annum across the globe which may rise to75% in the female population by the age of 24 and 15-25% of this group may suffer from the relapse of this diseases.(3) It has been observed that upto one-third of all women will experience UTI at some point during their lifetime. This finding has been attributed to three features that facilitate ascending infections into bladder, namely a short urethra, the proximity of urethra to anus and colonization of vagina by the fecal flora.(4)

UTIs are defined by the presence of a growth of more than 10^5colony forming units (CFU) of bacteria per ml of urine for asymptomatic individual and much lower for symptomatic individual (~10^3CFU/ml).(5) In urine sample obtained by supra pubic aspiration or in-and-out catheterization and in samples from a patient with an indwelling catheter, colony count of 10^2-10^4/ml generally indicates infection.(6)

UTI that occurs in a normal genitourinary tract with no prior instrumentation are considered as uncomplicated whereas complicated infections are diagnosed in genitourinary tract that have structural or functional abnormalities including instrumentation such as indwelling urethral catheters, and are frequently asymptomatic.(7) Complicated UTI exhibits a broader bacterial spectrum as the cause of infection.(8) Many organism can infect urinary tract, but by far the most important agents are the gram-negative bacilli. *Escherichia coli* cause 80% of acute infections. Other gram negative bacilli, *Proteus* and *Klebsiella* species and occasionally *Enterobacter* species accounts for uncomplicated UTI. Nosocomial infections are more likely to be caused by *Escherichia coli*, *Klebsiella* species, *Proteus* species, *Staphylococcus* species, *Pseudomonasaeruginosa* and*Enterococci* species.(13)

The introduction of antimicrobial therapy has contributed significantly to the management of UTIs.(9) The antimicrobial agents used in treatment of UTI include cell wall inhibitors like penicillin, third generation Cephalosporins (Cefotaxime, Cephradine, Ceftazidime and Cefaclor), DNA gyrase inhibiters like Floroquinolones(Ciprofloxacin, Ofloxacin, Sparfloxacin and Enoxacin) and Aminoglycosides (Amikacin, Gentamycin and Kanamycin) that are protein synthesis inhibitors. Inappropriate and extensive use of antibiotics has lead to the development of multidrug resistance among the pathogens[11].The most common antibiotic used for the

treatment of bacterial infections are the β-lactam antibiotics, but the production of

β-lactamases make the pathogen resistant to this drug.(10) β-lactamases are extracellular enzyme produced by large number of bacteria causing breakage of amide bond of β-lactam ring of Penicillins and capable of inactivating Oximino-cephalosporins and Aztreonam but are inactive against Cephamycins and Carbapenem.(10) ESBLs are chromosomal or plasmid mediated β-lactamases which have mutated from pre-existing broad-spectrum β-lactamases (TEM-1, TEM-2, SHV-1) as a consequence of widespread use of 3rd generation Cephalosporins as well as Aztreonam. .(19) These enzymes are coded by plasmids and their ability to spread to other bacteria has led to dramatic increase in their prevalence worldwide in a very short span of life.(13) They occur predominately in *Escherichia coli* and *Klebsiella* species, they have also been described in other genera of the *Enterobacter*iacea.(17) The prevalence of ESBL producing organisms among urinary isolates varies from 20-71% in India and 8-45% worldwide.(13)

This study was carried out,

- To determine the distribution of bacterial pathogens that cause urinary tract infections.
- To analyze the antibiotic sensitivity and resistance patterns of uropathogens and
- To identify Extended spectrum β-lactamases (E.S.B.L.)producers in different populations of uropathogens

2. Material and Methods

The present study was conducted at the microbiology laboratory of JJM Medical College, Davangere, Karnataka over a period of 2 months from April 2015 to June 2015.The study included patients fromboth out-patient clinics and in-patient units of various clinical departments (Medicine, Surgery, Gynecology) of Chigateri Government Hospital(C.G.H.) and Bapuji Hospital in Davangere.A total of 120 urine samples were collected from suspected UTI patients of age group 12 to 90 years.
Informed consent was taken from all the subjects participated in the study after explaining the study details in the subjects mother tongue.

Inclusion criteria-

- With fever (>38^{o}C) and chills
- Patient showing one or more of the following symptoms- Burning micturation, increased frequency, urgency of urine, dysuria and pain lowerabdomen / flank pain/ supra pubic pain.

Exclusion criteria-

- Asymptomatic patients.
- Patients already on antibiotic treatment(duration of 5-7 days)
- Age less than 12 years.
- Patients on indwelling catheters.

Specimen collection-
About 30 ml of clean catch midstream urine sample were collected in 100ml sterile, dry, leak-proof container with instructions on how to collect a clean catch midstream urine (MSU).(21) The MSU requires that the first 10-30 ml of the voided urine be discarded and the second midstream be sampled. In the female patients, adequate peiurethral cleansing is necessary to reduce the probability of contamination. For cleaning, water and soap solution without antibacterial activity was used.(22)Urine is an excellent culture media and bacteria will multiply if specimen is left at room temperature for any appreciable time.For this reason, urine specimen was transported to the laboratory immediately after obtaining and was processed within one hour or in case of delay they were refrigerated at 4^{o}C(upto 24 hours), until culture can be performed.(23)

Processing and Culture
At first, urine was examined microscopically as a wet preparation to detect significant pyuria that is WBCs in excess of 10 ^{7} WBC/ml of urine .Detecting bacteria in uncentrifuged urine indicates urinary infection, pyuria that can be quantified by counting WBC on estimating numbers by examining a drop of urine on a slide (1 WBC per lower power field corresponds to 3 cells per µl). The Gram's stain was another method used to estimate bacteriuria .The presence of $\geq$ 1 organism/oil emersion field in uncentrifuged urine reflects colony counts of >10^5 CFU/ml.(24)

Semi quantitative urine culture was done using a calibrated loo*p*.(15) The pathogens were isolated by following standard protocols using sterile bacteriological media, including Blood agar, MacConkey agar and Cystine-Lactose-Electrolyte–Deficient (CLED) agar.(10)For this, sterile standard nichrome loop of 28 SWG was used which had a internal diameter of 3.28mm and volume holding capacity of 0.004ml.(25) Culture plate was incubated at 35-37^{0}C for 18-24 h.(18) Before inoculation of the urine, there was prior incubation of the plates at 37^{o}C for 30minutes to dry the surface and eliminate contaminations.(12) After 18 to 24hr of incubation, the number of bacteria in urine sample is estimated by counting the number of colonies that appear on the surface of the media.(24) All plates showing significant growth (>10^5 CFU/ml) as per the Kass count were further processed.(26)But if the CFU is less than 10^5, it is considered as non significant bacteriuria or negative.(14)

The isolates were initially characterized on the basis of their gram staining reaction, morphology, growth and biochemical characteristics i.e. fermentation of lactose, ability to produce indole, reaction on triple sugar iron (TSI) medium, observation of hemolysis on blood agar, citrate utilization and motility of organism.(5)

Antimicrobial susceptibility testing-
Antibiotic sensitivity testing was done by emulsifying selected colonies in normal saline at a turbidity compared to 0.5 MacFarland's standard. Using sterile swabs, suspensions were inoculated on Muller-Hinton agar in accordance with Kirby Bauer as per recommendatation of CLSI guidelines and incubated at 35-37°C for 18-24 hours.(20) The inhibition zone size was interpreted by using the standard recommendations of the Clinical Laboratory Standard

Institute (CLSI) guidelines(2014).(16) The organisms were reported as susceptible, intermediate or resistant using standard referring table.

The antibiotics used in this study are Amikacin(30µg), Cefotaxime(30µg), Co-trimoxazole(25µg), Ceftazidime(30µg), Ceftazidime+ Clavulanic acid(30/10µg), Ciprofloxacin(5µg), Gentamycin(10µg), Norfloxacin(10µg) and Nitrofurantoin(300µg).

Detection of ESBL producing organisms-

According to CLSI guidelines (2014)

Screening test- Disc diffusion method by using Cefpodoxime(10µg)or Ceftazidime(30µg) or Cefotaxime (30µg)or Ceftriaxone (30µg).

Confirmatory test- Combined disc diffusion method by using Ceftazidime (30µg), Ceftazidime +Clavulanic acid (30µg/10µg) and Cefotaxime (30µg) and Cefotaxime +Clavulanic acid (30µg).

3. Results

A total of 120 symptomatic patients were included in the study.

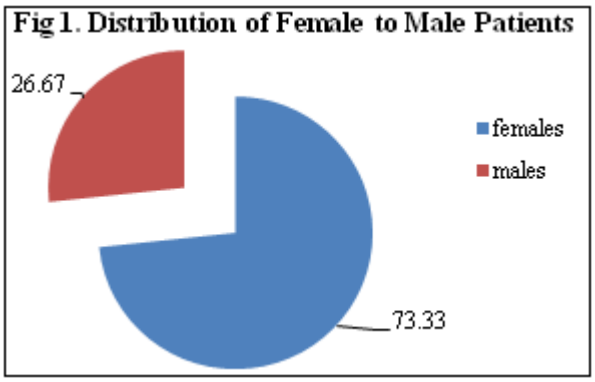

Figure 1: Shows distribution of ratio of female to male patients.

Of these 120 symptomatic patients in the age group of 12years to 90years, 88(73.33%) were female and 32(26.67 %) were male patients. Female and male patient ratio was 2.75:1.

Table 1: Shows the frequency of culture positives in males and females.

Sl No.	Total no. of samples	Total no. of culture positives	Males			Female		
			Total no. of patients	Culture positives	Percentage of culture positivity	Total no. of patients	Culture positives	Percentage of culture positivity
1.	120	48	32	11	34.38%	88	37	42.05%

Comparing sexes the present study reveals that there is higher frequency of culture positivity in female (42.05%) than male (34.38%) patients. Total no. of positive samples are 48(40%), out of which 37(30.83%) are female and 11(9.17%) are male patients.

Table 2: Shows age and gender wise distribution of UTI patients with positive cultures.

Sl no.	Age group	Total no. of patients	Males			Females		
			Total no. of patients	Patients with culture positives	Percentage of culture positivity	Total no. of patients	Patients with culture positives	Percentage of culture positivity
1.	12-20	8	4	0	0%	4	4	100.0%
2.	21-30	51	4	3	75.0%	47	19	40.43%
3.	31-40	22	8	3	37.5%	14	8	57.14%
4.	41-50	10	4	0	0%	6	1	16.67%
5.	51-60	17	5	2	40.0%	12	3	25.0%
6.	>60	12	7	3	42.86%	5	2	40.0%
Total		120	32	11	31.43%	88	37	42.05%

The study included both male and female patients in the age group of 12 to 90 years with 8 patients in the age group 12-20 years, 51 patients in the age group of 21-30 years, 22 patients in the age group 41-50 years, 17 patients in the age group 41-50 years and 12 patients in the age group >60 years.

Table 3: Shows the distribution patterns of uropathogens from the urinary specimens under study.

Sl no.	Isolates	Total no. of Isolates	Percentage of Isolates
1.	*Escherichia coli*	14	29.17%
2.	*Klebsiella sp.*	11	22.92%
3.	*Pseudomonas*	6	12.5%
4.	Coagulase negative *Staphylococcus*	8	16.67%
5.	*Staphylococcusaureus*	2	4.17%
6.	*Enterococcus*	6	12.5%
7.	*Acinetobacter*	1	2.08%
Total		48	100%

The isolates included both gram positive and gram negative organisms. Of the 48 isolates, gram negative bacteria accounts for 32 (66.67%) while gram positive bacteria accounts for 16(33.33%). *Escherichia coli* showed the highest prevalence of 29.17% followed by *Klebsiella* species with 22.92%, Coagulase negative *Staphylococci* (16.67%), *Pseudomonasaeruginosa* (12.5%), *Staphylococciaureus*(4.17%), *Acinetobacter*(2.08%). *Escherichia coli* was the predominant isolate among the gram negative organisms and coagulase negative *Staphylococcus* among the gram positive organisms.

Table 4: Summarizes the antimicrobial potency and spectrum of selected antimicrobial agents of different classes against UTI isolates.

Organisms	No. of isolates	Amikacin(30µg)	Cefotaxime(30µg)	Co-trimoxazole(25µg)	Ceftazidime(30µg)	Ceftazidime+ Clavulanic acid(30/10µg)	Ciprofloxacin(5µg)	Gentamycin(10µg)	Norfloxacin(10µg)	Nitrofurantoin(300µg)
Escherichia coli	14	8 (57.14%)	6 (42.86%)	4 (28.57%)	1 (7.143%)	1 (7.143%)	3 (21.43%)	9 (64.29%)	4 (28.57%)	5 (35.71%)
Klebsiella	11	6 (54.54%)	4 (36.36%)	7 (63.63%)	1 (9.09%)	2 (18.18%)	6 (54.54%)	6 (54.54%)	10 (90.9%)	1 (9.09%)
Pseudomonas	6	6 (100%)	4 (66.67%)	1 (16.67%)	1 (16.67%)	1 (16.67%)	4 (66.67%)	3 (50%)	3 (50%)	-
Coagulase negative *Staphylococci*	8	7 (87.5%)	7 (87.5%)	5 (62.5%)	1 (12.5%)	3 (37.5%)	6 (75%)	5 (62.5%)	-	1 (12.5%)
Enterococcus	6	2 (33.33%)	1 (16.67%)	-	-	1 (16.67%)	1 (16.67%)	3 (50%)	-	-
Staphylococcusaureus	2	-	-	-	-	-	-	-	-	-
Acinetobacter	1	-	-	-	-	-	-	1 (100%)	-	-

Table 5: Shows the antibiotics sensitivity and resistance patterns of isolates.

Sl no.	Antibiotic	Total no. of isolates	Sensitive		Moderatelysensitive		Resistant	
			No.	%	No.	%	No.	%
1.	Amikacin(30µg)	48	29	60.42	10	20.83	9	18.75
2.	Cefotaxime(30µg)	47	21	44.68	1	2.13	25	53.19
3.	Co-trimoxazole(25µg)	48	17	35.42	8	16.67	23	47.92
4.	Ceftazidime(30µg)	38	4	10.5	3	7.89	31	81.58
5.	Ceftazidime+ Clavulanic acid(30/10µg)	38	7	18.42	2	5.26	29	76.32
6.	Ciprofloxacin(5µg)	48	24	50.0	2	4.17	22	45.83
7.	Gentamycin(10µg)	48	27	56.25	10	20.83	11	22.97
8.	Norfloxacin(10µg)	30	17	56.67	0	0	13	43.33
9.	Nitrofurantoin(300µg)	29	7	24.13	11	37.93	11	37.93

Table 6: Shows the prevalence of ESBL producers

Sl no.	Isolates	Total no. of isolates	Extended spectrumβ-lactamase producers	
			No. of ESBL	% of ESBL
1.	*Escherichia coli*	14	5	35.71%
2.	*Klebsiella*	11	3	27.71%
3.	*Pseudomonas*	6	1	16.67%
Total		31	9	29.03%

The prevalence of ESBL producers, with a percentage of 35.71%, 27.27% and 16.67% among *Escherichia coli*, *Klebsiella* and *Pseudomonasaeruginosa* being ESBL producing. The overall prevalence of ESBL production was 9 (29.03%).

4. Discussion

UTI impose a huge burden on health care systems due to high prevalence of infection in both community and nosocomial settings.UTIis caused by variety of pathogens including *E. coli*, *K. pneumonia* and*P.aureginosa*. Continuous surveillance of antibiotic susceptibility patterns of uropathogens at local level is crucial in dealing with emerging problems of antibiotic resistance and provides assistance in managing effective initial therapy.(5)

Amoxicillin (a β-lactam antibiotic) was traditionally used in the first line therapy for UTIs, but with the spread of drug resistance, treatment options have now changed. Complicated cases of UTI usually require a longer course or intravenous antibiotics, and in case symptoms do not

improve in two to three days, further diagnostic testing is needed.(3)

The present study included 120 symptomatic patients.Urine culture was positive in 48(40%) patients which is very similar to study done by ShaistaBano*et al* 2014 (41.18%) and Anup Shah *et al* 2015(43.07%). Women accounted for 30.83%of all positive patients,which is similar to ShaistaBano*et al* 2014 (31.09%).

The gender and age wise analysis showed a higher incidence of urinary tract infection in 21-40yrs age group in females which can be explained by the fact that urinary tract infections are more common in the reproductive age group. Higher incidence of urinary tract infections among males was in the >60 years age group; which could be explained due to co-morbid conditions like prostrate hypertrophy and history of Diabetes mellitus among them.*E. coli* was the most common bacteria (29.17%) in UTI Patients, but with a different rate obtained from other populations(48.21% in Anup Shah *et al* 2015). Percentage of *Pseudomonas* 6(12.92%) was similar to study done by InamUllah Khan *et al* 2015(11.8%). Other isolates included *Klebsiella*11(22.92%), coagulase negative *Staphylococcus* 8(16.67%, *Staphylococcusaureus* 2(4.17%), Enteroccus 6(12.5%) and *Acinetobacter* 1(2.08%).
Antibiotic resistance represents a global challenge to public health. The intense use and misuse of antibiotics have been responsible for emergence of antibiotic resistance together with selection and spread of the antibiotic resistant strains of bacterial pathogens, including uropathogens. Knowledge of the local resistance and surveillance studies to monitor emerging trends of resistance through susceptibility testing of uropathogens,particularly *E. coli* is recommended.(5) This study provides current scenario of antibiotic resistance pattern in Davangere, Karnataka.

In the present study, antibiotic susceptibility patterns showed that more than 50% of the isolates(60.42%)show sensitivity to Amikacin, where all of the *Pseudomonas* isolates and more than 85% of Coagulase negative *Staphylococcus* are sensitive to Amikacin. Also, *E. coli* shows very less sensitivity towards Ceftazidime (7.14%), Ceftazidime + Clavulanic acid(7.14) and Ciprofloxacin (21.14%), which indicates that these drugs should not be chosen for treating UTI and should only be prescribed after the sensitivity report from microbiological laboratory keeping in mind the emerging antimicrobial resistance.

Rama Biswas et al found that 86.36% of all isolates were sensitive to Amikacin and 73.63% were sensitive to Nitrofurantoin. But in this study, it was found out that 60.42% of isolates were sensitive to Amikacin and just 24.13% were sensitive to Nitrofurantoin.

From the present study, it is shown that there is an increased resistance for 2nd and 3rd generation Cephalosporins like Cefotaxime, Ceftazidime and also Ceftazidime and Clavulanic acid. The resistance rates are 53.19%, 81.58% and 76.32% respectively. So, the increasing resistance to Cephalosporins promoted us to search for ESBL producers. The incidence of ESBL strains among clinical isolates have been steadily increasing over the past few years resulting in major problem for clinical therapeutics.
Detection of ESBL isolates is a challenge for microbiological laboratory because these ESBL producing gram negative bacilli appear susceptible in-vitro to certain β-Lactam antimicrobial agents, yet results in treatment failures. So, proper identification is necessary.

In this study, the frequency of ESBL producing organisms among gram negative bacterial isolates was found to be 9 (29.03%). A similar frequency of ESBL producing organisms (27.67%) was observed by Dugal *et al* 2013. The present study showed ESBLs production prevalence in 35.71% *Escherichia coli* followed by 27.27% *Klebsiella*species and 16.67% *Pseudomonas*. In comparison, study by Rama Biswas*et al* 2014 showed 46.87% of *E. coli* and 25% of *Klebsiella* species to be ESBL producers.

Many of the isolates were observed to be multidrug resistant. So the present study gives an idea about the common trend of increasing antibiotic resistance of uropathogens in this region which could be due to indiscriminate or under dose of antibiotic use. Thus this data may help the physician in proper treatment of urinary tract infections and avoid use of resistant antibiotics.

5. Conclusion

It was concluded that the incidence of UTI infections are higher among females with more prevalence in 20-30yrs age group.The study revealed that *E. coli* was the predominant bacterial pathogen of UTIs followed by *Klebsiella*. An increasing trend in production of ESBLs among UTI pathogens were noted which is more prevalent in *E.coli*, followed by *Klebsiella* and *Pseudomonas*. In the current study, a majority of isolates were sensitive to Amikacin, Gentamycin and Norfloxacin. Bacterial isolates showed more resistance against Ceftazidime and Ceftazidime + Clavulanic acid. So these drugs should not be used as first line treatment drugs and instead should be used only after antibiotic sensitivity testing.
For prevention of UTIs, implementation of strict infection control guidelines, effective hand washing and judicious use of antimicrobials is mandatory which to prevent the emergence of drug resistance among uropathogens.

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