Original Research Article

A Retrospective Study of the Culture and Sensitivity Pattern of Urinary Tract Infection Causing Organisms at RIMS Teaching Hospital, Raichur, India

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A retrospective study was done and culture and sensitivity reports were collected from RIMS, Teaching Hospital, Central Laboratory Microbiology Department, Raichur for a period of Sep 2014 to April 2016 were analysed. A total of 351 urine samples collected during a time period of 20 months from September 2014 to April 2016 and fulfilling the inclusion criteria were included in the study. The objective was to assess the distribution of urinary tract bacterial pathogens and to determine the antibiotic susceptibility profile of the isolates in our setting. Single positive cultures with a colony count of >105 CFU on CLED medium were identified and their antibiotic sensitivity and resistance profile was depicted in the form of an antibiogram. Out of 351 urine samples 217(61.8%) urine samples were culture positive with significance. 57.1% of the total isolates were identified as *E. coli*, followed by *Pseudomonas spp.* 16.1%, *Klebsiella pneumonia* 9.6%, Proteus spp 4.6%, *Citrobacter* spp 4.1%, *Staphylococcus aureus* 4.6%, Coagulase negative *Staphylococcus* 2.3% and *Candida spp* 1.4%. Among 199 gram negative organism isolated it was observed that 198/199(99.4%) of total tested isolates were sensitive to Imipenem followed by Piperacillin/Tazobactam 170/199(85.4%), Cefoperazone/sulbactam 128/199(64.3%), Ceftazidime 112/199(56.2%), Augmentin 107/199(53.7%), Amikacin 105/199(52.7%), Nitrofuration 101/199(50.7%), Cefipime 100/199(50.2%), Ceftriaxone 95/199(47.7%), Gentamicin 90/199(45.2%), Nalidixic acid 75/199(37.6%), Levofloxacin 70/199(35.1%), Ciprofloxacin 48/199(24.1%), Cotrimoxazole 28/199(14.1%). Also out of 18 gram positive organisms isolated 3 were Candida species. Sensitivity profile of gram positive organisms showed Vancomycin was most susceptible 18/18(100%) followed by Augmentin 14/18(77.7%), while Penicillin was least susceptible 4/18(22.2%). This is an alarmingly low sensitivity to Ciprofloxacin, Cotrimoxazole and Penicillin which is routinely used as empirical therapy for uncomplicated community acquired urinary tract infections and other infections. There is need for proper sensitivity testing to guide the management of UTIs with hospital antibiotic policies.

Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections in humans both in the community and hospital setting. [1–3] UTI remains second most common infectious disease and approximately about 150 million people were diagnosed with UTI each year.[4] UTI can be caused by different microorganisms including bacteria, protozoa, parasites, fungi and viruses are the major causative organisms. Among these bacteria account for more than 95% of UTI cases. [5] *E. coli* is the most common isolate

responsible for UTI.[6-9] Other important pathogens are *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.[10, 11] Distribution of uropathogens and their susceptibility to antibiotics is variable regionally[7,12], so it becomes necessary to have a knowledge of distribution of uropathogens and their susceptibility to antibiotics in a particular setting. Antimicrobial resistance is a growing problem and a cause of major concern in many countries.[13] This study is aimed to determine the distribution of bacterial uropathogens and their susceptibility to antimicrobials in patients who attended outpatient and inpatient from various departments of RIMS, Teaching Hospital, RAICHUR, Karnataka from September 2014 to April 2016.

Materials and Methods

This was a retrospective study conducted at RIMS, Teaching Hospital, Central Laboratory Microbiology Department, RAICHUR, Karnataka. The data of all cases with urine culture positive reports were analysed from Sep 2014 to April 2016. Over the course of study, total of 351 urine samples were collected. Out of those 351 successful samples 176 females and 175 males and were in the age group of 1 to above 60 years old. There were higher numbers of samples collected from the extreme of ages 1-9 years and above 60 years (Table 1).

Collection of urine specimen

The specimens were collected into sterilized, wide necked, leak proof, plastic universal containers. Clean-voided mid-stream urine specimens were collected. Each of the study participant was instructed on the mode of collection of the mid-stream urine that was during forceful urination after the first 10 -20 ml has been voided.[14]

Sample preparation and microscopy

The collected urine sample was prepared for microscopy according to standard method. The urine samples were mixed and aliquots centrifuged at 5000 rpm for 5 min. The deposits were examined using both 10X and 40X objectives. Samples with ≥ 6 white blood cells/mm were regarded as pyuric.[15]

Media preparation

The media used were Blood Agar (BA), Nutrient Agar (NA) and Cysteine Lactose Electrolyte Deficient (CLED) Agar were prepared according to the manufacturer's instructions.

Sample culturing

The collected urine samples were processed for culture and report according to standard method procedure and protocol. For the visual examination and calibrated Loop Streak method for urine culture performed 1 µl wire loop was sterilized and dipped of into urine sample. The loop was then streaked on the plate of Cysteine Lactose Electrolyte Deficient (CLED) Agar. The plates were incubated over night at 37°C for 24 to 48 hours. Following the appearance growth well-isolated bacterial colonies were processed for antibiotic sensitivity and biochemical tests.[16]

Identification of microorganisms

All bacterial isolates were characterized on the basis of colony morphology, Gram staining, Catalase test, Coagulase test, and oxidase tests. From the results of preliminary identification test the gram negative rods were subjected to the following routine biochemical tests such as mannitol motility test, indole production, urease production, citrate utilization test and Triple sugar iron reaction.[17]

Sensitivity test

All isolated organisms were tested against various antibiotics in vitro, antibiotic susceptibility was tested in laboratory by disc diffusion method[18] on Muller Hinton Agar by Kirby-Bauer method[18] using the following antibiotic discs, Imipenem, Piperacillin/Tazobactam, Cefoperazone/ sulbactam, Ceftazidime, Augmentin, Amikacin, Nitrofuration, Cefipime, Ceftriaxone, Gentamicin, Nalidixic acid, Levofloxacin, Ciprofloxacin, Cotrimoxazole, Vancomycin, Novobiocin, Penicillin, Erythromycin and Tetracycline accordingly.

Statistical analysis

The data were entered and analysed using Statistical Package for Social Sciences SPSS software version 21.0. P - Value < 0.05 was considered statistically significant.

Results and Discussion

Out of total collected 351 successful samples 176 females and 175 males and were in the study group of 1 to above 60 years old. There were higher numbers of samples collected from the extreme of ages 1-9 years both males/females (23.5%)/(20.5%) and above 60 years both males/females (20.6%)/(24.5). In age groups of 20-29 years and above 60 years there were higher numbers of negative samples of males and females respectively showing no growth on culture plates. That could be due to indiscriminate consumption of non-prescribed antibiotics by the peoples whenever difficulty in urination was noticed. UTIs were higher in numbers of females than males in age group of 1-9 years because of continuous wetting and due to difficulty in keeping dry under wears and may be due to short distance between vagina and anus which make easy excess of pathogens in female urinary tract. The male positive samples

above 60 years old showed high risk of infections due to bladder outlet obstruction in older men there might be bacterial prostatitis and non-bacterial prostatitis. For the age group 30-39 years again females showed higher risk of infections than males because younger females represented complication of sexually transmitted urethritis. However, in 10-19 years age groups both males and females samples there were a slight difference of positive and negative samples. Both the extremes of age groups have risk of UTIs infections due to low immunity (Table 2).

Out of total 351 collected samples 134 samples showed no growth on culture plates. 217 were positive samples. Pathogenic microbes were isolated from 217 positive samples (61.8%) which in the vast majority of cases *E. coli* (57.2%) followed by Pseudomonas (16.1%) and *Klebsiella* species (9.6%) were among the most frequent uropathogen bacteria. That was similar to other studies where it was the most frequent pathogen causing UTI as studied by Mehar where 62.6% cultures grew *E. coli* 16 and 66% *E. coli* studied by Naeem in Islamabad[17].

Those results were also similar with a study conducted by Dilnawaz in 2005 in India which reflects that first two common organisms were *E. coli* and *Klebsiella pneumoniae* 18; but in this study first two common organisms were E. coli and *Pseudomonas* sps. In our study most of cases of UTI were caused by gram negative organisms (91.1%), in which female case prevalence was high, similar to other studies (Table 3).

Consequently higher prevalence of *gram negative organisms* might be due to fecal contamination, the predilection of the organisms from the toilets and the shortness of the female urethra in females. That prevalence however was also reported in

earlier works by Smith in Australia.[19] There was also a possible link between the prevalence of UTI among population and the level of personal hygiene or the state of toilet facilities. Sexual activity was another feature that predisposes population to UTI. For example *Staphylococcus aureus* (4.6%) and CONS which was a member of skin flora might stay on the skin and get transmitted during sexual intercourse. On examination of demographic state of the cases by age, it was observed that both the extremes of age groups have risk of UTIs.

Table.1 Showing age and gender wise distribution of study participants

Age groups (years)	Males No. (Percentage)	Females No. (Percentage)	Total No. (Percentage)
1-9	41 (23.5)	36 (20.5)	77 (22.0)
10-19	30 (17.1)	22(12.5)	52 (14.8)
20-29	31 (17.7)	30 (17.0)	61 (17.4)
30-39	10 (05.7)	20 (11.4)	30 (08.5)
40-49	10 (05.7)	10 (05.6)	20 (05.7)
50-59	17 (09.7)	15 (08.5)	32 (09.1)
>60	36 (20.6)	43 (24.5)	79 (22.5)
Total	175 (100)	176 (100)	351 (100)

Table.2 Showing age and gender wise distribution of positive and negative samples of study participants

Age groups (years)	Positive samples of Males No. (% age)	Negative samples of Males No. (% age)	Positive samples of Females No. (% age)	Negative samples of Females No. (% age)
1-9	22 (22.4)	19 (24.7)	32 (26.9)	04 (07.0)
10-19	15 (15.3)	15 (19.5)	10 (08.4)	12 (21.0)
20-29	07 (07.2)	24 (31.2)	20 (16.8)	10 (17.6)
30-39	09 (09.2)	01 (01.3)	19 (16.0)	01 (01.8)
40-49	08 (08.2)	02 (02.5)	04 (03.3)	06 (10.5)
50-59	16 (16.3)	01 (01.3)	08 (06.7)	07 (12.3)
>60	21 (21.4)	15 (19.5)	26 (21.9)	17 (29.8)
Total	98 (100)	77 (100)	119 (100)	57 (100)

Table.3 Showing percentage of isolation of microbes from UTIs

Organisms Isolated	Males (%)	Females (%)	Total (%)
E. coli	48(57.8)	76(56.8)	124(57.2)
Pseudomonas *Sps*	14(16.9)	21(15.7)	35(16.1)
Klebsiella *Sps*	10(12.0)	11(8.2)	21(9.7)
Proteus *Sps*	04(4.8)	06(4.5)	10(4.6)
Citrobacter *Sps*	03(3.6)	06(4.5)	09(4.1)
Staphylococcus aureus	03(3.6)	07(5.2)	10(4.6)
Coagulase negative Staphylococcus	01(1.2)	04(2.9)	05(2.3)
Candida *Sps*	00(0.0)	03(2.2)	03(1.4)
Total	83	134	217(100)

Table.4 Percentage susceptibility of Gram negative isolates of UTIs

Antibiotics	Susceptibility	Percentage
Imipenem	198/199	99.4%
Piperacillin/Tazobactam	170/199	85.4%
Cefoperazone/ Sulbactam	128/199	64.4%
Ceftazidime	112/199	56.2%
Augmentin	107/199	53.7%
Amikacin	105/199	52.7%
Nitrofurantion	101/199	50.7%
Cefipime	100/199	50.2%
Ceftriaxone	95/199	47.7%
Gentamicin	90/199	45.2%
Nalidixic acid	75/199	37.6%
Levofloxacin	70/199	35.1%
Ciprofloxacin	48/199	24.1%
Cotrimoxazole	28/199	14.1%

Table.5 Percentage susceptibility of Gram positive isolates of UTIs

Antibiotics	Susceptibility	Percentage
Vancomycin	18/18	100%
Augmentin	14/18	77.7%
Nitrofurantion	13/18	72.2%
Novobiocin	13/18	72.2%
Tetracycline	12/18	66.6%
Erythromycin	12/18	66.6%
Ciprofloxacin	12/18	66.6%
Nalidixic acid	12/18	66.6%
Gentamicin	12/18	66.6%
Cefotaxime	11/18	61.1%
Cefoperazone	11/18	61.1%
Penicillin	4/18	22.2%

Antibiogram in our study showed that Imipenem and Piperacillin/Tazobactam were most susceptible drugs for UTI infection while Ciprofloxacin and Cotrimoxazole showed least susceptibility for the isolates, which is alarming because these drugs are used as empirical treatment for UTI (Table 4).

Vancomycin and Augmentin were most effective antibiotics for isolated gram positive bacteria, while Penicillin is most resistant drug for gram positive bacteria. Those antibiotics are still effective for treatment of urinary tract infections. The majority of antibiotics tested according to tables 4 and 5 resistance of uropathogens against majority of commonly used antibiotics increased. The implication of that was the possibility of easy access causing self-medication, misuse and abuse, leading to the development of resistance. The selection of antibiotic against any urinary tract pathogen depends on the

antibiotic resistance pattern, its pharmacokinetic properties, dose quantity and timings, its effect on gastrointestinal tract and vaginal flora allergies and adverse effects caused by that drug.[20] Antimicrobial resistance was a big problem cause of great concern throughout the world. Knowledge of the antibacterial resistance among uropathogens was essential to provide appropriate cost effective therapy.

In our study *E. coli* showed to be the most predominant uropathogen which shows that there is an increasing need for selection of appropriate and effective antibiotics, as the antibiotic resistance patterns are increasing against microorganisms causing UTI. Unsystematic recommendation and use of antibiotics should be checked in both the public and hospital setting by increasing public awareness on rational antibiotics use as well as taking on stern national and local antibiotic policy to standardize the recommendation, sale and use of antibiotics. Appropriate use of empirical antibiotics based on the bacterial susceptibility of that particular area is of utmost importance because most of the drugs are gaining resistance.

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