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Original Research Article

PREVALENCE AND ANTIBIOGRAM OF BACTERIAL ISOLATES FROM URINARY TRACT INFECTIONS IN EASTERN PART OF UTTAR PRADESH

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ABSTRACT

Background: Urinary tract infection (UTI) is one of the most frequently reported bacterial infections in the community coming second to respiratory tract infections. Empirical antibiotic therapy is usually applied in the management of UTIs, which has resulted in rapidly emerging antimicrobial resistance in hospitals and the community. Hence, the present study was designed to study the prevalence of uropathogens and to determine their antibiotic resistance patterns in our hospital.

Material and Methods: This retrospective study was done among patients attending a tertiary care hospital in eastern part of Uttar Pradesh, from November 2018 to June 2019 with the aim to investigate the prevalence of uropathogens and their antibiotic susceptibility pattern. A total of 1845 urine samples from patients with clinical symptoms of UTI were received in the Microbiology laboratory. All the samples were cultured and identified using conventional biochemical tests and antimicrobial susceptibility was performed by Kirby-Bauer disk diffusion method.

Results: Among a total of 1845 urine samples received in laboratory, 366 (19.8%) had significant bateriuria. Majority of the pathogens were isolated from females with isolation rate of 60.4% compared to males with isolation rate of 39.6%. E. coli was the most predominant pathogen isolated from urine samples with prevalence of 41.2% followed by Klebsiella spp. 15.84 %, P. aeruginosa 10.1 %, Enterococcus 9.8 %, S. aureus 8.7 % Acinetobacter 7.1 %, Candida 4.9 %, Citrobacter 1.6 % and Proteus spp. 0.54%. The three most frequently isolated bacteria had sensitivity rates of 70.2% to 100% to Amikacin; 0% to 98.7% to nitrofurantoin; 24.5% to 86.4% to ciprofloxacin and 70.6% to 75.7% to gentamicin. All the Gram negative bacilli were found susceptible to Amikacin whereas, among the Gram positive cocci susceptibility to Amikacin was 97.1% followed by Vancomycin 94.1%, Teicoplanin 83.8%, Levofloxacin 83.8%, Nitrofurantoin 80.9% and Gentamycin 75%. Cephlosporins and Nalidixic acid were resistant to all the isolated pathogens by 50% and more.

Conclusion: This study concludes that Gram-negative organisms are the leading cause of UTIs among adult population,in which E.coli is the principal uropathogen. Since most isolates were susceptible to nitrofurantoin and amikacin, they are considered as appropriate antimicrobials for empirical treatment of urinary tract infections.

Key Words: Urinary Tract infections, Significant bacteriuria, E. coli.

INTRODUCTION

Urinary tract infection (UTI) is commonly caused by bacterial pathogens that often affect the urinary system including the kidney, ureters, bladder, and urethra. The bacterial pathogens invade the urinary tract and may sometimes spread to the bloodstream resulting in several clinical syndromes such as fever, flank pain, dysuria and hematuria classified as cystitis (bladder infection) and pyelonephritis (kidney infection).[1] Pregnancy and the perinatal period are other characteristic time points marked by frequent urinary tract infections.[2] Urinary tract infections affecting the kidney brought a significant and serious health-related problem,[3,4] in all sex and age groups beginning from neonates to the geriatric age group but especially women are at high risk for developing UTI than men due to the anatomical structure and shorter distance of the urethra.[5]

Gram-negative bacteria such as Escherichia coli, Klebsiella species, Proteus mirabilis, Pseudomonas aeruginosa, and Acinetobacter species cause most of the UTIs, and Gram-positive bacteria such as Enterococcus species and Staphylococcus species also contribute to causing UTIs.[12]

In general, there is a need to start treatment before the final microbiological reports are available, which may lead to frequent misuse of antibiotics. This therapy, without rational drug prescription may lead to antibiotic resistance and treatment failure.[7]

As the etiological agents and the drug resistant pattern of the uropathogens varies according to the geographical area and changes through time, the selection of appropriate drug for UTIs should be assured after susceptibility pattern analysis of the urinary isolates. Knowledge of current local trend and susceptibility patterns in our hospital is important to update appropriate empirical therapy and to prevent development of multi drug resistant organisms. However, no data have been reported from the present study area.Thus it is in this light that the present study was conducted to observe the recent trend of prevalence of local bacterial isolates from suspected UTI and antimicrobial susceptibility patterns of uropathogenic bacteria causing UTI infections.

MATERIALS AND METHODS

Study Design

A retrospective cross sectional study of 1845 urine samples collected from patients both inpatient and outpatient from suspected UTIs who had a presumptive diagnosis of UTI, was performed in the department of microbiology at MRA medical college, Ambedkar nagar from November 2018 to June 2019.

Methods

Collection of Sample

Total of 1845 patients suspected of having UTI were instructed to give four ml of midstream, clean catch urine samples in a wide mouth sterile container and immediately transferred to the laboratory for investigation. Proper sampling instructions were given to each patient. The sample was collected before starting the antibiotics. Urine samples were examined and processed for bacteriuria in the laboratory as soon as possible after collection[8].

Microscopy

Urine specimens were examined by wet mounts for the presence of any pus cells, microorganisms, red blood cells, cast and crystals, or any other findings.

Culture and identification of isolates a modified semi-quantitative technique were employed (standard wire loop method). A standard bacteriological loopful of urine (0.01 ml) was inoculated over the surface of cystine lactose electrolyte deficient agar plate, blood agar and MacConkey agar (incubated at 37 degree C for 24 hours) from November 2018 to Jan 2020. . The plates were then incubated at 37°C for 18–24 h. Single species count of more than 10^5 organisms per ml of urine was considered as significant. Identification of isolates from positive cultures was done on the basis of Gram's staining, biochemical reactions according to the standard operational procedures as per the standard microbiological methods[9] and antimicrobial susceptibility testing was performed by Kirby Bauer disc diffusion method, using Muller – Hinton agar as per CLSI 2019 guidelines.[10]

Antimicrobial susceptibility tests

Antimicrobial susceptibility tests were done on Mueller-Hinton agar using Kirby-Bauer disk diffusion method. The antimicrobial agents tested were:

Amikacin (AN, 30 µg), ampicillin (10 µg), amoxicillin-clavulanic acid (AMC, 20/10 µg), aztreonam (AT 30µg), bacitracin (B) 10 units, ceftriaxone CRO (30 µg), Cefixime (CFM 5µg), cefuroxime, ceftazidime(CAZ, 30 µg), clindamycin (CC, 2 µg), ciprofloxacin(CP, 5 µg), erythromycin (15 µg), gentamycin (GM, 10 µg), imipenem (IPM, 10 µg), levofloxacin (LE 5µg), nalidixic acid (NA, 30 µg), nitrofurantoin (FM 300 µg), norfloxacin (NOR 10µg), Piperacilln/Tazobactum (PIT 100/10µg), Teicoplanin (TEI 30µg), Tobramycin (TOB 10µg), vancomycin (V, 30 µg) that were available and routinely used in hospital.

RESULTS

Among the 1845 patients with clinical symptoms of UTI, significant bacteriuria was detected in 366 patients that constitute 19.8% of the sample tested. The age of patients ranged from 10 month to 87 years. Two hundred twenty one (60.4%) urines samples were from female and 145 (39.6%) were from male patients. Age wise distribution is shown in table 1. Large numbers of isolates were found in reproductive age group. The highest isolation rate was observed in the age group > 44 years of age

(Table 1). Among females, age groups >44 years and among male's age group 5-14 years were predominant age groups in terms of incidence. [Table 1]

Among the 366 pathogens isolated from the samples, the Gram Negative Bacilli (GNB) with 280 isolates (76.5%) was the major cause for UTI while only 68 isolates(18.6%) were Gram positive cocci (GPC); candida accounts for 4.9%. Among the 280 GNB, the most commonly reported organism was E. coli accounting for 41.2% (n = 151) of the samples followed by Klebsiella spp. 15.8% (n = 58). The other less commonly isolated organisms were - Pseudomonas spp. from 10.1%, Acinetobacter from 7.1%, Citrobacter spp. from 1.6%, Proteus spp. from 0.54% of the culture-positive samples. Among the 68 isolates of GPC, 36 isolates were Enterococcus spp and 32 were Staphylococcus aureus.[Table 2]

Antimicrobial susceptibility pattern: The antibiogram revealed that, all the isolated bacteria such as E.coli, Klebsiella spp, Pseudomonas aurogenosa, Acinetobacter, Citrobacter, Proteus had a maximum sensitivity pattern to Amikacin. Isolated Gram-negative bacteria indicated the highest antibiotic resistance to cefuoxime(93.6%) followed by cefixime (86.1%)and also revealed the most sensitivity to Amikacin(93.2%) and piperacillin tazobactam(93.2%). [Table 3] It was also observed that the patients responded effectively to sensitive antimicrobial agents against Gram-negative bacilli. Majority (98.7%) of E. coli isolates were susceptible to nitrofurantoin with resistance rate of 1.3%. E. coli, the most frequently isolated bacterium, showed high resistance rates (>80%) to cephalosporins. K. pneumoniae (100%) isolates showed resistance to Amikacin and Piperacillin tazobactum.

Furthermore, the most effective antibiotics against Gram-positive cocci was found to be Amikacin (97.1%) followed by Vancomycin (94.1%), Teicoplanin (83.8%), Levofloxacin (83.8%), Nitrofurantoin (80.9%), Gentamycin (75%), Imipenem (70.6%) and Norfloxaciin (69.1%). Lower sensitivity pattern observed in Amoxyclav (32.4%), ampicillin (39.7%), cefixime (23.5%), Ciprofloxacin (38.2%), Clindamycin (26.5%), Erythromycin (30.9%), PT (52.9%), Tobramycin (47.1%). [Table 4]

Data Analysis: Chi-square test was employed to compare the proportion of bacterial isolates between gender and age and comparison of antimicrobial resistances. P-value of less than 0.05 was considered to indicate statistically significant difference.

Table 1: Age and gender wise prevalence of UTI

Age Group	Male (%)	Female (%)	Total (%)
<4	23(6.3%)	31 (8.5%)	54 (14.8%)
5-14	38 (10.4%)	27 (7.4%)	65 (17.8%)
15-25	24 (6.6%)	54 (14.8%)	78 (21.3%)
26-44	16 (4.4%)	54 (14.8%)	70 (19.1%)
>44	44 (12%)	55 (15%)	99 (27.1%)
Total	145 (39.6%)	221 (60.4%)	366 (100%)

Table 2: Bacterial isolates from urine samples of patients with suspected UTI

Bacteria isolated	Frequency (%)
E. coli	151(41.2%)
Klebsiella spp	58(15.84%)
Pseudomonas spp	37(10.1%)
Enterococcus	36(9.8%)
S. aureus	32(8.7%)
Acinetobacter	26(7.1%)
Candida	18(4.9%)
Citrobacter spp	6(1.6%)
Proteus	2(0.54%)
Total	366(100%)

Table 3: Antibiotic susceptibility percentage for Gram-negative bacilli

Antimicrobial agents	*E.coli* 151(%)	*Klebsiella* 58 (%)	*Pseudomonas* 37 (%)	*Acinetobacter* 26 (%)	*Citrobacter* 6 (%)	*Proteus* 2 (%)	Total 280(%)
Amikacin	143(94.7%)	58(100%)	26(70.2%)	26(100%)	6(100%)	2(100%)	261(93.2%)
Amoxy/clav	34 (22.5%)	27 (46.6%)	37 (100%)	13(50%)	3(50%)	0 (0%)	114(40.7%)
Aztreonam	40(26.5%)	8(13.8%)	25(67.6%)	0(0%)	6(100%)	2(100%)	81(28.9%)
Ceftriaxone	18(11.9%)	11(18.9%)	15(40.5%)	7(26.9%)	0(0%)	1(50%)	52(18.6%)
Cefixime	32(21.2%)	7(12.1%)	0(0%)	0(0%)	0(0%)	0(0%)	39(13.9%)
Ciprofloxacin	37(24.5%)	18(31%)	32(86.4%)	0(0%)	6(100%)	1(50%)	94(33.6%)
Cefuroxime	18(11.9%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	18(6.4%)
Ceftazidime	29(19.2%)	0(0%)	23(62.2%)	0(0%)	0(0%)	1(50%)	53(18.9%)
Gentamycin	108(71.5%)	41(70.6%)	28(75.7%)	13(50%)	2(33.3%)	2(100%)	194(69.3%)
Imipenem	111(73.5%)	40(68.9%)	30(81.1%)	20(76.9%)	6(100%)	2(100%)	209(74.6%)
Levofloxacin	64(42.4%)	12(20.6%)	33(89.2%)	22(84.6%)	6(100%)	0(0%)	137(48.9%)
Nalidixic acid	48(31.8%)	0(0%)	0(0%)	0(0%)	0(0%)	1(50%)	49(17.5%)
Nitrofurantoin	149(98.7%)	54(93%)	0(0%)	13(50%)	6(100%)	1(50%)	223(79.6%)
Norfloxacin	40(26.5%)	31(53.4%)	37(100%)	13(50%)	1(16.7%)	1(50%)	123(43.9%)

PT	132(87.4%)	58(100%)	37(100%)	26(100%)	6(100%)	2(100%)	261(93.2%)
Tobramycin	111(73.5%)	41(70.6%)	37(100%)	21(80.8%)	6(100%)	2(100%)	218(77.9%)

Table 4: Antibiotic susceptibility percentage for Gram-positive cocci

Antimicrobial agents	*Enterococcus* 36(%)	*S.aureus* 32 (%)	Total 68(%)
Amikacin	36(100%)	30(93.8%)	66(97.05%)
Amoxy/clav	22(61.1%)	0(0%)	22(32.4%)
Ampicillin	18(50%)	9(28.1%)	27(39.7%)
Cefixime	0(0%)	16(50%)	16(23.5%)
Ciprofloxacin	16 (44.4%)	10(31.2%)	26(38.2%)
Clindamycin	0(0%)	18(56.3%)	18(26.5%)
Erythromycin	9(25%)	12(37.5%)	21(30.9%)
Gentamycin	29(81%)	22(68.8%)	51(75%)
Imipenem	29 (81%)	19(59.4%)	48(70.6%)
Levofloxacin	36(100%)	21(65.6%)	57(83.8%)
Nitrofurantoin	26(72.2%)	29(90.6%)	55(80.9%)
Norfloxacin	36(100%)	11(34.4%)	47(69.1%)
PT	36(100%)	0(0%)	36(52.9%)
Teicoplanin	29(80.5%)	28(87.5%)	57(83.8%)
Tobramycin	0(0%)	32(100%)	32(47.1%)
Vancomycin	33(91.6%)	31(96.9%)	64(94.1%)

DISCUSSION

UTIs are one of the most common diseases diagnosed worldwide among humans. It continue to be problematic in clinical practice where empirical treatment of infections is routine. Availability of new antimicrobials has improved the management of UTIs. However, the management of UTI infections has been jeopardized by increase in emergence of antimicrobial drug resistance due to the improper use of the antimicrobials for the treatment of the infections. Also causes adverse effects on public health organization of a country both in economic impact. Hence, it is essential to continuously evaluate causative organisms and their susceptibility pattern that vary in regions and change through times which was the first purpose of the present study, particularly in the case of UTIs.

This retrospective study is based on the results of routine microbiological tests carried out from Nov 2018 to June 2019. Due to the nature of the retrospective analysis we couldn't trace patients' clinical settings. Thus the study did not consider such features as inpatient and outpatients, catheterized and non-catheterized patients.

The overall isolation rate of uropathogens in this study was 19.8% which is relatively lower than the prevalence rate of 22.7% in Ethiopia.[11] However the rate was higher than other studies.[12]

Out of 366 bacterial isolates 39.6% were males and females were 60.4% with the male to female ratio of 2:3. Statistically significant difference was observed between genders as majority of the pathogens were isolated from females (P<0.001).

In the present study higher positivity was observed in the age group of >44 (12%) in males as compared with age group of 26-44 (4.4%), and overall female subjects predominated over males in terms of urine culture positivity especially the age group of 15-25, 26-44 and >44 with positivity of 15%.

The predominated isolates were gram-negative bacteria during this study and consistent findings were reported from Uganda, Libya, and three other studies from India.[13,14,15,16]

In the present study, E. coli (41.2%) was the commonest organism isolated followed by Klebsiella (15.84%), Pseudomonas (10.1%) and the least isolated was Citrobacter, and Proteus total accounting 2.1%. This finding was in agreement with studies done by Indian authors.[17] The study conducted at Bangladesh showed 48.1% by Mouse et al. (2015),[18] and Ghadage et al. (2016),[19] reported 41.3% in Pune. Pseudomonas spp. accounted for 10.1% of the total cases in our study which was higher as compared to 4.9% as reported from Northeast India by Chongtham et al.[20] Acinetobacter spp. was isolated in 7.1% of cases in our study, which was higher to other studies done in India.[17]

In our study, the most sensitive antibiotic for E. coli and Klebsiella spp. was aminoglycosides similar to the study done by Chongtham et al.[20] In our study only 93.2 % of the isolates were sensitive to amikacin; Other studies, however, have shown similar sensitivity pattern - Pandey et al. from Nepal reported 20% of the isolates were resistant to amikacin.[6]

Of the total E. coli and Klebsiella spp. isolates, 1.3% and 7%, respectively, showed resistance to nitrofurantoin, which could be because of less use of the drug to treat UTI in the region. Thus it is found to be effective against E. coli. Nitrofurantoin is still a sensitive antibiotic as compared to the other commonly used drugs which show significant resistance because it acts on multiple sites unlike Ampicillin which has a single target and Co-trimoxazole which has two targets. Thus, resulting in few side effects and being a safe drug even in pregnancy.[21] Several studies have shown that ESBL producing E.coli isolates are also susceptible to Nitrofurantoin ranging from 70-95%.[21]

However, E. coli and Klebsiella isolates were highly resistant against norfloxacin (73.5% and 46.6% resistant, respectively) similar to study conducted by Thass et al.[8]

Norfloxacin, Piperacillin- tazobactum and Tobramycin were the effective drug against P. aeruginosa in the present study where the sensitivity was 100%. Moderate sensitivity was observed for quinolones (86-100%) and Imipenem (81%).

The other isolates were sensitive to gentamicin and ciprofloxacin with resistance rates of 0-66.7% and 0-100% respectively. High rates of sensitivity to nitrofurantoin,[22] ciprofloxacin,[22] and gentamicin.[23] have been documented from earlier studies. A previous study in Ethiopia has demonstrated a comparable result.[24]

As bacterial resistance increased in recent decades, the isolates of the present study recovered from UTIs showed high resistance. This is quite alarming that the First, second and third generation cephlosporins, nalidixic acid and norfloxacin were resistant to all the isolated pathogen by 50% and more similar to the previous study.[26] This is because First generation Cephalosporins and Fluoroquinolones are commonly prescribed antibiotics in a tertiary care hospital resulting in frequent antibiotic resistance.[20,21] Resistance to cephalosporins was seen in 47.3–47.5% of the isolates in other study conducted8.

Resistance among the Gram-negative isolates was 25.4% in case of carbapenem which is higher than a study done by Devmurari et al,[25] and some other studies8. Carbapenems are resistant to the β-lactamase enzymes produced by numerous MDR Gram-negative bacteria, so, playing a significant role in the treatment of infections not cured with other antibiotics. Hence, probable increase of the imipenem-resistant strains can be an emerging concern for health control systems of a country. Some Gram-negative bacteria were resistant to these antibiotics, which are widely used for treating hospital-acquired infections with MDR Gram-negative bacteria such as Pseudomonas and Acinetobacter.

The gram positive organisms were highly resistant to Ampicillin, Ciprofloxacin and erythromycin and showed >94% sensitivity to Amikacin and Vancomycin. But another studies from Bangladesh reported 100% susceptibility to Amikacin.[27] S.aureus showed 100% to Tobramycin, Vancomycin (96.9%) and Amikacin(93.8%) whereas most resistant to macrolides and fluoroquinolones.

An overall 8.4% resistance for vancomycin was observed in Gram-positive cocci belonging to the Enterococcus species, whereas other studies have reported much lower resistance rate.[8,12,28] Resistance to amikacin was reported among 0% of Enterococcus spp., whereas a study done in North India by Vohra et al,[28] reported 50% resistance for amikacin. Only 27.8% of the Enterococcus spp. were found to be resistant to nitrofurantoin contrary to a study done by Vohra et al,[28] that reported a high (62.5%) resistance.

CONCLUSION

In this study, high prevalence of UTI was found in adult age group and in female gender. The study shows that Gram-negative organisms are the leading cause of UTIs among adult population and they have developed resistance mechanisms against the routinely prescribed drugs. It concludes that E. coli (41.2%) was the principal pathogen. Our study points out emerging high resistance rate among UTI patients. Nitrofurantoin and Amikacin are considered as appropriate antimicrobials for empirical treatment of UTI in the area. Periodic monitoring and surveillance need to be done to prevent the development of emerging resistance among uropathogens so that more appropriate regimen can be given to the patient.

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