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Antimicrobial Susceptibility Pattern and Microbiological Profile of Uropathogens among Patients in a Tertiary Care Hospital in Bihar

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Abstract: ***Background: Urinary tract infection is one of the most prevalent infections. Microorganisms causing UTI vary in their susceptibility to antimicrobials due to widespread use of inappropriate and empirical antibiotic therapy. Urinary tract infection (UTI) is also the most common nosocomial infection. Objective: This study was designed to determine the microbiological profile and antimicrobial susceptibility patterns of uropathogens among patients referred to Patna medical college and hospital Patna .Material and Method: A total of two hundred and fifty (250) samples of urine from patients attending different inpatient and outpatient departments were included in the study. Urine samples were inoculated on Nutrient agar, Blood agar and McConkey agar plates by streaking. Inoculated plates were then incubated aerobically at 37˚C for 24 hours. After 24 hours of incubation, isolated colonies were picked up and Gram staining was done. Motility test and other biochemical tests were done for further identification of bacterial isolates using suitable Controls. Finally Antibiotic Susceptibility Test (AST) was performed to detect the degree of sensitivity or resistance of the pathogen isolated from the patient to an appropriate range of antimicrobial drugs on Mueller-Hinton agar (MHA) plates by Kirby-Bauer disc diffusion method. Results and Conclusion: Out of the total of two hundred and fifty (250)samples examined in the laboratory during the study period only one hundred two (102) patients were found to have bacteriological infection. Among the bacteriologically positive cases, UTI was more common in females. Among the bacterial isolates Escherichia coli was the commonest pathogen in both males and females, followed by Klebsiella species. Sparfloxacin and Gatifloxacin were the most effective antibiotics in vitro for the Gram Negative bacilli isolated, while Azithromycin was most effective against the Gram Positive cocci. The Gram Negative uropathogens showed a high degree of resistance to cephalosporins. While the Gram Positive cocci showed highest resistance to Norfloxacin and ofloxacin. It is due to the excessive use of antimicrobials for all sorts of infections that uropathogens responsible for UTI are increasingly showing resistance to antibiotics.***

Keywords: Uropathogen, Urinary Tract Infection, AST , *E.coli*, Cephalosporin

1. Introduction

Urinary tract infections (UTIs) are one of the most common infectious diseases in clinical practice both in community and hospital settings. Due to its frequency and recurrence ,UTI poses a real challenge to the medical professionals. UTI is classified into uncomplicated and complicated infections. The empirical choice of antimicrobial treatment is generally guided by susceptibility data provided by regional microbiological laboratories, however, since samples of uncomplicated UTIs are rarely sent for culture, these data are mainly found in complicated UTIs [1, 2].

Etiology of UTI is influenced by factors like age,sex, diabetes, urinary catheterization and others. Urinary tract infection are more common in women than men, It could be due to the proximity of genital tract and urethra /anus . Nearly 10% of women experience a UTI during their lifetime[3]. The spectrum of bacteria which causes complicated UTI is much broader than uncomplicated ones. Resistances to antimicrobial agents have undergone dramatic variations and consequently the empirical treatment of UTI needs constant updating of the antibiotic sensitivity of the main uropathogens of that area.

A patient is said to have a urinary tract infection, when there is the presence of over $1x10^5$ organisms per ml in the midstream sample of urine [4]. The human urinary tract system comprises of the kidneys, ureters, bladder and urethra. Infections in any of these anatomical sites are referred to as UTI. Infections extending to the bladder leads to cystitis while those involving the kidneys leads to pyelonephritis [5]. *Escherichia coli* is the most common cause of urinary tract infection [6,7] and accounts for approximately 90% of first urinary tract infection in young women [7]. The symptoms and signs include increased urinary frequency, dysuria, hematuria and pyuria. Flank pain is associated with upper tract infections. None of these symptoms or signs is specific for *Escherichia coli* infection [8].

Antimicrobial susceptibility profile in respect to causative microbes may significantly reduce morbidity and mortality, cost of treatment, and duration of hospitalization if duly provided to medical practitioners and clinicians in a rapid and timely fashion [9].

2. Material and Method

This study was carried out in Department of Microbiology Patna Medical College, Patna for 6 months, from march 2014 to September 2014. A total of two hundred fifty (250) midstream, clean catch urine samples were collected in wide-mouthed, sterile, screwcapped universal plastic containers and received in the laboratory routinely from patients attending OPD (Out patient department) and also from indoor.A brief clinical history of the patients and antibiotic intake was also taken.

The specimens collected were examined by microscopy and then put up for culture. The samples were inoculated on Nutrient agar, Blood agar and McConkey agar plates by streaking. Inoculated plates were then incubated aerobically at 37^0C for 24 hours. After 24 hours of incubation, isolated colonies were picked up and Gram staining was done. Motility test and other biochemical tests were done for further identification of bacterial isolates. Control strains used were as follows:
Escherichia coli: ATCC 25922
Staphylococcus aureus: ATCC 25923
Klebsiella pneumonia: ATCC 700603
Staphylococcus saprophyticus: ATCC 15305
Proteus mirabilis: ATCC 7002
Staphylococcus epidermidis: ATCC 14990
Pseudomonas aeruginosa: ATCC 27853
Enterococcus faecalis: ATCC 29212

Finally, Antibiotic Susceptibility Test (AST) was performed to detect the degree of sensitivity or resistance of the pathogen isolated from the patient to an appropriate range of antimicrobial drugs. AST was done on Mueller-Hinton agar (MHA) plates by Kirby-Bauer disc diffusion technique [9] using commercially available antibiotic discs (HiMedia, Mumbai). Interpretation of results was done based on the diameter of the zone of inhibition as per guidelines laid down by CLSI (Clinical and Laboratory Standards Institutes). The antibiotics and the concentrations at which they were used were as follows:

Sparfloxacin (5 µg) Norfloxacin (10 µg) Cefuroxime (30 µg) Cephalexin (30 µg) Ofloxacin (5 µg) Gentamycin (10 µg) Azithromycin (15 µg) Cotrimoxazole (25 µg) Gatifloxacin (5 µg) Ciprofloxacin (5 µg) Amoxicillin (30 µg) Ceftriaxone (30 µg) Amikacin (30 µg)

3. Results

Out of the total of two hundred fifty (250) samples received and examined in the laboratory during the study period only one hundred two (102) patients were found to have bacteriological infection (40.80%). The remaining samples of one hundred forty eight (148) patients were found to be sterile (59.20%) .

Out of the one hundred two (102) positive samples, 36 (35.29%) were male patients and 66 (64.71%) were female patients [Table 1].

Table -2 shows that amongst the one hundred two (102) bacteriologically infected samples, *Escherichia coli* was the commonest pathogen (59.81%) responsible for urinary tract infection followed by *Klebsiella pneumoniae* (18.62%),*Staphylococcus aureus* & CONS (Coagulase negative staphylococcus)(15.68%), *Proteus mirabilis* (2.95%) , *Pseudomonas aeruginosa* (1.96%) and mixed - Staph/ E.coli (0.98).

Out of sixty one (61) strains of *Escherichia coli* isolated from the urine culture, 45 strains were found to be sensitive to Gatifloxacin (73.77%) and Sparfloxacin (73.77%), Amikacin (59%), Cotrimoxazole (54.09%), Gentamycin (49.18%), Ceftriaxone(45.90) ,Ciprofloxacin (41%)each) and Ofloxacin (39.34%). The remaining antibiotics were less effective.

Out of sixteen (16) strains of Staphylococcus aureus and CONS isolated from the urine culture, 15 were found to be sensitive to Azithromycin (93.75%), followed by Sparfloxacin (81.25%), Ciprofloxacin (68.75%), Ceftriaxone (68.75%), Gatifloxacin (68.75%), Cotrimoxazole (50%), Cefuroxime (50%), Amikacin(50%),Gentamycin (50%) and Cephalexin (25.00%). The remaining antibiotics were effective in less than 25% strains.

High degree of resistance was seen in *E. coli* with reference to Azithromycin (85.25%), Cephalexin (70.5%), Norfloxacin (67.22%), Amoxicillin (65.56%), Cefuroxime (63.96%), Ofloxacin (60.66%), Ceftriaxone(54.10%) and Ciprofloxacin (59%). Ciprofloxacin resistance was more in *E. coli* as compared to resistance seen in the other Gram Negative uropathogens.

High rates of resistance was seen among the Gram Negative uropathogens isolates to cephalosporins like Cephalexin, Cefuroxime and Ceftriaxone . Ceftriaxone resistance was 54.10% in *E. coli*, 42.11% in among Klebsiella, 66.66% in Proteus and 50% in Pseudomonas.

Amongst the Gram Positive isolates the commonest uropathogen was *Staphylococcus aureus* , While most isolates of *Staphylococcus aureus* and CONS were resistant to Norfloxacin (87.50%).

Table 1: Gender-wise distribution among the cases suffering from UTI

Sex	Total number of isolates under study (n=102)	
	Number	Percentage(%)
Male	36	35.29
Female	66	64.71

Table 2: Distibution of different pathogenic organism among cases of UTI

Pathogenic organisms	Total isolates under study	
	Number	Percentage (%)
Escherichia coli	61	59.81
Klebsiella pneumoniae	19	18.62
Proteus mirabilis	3	2.95
Pseudomonas aeruginosa	2	1.96
Staphylococcus aureus and CONS	16	15.68
Mixed (staph/E. coli)	1	0.98

4. Discussion

This study showed that *E. coli* was the commonest pathogen causing complicated and uncomplicated UTI amongst the several organisms known to cause UTI, including *P. aeruginosa, S. saprophyticus, S. epidermidis, Enterococcus spp., P. mirabilis, Klebsiella* pneumonia etc. as reported by earlier workers [10-14]. Among the non-fermenters *Pseudomonas aeruginosa* was isolated as an uropathogen particularly in the intensive care units, although in a very few cases (1.96%). Furthermore, most other workers elsewhere have reported the involvement of Klebsiella as the second most important pathogen in UTI cases [15-26, 27-

30],in the same way present study shows a greater incidence of Klebsiella(18.62%).

While ciprofloxacin and ofloxacin are the most extensively used fluoroquinolones for the treatment of UTIs, the emergence of resistance for fluoroquinolones is based on several factors [14, 18, 28, 29]. Resistance to ciprofloxacin has emerged in a variety of genera belonging to the family Enterobacteriaceae. Our findings concur with such findings reported earlier [31, 32]. Apart from the notable resistance of E. coli to ciprofloxacin, other organisms were also found to be resistant to ciprofloxacin especially *K. pneumoniae*, Pseudomonas spp., Proteus spp. etc. Also, fluoroquinolone resistance in E. coli has emerged particularly in patients with urinary tract infections who have received fluoroquinolone prophylaxis [16-23]. An association between the increase in quinolone prescriptions and an increase in bacterial resistance has been reported from several countries [16-19, 23]. Usually, the prevalence of fluoroquinolone resistance is related to the intensity of antibiotic use [16].

5. Conclusion

The present study shows an increasing antibiotic resistance trends amongst UTI patients. Due to excessive use of antimicrobials for all sorts of infections, uropathogens responsible for UTI are increasingly showing resistance to antibiotics. So it is imperative to rationalize the use of antimicrobials and to use these conservatively. The present study also provides the knowledge of uropathogens and their antimicrobial susceptibility pattern which will help in appropriate and judicious antibiotic usage in our health care setup.

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