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Antibiotic Sensitivity Patterns of Different Uropathogens at a Tertiary Hospital in Southern Orissa of India

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Urinary tract infections are some of the most common infections experienced by humans particularly in adult women. A total of 181 urine samples were collected in sterile universal containers from patients attending the MKCG Medical College Hospital, Berhampur, for antibiotic sensitivity pattern study in the Microbiology Department Laboratory. Upon culture for 24 hours, microscopic and biochemical examinations, six bacterial species were isolated. Among them *Escherichia coli* was found to be the most frequent bacterial species accounting highest percentage of infections followed by *Staphylococcus aureus* and *Enterococcus faecalis.* Out of 13 antibiotic discs tested, highest sensitivity towards major causative bacterial species was noticed for Linezolid followed by Amikacin and Gentamycin. In order to avoid renal complicacy and successful treatment of urinary tract infections, updated information of antibiogram is essential.

Key words: Urinary tract infections, Uropathogens, Antibiotics, Antibiotic Sensitivity tests, Drug resistance.

Urinary tract infection (UTI) is defined as bacteriuria along with clinical urinary symptoms (Zelikovic *et al.*, 1992). It is an important cause of morbidity and mortality in Indian subjects, affecting all age groups across the life span (Farhana *et al*. 2004; Vincet and Andriol, 1987). Kass (1956) first introduced the concept of significant bacteriuria and demonstrated the presence of more than 10^5 CFU of bacteria per ml. of urine in a single specimen indicated bacteriuria with a probability of greater than 80%. Sometimes increased fluid intake or antibiotic therapy in the recent past shows bacterial count between 10^3 – 10^5 with clinical symptoms is also considered to be significant bacteriuria. It is a typical example of host-microbe interaction. Upon literature survey, it is observed that, major urobacterial pathogens responsible for UTI in Indian continent include *Escherichia coli, Staphylococcus aureus, Klebsiella spp., Proteus spp., Citrobacter spp., Pseudomonas spp., Streptococcus faecalis, Streptococcus viridans* etc. Under normal circumstances, the urine is sterile until it reaches the distal urethra (Ramzan, 2004).There are a number of factors responsible for developing UTI.Some of these are age, sex, pregnancy, catheterization, kidney stones, tumors, urethral strictures, neurological diseases, congenital or acquired anomalies of bladder, vesico-ureteral

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reflex, suppressed immune system, diabetes mellitus, enlarged prostrate, ureteric stresses etc (Sklar,1987).Women are especially prone to this infection and in men, this is of less frequent but can be very serious when they do occur. Recurrent UTI is common among young married women, even though they generally have anatomically and physiologically normal urinary tract (Nicolle and Ronald, 1987).early recognition and prompt treatment of UTI prevents development of pyelonephritis and late sequelae such as renal scarring or renal failure (Al-Mardeni *et al*., 2009). Some of the commonly used antibiotics to treat UTIs are Amoxyclav, Amikacin, Cephadroxyl, Gatifloxacin, Linezolid, Cefotaxime, Ciprofloxacin, Ampicillin etc. However, due to incessant and misuse of antibiotics, continuous change in antibiotic spectrum and increasing resistance of bacterial pathogens, an updated information of antibiogram is essential. This study was conducted to identify the major bacterial uropathogens responsible for UTI in this region, their antibiotic sensitivity pattern analysis and to select an effective first line therapy for the treatment of UTIs in this region, of patients attending MKCG Medical College Hospital, Berhampur, a prime health centre covering over ten districts of southern Odisha along with border villages of Andhra Pradesh.

MATERIAL AND METHODS

Midstream early morning urine samples were collected from 181 patients (65 males and 116 females) attending outdoor department of male and female Medicine of MKCG. Medical College Hospital, Berhampur, Odisha, in sterile universal containers, following adequate cleansing of the external genitalia.All urine samples were cultured immediately by streaking over the surface of Cystine Lactose Electrolyte Deficient (CLED) agar media (HiMedia) with a special inoculation loop calibrated to deliver 0.1 ml. of urine sample. For identification of bacteria, thereafter selectively cultivated in MacConkey and 5% sheep blood agar while for fungi, Sabouraud Dextrose agar was used. Plates were then incubated at 37 ^{o} C for 24 hour under aerobic conditions. The bacteria were sub cultured into freshly prepared media for pure culture isolations and identified by cultural, microscopic and biochemical characterization using Gram staining, catalase, indole, methyl red (MR), cytochrome oxidase, coagulase, urease, Voges-Proskauer (VP), H_2S production and sugar fermentation tests. Confirmation of *Escherichia coli* was done by positive indole and MR test, negative urease test and by pink colour colony in MacConkey agar plates with lactose fermenters. *Klebsiella* was confirmed by pink colour mucoid colony on MacConkey agar, negative indole and MR test, positive VP and urease test. *Enterococcus* was confirmed by their ability to grow on 6.5% NaCl medium, production of black colony on tellurite blood agar and conferment sucrose and mannitol. *Staphylococcus* was confirmed by catalase and coagulase positive test and production of acid by fermenting lactose, maltose and mannitol. *Pseudomonas* was confirmed by large, non-lactose fermenting colonies on MacConkey agar, negative reactions for MR and VP tests and positive oxidase test while *Citrobacter* by H_2S, MR and citrate positive and very slow lactose fermentation. Antibiotic sensitivity tests of isolated bacterial strains was performed on nutrient agar plate that was heavily and uniformly inoculated with an actively growing bacterial strain by disc diffusion method as described by Bauer *et al*. (1966), in accordance with the National Committee for Clinical Interpretation as 'Sensitive' (S) or 'Resistant'(R) on the basis of diameters of zones of inhibition recommended by the disc manufacturer. Different commercial antibiotic discs such as Amikacin, Gentamycin, Linezolid, Gatifloxacin, Netillin, Ofloxacin, Amoxyclav, Ceferoxime sodium, Norfloxacin, Cefadroxyl,Cefixime, Plymyxin B and Cotrimaxozole were placed on the plates and incubated at 37 ^{0} C for 24 hour. The zones of inhibitions were measured and recorded for correlation.

RESULTS

Among the total 181 urine samples, 128 (70.72 %) showed growth of bacterial strain in pure culture, while rest 53 (29.28%) did not show any growth. Out of 128 culture positive cases 84 female (65.62%) and 44 male (34.38%) was detected; while 35 female (66.04%) and 18 male (33.96%) patients urine sample showed culture negativity. The cultural, microscopic and biochemical

characterization revealed the growth of *Escherichia coli, Staphylococcus aureus, Enterococcus* sp., *Klebscilla* sp., *Pseudomonas* sp., *Citrobacter* sp. and others. *E.coli* was found to be the most frequent organism (36.71%), *Staphylococcus aureus* and *Enterococcus* sp., were isolated as the second (35.93%) and third (10.93%) highest organism respectively. *Klebsiella* sp., and *Pseudomonas* sp., were less frequently isolated. *Citrobacter* sp. (1.56%) is the least isolated organism. (Table 1).

Among the 128 culture positive cases, 41 male (37.61%) and 68 female (62.39%) were symptomatic while 3 male (15.79%) and 16 female (84.21%) were asymptomatic. Out of 53 culture negative cases 5 male (26.32%) and 14 female (73.68%) were symptomatic and 11 male (32.35%) and 23 female (67.65%) were asymptomatic. The antibiotic sensitivity of different urinary isolates revealed differential drug sensitivity pattern of bacterial strains (Table 2).

Most of the *E.coli* strain and *S.aureus* showed high degree of sensitivity to Amikacin and Gatifloxacin respectively. *Klebsiella* strain was highly sensitive to Gentamycin and Polymyxin B. *Pseudomonas* was highly sensitive to Amikacin and Gatifloxacin. *Enterococcus* was highly sensitive to Linezolid. *Citrobacter* was observed

Table 1. Distribution of isolated uropathogens according to sex and culture positivity

Isolate	Male	Female	Total
Escherichi coli	14 (7.73)	33 (18.23)	47 (36.71)
Staphylococcus aureus	18 (9.94)	28 (15.46)	46 (35.93)
Enterococcus sp.	4 (2.20)	10 (5.52)	14 (10.93)
Klebsiella sp.	5 (2.76)	3 (4.65)	8 (6.25)
Pseudomonas sp.	2 (1.10)	2 (1.10)	4 (3.13)
Citrobacter sp.	1 (0.55)	1 (0.55)	2 (1.56)
Others	0 (0)	7 (3.86)	7 (5.46)
Total	44 (34.38)	84 (65.62)	128

Table 2. Antibiotic sensitivity pattern of different uropathogenic bacterial isolates

Antibiotics	Bacterial Strains*						
	E. coli	*S. aureus*	*Klebsiella* sp.	*Pseudomonas* sp.	*Enterococcus* sp.	*Citrobacter* sp.	Total
Amikacin	39(88.66)	37(88.07)	5(62.5)	2(100)	11(84.61)	2(100)	96(86.48)
Gentamycin	31(74.41)	33(84.61)	4(66.66)	1(66.66)	11(84.61)	2(100)	82(78.09)
Linezolid	3 (100)	33(91.66)	0(0)	0(0)	8(88.88)	0(0)	44(89.79)
Gatifloxacin	12(48.48)	33(97.05)	2(33.33)	2(100)	5(55.55)	1(50)	
55(64.70)							
Netillin	9(83.33)	11(77.33)	0(0)	0(0)	1(33.33)	0(0)	21(70)
Ofloxacin	3(50)	6(75)	0(0)	0(0)	0(0)	2(100)	11(57.89)
Amoxyclav	6(22.22)	11(33.33)	1(14.28)	0(0)	3(50)	0(0)	21(25)
Cefuroxime	2(11.11)	2(20)	0(0)	1(50)	5(83.33)	1(50)	11(28.94)
Sodium							
Norfloxacin	6(20)	3(12.50)	1(14.28)	0(0)	1(9.09)	2(100)	13(17.10)
Cefadroxyl	2(16.66)	1(5)	0(0)	0(0)	0(0)	0(0)	3(6.66)
Cefixime	5(35.29)	1(6.25)	1(33.33)	0(0)	0(0)	0(0)	7(15.90)
PolymyxineB	7(50)	0(0)	2(66.66)	0(0)	0(0)	1(50)	10(41.66)
Cotrimaxazole	9(35.75)	0(0)	1(50)	1(50)	0(0)	0(0)	11(32.35)

*The digit in parenthesis represents the percentage value.

in few cases, which was sensitive to Amikacin, Gentamycin, Ofloxacin and Norfloxacin. Among all the antibiotics tested, highest sensitivity was observed for Linezolid (89.79%) followed by Amikacin (86.48%), Gentamycin (79.09%), Netillin (70%), Gatifloxacin (64.70%), Ofloxacin (57.89%), Polymyxin B (41.66%), Cotrimaxazole (32.35%), Cefuroxim sodium (28.49%), Amoxyclav (25%), Norfloxacin (17.10%), Cefixime (15.90%) and Cefadroxyl (6.66%).

DISCUSSION

The present study revealed that, *E.coli* was the predominant urinary bacterial pathogen followed by *Staphylococcus aureus* and *Enterococcus* sp.,which is in accordance with the earlier observations of Al-Mardeni *et al.* (2009). The role of *Staphylococcus aureus* is also significant like that of *E.coli* and this may be due to an increasing virulence. Antibiotic resistance by the bacterial pathogens is an important inhibiting factor for the successful treatment of UTI. Hence, empiric antibiotic therapy for UTI necessitates the adequate knowledge of the predominant uropathogens and their antimicrobial susceptibility pattern study in actual practice (Schlager, 2003; Shao *et al*. 2003), apart from age, clinical severity, location of infection, presence of structural abnormalities and allergy to certain antibiotics is also important (Anthony & Brian, 2006).

In this study 84 (65.62%) females, almost double the number of males were found to be culture positive. Due to several anatomical reasons, female are more prone to develop UTI, which is also corroborating with the early result of Nicolle and Ronald (1987). There is regular increase in the rate of UTI with age in women (Ramzan *et al*. 2004). Women with recurrent UTI have increased susceptibility to vaginal colonisation with uropathogens, which is due to greater propensity for uropathogenic *E.coli* forms to adhere uroepithelial cells (Nicolle & Ronald, 1987). The presence of simple bacteriuria particularly in females should not be neglected. It may act as a risk factor for UTI (Zhanel *et al*. 1990).

Among the culture positive cases, 109 patients were symptomatic and 19 were asymptomatic. Beside this, among the symptomatic patients, some urine culture showed no growth of any organism. This may be due to those uropathogenic agents that are unable to grow in ordinary media or due to urethritis. Since UTI may cause serious health problems with some unwanted complications, a timely treatment is considered to be important. To start the prognosis and diagnosis, antibiogram information is highly essential for sensitivity testing and proper treatment (Taiwo and Aderounmu, 2006). This will also help in turn diagnose the asymptomatic bacterial urinary tract infection patients. UTI treatment generally begins with broad- spectrum antibiotics and its recommendations should based on individual hospitals urine culture and sensitivity results (Al-Mardeni *et al.* 2009). The present investigation suggests that, the prevalence of *E.coli* was alarmingly high followed by *S.aureus* and the most appropriate first line oral antibiotic for empiric treatment of UTI at our centre was Linezolid and Amikacin was the second line agent.

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REFERENCES

1. Al-Mardeni, R.I.,Batarseh, A., Omaish,L.,Shraideh, M., Batarseh, B. and Unis, N., Empirical treatment for pediatric urinary tract infection and resistance patterns of uropathogens in Queen Alia Hospital and prince A'jsha military Centre, Jordan, *Saudi J. Kidney Dis. Treatment*. 2009; **20**(1): 135-139.
2. Anthony,P. and Brian , J., Review of urinary tract infection, *US Pharm*. 2006; **31**: 6.
3. Bauer,A.W., Kirby, W.M.M., Sherris, J.C. and Truck, M., Antibiotic susceptibility testing by a standard disc method. *Am. J. Clin. Path*. 1966; **45**: 493-496.
4. Farhana, A.,Kadri, S.M., Irfan, A. and Shafiq, A., A study of recurrent urinary tract infection among women attending out patient department in SMHS Hospital, Srinagar,Kashmir, India. *JK Practitioner*. 2004; **11**(4): 272-273.
5. Kass, E.H., Asymptomatic infections of the urinary tract. *Trans. Assoc. Am. Phys*. 1956; **69**: 56-63.
6. Nicolle, L.E. and Ronald, A.R., Recurrent urinary

tract infection in adult women, diagnosis and treatment. *Infec. Dis. Clin. N. Am.* 1987; **1**: 793-806.

7. Ramzan, M., Bakhsh, S., Salam, A., Khan, G. M. and Mustafa, G., Risk factors in urinary tract infection. *Gomal J. Med. Sci*. 2004; **2**(2): 50-53.
8. Sklar, A. H., Caruana, R.J., Lammers, J.E. and Strauser, G.D., Renal infections in autosomal polycystic kidney diseases. *Am. J. Kidney Dis*. 1987; **10**: 81-88.
9. Schlager, T.A., Urinary tract infections in infants and children. *Infec. Dis. Clin. N. Am.* 2003; **17**: 353-365.
10. Shao, H.F., Wang,W.P., Zhang, X.W. and Li,Z.D., Distribution and resistance trends of pathogens from urinary tract infections and impact on management. *Zhonghua Nan. Ke. Xue*. 2003; **9**(9): 690-696.
11. Taiwo, S.S. and Aderounmu, A.O.A., Catheter associated urinary tract infection: aetiologic agents and antimicrobial susceptibility pattern in Ladoke Akintola University Teaching Hospital, Osogbo, Nigeria. *African J. Biomed. Res*. 2006; **9**: 141-148.
12. Vincet, T. and Andriol, A. Urinary tract infection: recent developments. *J. Infec. Dis.* 1987; **156**: 865-868.
13. Zelikovic,I., Adelman, R.D. and Nancarrow, P.A., Urinary tract infections in children : an update. *West. J. Med*. 1992; **157**: 554-561.
14. Zhanel, G.G., Harding, G.K. and Guay, D.R., Asymptomatic bacteriuria. Which patient should be treated? *Arch. Intern. Med*. 1990; **150:** 1389-1396.