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Prevalence and Antibacterial Susceptibility Pattern of Aerobic Bacteria Causing Urinary Tract Infection in Tribal Population in Rural Part of West Bengal, India

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A B S T R A C T

To observe the prevalence rate and pattern of drug sensitivity of bacterial infection among urinary isolates from rural part of West Bengal, India. A total of 9,763 urine samples of clinically suspected UTI were collected. The samples were inoculated on Blood agar, Mac Conkey's agar, and cystine lactose electrolyte deficient (CLED) agar and incubated at 37 °C for 24 hr and extended up to 48 hr in cases of no growth of any organism. Antibiotic susceptibility testing against the isolated aerobic bacteria was performed by Kirby Bauer's disc diffusion method. Altogether 9,763 urine samples from tribal areas were collected for this study from January, 2013 to December, 2015. Total 2,710 (27.75%) samples were shown to be positive for growth of any bacteria. The dominant aerobic microorganisms isolated as the causative agents were *E. coli* (64.65%), *Klebsiella spp.* (16.27%), *Pseudomonas aeruginosa* (4.09%), *Proteus spp.* (3.21%), *Staphylococcus aureus* including coagulage negative Staphylococci (6.46%), *Enterococcus spp.* (1.00%) and other gram negative bacteria (4.32%). *E. coli* was the most common gram negative aerobic pathogen of UTI isolated in tribal population. In-vitro antibiotic susceptibility testing showed that the gram negative bacteria were sensitive to aminoglycosides (amikacin) and carbapenems (meropenem), while the gram positive isolates were sensitive to norfloxacin, gatifloxacin and levofloxacin. Isolation of the aerobic microorganisms from UTI patients of tribal population in rural part of West Bengal revealed *E. coli* as the most common bacterium in significant bacteriuria and amikacin is the most effective drug against the clinically isolated pathogens.

Introduction

A bacterial urinary tract infection (UTI) is the most common type of infection affecting the urinary tract including the bladder and kidneys (Naeem A, 2000). Urine is a favorable medium for growth of bacteria

due to its enriched chemical composition (Acharya VN, 1992; Asscher AW *et al.*, 1966; Asscher AW *et al.*, 1968).

UTI is the most common bacterial infection which is generally associated with minimal morbidity except among specific sub-populations. However, there is paucity of data regarding its incidence, factors that increase susceptibility, and long-term sequel of UTI. There is a need for data collection for computing the incidence of symptomatic UTI and pyelonephritis among tribal populations.

Several studies have shown that the incidence of urinary tract infection is higher among the underprivileged communities. Over 84 million people belonging to 698 communities are identified as members of scheduled tribes (IMTA, 2004), constituting 8.2% of the total Indian population (ORGCC, 2001) and is larger than that of any other country in the world. In West Bengal, India majority of tribal population resides in districts of Purba Medinipur, Paschim Medinipur, Bankura and Purulia. The groups like *Lodha, Kheria* (*Sabar*), *Munda, Santhal, Kohl, Oraon, Mahali* and *Bhumij* are major primitive tribes and santhals represent 54.27% of total tribal population (Bagchi T, 1994). The Midnapore district (location: 21°36′35″N - 22°57′10″N and 86°35′50″E - 88°12′40″E) is one of tribal rich districts in India (Maji SK *et al.*, 2013). The groups like *Lodha, Kheria* (*Sabar*), *Munda, Santhal* are primitive tribes (Maji S *et al.*, 2010). These tribal people mostly live in the remotest places in rural areas with poor health status complicated by poverty, illiteracy, and nutritional problems. The predominance of urinary tract infection of tribal population is an indicator of community health status which mostly remains unreported.

UTI is the third most common cause of hospital admission in India. It has been estimated that about 6 million patients per year are visiting outpatient departments (OPD) worldwide for UTI out of which around 30,000 are treated in the indoor wards (Bano K *et al.*, 2012). UTI accounts for a significant work load in clinical microbiology laboratories and *Escherichia coli* remains the most frequent cause of UTI (Cheesbrough M, 2000). The factors that interfere with its natural resistance to infection include age, sex, duration of hospitalization and obstruction in urinary tract. Females are more affected than males (Bano K *et al.*, 2012; Chernew I *et al.*, 1962). Antimicrobial susceptibility testing profile in respect of causative microbes may significantly reduce morbidity and mortality, cost of treatment and duration of hospitalization if diagnosed in a rapid and timely fashion (Ataee RA *et al.*, 2011; CLSI, 2011).

Materials and Methods

Study Design

Urine samples of clinically suspected UTI cases attending OPD and hospitalized patients were collected. A detailed history was taken and complete clinical examination was carried out. Clean catch midstream urine sampled from adult patients. In neonates, the samples were collected through suprapubic approach and in children (less than 3 years) sampled by using sterile urine bags. Urine samples were delivered to the laboratory within 1 hour of collection and processed within 24 hours. Each sample examined for the presence of pus cells, RBCs, epithelial cells, casts and crystals. A total of 9,763 urine samples of patients from different tribal areas were collected from January, 2013 to December, 2015.

Isolation and Identification of Organisms

A standard loop technique (CLSI, 2011) was used to place 0.01ml of urine for inoculation on Blood agar, MacConkey's agar and CLED agar at 37^{o}C for 24 hrs and extended up to 48 hrs in cases of no growth of any organism. The number of colonies was counted to quantify the organism. The diagnosis of UTI was made based on the significant colony count of a single pathogen like >10^5 CFU/ml for gram negative bacteria (GNB) and >10^4 CFU/ml for a Gram positive bacteria (GPB). The organisms were identified by following the general biochemical tests like Catalase test, Oxidase test, Triple Sugar Iron agar (TSI) test, citrate utilization test (Simmon's citrates medium), urease test (Christensen's Urea Agar), indole motility H_2S production test (Sulphide Indole Motility Medium), esculin hydrolysis test (Bile esculin agar) and sugar fermentation tests (Ataee RA *et al.*, 2011; CLSI, 2011; Colle JG *et al.*, 1996). All culture media were provided by Himedia Laboratories Pvt. Ltd., India.

Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing against the isolated aerobic bacteria was done according to Kirby Bauer's method as recommended by Clinical and Laboratory Standard Institute (CLSI, 2009). The different antibiotic discs (each 6.3mm diameter) used were Amoxicillin with clavulanic acid (30 mcg), Ceftazidime (30 mcg), Cefoperazone (75 mcg), Cefotaxime (30 mcg), Ceftriaxone (30 mcg), Tobramycin (10 mcg), Gentamycin (10 mcg), Amikacin (30 mcg), Co-trimoxazole (25 mcg), Gatifloxacin (5 mcg), Norfloxacin (10 mcg), Levofloxacin (5 mcg), Ofloxacin (5 mcg), and Meropenem (10 mcg). The zones of inhibition of each antibiotic against the causative bacteria were compared using standard CLSI protocol (CLSI, 2011; CLSI, 2009).

Results and Discussion

Present study included a total of 9,763 samples of urine collected from January, 2013 to December, 2015 from patients suspected to be suffering from UTI. Overall positivity was 27.75% (Table-1). More than one fourth (26.77%) male and about one third (28.65%) female had positive test result (Table-2). More than half cases (64.65%) reported as *E.coli* followed by *Klebsiella spp*. (16.27%), *Pseudomonas aeruginosa* (4.09%), *Proteus spp*. (3.21%) and other Gram negative bacteria (4.32%). Antibacterial sensitivity testing (Table-3) in the positive cases revealed highest sensitivity of *E. coli* & *Klebsiella spp.* with amikacin (86% & 79%) followed by meropenem (82% & 78%); *P. aeruginosa* & *Proteus spp* with gatifloxacin (78% & 67%); GNB with levofloxacin (78%) and Enterococcus spp. with Ofloxacin (66%). Staphylococcus spp. was most sensitive to Norfloxacin (87%) followed by ofloxacin (75%).

The prevalence of culture proven UTI in the population was 27.75%. This is lower than prevalence rate of 31.35% significant bacteriuria recorded by Savitha T et al (Savitha T, 2011) and that of 66.78% as recorded by Mahesh E et al (Mahesh E *et al.*, 2010). The wide variation in the prevalence rate among different population may be due to factors like sexual intercourse, peer group influence, pregnancy, low socio-economic status.

Table.1 Distribution of urine culture reports (n=9,763).

No growth: **6,172** (63.22%)	Positive growth: **3,591** (36.78%)	
	Mixed growth: **44**	
	Insignificant growth: **837**	
	Significant growth: **2,710**	
	E.coli	**1,752** (64.61%)
	Klebsiella spp.	**441**(16.31%)
	Pseudomonas aeruginosa.	**111** (4.10%)
	Proteus spp.	**87** (3.21%)
	Staphylococcus spp.	**175** (6.46%)
	Enterococcus spp.	**27** (1.00%)
	Other gram negative bacteria	**117** (4.31%)

Table.2 Sex distribution of patients according to different organisms isolated.

Bacteria isolated	**Patients showing significant positive growth**	**Male, number (%)**	**Female, number (%)**
E.coli	1752	794 (45.31%)	958 (54.68%)
Klebsiella spp.	441	178 (40.36%)	263 (59.63%)
Pseudomonas aeruginosa.	111	79 (71.17%)	32 (28.82%)
Proteus spp.	87	48 (55.17%)	39 (44.82%)
Staphylococcus spp.	175	76 (43.42%)	99 (56.57%)
Enterococcus spp.	27	12 (44.44%)	15 (55.55%)
Other gram negative bacteria	117	55 (47.00%)	62 (52.99%)
	Total = 2710	**1242 (45.83%)**	**1468 (54.17%)**

Table.3 Antibacterial sensitivity testing in organisms isolated from urine samples (n=2710)

Name of Drug	**E. coli, n=1752, (%)**	**Klebsiella spp. , n=441, (%)**	**Psedomonas aeruginosa. n=111, (%)**	**Proteus spp. , n=87, (%)**	**Staphylo-coccus spp. , n=175, (%)**	**Entero-coccus spp. n=27, (%)**	**Other Gram negative organisms. n=117, (%)**
Amoxycillin + Clavulanic acid	27	19	-	14	48	23	11
Ceftazidime	35	33	9	26	39	-	35
Cefoperazone	31	47	14	42	21	-	49
Cefotaxime	33	51	-	52	27	-	48
Ceftriaxone	44	73	-	59	45	-	54
Tobramycin	55	56	38	51	63	-	47
Gentamycin	67	61	64	53	79	53	55
Amikacin	86	79	74	73	64	-	70
Co-trimoxazole	29	35	-	25	58	22	37
Gatifloxacin	76	73	78	67	53	37	75
Norfloxacin	48	39	48	49	87	54	50
Levofloxacin	57	54	72	57	56	46	78
Ofloxacin	55	57	55	31	75	66	46
Meropenem	82	78	63	64	13	17	76

The most common organisms isolated in this study was *E. coli* (64.61%) followed by *Klebsella spp.* (16.31%), *P. aeruginosa* (4.1%), *Proteus spp.* (3.21%), other gram

negative bacteria (4.31%), *Staphylococcus spp.* (6.46%) and *Enterococcus spp.*(1.00%). In this study, the prevalence of UTI in females is more than in males. Of the 2,710 cases of significant positive growth, 1468 (54.17%) were from female patients while 1242 (45.83%) were from males. In studies by Kolawole AS *et al.,* 2009 also showed a higher prevalence of UTI in female (66.67%) in comparison to male (33.33%).

There is high incidence of symptomatic UTI necessitating antimicrobial therapy, as well as an increasing population of highly susceptible patients who require antimicrobials for UTI and/or other infections, resulting in an increased risk of developing antimicrobial resistance among common uro-pathogens. As a result, there is a growing need to ensure appropriate therapy with agents that maximize success for both community-acquired and nosocomial UTI while minimizing risk of the development of antimicrobial resistance.

The most useful antibiotics in this study were amikacin, meropenem and fluroquinolones (norfloxacin, gatifloxacin, levofloxacin), gentamycin & ofloxacin (in Gram positive isolates) because they inhibit most commonly isolated UTI pathogens. These drugs are relatively expensive when compared to most antibiotics frequently used. Amoxycillin with Clavulanic acid and Cefoperazone which are commonly used antibiotics showed poor in-vitro sensitivity against majority of the organisms isolated in this study. Very high antimicrobial resistance patterns of urinary isolates shown in a tertiary care hospital in India (Hasan AS *et al.,* 2007). In view of the increasing bacterial resistance, regular monitoring of resistance patterns is necessary to improve guidelines for empirical antibiotic therapy (Kolawole AS *et al.*, 2009; Kripke C, 2005). In conclusion, isolation of the aerobic microorganisms from UTI patients of tribal population in West Bengal, India revealed *E. coli* as the most common bacterium in significant bacteriuria. This result enriched the fact that females are more susceptible to UTI than males and the most effective drug against the clinically isolated pathogens is amikacin belonging to aminoglycoside class of antibacterial agents. The emphasis should be given on education and awareness programs explaining the consequences of misuse of broad spectrum antibiotics. So culture & antimicrobial drug sensitivity testing are needed for surveillance purposes to guide the clinicians on the proper management of cases of asymptomatic & symptomatic bacteriuria.

This work may be considered unique to define the prevalence of urinary tract infection by aerobic bacteria in tribal population in West Bengal, which can be effective for reducing the morbidity and mortality related to UTI, among the tribal inhabitants in rural part of Eastern India.

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