Original Article

Antimicrobial resistance in pathogens causing urinary tract infections in a rural community of Odisha, India

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ABSTRACT

Background: Antimicrobial resistance of urinary tract pathogens has increased worldwide. Empiric treatment of community-acquired urinary tract infection (CA-UTI) is determined by antimicrobial resistance patterns of uropathogens in a population of specific geographical location. **Objectives:** This study was conducted to determine the prevalence of CA-UTI in rural Odisha, India, and the effect of gender and age on its prevalence as well as etiologic agents and the resistance profile of the bacterial isolates. **Materials and Methods:** Consecutive clean-catch mid-stream urine samples were collected from 1670 adult patients. The urine samples were processed and microbial isolates were identified by conventional methods. Antimicrobial susceptibility testing was performed on all bacterial isolates by Kirby Bauer's disc diffusion method. **Results:** The prevalence of UTI was significantly higher in females compared with males (females 45.2%, males 18.4%, OR = 2.041, 95% CI = 1.64-2.52, $P \leq 0.0001$). Young females within the age group of 18-37 years and elderly males (≥68 years) showed high prevalence of UTI. *Escherichia coli* (68.8%) was the most prevalent isolate followed by *Enterococcus spp*. (9.7%). Amikacin and nitrofurantoin were the most active antimicrobial agents which showed low resistance rate of 5.8% and 9.8%, respectively. **Conclusion:** Our study revealed *E. coli* as the pre-dominant bacterial pathogen. Nitrofurantoin should be used as empirical therapy for uncomplicated CA-UTIs. In the Indian setting, routine urine cultures may be advisable, since treatment failure is likely to occur with commonly used antimicrobials. Therefore, development of regional surveillance programs is necessary for implementation of national CA-UTI guidelines.

Key words: Antimicrobial, community-acquired urinary tract infection, *Escherichia coli*, prevalence, resistance

INTRODUCTION

Urinary tract infection (UTI) is most common infectious disease after respiratory tract infection in community practice. It remains a major public health problem in terms of morbidity and financial cost with an estimated 150 million cases per annum worldwide, costing global economy in excess of 6 billion US dollars.[1] Although UTIs occur in all age groups including men and women, clinical studies suggest that the overall prevalence of UTI is higher in women. An estimated 50% of women experience at least one episode of UTI at some point of their lifetime and between 20% and 40% of women can have recurrent episodes.[2,3] Approximately 20% of all UTIs occur in men.[4]

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UTI is defined as bacteriuria along with urinary symptoms.[5] It may involve only the lower urinary tract or may involve both the upper and lower tract. The term cystitis has been used to describe lower UTI, which is characterized by a syndrome involving dysuria, frequency, urgency and occasionally suprapubic tenderness. However, the presence of symptoms of lower tract without upper tract symptoms does not exclude upper tract infection, which is also often present.[6]

Malnutrition, poor hygiene, low socio-economic status are associated with UTIs and these factors are usually found in rural settings.[7] Although *Escherichia coli* has been reported as the commonest isolate causing UTI, few authors have reported changing patterns in the prevalence of uropathogens.[8,9]

The introduction of antimicrobial therapy has contributed significantly to the management of UTIs. In almost all cases of community-acquired UTI (CA-UTI), empirical antimicrobial treatment is initiated before the laboratory results of urine culture are available; thus resistance may increase in uropathogens due to frequent misuse of antimicrobials.[10] The resistance pattern of community-acquired uropathogens has not been extensively studied in the eastern India.[11-13] To the best of our knowledge, no data concerning resistance of bacteria isolated from UTIs from rural Odisha, India has been documented till date. Since most CA-UTIs are treated empirically, the selection of appropriate antimicrobial agents should be determined by the most likely pathogen and its expected resistance pattern in a geographic area. Therefore there is need for periodic monitoring of etiologic agents of UTI, and their resistance pattern in the community.

This study was undertaken in view of paucity of reports of UTIs in our adult community of Odisha state, India. The aim of the study is to determine the prevalence of UTI as well as the effect of gender and age on its prevalence. The etiologic agents and their susceptibility pattern will also be determined.

MATERIALS AND METHODS

Study area

The present retrospective study was carried out in the central clinical microbiology laboratory of a tertiary care hospital which is located in southern Odisha, India and catering patients mostly from rural to tribal areas. The duration of the study was two and half year period from January 2010 to July 2012.

Study population

A total of 1670 adult patients from rural areas with signs to symptoms of UTI who attended the outpatient departments (OPDs) of our hospital were recruited for this study. They consisted of 1006 females and 664 males with age ranging from 18 to 85 years. Exclusion criteria included (a) patient's age <18 years (b) patients with history of inpatient admission a week prior to their presentation in our OPDs to rule out hospital acquired infections (c) antibiotics usage within week and (d) patients from urban areas. Verbal informed consent was obtained from all patients prior to specimen collection. The study was conducted after due approval from institutional ethical committee.

Sample collection and processing

Freshly voided, clean-catch midstream urine sample was collected from each patient into sterile screw-capped universal container in the OPDs. The specimen was labeled and transported to the microbiology laboratory for processing within 2 h.

Semi quantitative urine culture was done using a calibrated loop. A loopful (0.001 mL) of well mixed un-centrifuged urine was inoculated onto the surface of cysteine lactose electrolyte deficient medium. The culture plates were incubated aerobically at 37°C for 18-24 h and count were expressed as colony forming units (cfu) per milliliter (ml). For this study, significant bacteriuria was defined as culture of a single bacterial species from the urine sample at a concentration of 10^5 cfu/ml associated with microscopy findings of >10 white blood cells (WBCs) per high power field.[14] Only patients with significant bacteriuria ($\geq 10^5$ cfu/ml) were included for microbiological analysis. The culture isolates were identified by standard microbiological methods.[15] All culture media were procured from HiMedia Laboratories, Mumbai, India.

Isolates were tested for antimicrobial susceptibility testing by the standard Kirby-Bauer disc diffusion method according to Bauer *et al*.[16] Mueller-Hinton agar plates were incubated for 24 h after inoculation with organisms and placement of discs. After 24 h the inhibition zones were measured. The following standard antibiotic discs for the isolates were used; ampicillin (10 mcg), augmentin i.e. amoxicillin + clavulinic acid (20/10 mcg), co-trimoxazole (23.75/1.25 mcg), nitrofurantoin (300 mcg), ciprofloxacin (5 mcg), ofloxacin (5 mcg), cefaclor (30 mcg), cefpodoxime cefpodoxime proxetil (10 mcg), gentamicin (10 mcg) and amikacin (30 mcg). Antibiotic discs were obtained from HiMedia Laboratories, Mumbai, India. The results were interpreted according to Clinical and Laboratory Standards Institute guidelines.[17] The quality control strains used were *E. coli* American type culture collection (ATCC) 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus fecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 25923 for antimicrobial discs.

Statistical analysis

The data were analyzed using Chi-square (χ^2) test, confidence interval (CI), odds ratio (OR) analysis and P value by GraphPad® Software, Inc. 2236 Avenida de la Playa La Jolla, CA 92037 USA, InStat statistical software. Statistical significance was defined when P value is <0.05.

RESULTS

The age distribution of the patients in the sample set was 18-85 years (mean 43.6 years, standard deviation 16.2 years). A total of 1670 urine samples from clinically suspected patients were analyzed for CA-UTI. Of these, 577 (34.5%)

samples were found to be culture positive showing significant bacteriuria and the remaining 1093 (65.5%) samples were either non-significant bacteriuria or had sterile urine [Table 1].

From total 1670 patients, 1006 (60.2%) were female to among these 455 (45.2%) showed culture positive significant bacteriuria. Out of 664 male patients, only 122 (18.4%) had CA-UTI. Female gender was a significant risk factor for acquiring CA-UTI (OR = 2.041, 95% CI = 1.64-2.52, and statistically significant P value of <0.0001, Table 1).

The prevalence of CA-UTI was highest within 18-27 years of age group (29.2%), followed by 28-37 years (26.2%), among the female patients. Whereas majority of the isolates (41.8%) were obtained from male patients aged ≥68 years [Table 2].

Table 3 illustrates the overall frequency of community-acquired uropathogens. From total 577 significant isolates, Gram-negative aerobic rods accounted for 451 (78.2%), while Gram-positive cocci accounted for 120 (20.8%) and *Candida spp.* in 6 (1%) cases. *Escherichia coli* was the most pre-dominant isolate causing CA-UTI, followed by *Enterococcus spp., coagulase negative Staphylococci (CONS), Staphylococcus aureus, Klebsiella spp., Citrobacter spp.* and *Pseudomonas spp.* (9.7, 6.2, 4.9, 2.9, 2.3, and 1.6%, respectively).

Antimicrobial resistance profiles of the bacterial isolates are summarized in Table 4. Overall Gram-negative isolates showed higher resistant pattern in comparison with Gram-positive isolates. Gram-positive isolates showed least resistant to nitrofurantoin, augmentin, ciprofloxacin, ofloxacin, gentamicin, and amikacin. Among all Gram-negative isolates, *E. coli* showed highest resistance to most commonly used antimicrobials. *E. coli* isolates were least resistant to amikacin (5.8%), followed by nitrofurantoin (9.8%) and gentamicin (15.9%). Importantly for *E. coli*, the commonly recommended antimicrobials i.e. augmentin, co-trimoxazole, ampicillin, cefaclor cefpodoxime, ciprofloxacin and ofloxacin showed high resistant rates (63.7, 51.9, 94.7, 66.7, 58.2, 53.4, and 47.1%, respectively). The presence of *Pseudomonas spp.* i.e., 1.6% of all isolates was striking since it is considered to be a nosocomial pathogen. It showed highest sensitivity to ciprofloxacin, gentamicin and amikacin.

DISCUSSION

Against the background of paucity of reports of CA-UTI in rural setting of India, this is the first study conducted to determine the prevalence of UTI, the effect of gender and age on its prevalence and their susceptibility profile in a rural community of Odisha state, India. This study provides valuable laboratory data to monitor the status of antimicrobial resistance among uropathogens and to improve treatment recommendations in a specific geographical region. The study also allows comparison of the situation in Odisha with other regions within and outside the country. Our data was restricted to patients who can afford laboratory analysis; therefore this study may not reflect the true prevalence of UTI among patients in Odisha as most patients are initially treated empirically for their symptoms of UTI.

From total 1670 urine samples collected from CA-UTI patients 577 (34.5%) yielded significant pathogens. The similar value of 39.7% was obtained by Oladeinde *et al*. in rural community of Nigeria.[18] The culture positive rate for CA-UTI was higher in our study in comparison with studies conducted in Jaipur, India (17.19%) and Aligarh, India (10.86%).[12,19] Orrett in south Trinidad and Garcia-Morŭa *et al*. in Mexican population had obtained higher significant uropathogens (49% and 97.3%, respectively).[20,21] Geographical location may be the reason for this wide difference.

The finding showed that females (45.2%) had higher prevalence of UTI in comparison with males (18.4%) in agreement with earlier studies.[18,19,21,22] Close proximity of the female urethral meatus to anus, short urethra, and sexual intercourse have been reported as factors which influence the higher prevalence in women.[8]

The age group analysis showed that young female patients in the range of 18-37 years had highest prevalence rate (55.4%) of CA-UTI. This result is in agreement with previous studies.[12,19,23] Among sexually active young

Table 1: Effect of gender on prevalence of urinary tract infection in rural Odisha, India

Gender	Total no. of urine specimen			Odds ratio	95% CI	*P* value
	No. tested	No. not infected (%)	No. infected (%)			
Male	664	542 (81.6)	122 (18.4)	2.041	1.64-2.52	$P \leq 0.0001$ ($P<0.05$, significant)
Female	1006	551 (54.8)	455 (45.2)			
Total	1670	1093	577			

CI, Confidence interval

women the incidence of symptomatic UTI is high, and the risk is strongly associated with recent sexual intercourse, recent use of diaphragm with spermicide, and a history of recurrent UTIs.[24] Elderly males (≥68 years) had a higher incidence of CA-UTI (41.8%) when compared with the elderly females (10.3%). This finding is similar to study conducted by Sood *et al*.[19] This is probably because with advancing age, the incidence of UTI increases among males due to prostate enlargement and neurogenic bladder.[25]

Table 2: Effect of age on prevalence of urinary tract infection in rural Odisha, India

Age groups in years	No. of females infected (%)	No. of males infected (%)	*P* value
18-27	133 (29.2)	12 (9.8)	Chi-Square (χ^2)=9.105 *P* value=0.1049 (*P*<0.05, Significant)
28-37	119 (26.2)	11 (9)	
38-47	65 (14.3)	09 (7.4)	
48-57	58 (12.7)	17 (13.9)	
58-67	45 (9.9)	22 (18.1)	
≥68	35 (7.7)	51 (41.8)	
Total	455 (100)	122 (100)	

Table 3: Prevalence of uropathogens in rural Odisha, India

Microorganisms	Frequency	Percentage
Escherichia coli	397	68.8
Klebsiella spp.	17	2.9
Citrobacter spp.	13	2.3
Pseudomonas spp.	09	1.6
Proteus spp.	08	1.4
Enterobacter spp.	07	1.2
Total Gram-negative organisms isolated	451	78.2
Enterococcus spp.	56	9.7
Coagulase negative staphylococci (CONS)	36	6.2
Staphylococcus aureus	28	4.9
Candida spp.	06	1
Total Gram-positive organisms isolated	126	21.8
Total organisms (Both Gram negative and positive)	577	100

In our study Gram-negative aerobic rods (78.2%) pre-dominated, among which *E. coli* was the commonest uropathogen responsible for CA-UTI. It accounted for 68.8% of all culture-positive isolates, while *Enterococcus spp.* was the next most common organism, accounting for 9.7% of isolates in the present study. The proportion of bacterial species isolated was similar to those described in previous studies.[19,26,27] Our study significantly differed from Garcia-Morŭa *et al*., who found out that *E. coli* was the commonest organism in UTI (24.7%), followed by *Candida albicans* (23.7%) among Mexican population group.[21] The data collected from other places around the world, also showed that *E. coli* and *Klebsiella spp*. are still the commonest uropathogens isolated in CA-UTI patients[12,13,28-30] [Table 5]. Gram-negative aerobic bacteria including Enterobacteriaceae have several factors responsible for their attachment to uroepithelium. They colonize in the urogenital mucosa with adhesins, pili, fimbriae, and P-1 blood group phenotype receptor.[25]

The infectious disease society of America guidelines consider co-trimoxazole, fluoroquinolones, nitrofurantoin, and β-lactams including augmentin, cefdinir, cefaclor, cephalexin, cefpodoxime-proxetil as current standard empirical therapy for uncomplicated UTI in women.[31] However, the guidelines have a suggestion that local antimicrobial susceptibility patterns must be taken into account before choosing an agent.

Generally, uncomplicated UTIs are treated empirically in the community with short courses of oral antibiotics. In most cases, microbiological evaluation of UTI cases were conducted only following treatment failure, recurrent or relapsing infection. Overall, Gram-negative isolates showed higher resistant pattern in comparison to Gram-positive isolates in the present study.

Table 4: Resistance patterns of *Escherichia coli*, Gram-negative isolates and Gram-positive isolates

Antimicrobial	Number (%) of isolates resistant for *Escherichia coli* (*N*=397)	Number (%) of isolates resistant for all Gram-negative isolates (*N*=451)	Number (%) of isolates resistant for all Gram-positive isolates (*N*=120)
Ampicillin	376 (94.7)	419 (92.9)	78 (65)
Augmentin	253 (63.7)	274 (60.7)	23 (19.2)
Co-trimoxazole	206 (51.9)	241 (53.4)	46 (38.3)
Nitrofurantoin	39 (9.8)	43 (9.5)	07 (5.8)
Cefaclor	265 (66.7)	288 (63.8)	43 (35.8)
Cefpodoxime	231 (58.2)	253 (56.1)	47 (39.2)
Ciprofloxacin	212 (53.4)	231 (51.2)	16 (13.3)
Ofloxacin	187 (47.1)	201 (44.6)	18 (15)
Gentamicin	63 (15.9)	66 (14.6)	11 (9.2)
Amikacin	23 (5.8)	24 (5.3)	12 (10)

Table 5: Worldwide distribution of pathogens isolated among community-acquired urinary tract infection patients

Author, year and place of study	Percentage (%) of bacterial species isolated										
	Escherichia coli	*Klebsiella* spp.	*Proteus* spp.	*Pseudomonas* spp.	*Citrobacter* spp.	*Enterobacter* spp.	CONS	*Staphylococcus aureus*	*Enterococcus* spp.	*Candida* spp.	Reference no
Akram *et al.* (2007) in Aligarh, India	61	22	-	4	2	-	-	7	1	-	[12]
Selvakumar *et al.* (2007) in southern India	44	14.5	0.6	6.2	3.8	3	-	-	-	-	[28]
Kothari *et al.* (2008) in Delhi, India	68	16.9	5.5	-	-	5.3	2.8	-	1.5	-	[13]
Khameneh *et al.* (2009) in Iran	78.6	5.5	3.4	1.6	1.5	2.2	5.2	1.7	-	-	[27]
Garcia-Morúa *et al.* (2009) in Mexico	24.7	6.5	-	6.1	-	-	-	6.8	13.4	23.7	[21]
Kashaf *et al.* (2010) in Tehran, Iran	68.8	9.6	12.4	3.3	0.2	0.4	1.1	0.6	1.3	-	[22]
Sood *et al.* (2011) in Jaipur, india	61.8	6.6	1.4	4.6	2.3	3.7	0.9	5.5	9.2	0.3	[19]
Oladeinde *et al.* (2011) in Nigeria	41.2	4.4	6.7	2.4	-	-	-	32.3	-	12.2	[18]
Bahadin *et al.* (2011) in Singapore	74.5	8.7	3	1.5	2.7	0.6	1.2	3.9	2.4	0.6	[29]
Sabharwal *et al.* (2012) in Rajasthan, India	63.3	8.3	3.4	1.7	-	-	15	8.3	-	-	[32]
Shaifali *et al.* (2012) in Lucknow, India	33.1	7.9	0.7	-	-	-	-	2.2	-	-	[23]
Bano *et al.* (2012) in Pakistan	47	18.1	0.2	0.2	-	0.1	3.6	13.2	3.6	4.8	[30]
Present study in Odisha, India	68.8	2.9	1.4	1.6	2.3	1.2	6.2	4.9	9.7	1	

CONS: Coagulase negative staphylococci includes S.=saprophyticus and S.=epidermidis

Our study revealed that among Gram-negative bacteria, the most common isolate *E. coli* showed high level of resistance to commonly used empirical antibiotics β-lactams (ampicillin, augmentin, cefaclor, and cefpodoxime), fluoroquinolones (ciprofloxacin and ofloxacin) and co-trimoxazole. This value is similar to previous community based studies in India[13,19,32] [Table 6]. These high resistant rates among uropathogenic isolates from a rural population with poor access to health care raises question about selection pressures that generate, maintain and spread resistant strains in the community. It is also possible that due to poor access to health care services, irrational prescription of antimicrobials which are available over-the-counter in India, has contributed to this alarming situation. Unqualified practitioners, untrained pharmacists and nurses all over the country use antimicrobials indiscriminately.[33] Similar practices have also been reported from other developing countries, including Nepal and Vietnam.[34,35] The widespread use of antimicrobials in veterinary practice may be another possible factor for the emergence of resistant strains. Our findings thus suggest that empirical treatment with these drugs should no longer be appropriate.

Aminoglycosides i.e., gentamicin and amikacin showed low resistant rate of 15.9% and 5.8%, respectively for *E. coli.* Aminoglycosides being injectables are used less commonly in the community-care setting and hence have shown better sensitivity rates.

In our study, nitrofurantoin has shown better activity against most uropathogens with resistant rate of less than 10%. Similar results had been reported from studies conducted in different parts of India.[19,23,32] Nitrofurantoin should therefore be the ideal antibiotic of choice for uncomplicated UTI. The limitation of orally available Nitrofurantoin formulation is that it cannot be recommended for serious upper UTI or for those patients with systemic involvement.[3]

CONCLUSIONS

The worldwide trend of empirically treating CA-UTI may not apply for specific geographical regions, where decreased susceptibility rates are documented for common uropathogens. As more than two thirds of all pathogens are *E. coli*, local antimicrobial susceptibility patterns of *E. coli* in particular should be considered in antimicrobial selection for CA-UTIs. In the Indian setting, routine urine cultures may be advisable, since treatment failure likely to occur with commonly used antimicrobials. Therefore, development of regional surveillance programs is necessary for implementation of Indian CA-UTI guidelines.

Table 6: Regional antimicrobial resistance patterns of *Escherichia coli* isolates

Author, year and place of study	Percentage of *Escherichia coli* isolates resistant to antimicrobials										
	Ampicillin	Augmentin	Co-trimoxazole	Nitrofurantoin	Ciprofloxacin	Cefpodoxime	Cefaclor	Ofloxacin	Gentamicin	Amikacin	Reference no.
Akram *et al*. (2007) in Aligarh, India	-	-	76	80	69	85	-	-	64	51	[12]
Selvakumar *et al*. (2007) in southern India	96	72	71	62	92	-	-	62	73	21	[28]
Kothari *et al*. (2008) in Delhi, India	-	59.2	74	24.4	72	-	-	-	-	33	[13]
Khameneh *et al*. (2009) in Iran	-	-	-	69.8	83.2	-	-	-	83.9	-	[27]
Garcia-Morŭa *et al*. (2009) in Mexico	-	-	-	19.5	73.2	-	-	-	-	14.7	[21]
Kashaf *et al*. (2010) in Teheran, Iran	96.4	-	61.8	28.7	31.9	-	-	-	50.7	-	[22]
Sood *et al*. (2011) in Jaipur, India	81.2	80.7	67.8	5.8	74.7	-	-	-	28	8.2	[19]
Oladeinde *et al*. (2011) in Nigeria	-	87.1	75.3	81.2	9.5	-	-	29.5	47.1	-	[18]
Bahadin *et al*. (2011) in Singapore	46.3	9.3	37.8	5.3	24.4	-	-	-	8.5	-	[29]
Sabharwal *et al*. (2012) in Rajasthan, India	90	78	75	10	80	-	-	-	15	00	[32]
Shaifali *et al*. (2012) in Lucknow, India	52.2	-	39.2	13.1	60.9	-	65.2	-	54.4	95.7	[23]
Bano *et al*. (2012) in Pakistan	90	62	88	75	95	-	-	-	77	44	[30]
Present study in Odisha, India	94.7	63.7	51.9	9.8	53.4	58.2	66.7	44.6	38.3	5.3	

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