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Original Article

Microbiology Section

Comparative Prevalence of Antimicrobial Resistance in Community-Acquired Urinary Tract Infection Cases from Representative States of Northern and Southern India

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ABSTRACT

Context: Urinary tract infections (UTIs) are amongst the most common infections described in outpatient settings. Increased antimicrobial resistance (AMR) of urinary tract pathogens is a matter of global public health concern. Treatment of UTI depends on both prevalence and antimicrobial resistance (AMR) of causative bacteria at any specific geographical location.

Aim: This study was undertaken to compare the prevalence of uropathogens and their AMR profile in two different geographical parts of India.

Materials and Methods: Clean-catch mid-stream urine samples were collected from adult patients, bacterial flora isolated from human urine was evaluated for antimicrobial susceptibility profile using Kirby Bauer's disc diffusion method among patients from Hyderabad (Southern India), Rajasthan and Punjab (Northern India). The data were analysed using Chi-square ($\chi 2$) test, confidence interval (CI), odds ratio (OR) analysis and p-value using SPSS 16 software.

Results: *Escherichia coli* (55.1%) were the most prevalent isolates followed by *Enterococcus faecalis* (15.8%). Amikacin was the most active antimicrobial agents which showed low resistance rate of 14%. The present study revealed the geographical difference in prevalence of uropathogens with *Klebsiella pneumoniae* being the second most common uropathogen followed by *E. faecalis* in the states from northern India while no *K. pneumoniae* was seen in samples from southern India but *E. faecalis* was the second most prevalent organism.

Conclusion: Therefore, development of regional surveillance programs is highly recommended for implementation of national CA-UTI guidelines in Indian settings.

Keywords: Antimicrobial resistance, Bacteriuria and antibiotics, Community-acquired urinary yract infections, Uropathogens

INTRODUCTION

Urinary tract infection (UTI) is the 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 six billion US dollars [1,2]. UTI is defined as bacteriuria along with urinary symptoms [3]. It may involve only the lower urinary tract or may involve both the upper and lower tract. Malnutrition, poor hygiene, low socio-economic status is important factors associated with UTIs [4].

The most episodes of UTI are caused by *Escherichia coli* (*E.coli*) and *Enterococcus faecalis* (*E. faecalis*), while *Klebsiella pneumoniae* (*K. pneumoniae*) accounts for most of the remaining infections [5]. Although *E.coli* has been reported as the commonest isolate causing UTI, recent reports suggest a changing pattern in the prevalence of uropathogens [6,7]. The introduction of antimicrobial therapy has contributed significantly to the management of UTIs along with other infectious diseases. In almost all cases of community-acquired UTI (CA-UTI), empirical antimicrobial treatment is initiated before the laboratory results for urine culture are available; contributing significantly to antimicrobial resistance (AMR) in uropathogens due to frequent and sometimes repeated misuse of antimicrobials [8]. The resistance pattern of community acquired uropathogens has not been extensively studied in the Indian subcontinent [9,10]. It is important to realize that there may be marked differences between various geographical areas. Since most UTIs are treated empirically the selection of antimicrobial agent should be determined not only by the most likely pathogen but also by its confirmed susceptibility pattern. Therefore, periodic monitoring of aetiological agents of UTI, and their resistance pattern in the community is essential for prudent empirical antibiotic therapy to control the menace of increasing AMR so as to maintain efficacy of available antibiotics. It was against this backdrop that the current study was undertaken to assess and compare the most frequent pathogens responsible for UTIs in outpatients and their AMR pattern in Southern and Northern Indian states. Additionally, the study also aimed at identifying the possible resistance trends.

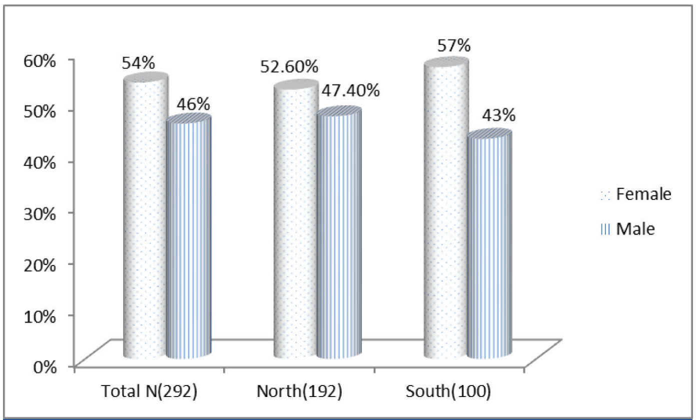
MATERIALS AND METHODS

Study area and study population

A retrospective study of all pathogens isolated from urine specimens of patients (both male and female; age 14-72 y) who attended the outpatient departments (OPDs) during the period January 2010 to June 2011 in Birla Sarvajanik hospital, Pilani (Rajasthan) and local diagnostic laboratories in Bathinda (Punjab) and Hyderabad (Andhra Pradesh). Patients were informed by the doctor about the test prior to collection of samples and the test for culture and sensitivity was conducted (based on prescription and doctor's advice). UTI was confirmed by positive urine culture reports. All patients who had significant bacteriuria (>10^5 cfu/ml) were included for further microbiological analysis in the present study. Only one specimen per patient was included.

Sample collection and processing

Discrete colonies obtained after culturing the urine sample on Luria's Broth (LB) agar plates were selected and these isolates were



[Table/Fig-1]: Gender distribution of UTI Incidence during the study period.

S.No	Organism	Frequency Total (N=292)	Frequency North (N=192)	Frequency South (N=100)
1	*E. coli*	161 (55.1)	107 (55.7)	54 (54)
2	*E. faecalis*	46 (15.8)	17 (8.9)	29 (29)
3	K. pneumoniae	40 (13.7)	40 (21)	0 (0)
4	*S. aureus*	18 (6.2)	13 (6.8)	5 (5)
5	*P. aeruginosa*	13 (4.5)	3 (1.6)	10 (10)
6	P. mirabilis	12 (4.1)	12 (6.3)	0 (0)
7	Others	2 (0.7)	0 (0)	2 (2)

[Table/Fig-2]: Distribution of microbiological flora causing urinary tract infections in OPD patients (Percentages given in parentheses)

used to grow new colonies on the same media to ensure purity of isolated bacterial strains. Bacterial inocula were then prepared by suspending the freshly-grown bacterial colonies in 10 mL sterile LB and incubated at 37oC; which were then inoculated in both Hichrome UTI agar and MacConkey agar plates followed by incubation at 37oC for 24-48 h for bacterial identification based on specific metabolism of chromogenic substrates. Susceptibility of the isolated UTI causing bacteria to commonly used antimicrobial agents was then examined.

Antibiotic sensitivity testing

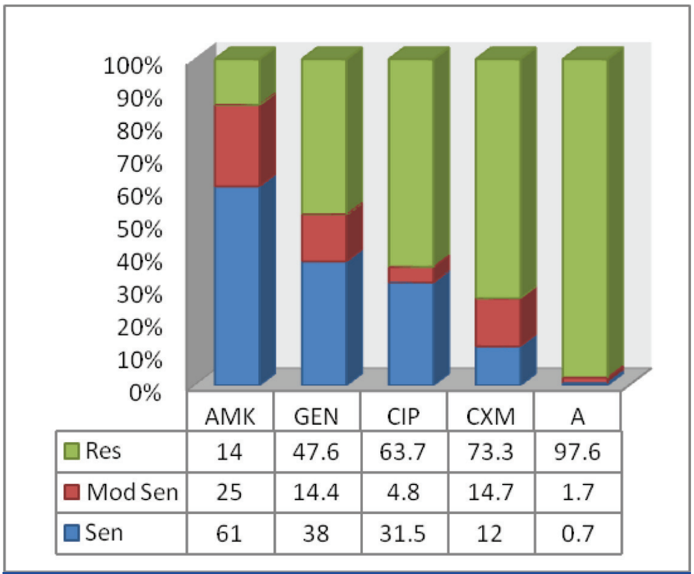
All antibiotic discs (Ampicillin 10μg; Gentamicin 30μg; Cefuroxime 30μg; Amikacin 30μg; Ciprofloxacin 5μg) and media used were obtained from Himedia Labs; India. The isolates were tested for antimicrobial susceptibility testing by the standard Kirby-Bauer disc diffusion method [11]. LB agar plates were incubated for 24h after inoculation with organisms and placement of discs. After 24h the inhibition zones were measured. Results were interpreted based on the diameter of the observed zone of inhibition. Following the Clinical and Laboratory Standards Institute Guidelines; the obtained results were categorized into three groups namely Sensitive (S); Intermediate (I); Resistant (R) and results were interpreted accordingly [12].

STATISTICAL ANALYSIS

The data were analyzed using Chi-square ($\chi2$) test, confidence interval (CI), odds ratio (OR) analysis and p-value using SPSS 16 software. Statistical significance was defined when p-value was <0.05.

RESULTS

A total of 830 urine samples from clinically suspected patients were analysed for CA-UTI. Of these, 292 (35.1%) samples (192 from northern India and 100 from Southern India) were found to be culture positive showing significant bacteriuria and the remaining 538



[Table/Fig-3]: Overall antimicrobial resistance profiles of the bacterial isolates (AMK: Amikacin; GEN: Gentamicin; CIP: Ciprofloxacin; CXM: Cefuroxime; A: Ampicillin)

(64.9%) samples showed either non-significant bacteriuria or were sterile. The incidence of the bacteria implicated in UTI in women was found higher than men [Table/Fig-1]. The total incidence of infection in women and men was 54% and 46% respectively, same pattern was observed in both the geographical regions.

[Table/Fig-2] illustrates the overall frequency of community-acquired uropathogens. From total 292 significant isolates, *E.coli* was the most pre-dominant isolate causing CA-UTI (55.1%), followed by *E. faecalis*, *K. pneumoniae*, *Staphylococcus aureus* (*S. aureus*), Pseudomonas aeruginosa (*P. aeruginosa*) and *Proteus mirabilis* (P. mirabilis) in order. Our study shows clear variation in prevalence of causative agents with geographical locations as seen from the [Table/Fig-2]. *K. pneumoniae* is second most common uropathogen after *E. coli* in the states from northern India while no *K. pneumoniae* was seen in samples from southern Indian population. Similarly P. mirabilis infection was also seen only in samples from northern Indian patients.

Overall AMR profiles of the bacterial isolates are summarized in [Table/Fig-3]. All the clinical isolates showed highest resistance to ampicillin and least resistance towards amikacin (97.6% and 14% respectively).

Among all the isolates, *E. coli* and *P. mirabilis* showed highest resistance to most commonly used antimicrobials except amikacin. Importantly for *E. coli*, the commonly recommended antimicrobials i.e. ampicillin, cefuroxime, ciprofloxacin and gentamicin showed high resistance rates (98.1, 84.5, 80.7 and 63.2%, respectively). The presence of *P. aeruginosa*, only 4.5% of all isolates was striking since it is considered to be a nosocomial pathogen. It showed highest sensitivity to gentamicin, ciprofloxacin and amikacin [Table/Fig-4].

Statistical analysis showed significant variation in efficacy of using gentamicin, amikacin and ciprofloxcacin in southern and northern India while ampicillin and cefuroxime showed the same effect in both the geographical regions. The bacterial isolates from southern Indian patients as compared to north Indian patients were found to be 6 times and 2.5 times more susceptible to gentamicin and ciprofloxacin respectively. While north Indian isolates were about 3.5 times more susceptible to amikacin than south Indian isolates [Table/Fig-5].

DISCUSSION

This study provides valuable laboratory data to monitor the status of AMR among uropathogens and to improve treatment

Antibiotic	Gentamicin			Ampicilin			Amikacin			Cefuroxime			Ciprofloxacin		
Organism	S	MS	R	S	MS	R	S	MS	R	S	MS	R	S	MS	R
E.coli (161)	45 (28)	14 (8.7)	102 (63.4)	02 (1.2)	01 (0.6)	158 (98.1)	128 (79.5)	13 (8.1)	19 (11.8)	16 (9.9)	09 (5.6)	136 (84.5)	25 (15.5)	06 3.7)	130 (80.7)
E. faecalis (46)	29 (63)	09 (19.6)	08 (17.4)	00 (0.0)	01 (2.2)	45 (97.8)	13 (28.3)	23 (50)	10 (21.7)	05 (10.9)	13 (28.3)	28 (60.9)	26 (56.5)	01 (2.2)	19 (41.3)
K. pneumoniae (40)	17 (42.5)	18 (45)	05 (12.5)	0 (0.0)	02 (5.0)	38 (95.0)	06 (15.0)	33 (82.5)	01 (2.5)	08 (20.0)	18 (45.0)	14 (35.0)	16 (40)	04 (10)	20 (50)
S. aureus (18)	07 (38.9)	00 (0.0)	11 (61.1)	00 (0.0)	01 (5.6)	17 (94.4)	10 (55.6)	02 (11.1)	06 (33.3)	02 (11.1)	01 (5.6)	15 (83.3)	10 (55.6)	01 (5.6)	07 (38.9)
P. aeruginosa (13)	10 (76.9)	01 (7.7)	02 (15.4)	00 (0.0)	01 (0.0)	13 (100)	09 (69.2)	02 (15.4)	02 (15.4)	04 (30.8)	01 (7.7)	08 (31.5)	10 (76.9)	00 (0.0)	03 (23.1)
P.mirabilis (12)	01 (8.3)	00 (0.0)	11 (91.7)	00 (0.0)	00 (0.0)	12 (100)	11 (91.7)	00 (0.0)	01 8.3)	00 (0.0)	00 (0.0)	12 (100)	5 (41.7)	00 (0.0)	07 (58.3)

[Table/Fig-4]: Susceptibility profile of clinical isolates to commonly used antibiotics.

Total N=292	Resistant cases		Chi Square (p value)	OR (95% CI)*
	South N=100 (%)	North N=192 (%)		
Gentamicin	21 (21%)	118(61.4%)	<0.001	5.99 (3.41,10.52)
Ampicillin	98 (98%)	187 (97.3%)	0.749	0.763 (0.14, 4.00)
Amikacin	24 (24%)	17 (8.85%)	<0.001	0.308 (0.15, 0.60)
Cefuroxime	69 (69%)	145 (75.5%)	0.178	1.44 (0.844, 2.48)
Ciprofloxacin	50(50%)	136 (70.8%)	<0.001	2.43 (1.47, 4.00)

[Table/Fig-5]: Table showing the variation in antimicrobial resistance pattern of clinical isolates, **Comparison of antibiotic resistance of uropathogens in South vs North India. Resistance % out of total in parentheses*

recommendations in a specific geographical region. Our data were restricted to patients who can afford laboratory analysis; therefore this study may not reflect the true prevalence of UTI among patients in a particular geographical area. From total 830 urine samples collected from CA-UTI patients 292 (35.1%) yielded significant pathogens. A similar value of 39.7% was obtained by Oladeinde et al. in rural community from Nigeria [13]. The culture positive rate for CA-UTI was higher in our study in comparison with studies reported from Aligarh, India (10.86%) [5]. Another study has reported even higher incidence of uropathogens, 49% [14]. In the present cohort *E. coli* was the commonest uropathogen responsible for CA-UTI followed by *E. faecalis* and K. pneumoniae. The proportion of bacterial species isolated was similar to those described in previous studies [15-18]. The data collected from other places around the world, also shows that *E. coli* and K. pneumoniae are still the commonest uropathogens isolated in CA-UTI patients [19-21]. Our study showed statistically strong correlation between efficacy of an antibiotic and variation in geographical region. Hence, monitoring of antibiotic susceptibility of bacterial isolates in the community should be made mandatory for disease surveillance programs in a given area.

Our data shows that the most common isolate *E. coli* has become highly resistant to several commonly used antibiotics ampicillin, ciprofloxacin, ciprofloxacin and gentamicin. These high resistant rates among uropathogenic isolates from a particular community points to the selection pressures that generate, maintain and spread resistant strains in the community. It is also a fact that inappropriate clinical practices; mismanagement; unsupervised use; overuse; over the counter availability; lack of awareness and self-medication have worsened the condition in developing counties like India. Unqualified practitioners, untrained pharmacists and nurses all over the country use antimicrobials indiscriminately [22]. Similar practices have also been reported from other developing countries, such as Nepal and Vietnam [23-25]. Our findings strongly suggest that empirical treatment with these drugs should no longer be practiced.

CONCLUSIONS

The worldwide trend of empirically treating CA-UTI may worsen the debacle of growing AMR and certainly does not apply for specific geographical regions, where decreased susceptibility rates are documented for common uropathogens. Therefore, development of regional surveillance programs is necessary for implementation of CA-UTI guidelines.

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