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BACTERIOLOGICAL PROFILING AND ANTIBIOGRAM OF UROPATHOGENS FROM URINE CULTURE OF SUSPECTED UTI PATIENTS IN RANCHI, JHARKHAND

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KEYWORDS

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ABSTRACT

Urinary tract infection (UTI) is one of the most common bacterial infectious diseases all over the world and people of all age groups and geographical locations are affected. Area-specific monitoring studies aimed to gain knowledge about the type of pathogens responsible for urinary tract infections and their resistance patterns may help the clinician to choose the correct empirical treatment. During this surveillance study, a total number of 854 urinary samples were collected from clinically-suspected cases of urinary tract infections and tested bacteriologically using standard procedures. Antimicrobial susceptibility test was performed for the isolated pathogens using Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standard Institute. Among 854 samples 205 (24%) gave positive culture. The common uropathogens encountered were Escherichia coli (72 %), Staphylococcus sp., (08 %), Klebsiella sp., (12 %), Enterococcus sp., (06 %) and Pseudomonas sp., (02%). Antibiotic resistance analysis revealed the multiple drug resistance nature of the isolates to the commonly used eighteen standard antibiotics. This study showed that *E. coli* isolates were the predominant pathogens and the presence of bacterial isolates with very high resistance to the commonly prescribed drugs emphasize an alternative options of drugs and awareness on antibiotic use for the effective UTI management. Therefore, the aim of this study was to determine the type, prevelance and antibiotic resistance pattern of the urinary pathogens isolated from patients from various age and sex groups.

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INTRODUCTION

Infections of urinary tract (UTI) is one of the most common diseases next to respiratory tract affecting peoples of all age worldwide (Blair, 2007). Urinary tract infections (UTIs) are a major public health problem in terms of morbidity and financial cost, and incur the highest total health care cost among urological diseases, exceeding that of chronic renal failure even when renal dialysis and renal transplantation are included (Gales *et al*., 2002). UTI represents one of the most common diseases encountered in medical practice today with an estimated 150 million UTIs per annum worldwide (Karlowsky *et al*., 2002). Although UTIs occur in both men and women, clinical studies suggest that the overall prevalence of UTI is higher in women. Uncomplicated UTIs in healthy women have an incidence of 50/1000/year (De Backer *et al*., 2008). 50% of women experience at least one episode of UTI at some point in their lifetime and between 20% and 40% of women have recurrent episodes (Rock *et al*., 2007, Vasquez and Hand, 2004). Approximately 20% of all UTIs occur in men (Griebling, 2007) In USA, they are accountable for 7 million clinic visits annually with a cost exceeding $1.6 billion (Sheerin, 2011). Among the several UTI implicated microorganisms, bacteria are the major causatives accounting more than 95% cases (Bonadio *et al*., 2001). Most episodes of UTI are caused by bacteria as *Escherichia coli* (up to 85%) and *Staphylococcus saprophyticus* (up to 10%), while *Klebsiella pneumoniae* and *Proteus* species account for most of the remaining infections (Dimitrov *et al*., 2004). The primary treatment of UTI may vary according to the patient's age and sex, any underlying diseases, lower or upper UTIs and the infecting agent (Wagenlehner and Naber, 2004). The currently recommended bacterial UTI treatment chemotherapeutic classes and anti-infective agent includes the β-lactams, fluoroquinolones, pyrimethamines, aminoglycoside, oxazolidinones glycopeptides and oxazolidinones (Andrade *et al*., 2006). As suggested by Andrade *et al. (2006) and* Banerjee (2012) the spectrum and antimicrobial resistance of UTI bacteria may vary temporally and geographically. So Bacteriological investigations of UTI are not complete without an antibiotic sensitivity test of the isolate. Microorganisms causing UTI vary in their susceptibility to antimicrobials from place to place and time to time (Landgren *et al*., 2005). Today UTIs became quite alarming as isolated uropathogens exhibits high percentage resistance to almost all antibiotics (Cuevas *et al*., 2010). Surveillance uropathogen's susceptibility profile studies are necessary to increase efficacy of empirical therapy. (Olufunmiso and Oluwaseun, 2011) which could help in target bacterial real antibiotic resistance data based antibiotic prescription. Monitoring antibiotic susceptibility patterns of uropathogens at a local level is imperative to be guided not only on emerging problems of antibiotic resistance but also to provide assistance in managing empirical therapy (Bhargavi *et al*., 2010). This information would be relevant not only for local area but also useful as regional reference data for physicians (Akram *et al*., 2007). Hence each institution must undertake its own local evaluation/drug policy. The resistance pattern of community acquired uropathogens has not been extensively studied in the Indian subcontinent (Koeijers *et al*., 2010, Kothari *et al*., 2008; Biswas *et al*.,

2006). No data concerning the antimicrobial resistance of bacteria isolated from UTIs from northern part of the country (i.e. the state of Jharkhand) has been documented till date. It is important to realize that there may be marked differences between various geographic areas within a vast country like India. 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 expected susceptibility pattern. Thus, knowledge of local antimicrobial susceptibility patterns of common uropathogens is essential for prudent empiric therapy of community acquired UTIs.

The present study was carried out to investigate and report the current scenario of growing multiple drug resistance among uropathogens. The study also aims to draw attention between uropathogen prevalence in male and female patients of different age groups and also documented the incidence of UTI with seasonal variations, to obtain guidance for selecting appropriate antibiotic empirical treatment and to device an up-dated treatment policy for the better management of UTI.

MATERIALS AND METHODS

Study Population

Between June 2011 to may 2012, a surveillance study on culture and sensitivity profiles of uropathogens was carried out at one point diagnostic centre, Ranchi Jharkhand, India. Therapeutic information of all outpatients with symptoms indicative of a UTI descriptive for age, gender, regional etc., was also collected. All patients received care as usual, *i.* e., diagnostic tests and empirical therapy according to the daily practice [Barrow et al 2003]. A total of 854 patients clinically suspected of having urinary tract infection were involved. The study group comprised of 632 females and 222 males with age range of zero to seventy years. All the samples were grouped into different age groups of the individual's patients (presented in Table1).

Collection of Sample and processing

The patients were properly instructed how to collect the sample under aseptic conditions. Clean Catch Mid Stream specimen of urine was collected from each patient. In case of children the specimen was collected by suprapubic aspiration and was immediately transported to laboratory for further processing. The name, age and sex were clearly mentioned on the universal container containing specimen. Uropathogens were isolated from a total 854 suspected UTI patient's according to NCCLS standard procedure. Growth of 10^5 colony forming units (CFU)/ml was considered a positive urine culture (CLSI DOCUMENT 2006). Using a sterile pipette 0.01 ml of urine sample was trasfered on MacConkey agar and Muller Hinton agar plates, spreaded thoroughly. After allowing the urine to be absorbed into the agar, the plates were then inverted and incubated at 37ºC for 18-24h. The colony count was done in duplicate setup for the present study.

Identification of isolates

Out of 854 only 205 (24%) showed culture positive. The obtained isolates were identified using standard morphological, cultural and biochemical methods as described (Mathai *et al*., 2001) and preserved at 4ºC in refrigerated condition**.**

Antibiotic susceptibility testing

An antibiogram was done by agar disc diffusion technique as Kirby – Bauer disc diffusion assay on Muller Hinton agar to determine the antimicrobial susceptibility profiles [Bours *et al.,* 2010]. The panel antimicrobials include β-lactam antibiotics (Amoxicillin 30μg), aminoglycoside (Amikacin 30μg, Gentamicin 30μg), cephalosporin antibiotics (Cefixime 5μg, Cefotaxime 30μg, Cefpodoxime10μg), synthetic quinolone fluoroquinolones antibiotics (Nalidixic acid 30μg, Ciprofloxacin 5μg, Ofloxacin 5μg, Norfloxacin 10μg, Levofloxacin 5μg), macrolide (Erythromycin 15 μg), glycopeptide antibiotic (Vancomycin 30μg), aminocoumarin

Table 1: Age and gender wise distribution of samples collected from suspected UTI patients of Ranchi

S.No.	Group of ages	Male	Female	+ve casemale	%value (M)	+ve case female	%value(F)	Total no. of +ve case
1	0-10 A	14	47	04	5.5	08	6.01	12
2	11-20 B	10	70	02	2.7	09	6.76	11
3	21-30 C	06	178	02	2.7	63	47.36	65
4	31 -40 D	16	156	04	5.5	33	24.81	37
5	41-50 E	88	78	36	50	10	7.51	46
6	51-60 F	76	67	22	30.55	06	4.5	28
7	61-70 G	12	36	02	2.7	04	3.0	06
	TotalSample(854)	222	632	72	100	133	100	205

Table 2: Distribution of urine culture bacterial isolates in different age group of 205 samples from UTI Patients of Ranchi (Jkd). Age Groups in Years

0-10 A ,	11-20 B,	21-30 C,	31 -40	D ,41-50 E ,	51- 60 F ,	61-70 G

Period(2011-2012)	Male							Female							Total	%
	s1	2	3	4	5	Total	%	1	2	3	4	5	Total	%		
Juneto Aug	21	02	02	01	-	26	35.06	32	03	03	03	01	42	32.81	69	33.65
Septo Nov	28	02	03	03	01	37	53.26	49	15	06	04	02	76	55.46	112	54.63
Dec toFeb	04	01	01	-	-	06	7.79	06	01	01	01	-	09	07.05	15	7.33
MartoMay	03	-	-	-	-	03	3.89	05	01	-	-	-	06	04.68	09	4.39
Total	56	05	06	04	01	72	100	92	20	10	08	03	133	100	205	100
Percentage (%)	28.29	3.90	2.92	1.95	0.48	37.54	-	43.92	8.29	4.88	3.90	1.47	62.46	-	100	-

(Novobiocin 30μg), nitrofuranes (Nitrofurantoin 30μg), sulfa drugs (Trimethoprim TMP 100ug),chloramphenicol 30ug and Tetracycline 30μg. The standard antibiotic discs (Himedia laboratories, Mumbai, India) available were used for this study. Appropriate antibiotic discs were tested on isolated gram positive or gram negative bacterias. Interpretation of results was done based on the diameter of the zone.

Statistical analysis

The obtained sensitivity profile statistics were analyzed using Microsoft excels ANOVA data analysis. Organism prevalence rate calculations, frequency distributions, susceptibility patterns and other descriptive statistics were computed and reviewed. The data was also analyzed for significant values and $p < 0.05$ was considered statistically significant [Kothari *et al.,* 2008].

RESULTS

Through the one year surveillance period (June 2011 to May 2012) a total number of 854 urinary samples were collected, wherein 205 were come up with a positive urine culture (24.00%). The positive samples were collected from both 72 (35.2%) male and 133 (64.8%) female patients of various age groups (Table 1). The *E.coli* (n = 148, 72%) is a main successfully isolated uropathogen from positive cases. The frequencies of other uropathogens include *Klebsiella* sp., (n = 25, 12%), *Pseudomonas* sp., (n = 04, 2%), *Staphylococcus* sp., (n = 16, 08%) and *Enterococcus* sp., (n = 12, 6%) as shown (Table 2). The obtained results upon statistical analysis (ANOVA and chi square test) revealed the insignificant difference in the gender and age group wise incidence of uropathogens. The seasonal variations in the incidence of UTI caused by them has also been determined among the present study population. From the season wise UTI incidences among male and female patients were presented in (Table 3), it is found that in female the highest rate 54.63 % of UTI has occurred during the months of September to November followed by 33.65 % between June to August**.** The major Gram positive uropathogenic isolates *Klebseilla* sp., (n = 25) showed resistance in the order of Nitrofurantoin (0%), Novobiocin (10.5%), Amikacin (12.0%), Gentamicin (20.4%), Vancomycin (10%) , Cefpodoxime (25.0%), Cefixime (30.2%), Tetracycline (30.2%) cefotaxime (35.1%), Erythromycin (45.5%), Ciprofloxacin (42.4), Levofloxacin (45.4%), TMP (100%), Nalidixic acid (82.0%), Ofloxacin (71.0%), Norfloxacin (75.4%), chleremphenicol (22.2%), Amoxicillin (80.5%) as presented (Table 4). The predominant uropathogenic *E.coli* is found to exhibit a dreadful resistance to number of antibiotics**.** The isolated uropathogens antibiotic resistance on the whole observed in this study is higher than the already available results from urban areas which is an alarming one.

DISCUSSION

The results of the present study deals with the distribution of microbial isolates in different age and sex groups. UTI patients and their susceptibility patter to most commonly used antibiotics. From various surveillance studies in India and some other nations, the limited controls on prescription practices, non-standardized antimicrobial manufacturing and the breakdown of infection control infrastructure due to

Table 3: Seasonal variation of UTI Incidence among gender1.*E.coli;* 2.*Klebsiella* sp.; 3. *Staphylococcus* sp; 4. *Enterococcus* sp. 5. *Pseudomonas* sp.

s.no		Age group							
	Bacteria	A	B	C	D	E	F	G	Total number of isolates
1.	*E.coli*	*06*	05	53	28	37	17	02	148
2.	*Staphylococcus sp.*	*02*	01	04	02	03	02	02	16
3.	*Kelebsiella sp.*	*03*	04	06	04	04	04	00	25
4.	*Pseudomonas sp.*	*00*	00	02	02	00	00	00	04
5.	*Enterococcus sp.*	*01*	01	00	01	02	05	02	12
	TOTAL	*12*	11	65	37	46	28	06	205

Table 4: Shows antibiotic resistant pattern (%value) of isolated uropathogens

S.No.	Antibiotics used	Gram negative organisms			Gram positive organisms	
		E .coli	*Klebsiella* sp.	*Pseudomonas* sp.	*Staphylococcus* sp.	*Enterococcus* sp.
1	Nitrofurantoin	2.5	0.0	8.5	7.5	5.4
2	Amikacin	10.5	12.0	18.6	15.2	12.2
3	Gentamycin	30.0	20.4	36.2	35.8	28.8
4	Novobiocin	50.0	10.5	52.8	42.2	38.4
5	Levofloxacin	67.5	45.4	72.2	68.4	72.6
6	Cefixime	10.1	30.2	16.4	10.2	15.4
7	Ciprofloxacin	70.5	42.4	72.2	80.6	76.6
8	Tetracycline	12.8	30.2	24.4	16.2	26.2
9	Cefpodoxime	40.5	25.0	48.4	30.4	45.8
10	Cefotaxim	75.0	35.1	72.6	72.4	70.2
11	Norfloxacin	75.5	75.4	78.2	82.6	84.6
12	Ofloxacin	80.0	71.0	80.4	80.2	82.8
13	amoxicillin	98.2	80.5	96.4	96.8	94.2
14	Nalidixicacid	98.5	80.0	96.2	98.2	96.4
15	Erythromycin	98.0	45.5	98.0	100	98.2
16	TMP(trimethoprim)	100	100	98.6	100	96.4
17	Vancomycin	30.0	10.0	28.2	32.2	32.4
18	Chlormphenicol	15.0	22.2	32.4	24.8	28.2

compromised resources present a high potential for resistant pathogens emergence and their unimpeded spread (Bhargavi *et al*., 2010). Poverty, inadequate access to drugs, increased use and misuse are major forces for the emergence and increase of this public health challenging antimicrobial resistance which hampers the clinical management of UTIs worldwide (Hassi *et al*., 2005). International guidelines are no longer applicable for treating community associated UTI in India, and development of specific guidelines based on local susceptibility patterns are necessary. Regional surveillance programs development are necessary to provide information for Indian CA-UTI guidelines development (Morgan *et al*., 2011). The present findings suggests that the major causative agent of UTI is *E.coli* which has also been observed by many workers (Landgren *et al*., 2005; Bara *et al*., 2012; Getnet *et al*., 2012; Murugan *et al*., 2012; Kalsoom *et al*., 2012). The studies of uropathogen profile and resistance pattern of UTI isolates of many authors coincides with the present study. The present study exhibited most common and higher prevalence of UTIs pathogen as *E.coli* (72%) followed by *Klebsiella sp* (12%) then *Staphylococcus* sp (08%). Though the seasonal influences on human health have been known for more than 2000 years its influence on UTI incidences throughout the world especially in India have not been appraised. To the best of our knowledge, this is the first report correlates UTI incidences with seasonal variation in Ranchi,Jkd, India also reported the partial associations between its occurrences and meteorological parameters. They also articulated that the data available in the literature regarding the subject of this study are not conclusive. The findings of this study shows that the incidence of UTI is remarkably high in females than males also from the present findings, we suggest that antibiotics, amikacin (89.5%), and nitrofurantoin (97.5%) are highly effective. A similar observation was made by (Curtis *et al*., 2005; Hummers *et al*., 2005; Landgren *et al*., 2005; Smita *et al*., 2012. Furthermore, national, state and hospital level programs of surveillance and intervention must be strengthened to prevent the continued emergence of multi drug resistant pathogens and to limit their spread into other communities or other institutions.

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