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Urinary Tract Infections and Antibiotics Sensitivity among Women of Allahabad Region of U.P. State

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The objective of this study was to explore the microorganism causing urinary tract infection (UTI) and to compare the safety and efficacy of antibiotics for these microorganisms in Allahabad region of U.P. State. Seventy urine samples of patients were analyzed and the microorganisms were identified by Gram´s Staining, biochemical tests like Growth on MacConkey and blood agar IMViC Test of Enteric bacteria- Indole test, Methyl Red, Vogues- Proskauer (VP) test, Citrate test for Citric Acid Utilization, Motility Test, Triple Sugar Iron Agar Test (TSI Test). Antimicrobial Susceptibility Testing performed by Disc diffusion methods and Dilution Methods followed by Minimum Inhibitory Concentration (MIC). Out of seventy samples sixty three were found to be infected by micro-organism like *E.coli*, *Staphylococcus epidermis*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Streptococcus faecalis*(enterococci) *Pseudomonas auruginosa*, *Proteus mirabilis*, *Citrobacter Serratia* and *Klebsiella pneumoniae*. These isolates of enteric and pathogenic bacteria were found to be Gram – positive bacteria: *Staphylococcus epidermis, Staphylococcus aureus, Staphylococcus saprophyticus, Streptococcus faecalis,* Gram – negative bacteria*: Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Citrobacter and Klebsiella pneumoniae*. Their resistance against antibiotic were also studied and recorded. There was high prevalence of asymptomatic bacteriuria among women in this setting regardless to women's age, parity and gestational age. *E. coli* with its multi resistance towards antibiotics was the most commonly isolated organism in Allahabad region of U.P. State. Thus, urine culture should be performed as screening and diagnostic tool of UTI in this setting.

Key words: Urinary tract infection (UTI), Uropathogenic *Escherichia coli* (UPEC), Antibiotics sensitivity.

Urinary tract infection (UTI) is the second most common infectious disease present in community practice. Worldwide, about 150 million people are diagnosed with UTI each year, costing the global economy in excess of 6 billion US dollars[1]. The women numbered 50 to 80% in the general population acquire at least once UTI during their lifetime.

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Recurrent UTI (RUTI), in healthy non pregnant women is defined as three or more episodes of UTI during a twelve month period. About 20 to 30% of women who have had one episode of UTI will have recurrent episode. The women have propensity to acquire RUTI due to various inherent, genetic factors making it a common infection, 30 times more than males. At least 40% of women have UTI at some point in their lives. The school girls aged 5 to 14 years are affected up to 3-5%. Adolescents and young adults make numbers to 4% and additional 1-2% increase per decade of age[2-4].

Conventional medical treatment of UTI involves the use of antibiotic drugs, which typically cure most infections within one or two days. Longer treatment is especially indicated in cases where symptoms have lasted more than a week, when the infection is recurrent, or when the individual has diabetes (or other diseases in which the immune system may be impaired). When women are subject to RUTI, many doctors prescribe daily low dose antibiotics for as long as *two or three years*! One problem with such long-term treatment is that it is guaranteed to seriously disrupt the body's normal ecological balance by eradicating friendly bacteria, including *E. coli* in the GI tract and *Lactobacilli* in the vagina[5, 6].

MATERIALS AND METHODS

Chemicals used in biochemical test and antibiotics sensitivity - Gram´s Stain, MacConkey Agar MM011, Blood agar base (Infusion agar) BM 014 , Triple agar iron agar (I) TM 014 , Mannitol Salt Agar MM 015, IMViC test- Indole Nitrate Medium (Tryptone Nitrate Medium) IM 011, MR-VP Medium (Glucose Phosphate Broth MRVP Broth) MM 018, Christensen Citrate agar CM 035, E.M.B (Eosin Methylene Blue Agar) EM 011, C.E.L.D (Cystine Lactose Electrolyte Deficient Agar) CM 015 , Motility test Medium MM 017 and Mueller- Hinton agar MM019 for antimicrobial susceptibility were obtained from Sisco Research Laboratories, India. The antibiotic sensitivity discs used for highly resistant UTI & Systemic Isolates, Gram Positive and Gram Negative Bacteria were from Pathoteq Biological Laboratories (PBL), India with their respective Code No. 412, Code No. 112, and Code No. 212.

Sample collection of urine from women's of age group 18 to75 years

Urine for a culture can be collected at any time. As patient starts to urinate, some urine is allowed to fall into toilet, followed by the collection of one to two ounces of urine in the sterile container provided, and the rest is voided into the toilet. This type of collection is called mid-stream catch urine[7]. It is important that specimens after collection should reach the laboratory with the minimum of delay, preferably within 6 hours after collection or refrigerated at 4^0C.

Urine culture and its Isolation

Urine samples were collected in sterile plastic bottles from City Nursing Home Hospital and Deep Ganga Hospital in Allahabad (U.P) from patients referred by Dr. Safia Suhail and Dr. R. C. Gupta and brought to the laboratory, stored in refrigerator and processed within 24 hrs for clinical analysis which involves the quantitative determination of the number of micro-organism per ml of urine sample and expressed as colony forming units (CFU). If the count is > 10^5 CFU/ml then it will be considered the case of UTI.

Procedure for isolation from urine sample

Streaking a urine sample for isolation of microorganism on the prepared plate of one with blood agar and other plate of MacConkey agar medium followed by incubation of the plate at 37^0C for 24 hours. Semi quantitative technique of inoculation by standard loop was used in Standard loop technique; the fixed volume loop used can hold 0.004ml urine (i.e. 250 loopfuls make 1 ml), the total viable bacterial count per ml sample = Number of colonies X 250. When number of colonies formed in plates say 400.

Total count = 400X 250 organism, i.e.100, 000 or 10^5 organisms per ml of urine sample.

The plates were examined for the presence of bacteria with the following criteria

(a) More than 100, 000 or 10^5 viable bacteria of a single species (CFU/ ml) were considered as the significant growth followed by sensitivity test. (b) Between 10,000 to 100.000 bacteria CFU/ ml was taken as doubtful significant which required further inoculation on the culture plate to recheck the growth. (c) Less than 10,000 bacteria CFU/ ml was considered as no significant growth and regarded as contaminants[8,9].

Identification of Microorganism

For identification of enteric bacteria Gram's stain was done to differentiate between Gram- positive cocci and Gram- negative cocci using the method of Christian Gram developed in 1884 by following method of P. Chakraborty *et.al.*(2005). Further identification were carried out by biochemical tests like Growth on MacConkey and blood agar, IMViC Test of Enteric bacteria- Indole test for production of Tryptophan, Methyl Red(MR), Vogues- Proskauer (VP) test, Citrate test for Citric Acid Utilization, Motility Test, Triple

Sugar Iron Agar Test (TSI Test). CLED Agar (Cystine lactose electrolyte deficient agar) test, E.M.B. Agar (Eosin Methylene Blue agar), Mannitol Salt agar test were done following the method described by Baily & Scotts (2002). Antibiotic tests to check the resistance of antibiotic were: Antimicrobial

Susceptibility Testing-Disc diffusion methods, Dilution Methods- Minimum Inhibitory Concentration

(MIC)- There were two methods of testing for MIC: (a) Broth dilution method, (b) Agar dilution method and Dilution and Diffusion methods were followed by M.K Lalitha.

Results interpretation for antibiotics sensitivity after 24 hours of incubating the culture plates with antibiotics discs were examined for the presence of growth inhibition which is indicated by a clear zone surrounding standards disc. The susceptibility of organism was determined using Clinical and Laboratory Institute recommendations and expressed the results in mm, accordingly the results were recorded in each investigation as from sensitive (S) to resistance (R) respectively.

RESULTS

Various results obtained during above experimentations are shown below. Out of seventy samples, sixty three were found to be infected by micro-organism like *E.coli* 47.61%, *Staphylococcus epidermis* 14.2% , *Staphylococcus aureus* 17.46%, *Staphylococcus saprophyticus* 7.9%, *Streptococcus faecalis* 3.17% , *Pseudomonas aeruginosa* 3.17% , *Proteus mirabilis* 3.17% , *Citrobacter* 1.58%, *Klebsiella pneumoniae* 1.58%.

Interpretation of the results for antibiotics sensitivity after 24 hours of incubating the culture plates with antibiotics discs were examined for the growth inhibition which is indicated by a clear zone surrounding standards disc. The susceptibility of organism as determined using Clinical and Laboratory Institute recommendations and expressed the results in mm, accordingly the results were recorded in each investigation as from sensitive (S) to resistance (R) respectively. It was found that the leading Gram Negative uropathogens *E.coli* showed higher sensitivity (56%) for amikacin and low susceptibility (5%) with ciprofloxacin. The highest sensitivity of *K.*

Table 1 . Various Biochemical Tests

1	2	3	4	5 slant/butt	6	7 MR/VP	8	9	10	11	12	
[eleven isolates]	+	-	+	Y/YG	+	MR+/VP-	-	+	+	+	*Staphylococcus aureus*
[five isolates]	+	-	+	Y/R	-	MR-/VP+	+	+	+	+	*Staphylococcus.saprophyticus*
[thirty isolates]	-	+	-	Y/YG	+	MR+/VP-	-	+	-	+	*E.coli*
[two isolates]	+	-	+	Y/YG	-	MR-/VP+	+	-	+	+	*Streptococcus faecalis*
[two isolates]	-	+	-	Y/R	+/-	MR+/VP-	-/+	+	-	+	*Proteus.mirabilis*
[two isolates]	+	-	+	R/R	-	MR-/VP+	+	-	+	+	*Pseudomonas aeruginosa*
[one isolates]	-	+	-	Y/YG	-	MR+/VP-	+	+	-	+	*Citrobacter*
[one isolates]	+	+	-	Y/R	-	MR-/VP-/+	-	+	-	-	*Klebsiella. pneumoniae*
[nine isolates]	+	-	+	Y/YG	-	MR-/VP+	+	+	+	+	*Staphylococcus epidermis*

1-Variable culture, 2- Gram´s Staining, 3- Growth on MacConkey, 4- Blood agar, 5-Triple agar iron test, 6- IMViC test- Indole test, 7- MR-VP test, 8-Citrate test, 9-E.M.B. test, 10-C.E.L.D test, 11-Motility test, 12- Result

pneumonia was 80% with cefaprime and low susceptibility of 13% for ciprofloxacin. The highest resistance recorded in case of gentamicin and cefuroxime and the lowest resistance recorded in our study was with *K. pneumonia.* In case of *P. mirabilis* the resistance and sensitivity resulted were 50% for all the used antibiotics.

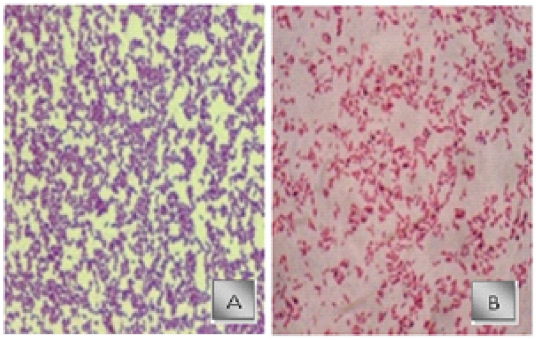
Susceptibility of *Staphylococcus species* was as high as 64% against amikacin and oxacillin but it showed lower sensitivity for Ampicillin and moderate for Roxythromycin, Ceftizoxime. *Streptococcus species, Pseudomonas aeruginosa* and *Citrobacter* organism showed sensitivity to all antibiotics.

DISCUSSION

There was high prevalence of asymptomatic bacteriuria among women in this setting regardless to women's age, parity and gestational age. *E. coli* was the commonest isolated organism with multi resistance toward different antibiotics. However, maternal age, parity and morbid obesity have been previously observed as risk factors for UTI among pregnant women[10]. Contrary to it, in our study UTI cases were also found in other age-groups beside pregnant women. Recently, it has been reported that, UTI develops in third trimester[11]. Perhaps the

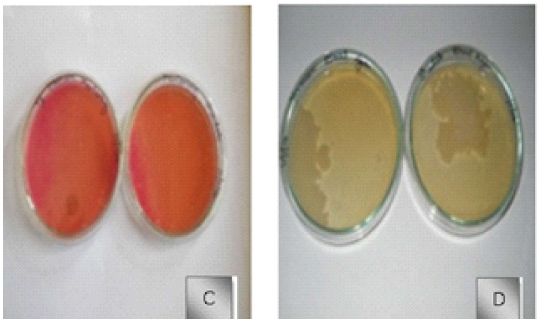
Table 2. Zone size interpretative chart for antibiotic used

		Zone size interpretative chart Diameter of zone inhibition					
S. No.	Antibiotic	Symbol	Strength	Resistant mm	Intermediate mm	Sensitivity mm	% 0f resistance of antibiotics
1.	Ampicillin	AS	20 mg	14	15-16	17	63.49%
2.	Co-trimoxazole	BA	25 mg	14	15-16	17	31.74%
3.	Cephalexin	PR	30 mg	14	15-16	17	9.52%
4.	Tetracycline	TE	30 mg	14	15-17	18	28.57%
5.	Cefotaxime	CF	30 mg	14	15-17	18	11.11%
6.	Ciprofloxacin	RC	5 mg	12	13-16	17	36.50%
7.	Levofloxacin	QB	5 mg	12	13-16	17	3.14%
8.	Linezolid	LZ	30 mg	14	15-17	18	7.93%
9.	Cloxacillin	CX	1 mg	13	14-20	21	0%
10.	Roxythromycin	AT	15 mg	14	15-17	18	30.15%
11.	Lincomycin	LM	2 mg	10	11-15	16	6.34%
12.	Gentamicin	GM	10 mg	15	16-20	21	33.33%
13.	Streptomycin	ST	30 mg	20	21-30	31	17.40%
14.	Piperacillin	PC	100 mg	20	21-30	31	0%
15.	Chloramphenicol	CH	30 mg	13	14-22	23	57%
16.	Ceftizoxime	CI	30 mg	14	13-15	16	12.6%
17.	Ofloxacin	ZN	5 mg	12	14-18	19	14.28%
18.	Amikacin	AK	30 mg	13	14-17	18	0%
19.	Gatifloxacin	GF	10 mg	15	16-20	21	1.5%
20.	Azithromycin	AZ	15 mg	14	15-17	18	4.7%
21.	Meropenem	MP	10 mg	15	16-20	21	0%
22.	Ticarcillin	TT	85 mg	20	21-30	31	4.7%
23.	Cefoperazone	CM	105 mg	20	21-30	31	0%
24	Cefpirome	CG	30 mg	14	15-17	18	1.5%
25	Teicoplanin	TF	30 mg	14	15-17	18	1.5%
26	Aztreonam	AC	30 mg	14	15-17	18	0%
27	Netilmicin	NT	30 mg	14	15-17	18	0%
28	Penicillin	PE	30 mg	14	15-17	18	22.2%
29	Vancomycin	VO	30 mg	14	15-17	18	26.98%
30	Norfloxcin	NO	30 mg	14	15-17	18	17.40%



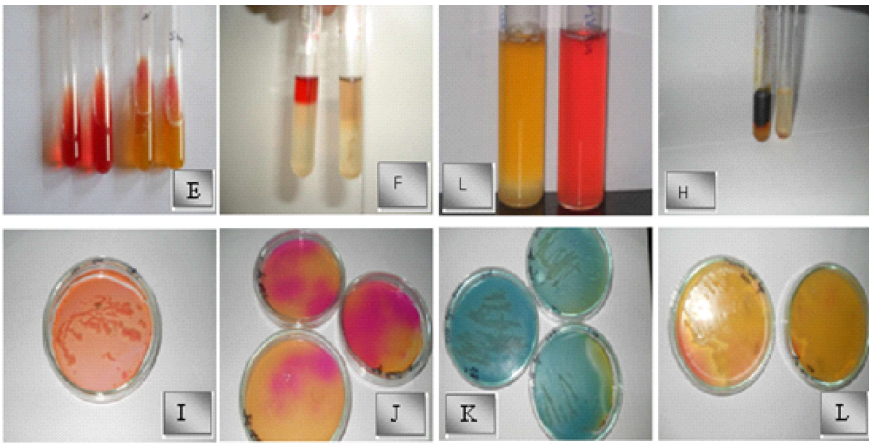
Bacteria can be differentiated based on how they react to a procedure of dying cells called Gram stain. Bacteria are divided into a group that turns purple (gram positive) [A] and a group that turns red (gram negative) [B]. Bacteria that are gram [+] include *Staphylococcus, Streptococcus, Bacillus and Micrococcus.* Gram [-] bacteria include *E.coli and Salmonella*.

Fig. 1. Differentiation of gram positive and gram negative bacteria



Growth on Mac Conkey (C) and Blood agar (D): if the blood agar plate has significant growth of bacteria, the causative organism is Gram- positive cocci and if the growth occurs on Mac Conkey plate then organism is a Gram negative.

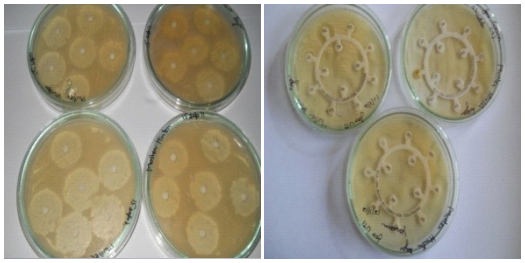
Fig. 2. Growth on Mac Conkey and Blood agar plates



(E) TSI
(F) Indole
(G) & (H) MR-VP

(I) EMB
(J) Citrate Test
(K) CLED
(L) Mannitol

Fig. 3. Various Biochemical tests



(M)Disc diffusion
(N) Prepared disc of antibiotic sensitivity test

Fig. 4. Antibiotic sensitivity test

susceptibility of UTI during this period is due to urethral dilatation which starts as early as 6th.week and reaching the maximum. In early infancy 58% of females as compared to 42% boys were detected to have urinary tract infection while in neonates 55% UTI prevalence occurred in boys and 45% in girls[13] showing that UTI affects a large proportion of the population and more prevalent in females. The high prevalence of infection in females is usually related to anatomical and pathogenic factors e.g. the short length of the urethra hence lesser distance of bacteria ascending up the tract lack of antimicrobial properties of prostatic fluid as in males, hormonal changes affecting the adherence of bacteria to the mucosa and urethra trauma during sexual intercourse.

Identification of causative organisms and its susceptibility to antimicrobials is important, so that proper drug is chosen to treat patient in easy stage of UTI. It is therefore recommended that routine microbial analysis and antibiotic sensitivity test of mid stream. Urine sample of patient be carried out before the treatment in the management of UTI, our results suggests that the following antibiotics Amikacin, Meropenem, Cloxacillin, Aztreonam, Netilmicin, Piperacillin, Cefpirome, Cefoperazone, Teicoplanin are chosen in management of UTI by the clinicians after having the culture sensitivity results over and above for prevention of UTIs implementation of strict infection control guideline effective hand washing and judicious use of antimicrobials is mandatory which goes a long way to cope up with the emergence of drug resistance among uropathogens.

As evident from our results that 47.61% of UTI was infected by *E.coli*. Our finding is consistent with those reported by F A Nakhjavani *et al* 2006 [15]. Further that antibiotic resistance was reported to be 50% of *E.coli* strains in Kuwait, U.S., South Europe, Israel and Bangladesh by Manges *et al*. 2001[14] which also corroborates our findings of 44% of *E. coli* resistance observed during our study.

It is quite alarming to note that almost all of the isolates including in our study were found resistant to four or more antibiotics. Antibiotics resistance is becoming a big problem for public health which threatens the lives of hospitalized individual as well as those with chronic conditions and adds considerably to health care cost.

CONCLUSION

There was high prevalence of asymptomatic bacteriuria among women in this setting regardless to women's age, parity and gestational age. *E. coli* with its multi resistance towards antibiotics was the most common isolated organism in Allahabad region of U.P. State. Thus, it is therefore recommended that urine culture should be performed as screening and diagnostic tool including routine microbial analysis and antibiotic sensitivity test for UTI treatment.

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