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Incidence of Urinary Tract Infections (UTI) and their Antibiotics Sensitivity Pattern

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Abstract: ***Urinary tract infection (UTI) is the second most common infection in community practice. It defines as to presence of bacteria in urinary tract, urinary bladder, urine collecting tubes and kidney. UTI causing organisms are mostly human intestinal commensals. These organisms showed virulent behaviors upon transmission to clinically significant biological sites. These organisms includes, Gram Negative Bacilli such as E. coli, Klebsiella pneumoniae, Citrobacter species, Acinetobacter species , Enterobacter species , pseudomonas aeruginosa , proteus species in the Gram Positive coccus include Staphylococcus aureus, staphylococcus saprophyticus, Staphylococcus epidermidis and Enterococcus faecalis and fungi like Candida albicans. Out of these, E coli is the most common organism causing UTI. Serotypes of E .coli consistently associated with UTI are designated as Uropathogenic E. coli or UPEC.***

Keywords: UTI, Uropathogens, Bacteriuria, Cystis and Antibiotic sensitivity

1. Introduction

Urinary tract infection UTI is a common bacterial infection and well known to affect the various part of the urinary tract and isolated from both the gender's including males and females. In this way the females are most susceptible to get this infection than male, because of their difference in anatomy and reproductive physiology. Urinary tract infection (UTI) can be also define as a conditions in which the urinary tract is infected with a bacterial pathogen and occasionally it will remain in the patient body for life time and causing inflammation. Various clinical features, including symptoms their diagnosis, antibiotic therapy, complications, and their long term significance vary depending on the site of infection and the presence or absence of structural and functional abnormality within the system. Urinary tract infection is not only common but the range of clinical effect varies from asymptomatic bacteriuria to acute pyelonephritis. Women are more susceptible to UTIs than males. Urinary tract infection is the common of all bacterial infections, affecting human beings throughout their life span especially in women. (Anayet Ullah, et al., 2007, Sumaira Zareef et al 2009).

The infection is usually caused as a consequence of Bacteriuria and bacterial invasion of the lower and upper wall of urinary tract. Neonates, girls, young women and older women are considered as most susceptible to UTIs. In women's, bacterial Cystis is most common bacterial infection and cause specific symptoms or without any symptoms (Levi et al 2005, Nicole and Jon 2008 and Jenson and Baltimore 2006). UTIs in children are significant source of morbidity, particularly when associated with abnormalities (Ross 1994). Vesico-ureteral reflux is the most commonly associated abnormality and reflux nephropathy is an important cause of end stage renal disease in children and adolescents (Bailey 1992).

2. Materials & Methods

Target group: The target groups are divided in five categories including- All the adolescents up to 15 years, 16 to 25 years 26 to 35 years, 36 to 45 years and women between 45 to 55 years and above.

Collection of urine samples: For collection of urine samples, clean voided mid-stream urine sample were collected from the person in a sterilized plastic sample bottle from various pathology labs of Ambikapur, Chhattisgarh. All the adolescents and young women were suffering with urinary tract infection and coming for their treatment. About 20 ml of urine sample were collected aseptically in a sterilized wide mouthed plastic bottle. Each sample in the bottle were properly labeled with patient details (Collee et al, 1996). Samples were collected during the January 2013-December 2014. Samples were brought to Microbiology lab and stored at 4^0C or ice begs, for the study of urine microbial profile and other analysis.(Forbes et al, 1998)

Culture media: For this, five different culture media with having different composition were used in the present study including: Nutrient agar (NA), MacConkey agar (MCA), Blood agar (BA) and Muller Hinton agar (MHA) from HiMedia. In Erlenmeyer flask, 100 ml media were prepared and sterilized by autoclaving at 121^0C temperature, 15 lbs pressure for 20 minutes then pour it in 9 cm dia Cornning Petri plates for inoculation of samples.

Urine microscopy: Five ml of urine sample were poured into a clean and dry glass centrifuge tube then centrifuge it at 3000rpm for 5-10 minutes. The supernatant fluid then allow to drain out and a drop of sediment was then taken on a clean glass slide and cover it with cover glass then examined under light microscope using 10X and 40X magnification. On the other hand, take one drop of un-centrifuged urine sample on a fresh and clean glass slide and fixed it, then observe it in microscope and count the bacterial propagules per focus of microscopic field. For

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characterization and identification of isolates the following methods were used (Chowdhury, 1998).

Screening through the Gram's staining: Gram's staining of isolated bacteria was performed as suggested by Duguid (1996). For this, bacterial smear was prepared from broth culture on a glass slide and heated up it carefully to fix the bacteria on glass plate. Then the slide was flooded with 0.5% Gram's crystals violate solution and left it for 30 second. Then allowed to poured Lugol's iodine solution and drain out the excess stain, the slide was washed off with 90-100% ethanol until discoloration. The slide was rinsed with water and then 0.1% safranin a counter stain for 60-90 second. The slide then wash with distilled water and air blotted to dry and observed under microscope.

Microscopic examination of bacteria for their external morphology, colonial color, shape, size and mobility etc, were also consider for confirmation test of identification. Finally, biochemical test performed for confirmation by using API kit from Biomerieus.

Frequency & Density: In the present study, microbial diversity analysis carried out by the direct counting method and the parameters such as frequency and density was calculated as the following formula:

$$Frequency = \frac{Number\ of\ samples\ in\ which\ bacterial\ species\ present}{Total\ number\ of\ sample\ studied}$$

$$Density = \frac{Number\ of\ individuals\ of\ bacterial\ species\ present}{Total\ number\ of\ sample\ studied}$$

Antibiotics sensitivity test: Bacteria sensitivity against antibiotics were tested for each isolates by using the agar disc diffusion method as described by Bauer et al.(1966). The turbidity of the bacterial suspension were then compared in NTU using Mac Farlands Barium sulphate standard solution corresponding to 1NTU = about $4.5x10^4$ cells/ml. The bacterial suspension standardized by Haemocytometer. Standardized bacterial suspensions of ($2x10^6$ spore/ml) each test bacteria were seeded over the Muller Hinton agar by using pre sterilized cotton swabs (ATCC No. 25923), over the surface of inoculated plates. Then leave the inoculated plates for one or two hour for settlement of bacteria on the upper surface of medium properly. Then the antibiotics impregnated discs of 6mm diameter (0xoid & HiMedia) were placed over the surface of inoculated plates and incubated for 24-48hrs. After the incubation plates were taken out and diameter of zone of inhibition around the discs were measured and compared with a zone of inhibition in standard chart to determine the sensitivity of tested isolates to the antibiotics.

In order to prepare standard chart of disc potency (HiMedia) were tested against the reference strain of *S. aureus* (ATCC No. 25923), *E. coli* (ATCC No. 25922) and *P. aeruginosa* (ATCC No. 27853). The zone of inhibition was compared with standard value as recommended by CLSI (2007).

Table 1: Antibiotics and their concentration used in the study

S.No.	Antibiotics	Short form	Concentration
1	Amoxicillin	Amo	30µg/ml
2	Ciprofloxacin	Cpr	05µg/ml
3	Chloromphenicol	Chl	30µg/ml
4	Amikacin	Amk	30µg/ml
5	Ofloxacin	Ofl	05µg/ml
6	Tetracyclin	Tet	30µg/ml

Table 2: Percentage of occurrence of isolated pathogenic bacteria in collected urine sample.

S.No.	Name of organism	Number of sample studies	Number of colonies appeared	% Frequency
1	*Escherichia coli strain I*	300	187	50.40
2	*E. coli strain II*	300	59	15.90
3	*Enterobacter aerogens*	300	07	01.88
4	*Klebsiella spp.*	300	22	05.92
5	*Proteus mirabilis*	300	09	02.42
6	*Proteus valgris*	300	14	03.77
7	*Pseudomonas aeruginosa*	300	27	07.27
8	*Streptococcus faecalis*	300	46	12.39
Total number of colonies appeared			371 colonies	100.00

Table 3: Distribution of Uropathogens in relation to age, sex and life style

S. No.	Age group	Total no of urine samples found positive in various Gender		Life style
		Male	Female	
1	Up to 15 years	02	17	Student male/female
2	15-25 years	03	19	Student male/female
3	25-35 years	03	26	Working men/women
4	35-45 years	05	55	Working men/women
5	45-55 years	05	75	Working men/women
6	Above 55 years	05	85	working male/female
Total no of samples found positive		23	277	300 (Samples analyzed)

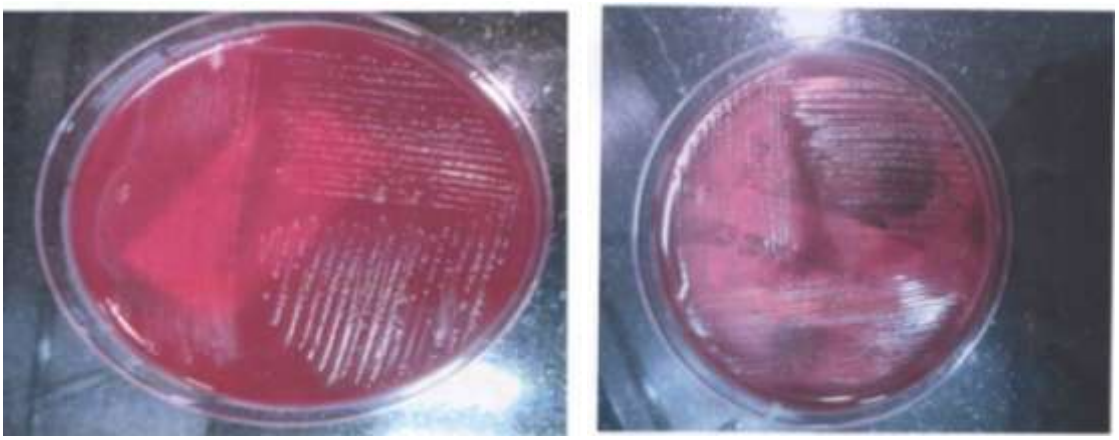
Table 4: Antibiotics sensitivity of isolated bacteria

S.no.	Name of organism	Antibiotics sensitivity					
		Amoxicillin	Ciprofloxacin	Chloramphenicol	Amikacin	Ofloxacin	Tetracycline
1	*Escherichia coli strain I*	09 mm (R)	12 mm (R)	O6 mm (R)	16 mm (S)	14 mm (S)	04 mm (R)
2	*E. coli strain II*	11 mm (R)	12 mm (R)	04 mm (R)	14 mm (S)	10 mm (R)	04 mm (R)
3	*Enterobacter aerogens*	14 mm (S)	15 mm (S)	04 mm (R)	17 mm (S)	17 mm (S)	06 mm (R)
4	*Proteus mirabilis*	15 mm (S)	15 mm (S)	06 mm (R)	16 mm (S)	14 mm (S)	04 mm (R)
5`	*Proteus valgris*	08 mm (R)	06 mm (R)	04 mm (R)	14 mm (S)	14 mm (S)	04 mm (R)
6	*Pseudomonas aeruginosa*	19 mm (S)	18 mm (S)	06 mm (R)	16 mm (S)	12 mm (S)	04 mm (R)
7	*Klebsiella sp.*	12 mm (R)	14 mm (S)	04 mm (R)	18 mm (S)	12 mm (S)	04 mm (R)
8	*Streptococcus faecalis*	22 mm (S)	16 mm (S)	14 mm (S)	26 mm (S)	16 mm (S)	18 mm (S)

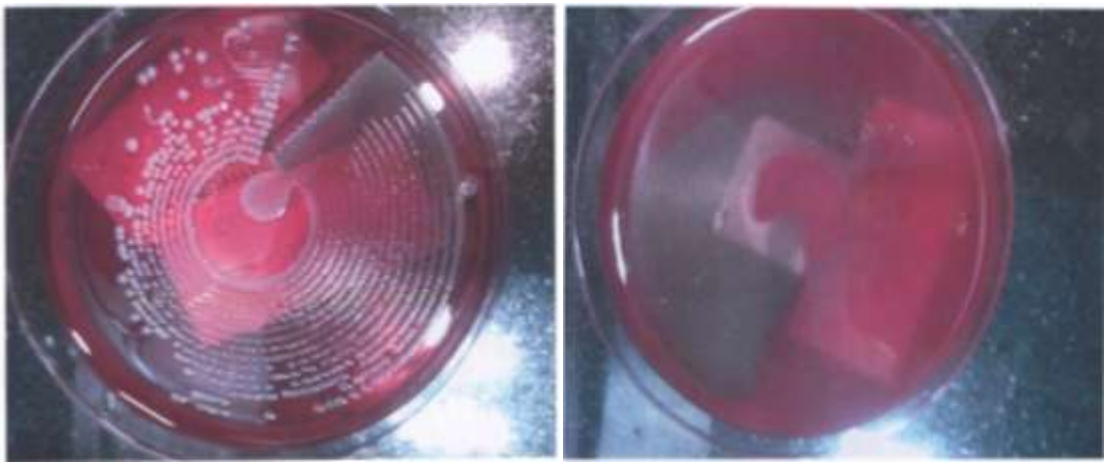
Note: Zone of inhibition recorded in mm and R-resistant ,S-sensitive

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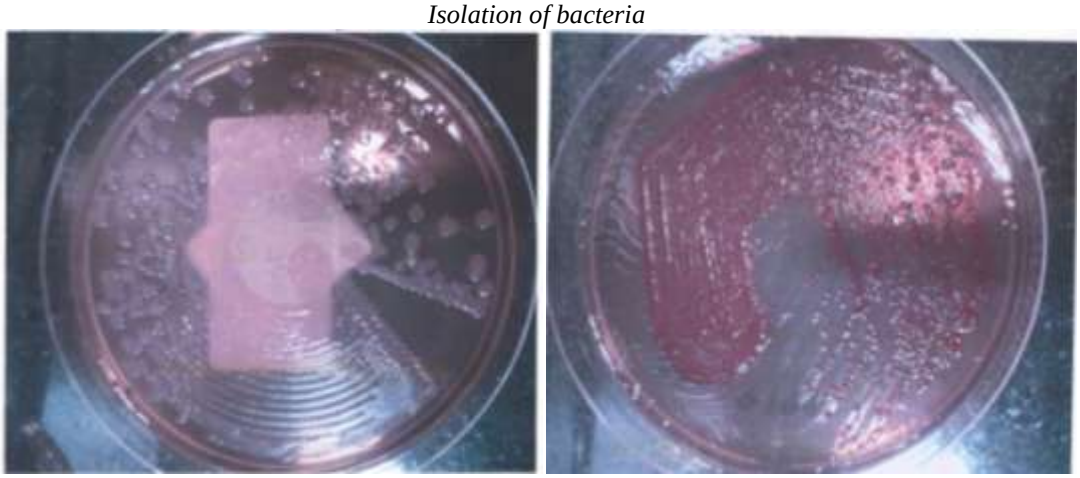
The drug resistance in bacteria increased, due to of over consumption of antibiotics. The pattern of resistance has also been reported from different states in India and other part of the world. Shukla (2014) also observed the drug resistance, particular to commonly available drug such as Ampicillin and Trimethoprim-sulfamethoxazole against bacteriuria. However Amikacin is found the drug of choice with highest sensitivity for all Gram Negative *Baciili* .



Isolation of bacteria



Isolation of bacteria

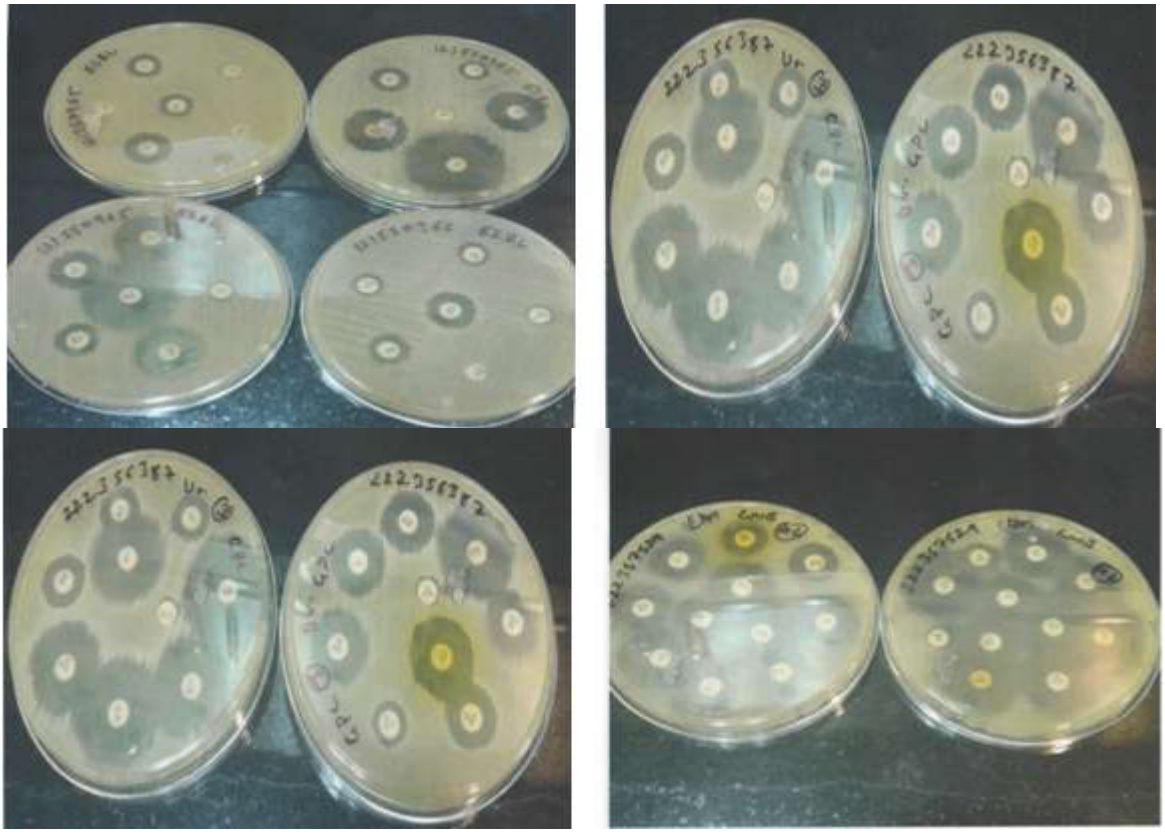


Isolation of bacteria

3. Results &Discussions

The current study makes an attempt to put forth the various bacteria that are responsible for the development of the urinary tract infection. If there is any change in physical condition including pH, sugar concentration, age group and life style may cause change bacterial flora of the body. Urinary tract infection is a common infection for both the gender but possibilities of infection are quite high in women due to their different physiology and anatomy.

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Antibiotics sensitivity of isolated bacteria

Urine is believed to be a sterile solution and considered as microbial free preparation of the body. Bacteria entered through urethra and initiate the consequences of infection. In this way, urinary tract is affected first then urinary system. Urinary system is a complex of various parts of urinary tract including the urethra, uterus, urinary bladder, kidney and the artery and veins those are related to involve in transportation of blood influx and out flux.

E. coli group are widely distributed in the intestine of humans and other warm blooded mammalians, and are the predominant facultative anaerobes in the bowel and part of essential intestinal flora that maintains the physiology of the healthy host. Although most strains of *E. coli* are not considered as pathogens, they can be opportunistic pathogens that cause infection in susceptible and immune-compromised hosts. Uropathogenic *E. coli* generally utilize *P fimbria* (pyelonephritis associated pili) to bind urinary tract especially endothelial cells and colonize the bladder. These adhesions specifically found between D-galactose-D-galactose moieties on the p blood group antigen of erythrocytes and uro-epithilial cells. *Klebsiella* sp, are second most common infectious agent and known as a residential species of the intestinal tract in mammalian system. *Klebsiella sp* are resistant to a number of antibiotics. Many strains have acquired an extended-spectrum beta-lactamase with resistance to carbenicillin, amphicillin, quinolones and increasingly to ceftazidime. On other hand pseudomonas infection of the urinary tract usually are hospital acquired and iatrogenic related to instrumentation and surgery. These infections may involve the urinary tract through a bacteremic spread and a frequent source of bacteremia.

The attachment of *Proteus species* on uroepithilial cell, generate various disorder in the mucosal endothelial cells, including secretion of interleukin-6 and interleukin-8. *Proteus species* are evolved in induction of apoptosis, and epithelial cell desquamation. Urease production and presence of bacterial motility and fimbriae, may favour the production of upper urinary tract infection. Resistance to Amphicillin and first generation Cephalosporins have acquired by more than 50% strains. On other hand *Staphylococcus species* and *pseudomonas species* are caused by community acquired infection and appeared as susceptible to most of the common antibiotics .Most of the cases, the UTI is caused by the single pathogenic bacteria usually are gram-negative bacteria contributing from the fecal micro flora of the host. The ***E. coli*** is most common in occurrence and accounting for more than 80% of the UTI infection.

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