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Antibiotic Susceptibility Profiling of Bacterial Biodiversity Associated with Urinary Tract Infections

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ABSTRACT

Urinary tract infections (UTI's) are a bacterial infection that affects any part of the urinary tract. Urinary tract infection is one of the major diseases that affect people of all age groups and sexes and can be separated into asymptomatic and symptomatic. The normal urinary tract is sterile but gets infected with the normal flora and opportunistic pathogens. Urinary tract infections (UTIs) are a serious health problem affecting millions of people each year. This is the second most common type of infection in the body. In the present study 20 morphologically different bacterial isolates were recovered from 50 different urine samples from UTI's patient. The prevalence of the isolates was varied with *E. coli* (40%), followed by *Staphylococcus* spp., *Pseudomonas* spp. *and Klebsiella* spp. each of (20%) respectively. All the isolates of different bacteria were tested for antibiotic sensitivity patterns against common antibiotics viz. Ampicillin, Tetracycline, Chloramphenicol, Vancomycin, Nitrofurontione and Ciprofloxacin with potencies of 30 µg each. The sensitive pattern of the isolates towards antibiotic Nitrofurontoine was (75%) followed by Tetracycline (55%) and Ciprofloxacin (45%). Intermediate activity was reported against Ampicillin (45%), Chloramphenicol and vancomycin (40%). Resistant pattern shown against vancomycin (60%), Chloramphenicol (45%), while both tetracycline and Ciprofloxacin shown (30%) against the UTI's isolates. Therefore, drugs like Nitrofurontoine, Tetracycline and Ciprofloxacin are the most effective choices against the common UTI's isolates.

Key words: Antibiotics, Sensitivity profiling, Urinary system, Urinary tract infections, *Escherichia coli, Staphylococcus, Pseudomonas, Klebsiella*

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1. INTRODUCTION

Urinary tract infection (UTI) is an infection that is associated with the urinary system. It is the second most common infection after respiratory infection. The human urinary system consists of the kidneys, ureters, bladder and the urethra [1]. The infection affects any part of urinary tract equally but most commonly the lower part as it exposed to environment. All areas of the urinary tract above the urethera in healthy humans are sterile, hence urine is normally sterile [2]. Infection generally arise when bacteria enters the opening of the urethra and multiply within and travelled to all part of the urinary tract.

The infection of urethra is called uritheritis, if bacteria travel to the bladder and multiply it causes bladder infection, cystitis. If the infection is not treated on time, bacteria may then promote up the ureters and infect the kidneys causing pyelonephritis. UTIs may also occur by the hematogenous or blood borne route.

This usually occurs due to bacteremia. Any systemic infection can lead to the seeding of the organism in the kidney [3]. The most common causes of UTIs are *Escherichia coli*, bacterial strain that usually inhabit the colon. However, many other bacteria viz. *Klebsiella, Pseudomonas, Enterobacter, Proteus*, *Staphylococcus, Mycoplasma, Chlamydia, Serratia* and *Neisseria* spp. also responsible for UTI's less frequently. In addition, fungi viz. *Candida* and *Cryptococcus* spp. and some parasites such as *Trichomonas, Schistosoma* also cause UTIs [4].

The diagnosis of urinary tract is usually confirmed by microscopy, culture, biochemical and serologic assays of properly collected urine samples. The treatment of infected person is based on the antibiotic sensitivity patterns to the bacteria isolated [5, 6].

Escherichia coli the most common etiological agent of UTI's (74.6%), followed by *Klebsiella spp.* (11.7%), *Staphylococcus saprophyticus* (6.4%), and *Pseudomonas aeruginosa* (2.2%) [7].

Keeping these considerations in mind the present study aimed to isolate the bacteria present in poly-microbial UTI samples from a population of elderly patients, and compare their prevalence, phenotypic activity and their antibiotic susceptibility profiling.

2. MATERIALS AND METHODS

2. 1. Materials used

All the chemicals used for preparing reagents and solutions were procured from Sd-fine chemicals and were of AR grade. For the isolation and biochemical characterization of isolates, dehydrated media used were procured from Hi media (India) and were used as per the manufacturer's direction. All the glassware's like Petri plates, flasks, spreader, test tubes, flasks etc used were made of borosililicate grade.

2. 2. Collection of samples

A total of 50 urine samples were collected from patients having clinical symptoms of UTI's infection IVY Hospital, Mohali (India). All the samples were collected in sterile plastic container and carried to the laboratory. All the samples were then stored at 4°C until further investigation.

2. 3. Recovery of the bacterial isolates

All the collected samples were then taken in calibrated inoculation loop (0.5 mm diameter) and streaked on nutrient agar. The inoculated plates were then incubated aerobically at 35 ±2 °C 24 hrs. After incubation, the plates were observed for appearance of colonies. Morphologically different colonies were selected for purification and characterization.

2. 4. Purification and preservation of the isolates

Single isolated colonies were picked up and streaked further on nutrient agar media to ensure purity of isolates. These plates were then incubated at 35 ±2 °C for 24 hours and later preserved at 4 °C in the refrigerators until further use. All the isolates were maintained on nutrient agar slants and stored at 4 °C.

2. 5. Cultural characterization of the isolates

Culture characterizations of isolates were recorded by growing on nutrient agar (NA), and MacConkey agar. The various cultural characteristics viz. colony morphology, shape, elevation, pigmentation, opacity, consistency etc. were recorded of each isolates.

2. 6. Morphological characterization of the isolates

Gram staining was done for studying Gram reaction and characteristics cell arrangement of isolates.

2. 7. Antibiotic susceptibility testing by disc diffusion method

Modified Kirby-Bauer disk diffusion method was used to test the susceptibility of the isolates to different antibiotics [8]. Ampicillin (30 µg), Tetracycline (30 µg), Chloramphenicol (30 µg), Vancomycin (30 µg), Nitrofurantoin (30 µg) and Ciprofloxacin (30 µg). The inocula were prepared by growing the various isolates on separate agar plates and colonies from the plate were transferred with inoculating loop into 3 ml of normal saline in a test tube.

The density of these suspensions was adjusted to 0.5 McFarland standards. The surface of Muller-Hinton agar plate was evenly inoculated with the organisms using a sterile swab. The swab was dipped into the suspension and pressed against the side of the test tube to remove excess fluid. The wet swab was then used to inoculate the Muller-Hinton agar, evenly streaked across the surface.

With the help of Disc Dispenser the antibiotic discs were applied to the surface of the inoculated agar and the plates were incubated overnight at 35 ±2 °C over night. The diameter of zone of inhibition was observed and measured and compared to the chart provided by National Committee for Clinical Laboratory Standard to find the susceptibility patterns.

3. RESULTS

3. 1. Recovery of the isolates

A total of 20 morphologically different bacterial isolates were recovered from 50 different urine samples.

3. 2. Cultural and Morphological Characterization

For the cultural characterization the colony morphology, shape, opacity and pigmentation were recorded for each isolates. Isolate US1, US2, US6, US8, US10, US14, US18 and US 20 have been observed with Oval, low convex, translucent, non pigmented small to medium sized colony appearance on nutrient agar. These isolates were gram negative, small rod and scattered,

While, isolates US9, US12, US17 and US19 were found to be with thick, opaque, moist golden yellow to white, small in size, gram positive, cocci, tetrad or in groups and isolates US3, US11, US13 and US15 have been observed with greyish-white to green fluorescent, mucoid, irregular, active, spreading occurs on agar with on nutrient agar also with cell morphology gram negative, small to medium bacilli. The isolates US4, US5, US7 and US17 were recorded for glistening moist colonies, small, and gram negative rod and arranged single to pair.

The result of the present study is shown in **Table 1**.

Table 1. Cultural and morphological Characterization of bacterial isolates

Isolates ID	**Colony Morphology**		**Gram rx^n**	**Arrangements**	**Shapes**
	Nutrient agar	Mac conkey agar			
US1	Low convex, colorless, translucent	Pink, mucoid, entire	Gram -	Scattered	Rod
US2	Round white	Pink, mucoid, entire	Gram -	Clusters	Rod
US3	Mucoid colonies, light green	Small colourless	Gram-	Singles to pairs	Rod
US4	Moist colonies, small,	Glistening colonies	Gram-	Single to clusters	Rod
US5	Moist colonies	Glistening colonies	Gram -	Single to pairs	Rod
US6	Smooth, shiny, white	Pink, mucoid, entire	Gram -	Single to pairs	Rod
US7	Glistening moist colonies	Transparent, small	Gram -	Single to clusters	Rod
US8	Low convex, irregular	Small Pink, mucoid, entire	Gram -	Scattered	Rod
US9	Circular, pinhead colonies	Opaque golden growth	Gram +	Single to clusters	Cocci
US10	Smooth, shiny, white	Pink, mucoid, entire	Gram -	Scattered	Rod
US11	Mucoid colonies, green fluorescent	Transparent, small, pin point	Gram -	Single to clusters	Rod
US12	Convex, smooth	Golden growth, colourles	Gram +	Single to clusters	Cocci

US13	Opaque, greyish white	Opalescent, small	Gram -	Scattered	Rod
US14	Low convex, irregular	Pink, mucoid, entire	Gram -	Single to clusters	Rod
US15	Smooth, white, non pigmented, green	Slightly opalescent	Gram -	Single to pairs	Rod
US16	Glistening colonies	Transparent, small, colourless	Gram -	Single to clusters	Rod
US17	Raised, glistening colonies	Opaque, transparent	Gram +	Single to clusters	Cocci
US18	Smooth, shiny, white	Pink, mucoid, entire	Gram -	Single to clusters	Rod
US19	Smooth, convex, golden yellow	Opaque, transparent	Gram +	Clusters	Cocci
US20	Low convex, colorless, translucent	Slightly oplascent, Pink, mucoid, entire	Gram -	Single to clusters	Rod

The Cell morphology and gram staining characteristics were determined by Gram staining. All the recovered isolates were gram negative rod except the isolates US9, US12, US17 and US19 were found gram positive cocci. The isolates US1, US2, US6, US8, US10, US14, US18 and US 20 were found to be lactose fermented and give pink colour colony on Mac conkey agar. The rest isolates were found to be non lactose fermented and gave transparent, colourless, poor growth over the same medium. The characteristics pattern of colony morphology of some isolates was depicted in **Figure 1a-d**.

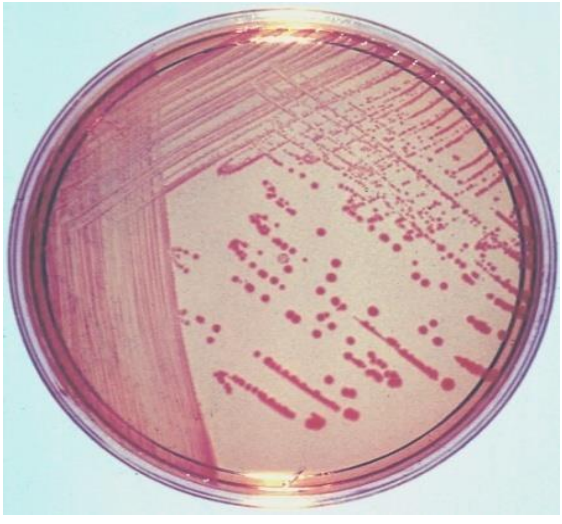

Fig. 1a. US1 on Mannitol salt agar

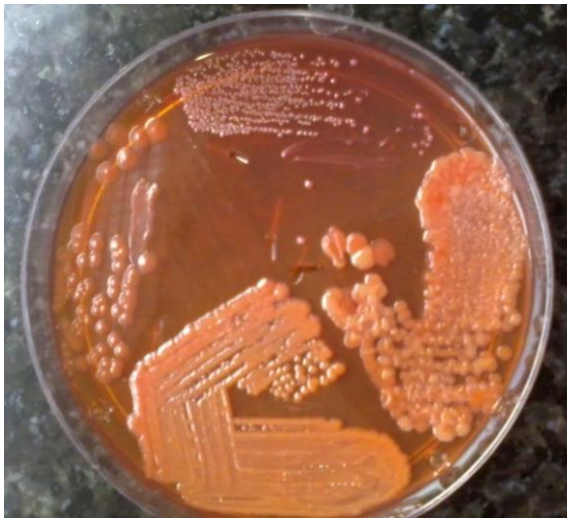

Fig. 1b. US1 on MacConkey agar

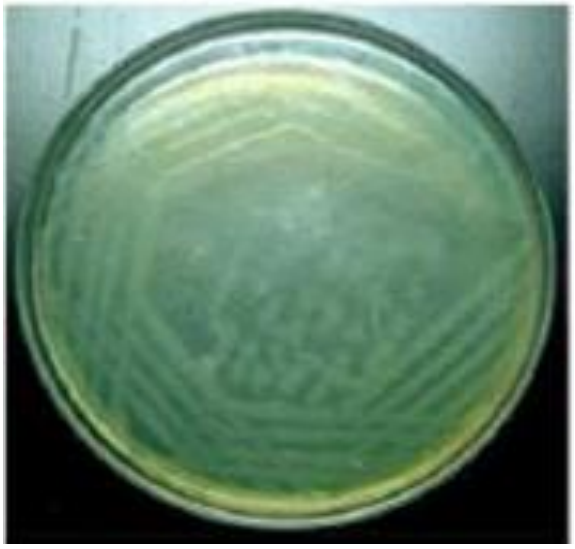

Fig. 1c. US1 on Nutrient agar

Fig. 1d. US1 on Mineral salt Agar

3. 3. Biochemical characterization of bacterial isolates

After morphological and cultural characterization all the 20 isolates were subjected to various biochemical tests viz. IMViC, Catalse, nitrate reduction test, gelatine liquefaction, casein hydrolysis and sugar fermentation. The result of various chemical characterizations is depicted in Table 1.

All the isolates show positive for catalsae test and nitrate reduction test. The isolates US9, US12, US17 and US19 were found to be shown positive for gelatine liquification test, rest remaining isolates shows negative for this test. The isolates US3, US11, US13 and US15 indicates positive test for casein hydrolysis and remaining all 16 isolates were found negative. The isolates US1, US2, US6, US8, US10, US14, US18 and US 20 were found to be positive for indole production. Out of 20 isolates eight indicates positive result and rest all were negative.

In methyl red test some isolates show positive result and some of them show negative result. For Voges-Proskeur test, the US4, US5, US7 and US17 were found to be positive and rest all were negative. The isolates US3, US11, US13, US15 and US4, US5, US7 and US17 show positive test for citrate utilization.

3. 4. Partial identification and prevalence of UTIs isolates

On the basis of cultural, morphological and biochemical characterization the urinary isolates were partially identified by comparing with Bergey's manual of systematic Bacteriology [18]. Isolate US1, US2, US6, US8, US10, US14, US18 and US 20 with similar characteristics were tentatively assigned as E. coli. Isolates US9, US12, US17 and US19 as Staphylococcus spp., isolates US3, US11, US13, US15 as Pseudomonas spp., and US4, US5, US7 and US17 as Klebsiella spp. respectively. The prevalence of the isolates was varied with E. coli (40%), followed by Staphylococcus spp., Pseudomonas spp. and Klebsiella spp. each of (20%). The prevalence is depicted in **Figure 2**.

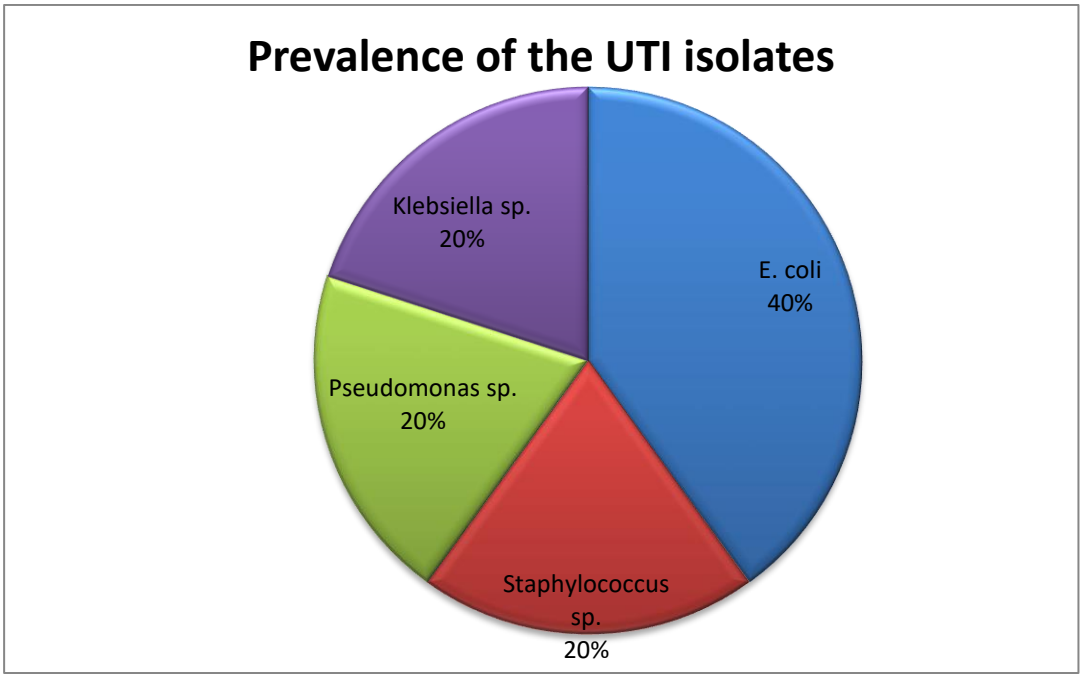

Figure 2. Prevalence of UTIs isolates

3. 5. Antibiotic susceptibility testing

Figure 3. Antibiotic susceptibility testing

All the isolates were tested for antibiotic sensitivity pattern against common antibiotics disc potency (30 µg) of Ampicillin, Tetracycline, Chloramphenicol, Vancomycin, Nitrofurantoin and Ciprofloxacin. All the recovered isolates were tested for their antibiotic sensitivity patterns by disc diffusion method. The result of the study is depicted in **Figures 3**

and **4**. The high sensitivity pattern was found towards Nitrofurantoin (75%) followed by Tetracycline (55%) and Ciprofloxacin (45%). Moderate sensitive pattern was found towrds Ampicillin (45%), Chloramphenicol and vancomycin (40%) each.

While the Resistant pattern was found towrds vancomycin (60%), Chloramphenicol (45%) and tetracycline and Ciprofloxacin both (30%) against all the UTI isolates. Therefore the drug like Nitrofurantoin, Tetracycline and Ciprofloxacin are the most effective drugs against the common UTI isolates.

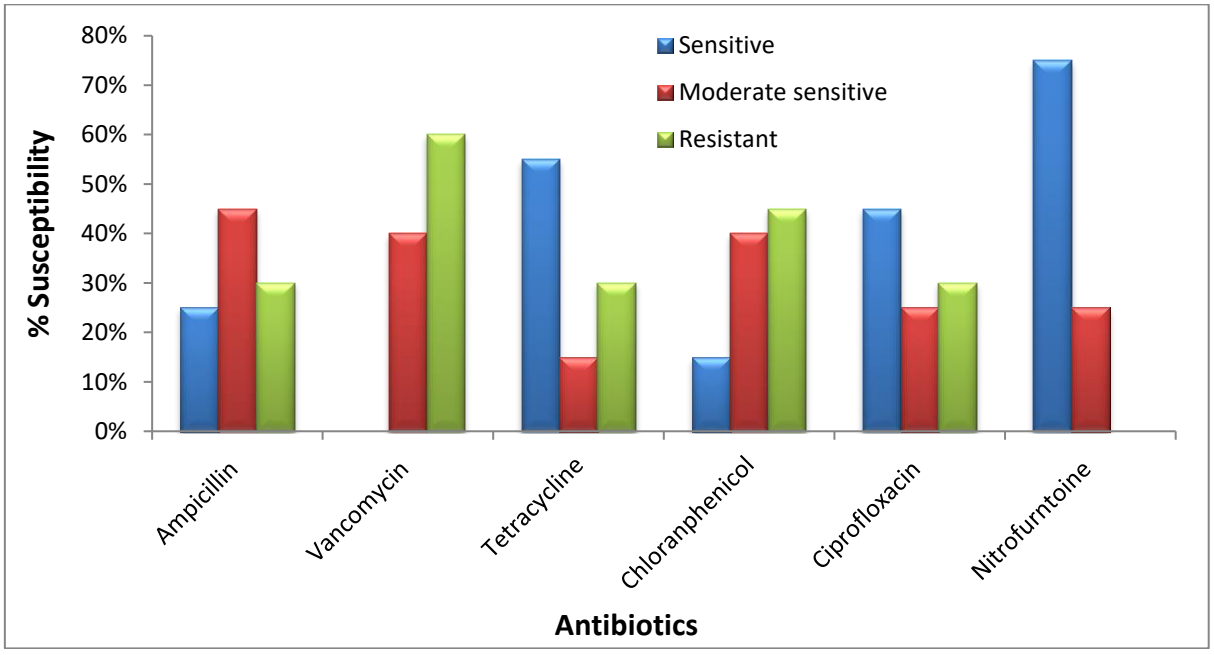

Figure 4. Susceptibility patterns of the isolates to different antibiotics

4. DISCUSSION

The isolated uropathogens characterised for their phenotypic properties and shown vide variability. The colony morphology and cell morphology varied with the isoalates. Similarly, some researcher recovered Gram negative and Gram positive bacteria from UTI patients. Some were rods and some were cocci and further characterized as *E. coli*, *Klebsiella* sp., *Pseudomonas* sp., *Staphylococcus* produced white shiny colonies, mucoid, glistening colonies [9]. In another study the recovered isolates from urine shows (83.17%) isolated bacteria were Gram negative bacilli while (21.73%) cases were Gram positive cocci [10]. Growth pattern of isolates was found to be different on Mac Conkey agar. Similarly, Das et al. found that the UTI isolate cultivated on MacConkey agar gave red-pink color colony, due to fermentation of lactose [11].

For catalase the test was significantly important. Some researchers found that, 29 uropathogens recovered from UTI's patient were found positive for catalase test [12]. Gelatin is a protein that is derived from collagen. The gelatin molecules is too large to enter the cell as a whole, therefore the exoenzyme gelatinase cleave gelatin to polypeptides and then further degrades polypeptide to amino acid which are taken up and utilized by the cells.

In a similar study, 20 isolates were screend for gelatinase production, most of them lack gelatinase enzyme. Thus it can be concluded that majority of the urinary isolates show negative results for gelatinase production [13].Casease is an exoenzyme that is produced by some bacteria in order to degrade casein. If the organism can produce casein, then there will be zone of clearing around bacterial growth. Some researchers tested 30 isolates of *E. coli* for nitrate reduction and found all the isolates were negative while some investigators reported that all the bacteria isolated from urine sample were positive for this test. Thus it can be concluded that isolates show variable results for the nitrate reduction test [14].

The IMViC test stands for combination of four test including Indole, Methyl red, Voges-Proskeur and Citrate utilization test. Similarly, some investigators characterise urinary isolates by IMViC test and found variable results [15-17].

Some co-investigators reported that various bacterial isolates associated with UTI, were found as *E. coli* (66.08%); *Staphyloccous* (11.3%); *Klebsiella* sp. (9.6%); *Straptococcus* (4.34%); *Enterococcus spp.* (4.34%); *S. aureus* (1.7%); *Proteus* spp. (1.7%), and *Enterobacter* spp. (0.86%) [10, 18]. Similarly, *E. coli* recovered from urine samples were tested for the antibiotic susceptibility with common drugs like Ampicillin, Tetracycline, Norfloxcin, penicillin, Cephalosporin and found that the high sensitive pattern of common drugs like Tetracycline (22%), Norfloxacin (18%) followed by Moderate sensitive pattern of common drugs like Ampicillin (14%), Cephalosporin (10%) and high Resistant pattern of common drugs like Penicillin (22%), Cephalosporin (18%) [19-21].

In similar study it was reported that the breadth of multidrug resistance among the urinary isolates varied. They employed this study by using ampicillin, fosfomycin, chloramphenicol, tetracycline, and three aminoglycosides (kanamycin, gentamicin, and streptomycin). Of these isolates, 30% offered multidrug resistance to three or more agents. Among multidrug resistant isolates, 100% were resistant to ampicillin, 47% to streptomycin, 41% to chloramphenicol, gentamicin and tetracycline, and 35% offered resistance to kanamycin while only 6% showed resistance to fosfomycin [22, 23].

5. CONCLUSIONS

Urinary tract infections (UTIs) are commonly encountered diseases by clinicians in developing countries with an estimated annual global incidence of at least 250 million. Many different microorganisms can cause UTIs though the most common pathogens are *Escherichia coli*, followed by *Staphylococcus, Proteus, Pseudomonas, Klebsiella* and other *Enterococcus sp.* in the recent study total of 20 morphologically different bacterial isolates were recovered from 50 different urine samples. The prevalence of the isolates was varied with *E. coli* (40%), followed by *Staphylococcus* sp., *Pseudomonas* sp. and *Klebsiella* sp. each of (20%). All the isolates were tested for antibiotic sensitivity pattern against common drugs like Ampicillin, Tetracycline, Chloramphenicol, Vancomycin, Nitrofurontione and Ciprofloxacin each of disc potency 30 mcg.

It was found that the high sensitive pattern was found towards Nitrofurontoine (75%) followed by Tetracycline (55%), Ciprofloxacin (45%). Moderate sensitive pattern was shown by Ampicillin (45%), Chloramphenicol and vancomycin (40%). vancomycin (60%), Chloramphenicol (45%) and tetracycline and Ciprofloxacin both (30%) shows resistant patterns. Therefore the drug like Nitrofurontoine, Tetracycline and Ciprofloxacin are the

effective drugs against the common UTI isolates. 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 that in turn leaves the clinicians with very few alternative options of drugs for the treatment of UTIs. As drug resistance among bacterial pathogens is an evolving process, routine surveillance and monitoring studies should be conducted to provide physicians knowledge on the updated and most effective empirical treatment of UTIs.

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