AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Isolation and Characterization of UTI Causing Bacteria from Local Population of Mansehra

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ABSTRACT

This study investigate uropathogenic species isolation and characterization in the population of Mansehra. The different physical chemical and microscopic properties of urine samples were examined. Isolated uropathogen organism were selected for antibiotic suceptibility and resistance pattren. Thirty seven clinics were visited to collect the data regarding the UTI patients among these nine Bacterial species were isolated from 32 urine samples which included bacterial species *Escherichia coli*, *Enterrobacter spp.* and *Staphylococcus aureus.* Different biochemicals tests were used for identification. The confirmed isolated bacteria were also tested against selected antibiotics for sensitivity and resistant strains. The result of present investigation revealed that UTI is most common infection among local population of Mansehra. The efficacy of antibiotics calculated and found that Ciprofloxacine, Chloramphenicol, Norfloxacine were sensitive against all isolated bacteria while Cefixime, Cefipime, Linkomycine, Sulfamethaxazole trimethoprim and Nitrofurantion were resistant while Tobramycine and Pipperacilline Tazobactum were intermediate. The efficacy of some antibiotics is also questionable because the efficacy of these antibiotics were found resistant and it was also found that common strains of UTI causing Bacteria were resistant against prescribed antibiotics in District Mansehra.

Keywords: UTI, *E.coli*, antibiotics, Resistance, uropathogen

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Received 06 August 2014, Accepted 28 August 2014

Please cite this article in press as: Muhammad L *et al*., Isolation and Characterization of UTI Causing Bacteria from Local Population of Mansehra American Journal of PharmTech Research 2014.

INTRODUCTION

The urinary tract is the body's drainage system used for removing wastes and extra water. It includes the organs which collect, store and excrete urine from the body.[1] Its infections most prevalent in extra-intestinal bacterial infections. UTI represents one of the most common diseases encountered in medical practices affecting people of all ages from the neonate to the old age group.[2] About 150 million people Worldwide are diagnosed with this infection every year. In United State about 8.3 million people have Urinary tract infection and are the second-most common site for infection.[3] Most infections were caused by retrograde ascent of bacteria from the fecal flora via the urethra to the bladder and kidney especially in the females.[4] Urinary tract infections are second most common type of infection in the human acounting about 8.1 million visits to health care providers each year.[5] Women are easily prone to UTIs due to shorter urethra which allow bacteria easily penetrate to the bladder and urethral opening. Women have lifetime risk of UTI which is 50 percent more than men.[6] An infection in urethra and bladder are called urethritis and cystitis, respectively. Bacteria may travel up the ureters to multiply and infect the kidneys called pyelonephritis.[7] The major groups of UTIs causing bacteria include Gram-negative bacilli and Gram-positive cocci. *Escherichia coli* (*E. coli*) was the major bacterial strain that cause 80% of UTIs while other gram negative *Klebsiella pneumonia, Proteus mirabilis* and *Enterobacter aerogenes* also considerable.[9] UTIs were treated conventionally with different antibiotics during acute episode and not commonly used for long term prophylaxis for individuals with recurrent infections. Commonly use antibiotics for treatment includes Trimethoprim, Sulfamethoxazole, Amoxicillin, Augmentin, Doxycycline and Fluoroquinolones. Fosfomycine trimethamine is a phosphoric acid agent effective as alternate treatment for urinary tract infection caused by enteric bacteria.[9] Drug resistance is a huge problem to treating major infetion causing diseases like malaria, tuberclosis (TB), diarreal diseases and UTIs. Goldman and Huskins in 1997[10] identified that improper and uncontrolled use of multidrug result in the occurence of antimicrobial resistance. Different kinds of resistance strains have been dicovered, as methiciline *Saphylococcus aureus* (MRSA),[11] *Pseudomonas aeruginosa*[12] and *Serratia marcescens* are multidrug resistance (MDR) vancomycin resistant enterococci (VRE) [13] and extended spectrum beta lactamase (ESBL) resistant enterococci.[14] In this study include isolation and identification of UTI causing bacteria from urine samples which were collected from different areas of District Mansehra. Also study their comparative biochemistry of urine samples of infected patient and in-vitro trials for sensitivity/ resistance of commonly used antibiotics against isolated bacterial strains.

MATERIALS AND METHODS

Study Area

The present study was carried out in District Mansehra, Pakistan. The incidence of UTI is obvious in many areas of Mansehra. The isolation and characterization of UTI causing bacteria was therefore necessary for future outbreak of UTIs in Mansehra.

Preliminary survey

A preliminary survey was conducted to find out the most common infections in District Mansehra. The data regarding the occurrence of UTI was collected from different areas of the district including Shinkiary, Bafa Doraha, Mansehra city, Chennai, Township and Oghi. Dunken multiple test was applied to find out the most common infections and antibiotics used against different types of infections. Frequency of infections was calculated along with percentage.

Sample collections

Total 32 midstream samples were collected from different Labs of Mansehra in sterile bottles from male and female patients of age group 18-60 years and transported to Microbiology laboratory of Hazara University for analysis.

In- Vitro examination of Urine samples

Physical, chemical and microscopic analysis were done by Routine Examination (RE) test. Samples were chemically examined by pH, protein and glucose level. With microscope pus cells, RBC's, epithelial cells, oxalate and phosphate crystals were identified. Samples had >10-15 pus cells were severe UTIs infected. The samples were stored at 4 ^{0}C and used dilutions according to Farooq, 2006.[15]

Preparation of Culture Media

Different culture media were used as, Nutrient agar, MacConkey Agar, Mannitol Salt Agar, Trypton Soya Broth, Methyl Red Voges Proskauer (MRVP) and Siemon's Citrate Agar for the isolation, growth and identification of bacterial species. The ingredients of respective media were taken in separate conical flasks and mixed thoroughly by vigorous shaking by using international criteria for Media preparation.[16] The equal amount of media (20ml/petri plate) was poured in each petri plate and allowed to cool and solidify. Some slants of media were also made in test tubes. The petri plates having poured media were placed in incubator at 30°C for 24 hours to check the microbial contamination in the petri plates.

Microbiological analysis

Primary isolation was done on Nutrient Agar to allow the growth of all bacteria from the urine.

Three fold serial dilutions were made of each sample and 1 ml of the appropriate dilutions was used as inoculum using the streake plate method.[17] Agar plates were incubated at 37°C for 24-48 hours for growth.

Pure Cultures

After counting the colonies on Nutrienr Agar, pure culture was made in order to differentiate the colonies on the basis of morphology and biochemical properties. Two different media were used as MacConkey Agar for G-ve bacteria and Mannitol Salt Agar (M.S.A) for G+ve bacteria. Colonies taken from difrential agar inoculated on both MacConkey Agar and Mannitol Salt Agar then incubated at 37 ^{0}C for 24-48 hours.

Biochemical Identification

Bacterial isolates were characterized on the basis of morphology, reaction to Gram's stain, motility test [18] and biochemical tests as Catalase test, Cougulase Test, IMVIC test, Indole, Methyl Red and Voges-Proskauer and The Citrate Test.

Evaluation of antibiotics (Disc Diffusion Assay)

The Kirby-Bauer Antibiotic Sensitivity test method was used for determining the antibiotic resistance in bacterial isolates. UTIs isolates confirmed by biochemical analysis were subjected to disc diffusion assay. Antibiotics used were Nitrofuratoin (F 300 ug), Norfloxacine (NOR 10 ug), sulfamethoxazole + Trimethoporim (SXT 25 ug), Choloramphenicol (C 30ug), ciprofloxacin (CIP 5ug), Tobramycine (TOB 30ug), Linkomycin (MY 10ug), Cefixime (CFM 5ug), Piperacilline Tazobactum (TZP 30ug) and Cefipime (FEP 300 ug). Inoculum was prepared from freshly grown culture on Nutrient Agar was inoculated in 1ml Phosphate Buffer Saline (PBS) made 0.5% McFarland standard. Antibiotic discs were placed on plates using sterile forceps and incubated for 24 hours at 37^0 C. On the basis of zone of inhibition around each antibiotic disc the isolates were classified as resistant, intermediate and sensitive by comparing the diameter values.[19]

RESULTS AND DISCUSSION

Common infections of District Mansehra

During present investigation a preliminary survey was conducted to find out the most common infections in District Mansehra. After a comprehensive survey it was found that Urinary Tract Infection, Typhoid, Gastero intestinal tract, Respiratory tract infections, Brucellosis, Enteric Fever, Pneumonia, Tuberculosis as shown in Figure. 1, Scabies, Malaria and Hepatitis are the common infections in Mansehra. The number of patients having a particular infection was counted and the

percentage of an infection was recorded on the base of average among all patients. The results of this survey are given in Figure. 1.

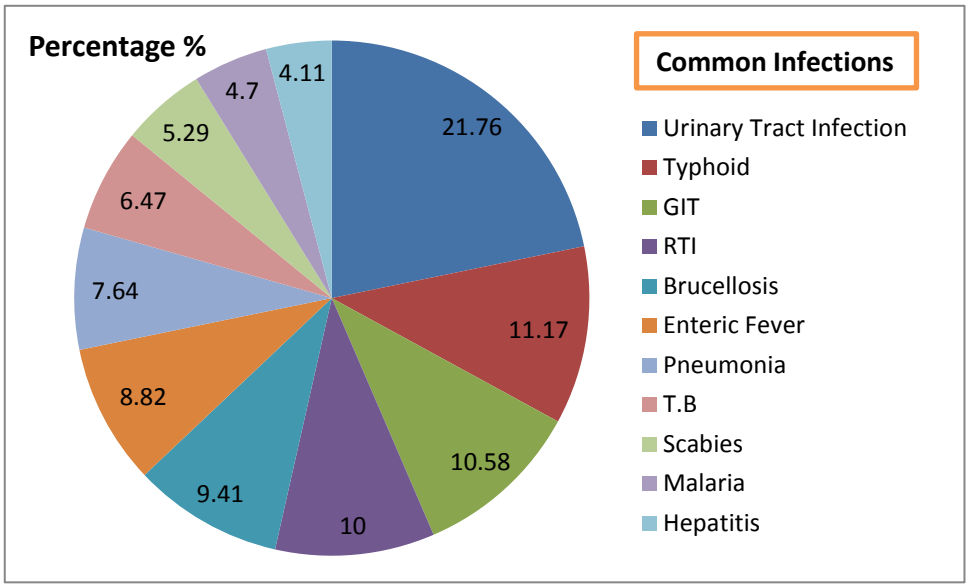

Figure.1. Percentages of different infections in Mansehra during March, 2012- July 2012

Laboratory analysis of urine samples

After collection all urine samples were examined for different physical, chemical and microscopic properties. The color, turbidity, pH, specific gravity, protein, glucose, pus cells etc. it has been found that in all samples the characteristics were different as compared to normal urine samples properties. For the specific identification of bacterial species the different biochemical test catalase, coagulase, indole, mehyl red, vogous proskauer and citrate were performed. Total nine bacterial species were isolated from 32 urine samples as shown in table. 1, *E. coli*, *E. faecalis* and *S. aureus* were dominant in all the samples tested in this investigation.

Table. 1 Isolation and identification of Bacteria

Sr. No	Bacteria	Number	Percentage
1	*Escherchia coli*	9	28.12%
2	*Enterrobacter spp*	6	18.75%
3	*Klebsiella pneumonia*	4	12.5%
4	*Pseudomonas aeroginosa*	6	18.75%
5	*Proteus mirabilis*	4	12.5%
6	*Enterrococcus faecalis*	9	28.12%
7	*Staphylococcus aureus*	9	28.12%
8	*Streptococcus spps*	8	25.0%
9	*Staphylococcus saprophyticus*	6	18.75%
10	Others species	3	9.37%

Evaluation of Antibiotic Resistance

For the efficacy of selected antibiotics zone of inhibition was measured. Their categories were

made on the base of zone of inhibition in mm. The antibiotics with zone of inhibition more than 15 mm were considered as sensitive while antibiotics with zone of inhibition less than 15 mm were considered as resistant and having 15 mm were intermediate. The results of antibiotic sensitivity of grame nagative bacteria (*E. coli, Enterrobacter spp, P.aeruginosa, K.pneumonia* and *P. mirabilis*) show high sensitivity (100%) against CIP, NOR and lower sensitivity (0%) against CFM, FEP, SXT and TOB antibiotics while these strains show highest resistance (nearly 100%) against CFM, FEP, SXT and TOB where as lowest resistance (0%) against CIP, NOR antibiotics, given in figure 2. While in case of gram positive bacteria (*E.faecalis, S. aureus, Streptococcus spps* and *S. saprophyticus*) they show high sensitivity (nearly 100%) against CIP, NOR and lower sensitivity (nealy 0%) against CFM, FEP, SXT and TOB antibiotics while these strains show highest resistance (nearly 100%) against CFM, FEP, SXT and TOB where as lowest resistance (nealy 0%) against CIP, NOR antibiotics, given in figure 3.

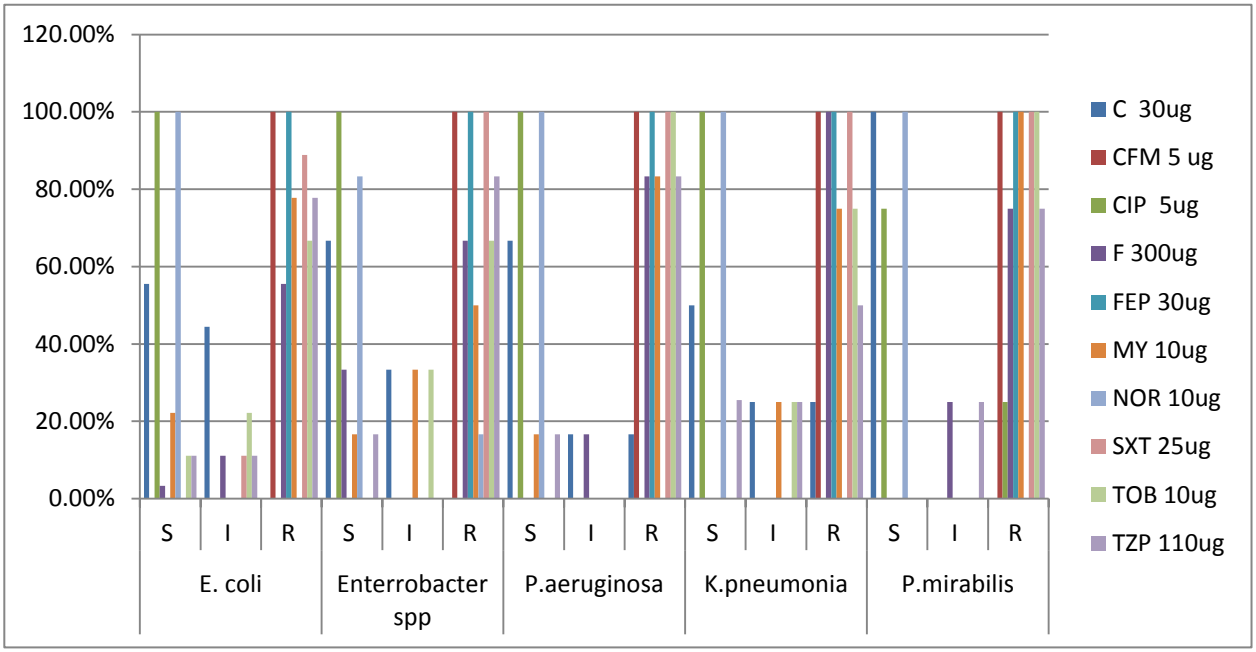

Figure 2: Antibiotic susceptibility and resistance pattern of Gram negative isolates

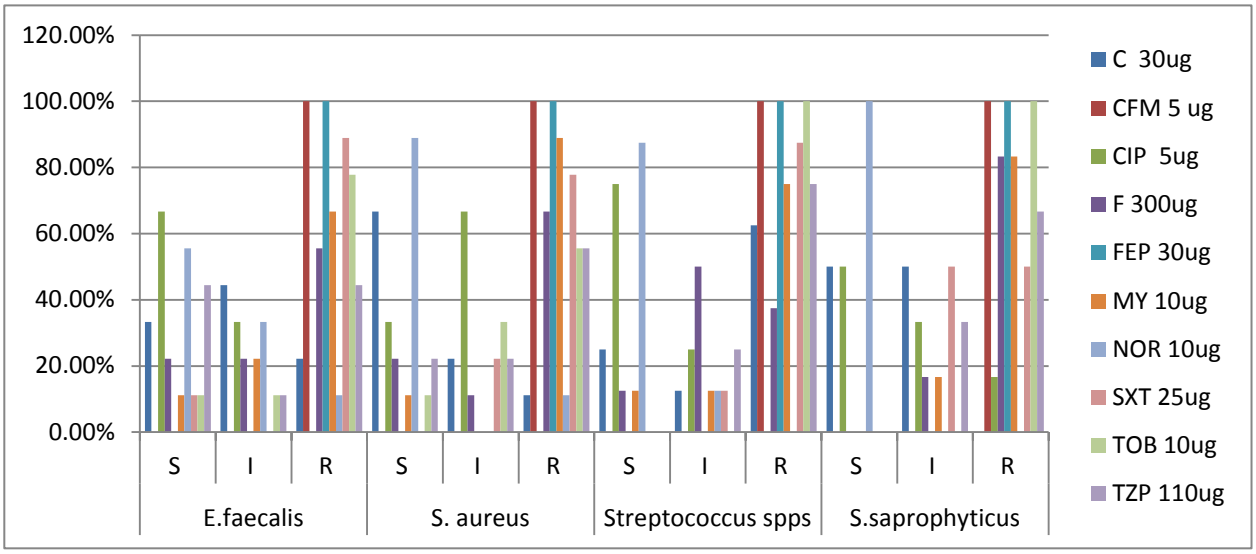

Figure 3: Antibiotic susceptibility and resistance pattern of Gram positive isolates

The results of present investigation clearly indicated that the UTI is the common bacterial infection among the local population of Mansehra and also revealed that the common utilization of different antibiotics against this infection were not sensitive but found to be resistant in many cases. The bacteria isolated were also identified common variations. Thirty two urine specimens were screened which were found positive for urinary tract infections. The study implicated nine microorganisms as possible aetiological agents of the UTI cases observed. These organisms; *E. coli, Proteus sp., Klebsiella sp., Staphylococcus sp., Streptococcus sp.* and *Pseudomonas sp.* were common causative agents of UTIs as reported.[20, 21] The prevalence reported by Smith et al.(2003) where they found out that *E. coli* accounts for 32% , *Proteus sp.* 17.25% as compare with other organisms which were *Klebsiella sp.* (13.79%), *Staphylococcus sp.* (12.07%), *Streptococcus sp.* (8.63%) and *Pseudomonas sp.* (5.17%).[22] Six of the positive cases were caused by a mixed culture of these organisms accounting for 10.34%. There was also a possible link between the prevalence of UTI among patients and the level of personal hygiene or the state of toilet facilities. Most patients examined rated the improper toilet. Antibiotics used in this study were Linkomycin, Cefixime, Cefipime, Pipperacilline tazobactum, Tobramycin and Trimethoprim sulfamethoxazole which were resistant to most of the tested antimicrobial agents. Ampicilline, tetracycline and streptomycin were commonly prescribed antibiotics in hopital even before the urine analysis for patients. This widespread and more often misuse of antimicrobial drugs led to general rise in the emergence of resistant bacterial strains. Ampicilline and cotrimoxazole resistant strain were reported in USAHigher resistant strains were reported in USA to ampicillin and cotrimoxazole.[23] Whereas few ciprofloxacin resistant strains were found in other countries.[24] This study also noticed

ciprofloxacin-resistant *E. coli* from UTIs. Ciprofloxacin as an option for therapy to UTIs has been considered, since its multiple mechanisms of action seem to have enabled it to retain potent activity against *E. coli*. Ciprofloxacin has high level of activity against UTI isolates of *E. coli* compared with other commonly used agents, such as Ampicillin and SXT.[25] Isolated UTI strains were tested for susceptibility against antibiotics, few of the antibiotics were sensitive, but most of antibiotics showed resistant to the MDR strains. Among these *E. coli*, *K. pneumoniae* and *P. aeruginosa* were highly resistance to most of the antibiotics, whereas *Staphylococcus spp*, and Serratia marcescens exhibited sensitive to Cephalexin, Cotrimoxazole, and Gentamycin. Moreover, most the UTI strains were highly resistance to nalidixic acid and SXT. Drug resistance is one of nature's never ending process by which the organisms develop tolerance to new environmental condition. It may be due to a pre-existing factor in the organisms or result from the acquired factor(s). The findings of current study coincide with the findings of Shittu and Manikandan, (2011) that *S. aureus* strains were highly resistant to naladixic acid.[26] All the isolates in this study showed resistance to at least 5 different antibiotics, indicating the presence of strong selective pressures from the antibiotics in the community. Brown et al.(2003) have reported that horizontal gene transfer is a factor in the occurrence of antibiotic resistance in clinical isolates and suggested that the high prevalence of resistance to a particular antibiotic does not always reflect antibiotic consumption as previously suggested byNwanze et al.(2007).[27, 28]Drugs like tetracycline, ampicillin and streptomycin have a relatively fair activity on isolates with 11, 11 and 10% activities respectively; though the recorded high level of resistance could be due to the cheap price and affordability of these drugs. It appears very common and closer to the students (and the general populace). The implication of this is the possibility of easy access causing self medication, misuse and abuse, leading to the development of resistance. On the other hand, Dalacine recorded a very poor performance with 1% sensitivity. Antibiotics like ciprofloxacin (Cp) at 1 mg and Gentamycin (G) at 10 mg showed excellent sensitive inhibitory actions while activity of Chlindamycin/Dalacine (D) at 10 mg is not commendable. Its inactivity in UTI cases is attributable to the fact that it was manufactured for activity against anaerobic microorganisms and not aerobes as the case is for the isolates. It is therefore not recommended.

CONCLUSION

The susceptibility and resistance profile of all isolates in this study have shown that Ciprofloxacin , Norfloxacin and Chlrophenicol possess the high efficacy while Linkomycin, , Cefixime, Cefipime, Pipperacilline tazobactum, Tobramycin and Trimethoprim sulfamethoxazole possess lower

efficacy. Despite this efficacy, there was a general increase in the resistance pattern of isolates to all the antibiotics used in this study. The present study confirms that bacterial resistance would be a greatest problem in the country. The findings of the present study confirms that still some bacteria are resistant to antibiotics especially Linkomycin, Cefixime, Cefipime, Pipperacilline tazobactum, Tobramycin and Trimethoprim sulfamethoxazole due to frequently use in District Mansehra.

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