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Screening of uropathogens and their antibiotic susceptibility

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Abstract

Urinary tract infections (UTIs) are the most prevalent infections across the world. The proper treatment of UTIs depend greatly on correct identification of the causing organisms as well as their antibiotic susceptibility pattern. The accurate diagnosis will then in turn help physicians in prescribing appropriate drugs. In this context, the present study was designed to determine prevalence and antibiotic susceptibility of uropathogens among the patients grouped on the basis of their gender and age. This is a retrospective study taking into account 130 urine cultures performed at a private hospital in Delhi out of which 97 samples were found positive with prominent bacterial growths. The culture tests showed 6 morphologically and biochemically distinct bacterial isolates out of which *Escherichia coli* (61 isolates) was the most frequently occurring bacteria followed by *Klebsiella pneumonia*e (17 isolates), *Pseudomonas aeruginosa* (7 isolates) and *Proteus mirabilis* (6 isolates) corresponding to 54, 13, 5 and 5%, respectively. It was observed that occurrence of uropathogens was greater in females and adult patients when compared to males and children, respectively. Further, sensitivity pattern of four major bacterial isolates was studied against different antibiotics and it was observed that all bacterial isolates are highly sensitive towards piperacillin tazobactum, amikacin, levofloxacin and gentamicin. The results also showed an alarming trend where the uropathogens under study exhibited high degree of resistance against ceftazidime, amoxicillin, ampicillin and cefotaxime. The observed trend of antibiotic resistance among bacterial isolates may attribute to the unprescribed and unregulated use of multiple antibiotics in the community.

Key words: - UTIs, uropathogens, antibiotic susceptibility, antibiotic resistance.

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1. Introduction

Urinary tract infections (UTIs) are the most common bacterial infections prevalent worldwide. Although most UTIs are mild and can be resolved by administering suitable antibiotics, more severe infections can be distressing, resulting in bacteriuria, sepsis and death [1]. In most cases of UTI (~ 95%) bacteria multiply at the opening of urethra and travel up to the bladder causing the infection. In rare cases, bacteria reaches kidneys from the bloodstream. UTIs can be categorized on the basis of anatomical and functional status of the urinary tract where uncomplicated UTIs correspond to the infection of structurally and functionally normal urinary tract e.g. acute cystitis and acute pyelonephritis [1, 2]. On the other hand, complicated infection refers to the infection of abnormal urinary tract (structurally or functionally), thus increasing the chances of acquiring bacteria e.g., prostate enlargement, renal calculi, diverticulitis [2]. There is a direct correlation between the host age and prevalence of UTIs with significantly high chances of the infection in older men and women [2, 3]. Male neonates during early months (2-3 months) of birth are more susceptible to UTIs [3]. Undoubtedly, *Escherichia coli* are the primary uropathogens causing nearly 80% of the infections but the involvement of other pathogenic bacteria such as *Klebsiella spp.*, *Enterobacter spp.* and *Proteus spp.* cannot be denied [1, 3]. One of the microbial agents responsible for conferring UTIs is Gram positive cocci which are gaining worldwide significance. Pathogens associated with UTIs are well known for their ability to form biofilm. Structural as well as functional abnormalities, foreign bodies, metabolic defects, impaired immunity, urological surgery and instrumentation act as major predisposing factors for the outbreak of UTIs [1 – 3]. Indwelling catheter use with duration of catheterization is the key risk factor which constitutes large proportion (upto 40%) of the healthcare-associated infections [5, 6]. Antimicrobial resistance (AMR) among major pathogens is spread worldwide across different geographical regions and is now considered as

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a serious threat from the prospective of global health [7, 8]. In the developing countries with high levels of poverty, illiteracy and poor hygienic practices, there is high circulation of fake and spurious drugs having questionable quality [9]. In majority of the cases, antibiotics are given empirically without actual lab based diagnostics. Therefore, it is mandatory to carry out area specific empirical studies to document the microorganisms causing urinary tract infections (UTI) and their antimicrobial susceptibility that would in turn help in the effective treatment [10]. Keeping in mind rapidly increasing UTIs and the damages caused by them, the current study was designed with primary objective of identifying most prevalent uropathogens in Nort-West Rohini, New Delhi (India) and evaluating most suitable antibiotics to eradicate them.

2. Methodology

2.1. Study design

The present study deals with UTI cases attending a private hospital in North-West Rohini, New Delhi (India) from January to June 2019. A total of 130 midstream urine samples were collected from study subjects in sterile containers. It was ensured that subjects have not taken antimicrobials within previous 15 days. The collected urine samples were examined for their color, pH and appearance and then processed to isolate uropathogens and test their antibiotic susceptibility.

2.2. Bacterial isolation and identification

The uropathogens were isolated by surface streaking the collected urine samples on MacConkey agar, nutrient agar and blood agar and incubating at 37°C for 24 h under aerobic conditions. The isolated bacterial strains were subjected to macroscopic and microscopic characterization to examine color, size, elevation, margins, texture, shape, arrangement and cell wall composition [11, 12]. The morphologically distinct colonies of bacteria were further identified using biochemical tests namely catalase, oxidase and urease [11, 12].

2.3. Antibiotic sensitivity testing of the isolated bacterial strains

The antibiotic sensitivity testing of isolates was carried out for all the isolated uropathogens using disk diffusion as per guidelines issued by Clinical Laboratory Standards Institute (CLSI) [11]. The antibiotic discs used in this study gentamicin, penicillin, amikacin, chloramphenicol, piperacillin-tazobactam, meropenem, cefixime, ceftriaxone, amoxicillin and levofloxacin were procured from HiMedia, India. Standard inoculum of all the isolates adjusted to 0.5 McFarland

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was swabbed onto Muller-Hinton agar (HiMedia, India). The antibiotic discs were placed gently on agar surface after drying plates for 5 min and incubated at 37°C for 24 h.

3. Results and Discussion

3.1. Prevalence of uropathogens

For this retrospective study, area of North-West Rohini in New Delhi, India was selected keeping in my high prevalence of UTIs in the locality. A total of 130 urine samples were collected from the patients visiting a secondary care hospital of Rohini in New Delhi (India) during the period of 1st January to 29th April, 2019. The data showing incidence of uropathogens in male and female patients has been summarized in Fig. 1. The total number of female samples were 81 out of which 75.3% of females exhibited urinary infections. On the other hand, number of male samples was 49 where 73.4% were found to be infected. The incidence of UTIs among different age groups was also determined. The results showed that UTIs were more predominant in adults with 74% infected samples followed by 26% infected samples in children. Further analysis of the collected samples revealed 4 morphologically distinct isolates with *Escherichia coli* (50.7%) being the most abundant bacterial strain followed by *Klebsiella pneumoniae* (13.07%), *Pseudomonas aeruginosa* (5.38%) and *Proteus mirabilis* (4.6%). The incidence of uropathogens in this study is well supported by another study carried out by Beyene and Tsegaye [11] which showed *E. coli* (33.3%) and *K. pneumoniae* (19%) as the significant bacteria and *P. mirabilis* (4.8%) as minor bacterium detected in the collected urine samples. *E. coli* has been reported to be the major aetiological agent that causes UTIs in nearly 90% of the cases irrespective of the gender [13 -16]. However, our findings for second most abundant bacteria *K. pneumoniae* were different from other reports by [17, 18] where *Staphylococcus* spp. is the second most frequent bacteria in urine samples.

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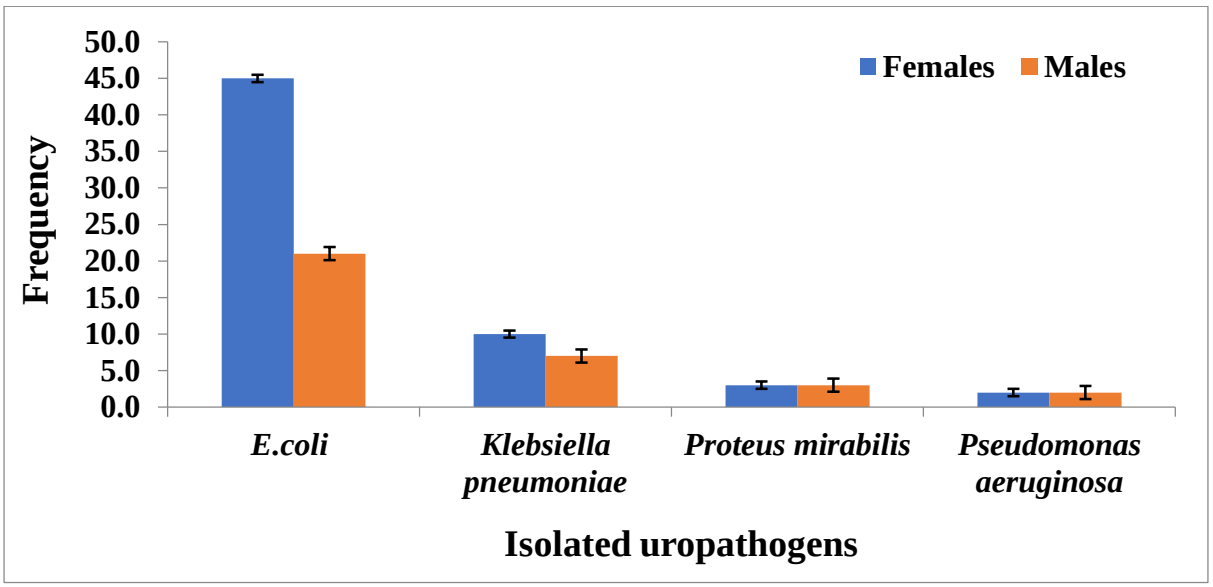

Fig. 1. Incidence of uropathogens in males and females.

3.2. Macroscopic and microscopic identification of the isolated uropathogens

The macroscopic as well as microscopic characteristics of the isolated uropathogens have been summarized in Table 1. It was observed that *E. coli* and *K. pneumoniae* appeared as small convex colonies on nutrient agar whereas *P. aeruginosa* showed large opaque colonies and *P. mirabilis* exhibited swarming white colonies. On MacConkey agar, *E. coli* and *K. pneumoniae* were found to be lactose fermenters, however, *P. mirabilis* and *P. aeruginosa* showed no signs of lactose fermentation. Further, *E. coli* and *P. aeruginosa* exhibited β-hemolysis on blood agar whereas *K. pneumoniae* showed ϒ-hemolysis. *P. mirabilis* on the other hand appeared as wavy swarming growth on blood agar. The observations on different media types for the bacteria under study were identical to what has been reported in literature [12, 14].

Table 1. Microscopic characteristics of isolated uropathogens

Isolate	Nutrient agar	MacConkey agar	Blood agar	Microscopic examination (Gram staining)
Escherichia coli	Convex, small colonies	Lactose-fermenting, shiny, rose pink	Shiny, opaque, creamy, β-hemolytic	Gram Negative Rods

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Klebsiella pneumonia	Convex, small mucoid colonies	Pink, colony, large, glistening and mucoid	Ɣ-hemolysis, hemolysis, grey color	Gram Negative Rods
Proteus mirabilis	Spreadable, swarming white to creamy colony	Lactose-non fermenting, colorless colony	Swarming spread film growth white to grey	Gram Negative Rods
Pseudomonas aeruginosa	Large, opaque, produce diffusible pigment	Flat, blue-green diffusible pigment, feathery	β-hemolysis Grayish colonies, oblique lighting	Gram Negative Rods

3.3. Biochemical testing

The biochemical characterization of the isolates has been summarized in Table 2. It was observed that all the isolates were catalase positive and oxidase negative. Further, *K. pneumoniae* and *P. mirabilis* were found to be exhibiting ureolytic activity whereas *E. coli and P. aeruginosa* showed negative results for urease test.

Table 2. The biochemical characterization of the isolates

Isolate	Catalase	Oxidase	Urease
Escherichia coli	Positive	Negative	Negative
Klebsiella pneumoniae	Positive	Negative	Positive
Proteus mirabilis	Positive	Negative	Positive
Pseudomonas aeruginosa	Positive	Negative	Negative

3.4 Antibiotic susceptibility testing of the isolates

Since resistance to antimicrobial agents is an increasing global problem, antibiotic susceptibility studies are need of the hour to combat with emerging resistant uropathogens [19]. In the present study, antibiotic susceptibility testing of all four isolates was carried out and results from the same are presented in Fig. 2 – 5. It was observed that *E.coli* is highly sensitive to amikacin (98%), pipercillin-tazobactam (83%) and spectinomycin (75%), however, very high degree of resistance was observed towards ampicillin (100%) and ceftazidime (100%) indicating the ineffectiveness of these antibiotics to treat *E. coli* infections (Fig. 2).

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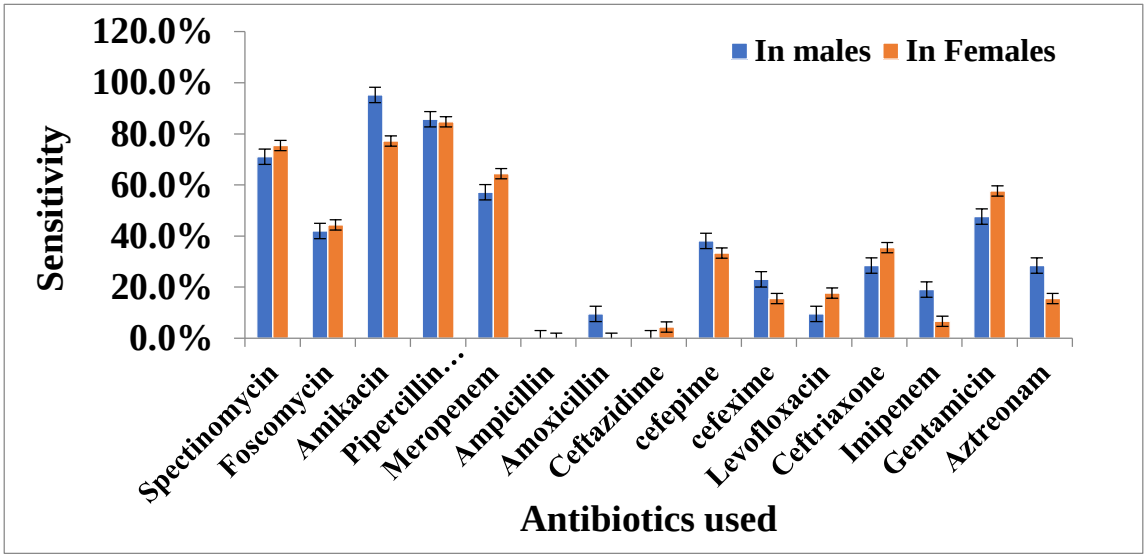

Fig. 2. Determination of antibiotic sensitivity of *E.coli* in males and females

The results for antibiotic susceptibility testing of *K. pneumoniae* showed that strain is highly sensitive to Doripenem (100%), Amikacin (89%) and Gentamicin (85%) whereas high degree of resistance of the stain towards ampicillin (100%) and amoxicillin (100%) was observed (Fig. 3).

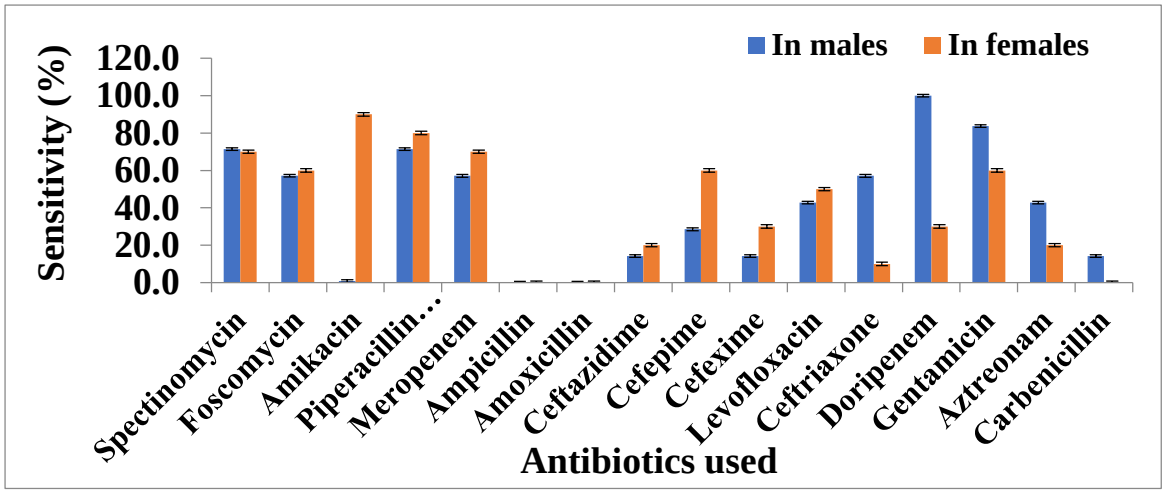

Fig. 3. Determination of antibiotic sensitivity of *Klebsiella pneumoniae* in males and females

P. aeruginosa showed high sensitivity to piperacillin-tazobactam (100%) followed by cefepime (89%) and gentamicin (85%) and it showed very high degree of resistance to

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amoxicillin (100%). Antibiotic susceptibility of *P. aeruginosa* in males and females has been summarized in Fig. 4.

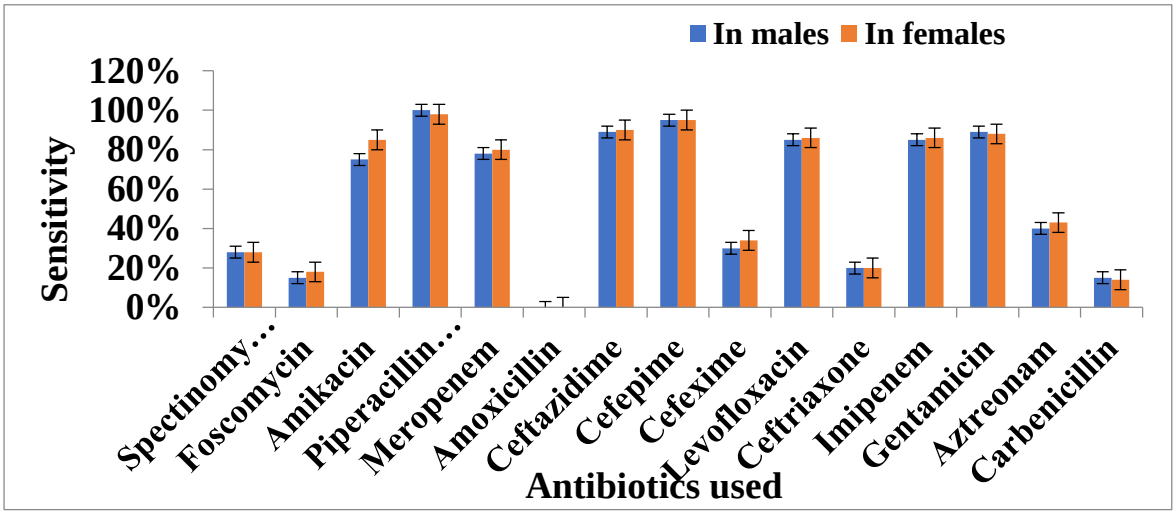

Fig. 4. Determination of antibiotic sensitivity of *Pseudomonas aeruginosa* in males and females

P. mirabilis showed high sensitivity to cefepime (95%), Piperacillin tazobactum (95%) followed by meropenem (85%), Levofloxacin (82%) and it showed very high resistivity to ampicillin (100%) and amoxicillin (98%). Antibiotic susceptibility of *P. mirabilis* in males and females has been summarized in Fig. 5.

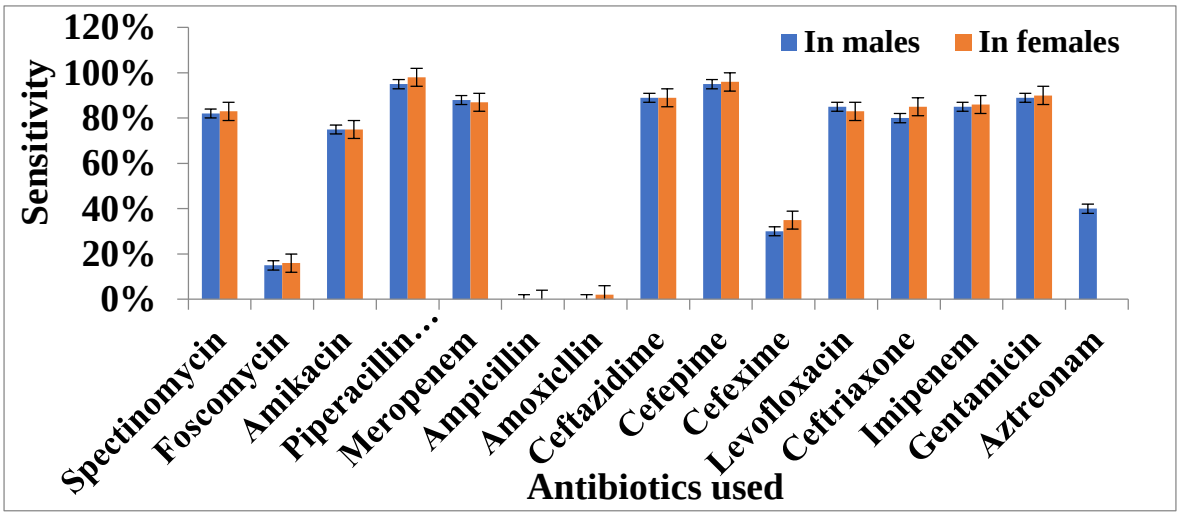

Fig. 5. Determination of antibiotic sensitivity of Proteus *mirabilis* in males and females

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There have been several reports in literature addressing antimicrobial susceptibility of uropathogens that proved helpful in providing options for empirical treatment of UTIs [14, 17, 20, 21, 22]. The current study is also an effort in this direction to determine most prevalent uropathogens in a specific community and suggest better empirical treatment for their associated infections.

Conclusions

Since drug resistance is an evolving phenomenon of pathogens, routine surveillance and monitoring is of utmost importance to equip physicians with most effective empirical treatment of UTIs. The present study conducted in this context revealed *E.coli* and *K. pneumonia* as the two most prevalent uropathogens in the patients visiting a private hospital in North-West Rohini, Delhi (India) which were found to be inhibited effectively by piperacillin-tazobactam, amikacin, spectinomycin, doripenem and gentamicin thus suggesting these antibiotics as a first line of treatment in patients effected with UTIs in the area of study.

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Through this section, we acknowledge the contribution of every contributing author. The first author contributed by performing lab experiments and data generation. The results were compiled and framed into manuscript by second author. The corresponding author was involved in designing experiments, analyzing results, guiding first and second author, in writing and polishing the manuscript.

Conflict of interest

The authors declare no conflict of interest among themselves.

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