

Prevalence and Antibiogram of Bacterial Uropathogens of Urinary Tract Infections from a Tertiary Care Hospital of Bangladesh

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Abstract

Urinary tract infections (UTIs) are one of the most frequently occurring infections majority of which are caused by multi-drug resistant (MDR) uropathogens. Hence, the present study was designed to find out the prevalence of bacterial pathogens causing UTIs and to determine their antibiotic resistance patterns against different classes of antibiotics. Clean-catch midstream urine samples were collected from 200 UTI patients of different sex and age groups. The uropathogens were isolated using Hi-Chrome UTI agar, Blood agar, MacConkey agar and then subjected to antibiotic susceptibility analysis against nine antibiotics of different classes using Kirby-Bauer's disc diffusion method. From 55.08% positive samples, it was found that females were more prone to UTIs than males and in both cases; the prevalence rate was higher in the age group 21-40 years (33%). Among the uropathogens, *E.coli* was the predominant etiological agent (57.38%) followed by *Enterococcus* sp. (36.06%), *Pseudomonas aeruginosa* (3.28%) and *Staphylococcus aureus* (3.28%). The pathogens showed remarkable amount of sensitivity against Gentamicin and Ciprofloxacin. The present experiment can be helpful for the clinicians in finding proper drugs in the developing countries like Bangladesh where multi-drug resistance problem has just complicated the treatment of UTIs.

Keywords: Urinary tract infections; Uropathogens; Antibiotics; Multi-drug resistance.

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1. Introduction

Urinary tract infection (UTIs) is defined as the invasion of pathogens to the urinary tract tissues extending from the renal cortex to the urethra which includes prostate, urinary bladder, kidney [1]. UTIs is the second most frequently occurring infections especially in

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female after respiratory tract infection which is 35% in hospitalized patients [2]. It is estimated that there are about 150 million urinary tract infections per annum worldwide [3]. UTIs is considered if there is presence of $>10^5$ cfu/mL bacteria or of a single strain of bacteria per mL in two consecutive midstream samples of urine [4]. It can occur in both the lower part (Cystitis) and the upper part (Pyelonephritis or kidney infection) of the urinary tract, and also varies according to age and sex [5]. The syndrome may be ranged from asymptomatic bacteriuria to perinephric abscess with sepsis or even death. Some factors which are associated with UTIs and accelerate the chance of increasing the infection are catheterization, pregnancy, sex, age, kidney tumors, neurological disorders, urethral structures, immune-suppression, enlargement of prostate, congenital/acquired anomalies of bladder, poor personal hygiene, obstruction of urinary tract, spermicidal contraception, sexual contraception, diabetes mellitus etc.

Among uropathogens, *E.coli* is the predominant etiological agent of UTIs [6]. The other gram negative pathogens causing UTIs are *Klebsiella spp.*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. However, *Enterococci* and coagulase negative *Staphylococci* are the gram positive bacteria most commonly responsible for UTIs [7]. Anaerobic organisms are rarely present in the urinary tract [8].

For proper diagnosis of UTI clinical signs, symptoms and urinalysis results play a vital role. As the main causative agent of UTIs is bacteria, the best choice for its treatment is use of antibiotics. Interestingly, uropathogens can change their physiologic features from time to time and place to place to induce multi-drug resistance [9]. The increase in multi drug resistance (MDR) among uropathogens is a global public health problem [10]. The Infectious Disease Society of America (IDSA) identified uropathogens called “ESKAPE pathogens” which include *Enterococcus faecium*, *S. aureus*, *Klebsiella spp.*, *Acinetobacter spp.*, *Pseudomonas spp.*, and *Enterobacter spp.* for new effective therapies [11]. In developing countries like Bangladesh, increased antibiotic resistance can be attributed to antibiotic abuse because in most of the cases, the antimicrobial treatment is initiated before the laboratory results; incomplete and under use of medications; and widespread practice of feeding livestock with low levels of antibiotics to promote growth [12]. For the empirical treatment of UTIs, it is highly important to have knowledge about the etiological agents of UTIs and also about their susceptibility to commonly prescribed antibiotics.

Therefore, the present study was aimed to finding out the bacterial uropathogens responsible for UTIs and to determine the antibiotic susceptibility pattern of the selected urinary cultures which may help the clinicians to choose the right empirical treatment.

2. Materials and Methods

2.1. Study area and population

The protocol for the study was approved by the individual Hospital’s Ethics Committee. The study was carried out in a tertiary care hospital (Al-Razi Islamia Hospital (Pvt) Ltd.) of Dhaka City, Bangladesh from October, 2015 to March, 2016. Two hundred clinical

samples were collected from different age and sex groups, among which, 59 samples from male and 141 samples from female patients.

2.2. Chemicals and media

HiChrome UTI agar, blood agar and MacConkey agar were used for the selective isolation and identification of microorganisms causing urinary tract infections and Mueller-Hinton Agar (MHA) was used for antibiotic susceptibility test of the selected urinary cultures. All the media and standard antibiotic discs were purchased from Himedia Laboratories, India and Oxoid Ltd., UK, respectively.

2.3. Sample collection

The clean catch midstream urine was collected from clinically suspected patients following the CLSI guidelines [13]. Urine samples were collected up to the fill line in clean sterile 50 mL screw-capped amber colored universal containers of wide base and an opening of at least 4 cm. Buffered boric acid (20 g/L) was added to the urine samples to prevent false positive culture or bacterial over growth if sample processing was delayed for more than 2 h [14]. Verbal informed consent was obtained from all patients and they were advised on how to collect proper sample in sterile container aseptically prior to collection. Urine samples were collected before the start of antibiotic therapy and the patient who had taken antibiotic was not included in the study.

2.4. Isolation and identification of uropathogens

Bacterial identification was done by phenotypic examination of the culture and biochemical characteristics on selective media specific for uropathogens. 100 μ L of urine samples were inoculated onto sterilized and solidified HiChrome UTI agar, blood agar and MacConkey agar media followed by incubation at 37°C for 24 h aerobically. After incubation, the colony number was counted for the diagnosis of UTI. If the number of colonies were greater than $>10^5$ cfu/mL, it was considered as positive culture and recorded as “significant growth” whereas in the case of $<10^5$ cfu/mL, it was considered as “non-significant growth” [5]. The culture plates were examined macroscopically to record the appearance, size, color, and morphology of the colonies.

2.5. Antimicrobial susceptibility test

Antibiotic susceptibility pattern of the selected bacterial isolates against nine antibiotics of different classes as shown in Table 1 were done by Kirby-Bauer agar disc diffusion method [15]. Briefly, bacterial cultures were grown in Luria-Bertani (LB) broth (Difco, USA) for 24 h at 37°C and inoculum suspension was adjusted to 0.5 McFarland standards (1.5×10^8 cfu/mL) to maintain uniform cell density. A sterile cotton swab was dipped into the standardized suspension and excess suspension was removed by pressing

the swabs against the inside of the test tube walls. Then the swab was used to inoculate evenly on pre-incubated MHA plate and left for 20 minutes for the surface moisture to dry. Next, the antibiotic impregnated discs were placed on the MHA plate with a sterile forcep and incubated at 37°C for 24 h aerobically. The diameters of zones of inhibition for individual antimicrobial agents were measured in millimeter (mm). The experiment was replicated three times and the isolates were recorded as resistant or susceptible by comparing mean zones of inhibition with that of Clinical and Laboratory Standard Institute (CLSI) guidelines [16]. Standard strains of *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923 were used as control strains for interpretations of AST.

Table 1. Antibiotics used throughout the study.

Class of Antibiotics	Antibiotics
Cephalosporin	Cefuroxime (30 µg), Ceftriaxone (30 µg)
Quinolone/Fluoroquinolone	Ciprofloxacin (30 µg), Nalidixic acid (30 µg)
Aminoglycosides	Gentamicin (10 µg), Amikacin (30 µg)
Sulfonamides	Co-trimoxazole (1.25/23.75 µg)
Carbapenem	Imipenem (10 µg)
Nitrofurans	Nitrofurantoin (30µg)
β lactam inhibitor combinations	Amoxiclave (20/10 µg)

3. Results and Discussion

Urinary tract infection continues to be one of the major community acquired public health issues around the world including Bangladesh due to the ongoing emergence of multi-drug resistant uropathogens. Consequently, the empirical treatment of UTIs becomes difficult and unpredictable due to the lack of alternative effective antibiotics. As local variations exist among urinary pathogens in different geographic settings, diagnosis of UTIs and antibiogram profiling of uropathogens is an urgent for close cooperation between the physician and the microbiologist to combat against multi-drug resistant bug. Hence, the investigation of bacterial pathogens causing uncomplicated UTIs and their antibiotic susceptibility pattern has been carried out in this study.

In the present setting, a total of 200 samples (141 samples from female and 59 samples from male) of different age and sex were collected from the suspected patients suffering from UTI. Out of the 200 samples, only 55.08 % samples showed positive result for UTIs (Table 2).

It is well stated that the incidence of UTI is more common in females as compared to men which may be either due to anatomical predisposition or other host factors [17]. From our investigation, highest frequency of infection was observed in females than males which are in agreement with this generalization [18]. Among the factors contributing to increasing frequency of UTI in female, major ones include vaginal colonization with

uropathogens, sexual activity, pregnancy and obstruction [19]. However, uncomplicated UTI may also occur in men because of insertive anal intercourse or lack of circumcision or having sexual partner with vaginal colonization with uropathogenic microorganisms or lack of immunity [20, 21]. Though, majority of uncomplicated UTIs don't cause any threat to the lives and irreparable damage, still there is a risk of serious tissue damage with prevalence of bacteremia, when kidneys are involved [22].

Table 2. Percentage (%) frequency of positive culture according to sex.

Sex	No. of Samples	% frequency	No. of positive cultures
Male	59	29.5	12 (20.33%)
Female	141	70.5	49 (34.75%)
Total	200	100	61 (55.08%)

In the present study, three age groups were formed to trace the distribution of uropathogens according to age (Fig. 1). The highest significant growth (33%) was observed in the age groups of 21-40 years followed by 40 years or above (24.62%) and 0-20 years (23.53%) age groups. According to a study in 2014 [23] UTIs was more prevalent in age group of 30–45 years which correlates our findings. Another, UTIs related study in Bangladesh reported that most of the UTIs cases occurred in 21-30 years old (44%) age groups, followed by 41-50 years old (37%) age groups [24]. However, variation of UTIs occurrence within different age groups may be attributed to the hormonal changes affecting the mucosal adherence of bacteria, frequent sexual activity, use of spermicidal agents, menopause for women and prostate gland enlargement of men [25].

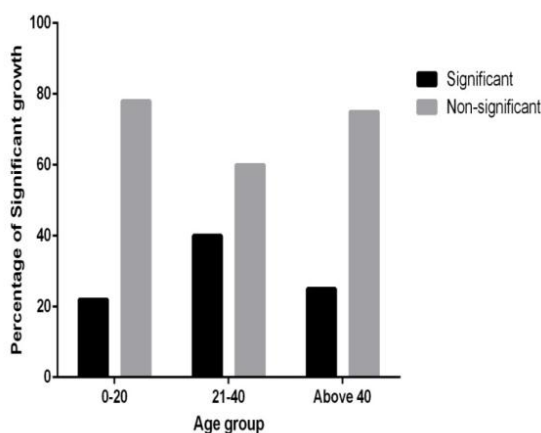


Fig. 1. Distribution of significant growth of urinary pathogens depending on age.

The organisms related with UTI were isolated and identified on the basis of colony morphology on HiChrome UTI agar, Blood agar and MacConkey agar (Table 3). Among

200 samples, *Escherichia coli* was found as the predominant uropathogen with a prevalence rate of 57.38% followed by *Enterococcus sp.* (36.06%), *Pseudomonas aeruginosa* (3.28%) and *Staphylococcus aureus* (3.28%) (Fig. 2). It was also observed that the prevalence of gram negative bacteria (60.66%) was much higher than the gram positive bacteria (39.34%) (Table 4).

The prevalence of uropathogens found in our study corroborates well with a few studies conducted in India, Pakistan and Korea [26–28]. All of those studies claimed *E. coli* as the most common cause of UTIs as also claimed from this study. The prevalence of *E. coli* as the most common uropathogen has also been found to be remarkably consistent with previous studies conducted in Bangladesh [18, 29, 30]. Our findings rightly coincided with a recent UTI related study of similar sample size which reported that *E. coli* was the predominant uropathogen followed by *Enterococcus spp.*, *Pseudomonas spp.*, *Enterobacter spp.*, *Klebsiella spp.*, *Acinetobacter spp.*, *Staphylococcus spp.*, *Citrobacter spp.*, and *Proteus spp.* [24]. The reason behind the highest prevalence of *E. coli* is that they are the normal fecal flora and possess several factors such as adhesion, pilli, fimbriae and P1-blood group phenotype receptor responsible for their attachment to the uroepithelial cells [31].

Table 3. Colony characteristics of selected uropathogens on different media.

Organisms	Macconkey Agar	HiChrome UTI agar	Blood agar
<i>E. coli</i>	Pink color (lactose positive)	Pink to Red	Non-hemolytic colony
<i>Enterococcus sp.</i>	No growth	Blue (pin headed, small)	Non-hemolytic colony
<i>P. aeruginosa</i>	Brown colonies (lactose negative)	Colorless (Large)	B-hemolytic colony
<i>S. aureus</i>	No growth	Golden Yellow	Clear B-hemolytic colony

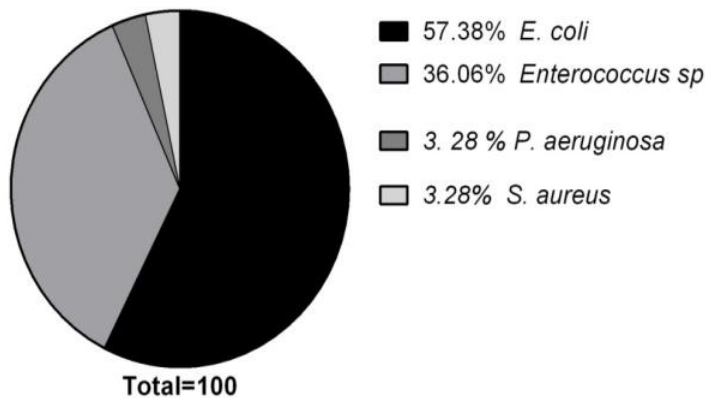


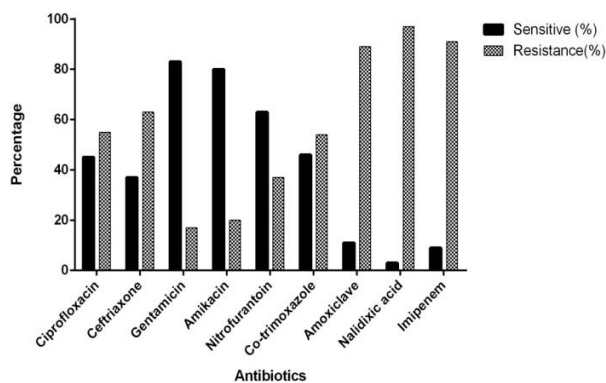
Fig. 2. Distribution of causative agents of UTIs.

Table 4. Prevalence of gram positive and gram negative bacteria in UTI.

Organisms		Percentage (%)	Total (Percentage)
Gram negative	<i>E. coli</i>	57.38	60.66
	<i>P. aeruginosa</i>	3.28	
Gram positive	<i>Enterococcus spp.</i>	36.06	39.34
	<i>S. aureus</i>	3.28	

Knowledge regarding the antibiogram profiling of the causative agents of UTI is necessary for the proper treatment. For this purpose, the isolates were examined for their susceptibility to commonly prescribed antibiotics. Nine classes of antibiotics were used viz. Quinolones/ Fluoroquinolones (Ciprofloxacin (30 µg), Nalidixic acid (30 µg)), Aminoglycosides (Gentamicin (10 µg), Amikacin (30 µg)), Carbapenem (Imipenem (10 µg)), Cephalosporin (Cefuroxime (30 µg), Ceftriaxone (30 µg)), Sulfonamides (Co-trimoxazole (1.25/23.75 µg), β lactam inhibitor combinations (Amoxiclave (20/10 µg)) and Nitrofurans (Nitrofurantoin (30 µg)).

The most common bacterial agent of UTI which is *E. coli*, showed maximum sensitivity to Gentamicin (82.86%) followed by Amikacin (80%) from the Aminoglycoside group. Previously, 94.1% and 100% sensitivity towards Gentamicin and Amikacin was reported from Bangladesh in 2014 [32]. The decreasing sensitivity of the antibiotics may complicate the empirical treatment of UTI. As the resistance of *E. coli* against B-lactam antibiotics was increasing day by day, Nalidixic acid were chosen for the treatment of UTI. But the present study showed a shocking result of only 2.86% sensitivity of *E. coli* to the antibiotics tested. 8.57% of the *E. coli* was sensitive towards Imipenem which is quite alarming as it is mostly prescribed antibiotic for the treatment of UTI now-a-days, which contradicts the findings of Jhora et al. [33] as they reported 95.02% sensitivity to Imipenem. Besides, the sensitivity towards Ciprofloxacin, Ceftriaxone, Nitrofurantoin, Co-trimoxazole and Amoxiclave were 45.71%, 37.14%, 62.86%, 45.71% and 11.41% respectively (Fig. 3).

Fig. 3. Antibiogram profiling of *Escherichia coli* (n=35).

The gram positive bacteria, *Enterococcus sp.* showed the maximum sensitivity (81.82%) towards Gentamicin whereas Amikacin, Nitrofurantoin and Ciprofloxacin were 68.18% sensitive followed by Ceftriaxone (45.45%) and Co-trimoxazole (45.45%) (Fig. 4). The lowest rate of susceptibility was found against Nalidixic acid (4.54%) followed by Imipenem (18.18%). But previous studies from Bangladesh reported about 100% sensitivity to Imipenem, 86.6% sensitivity to Nitrofurantoin and 53.33% to Gentamicin [33].

Ciprofloxacin and Gentamicin was the only effective drug against *P. aeruginosa* in the present study where the sensitivity was 100% (Fig. 5). Moderate sensitivity was observed for Amikacin (50%) and Imipenem (50%). This is quite alarming that the other antibiotics viz. Ceftriaxone, Nitrofurantoin, Co-trimoxazole, Amoxiclave and Nalidixic acid were totally useless in the present study.

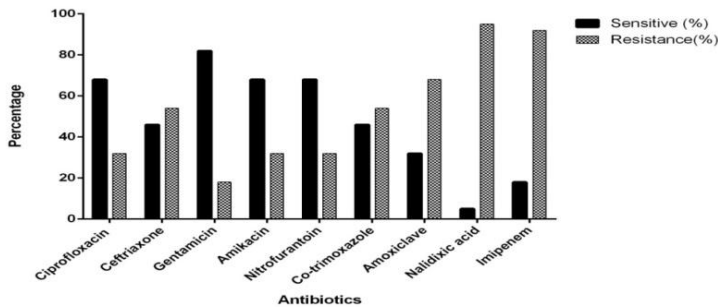


Fig. 4. Antibiogram profiling of *Enterococcus sp.* (n=22).

According to a study of Bangladesh in 2013 [34], Imipenem was considered as the most effective anti-pseudomonal drug which showed significant deviation from the findings of the present study.

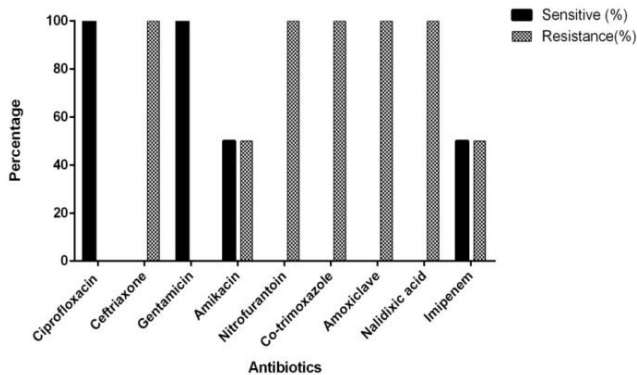


Fig. 5. Antibiogram profiling of *Pseudomonas aeruginosa* (n=2).

S. aureus also showed 100% sensitivity to Ciprofloxacin and Gentamicin like *P. aeruginosa* but showed 50% sensitivity to Ceftriaxone, Cefuroxime, Amoxiclave and Imipenem (Fig. 6). Co-trimoxazole and Amikacin were ineffective against *S. aureus* as the organism was completely resistant (100% resistant) to it. But another studies from Bangladesh reported 100% susceptibility to Amikacin and Co-trimoxazole [35].

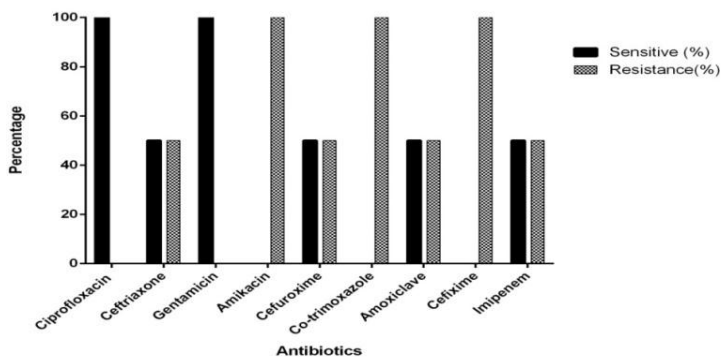


Fig. 6. Antibiogram profiling of *Staphylococcus aureus* (n=2).

In overall, it has been observed that most of the uropathogens showed remarkable amount of susceptibility to gentamicin from aminoglycoside group. In addition, amikacin was also found to be strongly effective only against *E. coli* whereas ciprofloxacin was against *P. aeruginosa* and *S. aureus*. It is very shocking to mention that, all other drugs (e.g. 3rd generation cephalosporins (cefuroxime, ceftriaxone), co-trimoxazole, amoxiclave and nitrofurantoin) were found to be ineffective against all uropathogens. Unfortunately, the drugs which found to be virtually useless against all uropathogens were nalidixic acid and imipenem, which is quite alarming for the future choice of drugs for empirical treatment of uncomplicated UTIs.

From this study, it is clear that the uropathogens are becoming resistant to the most commonly prescribed antibiotics for uncomplicated UTIs treatment. According to Haque *et al.*, extended spectrum β -lactamase (ESBL) of gram negative uropathogens help them to gain resistance against 3rd generation cephalosporins [18] whereas resistance to carbapenem antibiotic group is often due to loss of outer membrane proteins and up-regulation of active efflux pumps or production of metallo β (MBL) [36]. Major factors known to influence the evolution and transfer of multi drug resistance among microorganisms are incomplete doses, ease of access, over prescription, prescription of higher generation antimicrobials, prescribing antibiotics without laboratory results and indiscriminate use of antimicrobials in agriculture and livestock sectors [37]. As drug resistance is mainly an acquired property which can also be lost in any time [38]. For this reason, in many instances, the resistance profile of some drugs shows rises and downfalls

with course of time towards a particular pathogen, as observed when we discussed our findings with relation to previous UTIs related studies.

According to economist Jim O'Neill, who led a recent study "Tackling drug-resistant infections globally: Final report and recommendations" commissioned by the U.K. government, no fundamentally new classes of antibiotic drugs have been developed since the 1950s, and the means of diagnosing bacterial infections remains largely unchanged since the 19th century which accounts for dying 700,000 people each year from infection by drug-resistant pathogens and parasites. The report also found that if left unchecked, by the 2050 drug-resistant bacteria could kill 10 million people each year—more than currently die of cancer which knocking 2-3.5% off global GDP. In order to stop this coming plague, the report's outlined a 10-point plan like public awareness campaigns, improving sanitation and hygiene, improving global surveillance, and reducing pollution from agriculture and environment which have the potential to make a huge impact globally. As an example, the study estimates that just four countries *viz.* India, Nigeria, Indonesia, and Brazil use 500 million courses of antibiotics per year to combat diarrhea where only cleaning up water supplies could cut that figure by 60 percent [39]. Therefore, continuous surveillance is needed to track the emergence of MDR pathogens globally and proper recommendations like those of Jim O'Neil report could play a vital role in minimizing the spread of MDR properties globally.

Since the resistance pattern of the uropathogens are ever changing and continuous, dealing with a limited number of samples within a small part of the Dhaka city, Bangladesh, the present study emphasizes on the necessity of a broad-based longitudinal study that can reflect the authentic data and subsequent addition of reliable information's about the causative organisms and their antibiogram profiling which may serve as a basis for the development of the national antibiotic guideline for UTIs treatment or timely revision of the existing antibiotic guideline in response to the emerging MDR pathogens. Further studies on molecular level are required to understand the drug resistance mechanism combined with computational biology to identify potent drug target for designing novel therapeutics against MDR pathogens.

4. Conclusion

In poor resource settings where the availability of alternative effective antibiotics is limited, serious problems arise in the treatment of multidrug resistant uropathogens. This multi-drug resistance problem is not only a challenge for UTIs treatment but also for public health by threatening the lives of individuals. From this study, it has been concluded that *Escherichia coli* was the predominant uropathogens followed by *Enterococcus sp.*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The prevalence of UTIs was high in females than males and for both the incidence was frequent in the middle age groups (21-40 years). Gentamicin, Ciprofloxacin and Amikacin have been found as a reliable therapeutic intervention for the investigated uropathogens because of their broad spectrum activity in the current study. As the drug resistant pattern of the

uropathogens varies according to the geographical area and time, the selection of appropriate drug for UTIs should be assured after sensitivity pattern analysis of the urinary cultures.

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