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Incidence of Urinary Tract Infections (UTI) amongst Patients Attending Primary Health Centres in Anambra State

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Abstract

Urinary tract infections (UTIs) are one of the most prevalent extra-intestinal bacterial infections. It is a common disease encountered in medical practice affecting people of all ages, from neonate to geriatric age group. These infections are on the increase for outpatients attending Primary Health Centres in Anambra State, Nigeria, therefore the need for this study. The prevalence rate of urinary tract infection, age distribution and influence of sex were determined. Susceptibility pattern of the infectious organisms to antimicrobial agent were examined. Clean-catch midstream urine samples were collected and examined. Out of 3000 urine samples examined, 528 bacterial isolates were recovered and characterized. These include: *Escherichia coli* (24.2%), *Klebsiella* spp. (18.2%), *Staphylococcus aureus* (18.2%), *Proteus mirabilis* (9.1%), *Pseudomonas aeruginosa* (9.1%), *Enterococcus faecalis* (9.1%), *Citrobacter intermedius* (6.1%) and *Staphylococcus saprophyticus* (6.1%). More females (52%) were infected than males (48%) and in both sexes, the highest incidence was found amongst the age group, 26 - 38 years. Gram negative rods had the highest incidence in both sexes. Among the towns in Anambra state, Umunya in southern province was observed to have the highest incidence rate of UTI. Susceptibility test of the bacterial isolates to antimicrobial agents showed that *Staphylococcus aureus* was sensitive to Cephalexin, Penicillin V, Erythromycin and Gentamycin while *Pseudomonas aeruginosa* was resistant to all the antibiotics. *Escherichia coli* and *Klebsiella* spp. were resistant to all the antibiotics except Gentamycin while *Citrobacter intermedius* was resistant only to Cephalexin and Erythromycin. This study provides the evidence of urinary tract infections amongst outpatients of primary health centres and the drugs for their control.

Keywords

Urinary Tract Infection, Antimicrobial Agents, Bacterial Isolates

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1. Introduction

Urinary Tract Infection (UTI) is defined as the microbial invasion of any of the tissues of the urinary tract extending from the renal cortex to the urethral meatus [1]. The urinary tract includes the organs that collect and store urine and release it from the body, and these organs are the kidneys, ureters, bladder, urethra and accessory structures [2].

UTIs account for more than 8 million visits to physicians' offices, 1.5 million emergency room visits, and 300,000 hospital admissions in the United States annually [3]. Typical symptoms associated with UTI include the triad of dysuria (painful urination), urgency (the enhanced desire to void the bladder) and frequency (increased rate of urination). The evidence of UTI is confirmed by the presence of 10^5 microorganisms or of a single strain of bacterium per milliliter in two consecutive midstream samples of urine [4]. UTIs are caused by bacteria in the gastro intestinal tract that have colonized the periurethral area. Gram negative bacteria such as *Escherichia coli*, *Proteus* species, *Klebsiella* species, *Enterobacter* species, *Serratia* species and *Pseudomonas* species are usually detected in recurrent infections especially in association with stones, obstruction, urologic manipulation and nosocomial catheter-associated infections [5]-[7]. Other bacterial pathogens frequently isolated include *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus faecalis* [8].

It has been recognized that asymptomatic bacteriuria is common in pregnancy, thus women are of increased risk of UTIs [9]. The anatomical relationship of the female urethra to the vagina makes it liable to trauma during sexual intercourse; the moist environment of the females perineum favors microbial growth and predisposes females to bladder contamination [10] [11].

Antibiotics are used for prophylaxis of recurrent UTIs but can lead to emergence of drug-resistant bacteria [12]. Knowledge about the type of pathogens responsible for UTIs and their susceptibility patterns may help the clinicians to choose the right empirical treatment [13].

The study was conducted to isolate bacterial organisms capable of causing urinary tract infections from urine samples of patients and to determine the susceptibility pattern of antimicrobial agents used in therapy.

2. Materials and Methods

2.1. Study Population

The study was carried out at the Department of Applied Microbiology and Brewing Laboratory of Nnamdi Azikiwe University, Awka, Nigeria from March 2012 to March 2013. A total of 3000 urine samples were collected. The study subjects were outpatients attending Primary Health Centers in Umunya, Awkuzu, Ogbunike and Nteje in Oyi Local Government Area of Anambra State. These included 1500 females and 1500 males and were in the age group of 2 years and above. Verbal informed consent was obtained from all patients prior to specimen collection and the permission to that effect was obtained from the ethical committees of the health centers.

2.2. Sample Collection

Clean catch midstream urine samples were collected from each patient into a sterile screw capped universal container. The samples were labeled, transported to the laboratory and processed within two hours of collection, to ensure maximum recovery of the organisms.

2.3. Culturing of Urine Sample

The urine sample was examined for bacterial organisms following the methods described by [14] [15]. A loopful (0.001 ml) of thoroughly mixed, uncentrifuged urine sample was inoculated by spread plate method onto MacConkey-Inositol-Carbenicillin Agar. Duplicate plates were prepared and then incubated at 37˚C for 24 h. Pure cultures of the isolates were inoculated on Nutrient agar (Lab M) slants in test tubes and stored at 4˚C for further studies.

2.4. Identification of the Isolates

Identification of the working isolates were carried out based on the methods described by [16]. Tests carried out include Gram staining, Spore formation, oxidase, catalase, indole, sugar test, urease and citrate utilization, me-

thyl red, Voges Proskauer, motility test, NaCl tolerance, tyrosine, tween 80, starch hydrolysis, Triple Sugar Iron, Phenylalanine deaminase, nitrate reduction and oxidative fermentative test.

2.5. Antibiotic Susceptibility Test

Broth dilution technique described by National Committee for Clinical Laboratory Standard (2004) was used for the antibiotic susceptibility tests on the urine isolates. Antibiotics used include Cephalexin (500 mg), Penicillin (500 mg), Erythromycin (500 mg) and Gentamycin (40 mg).

2.6. Determination of Minimum Inhibitory Concentration

Minimum Inhibitory Concentrations (MICs) of the antibiotics on the test organisms were investigated. A two-fold serial dilution of the antibiotics was prepared in sterile nutrient broth. A 24 h culture of the test organism was diluted according to McFarland Standard to give a final inoculum concentration of 0.5×10^5 CFU/ml. In test tubes containing 1 ml each of the diluted antibiotic was added 1 ml each of the standard test organism and the tubes incubated at 37˚C for 24 h. Negative control contains only the broth and the antibiotic while the positive control has the broth and the test organism only. The MIC was determined as the lowest concentration of antibiotic completely inhibiting visible growth of the organism after 24 h incubation at 37˚C.

2.7. Determination of Minimum Bactericidal Concentration

Minimum Bactericidal Concentrations (MBC) of the antibiotics was studied. After determining the MIC, 0.1 ml of the culture medium from each tube showing no apparent growth was spread-inoculated on Muller-Hinton Agar (MHA) and the plates incubated at 37˚C for 24 h. The MBC was read as the lowest concentration of antimicrobial agent that allowed less than 0.1% of the original inoculum to survive.

3. Results

A total of 528 bacterial isolates were recovered from the urine samples, and the isolates were grouped as Gram negative rods and Gram positive cocci. The frequency of occurrence of the isolates and their strains is presented in **Figure 1**. The most frequently isolated organism in the urine samples of patients is *Escherichia coli* (24.2%) while *Citrobacter intermedius* (6.1%) and *Staphylococcus saprophyticus* (6.1%) were the least.

The age distribution of urinary tract infection in males and females are shown in **Figure 2(a)** and **Figure 2(b)**. Out of a total of 528 positive cases observed, 252 (48%) were males and 276 (52%) were females.

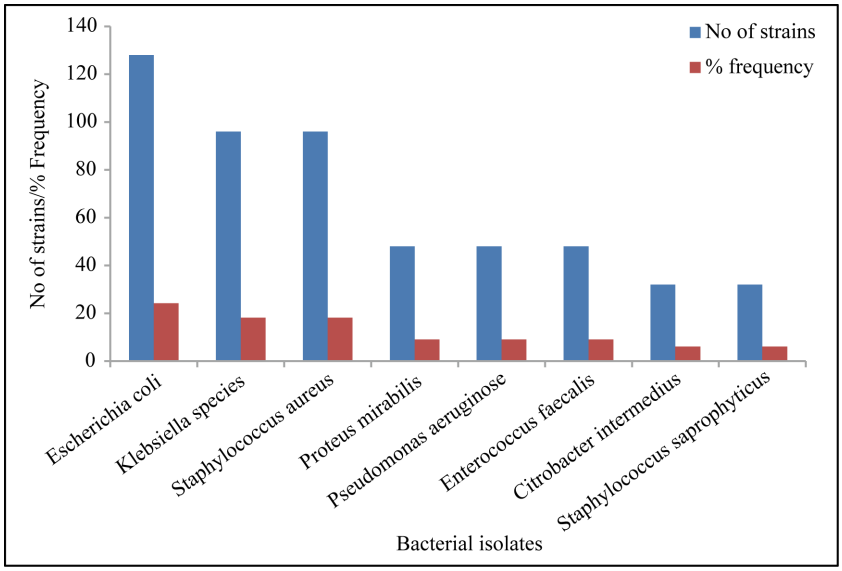


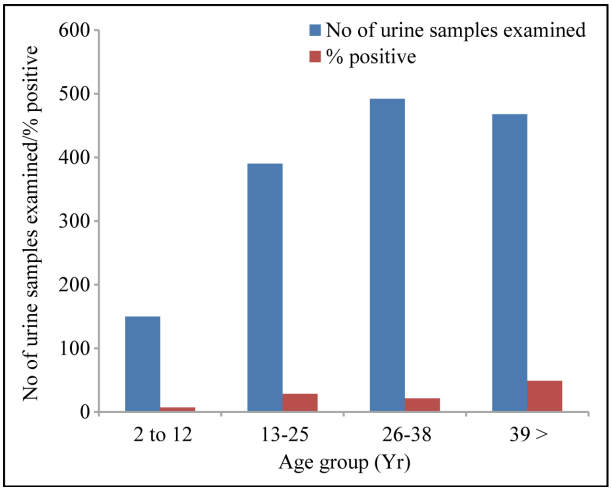
Figure 1. Frequency distribution of bacterial pathogens in midstream urine of outpatients studied.

The gender-related prevalence of the isolates among the patients (**Figure 3**) showed that 62% of the cases of UTIs were due to gram negative rods in males and 65.6% in females. Cases of UTIs with gram positive cocci were observed in 38% males and 34.4% females (**Figure 3**). **Figure 4(a)** and **Figure 4(b)** show the cases of UTIs in both sexes from different localities (Umunya, Awkuzu, Nteje and Ogbunike) in Oyi Local Government Area. Umunya had the highest percentage positive cases while Ogbunike had the least.

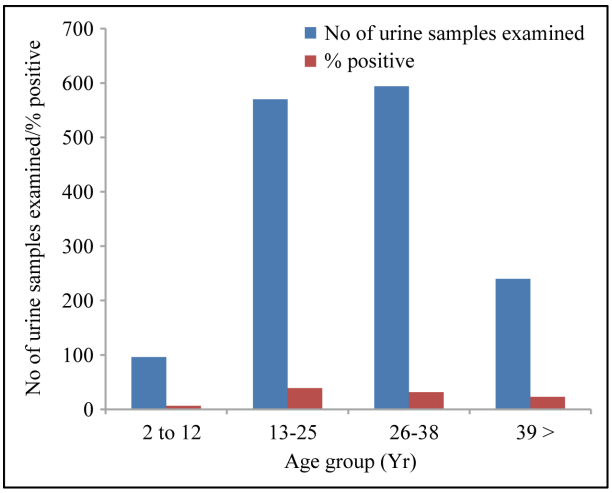
The bacterial species implicated in UTI, include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Citrobacter intermedius* and *Klebsiella* species. They showed varying susceptibility patterns to these antimicrobial drugs, cephalexin, Penicillin V, Erythromycin and Gentamycin (**Tables 1(a)-(d)**). *Staphylococcus aureus* was susceptible to cephalexin with Minimum Inhibitory Concentration (MIC) of 0.31 and Minimum Bactericidal Concentration (MBC) of 0.63 (**Table 1(a)**). All the other organisms were resistant to the antibiotic. As presented in **Table 1(b)**, *Citrobacter intermedius* was sensitive to Penicillin V, with MIC and MBC of 0.63 and 1.25 respectively. *Staphylococcus aureus* was inhibited at MIC of 5.0. As shown in **Table 1(c)**, only *Staphylococcus aureus* that was sensitive to Erythromycin with MIC and MBC of 1.25 and 2.50 respectively. All the organisms except *Pseudomonas aeruginosa* are sensitive to gentamycin (**Table 1(d)**).

4. Discussion

The incidence of urinary tract infections among patients attending Primary Health Centers in Oyi Local



(a)



(b)

Figure 2. (a) Age distribution of urinary tract infection in male; (b) Age distribution of urinary tract infection in female.

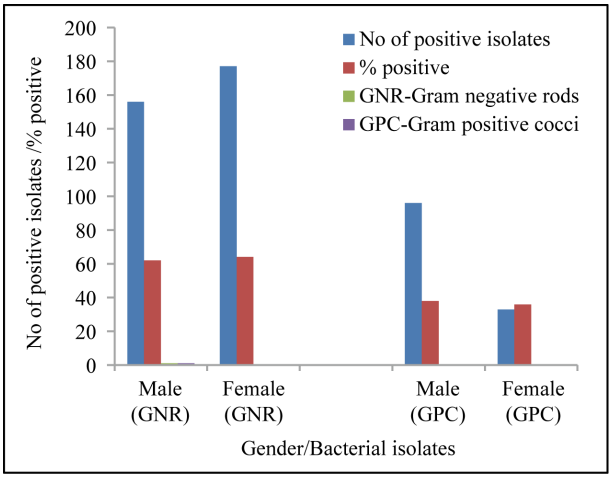
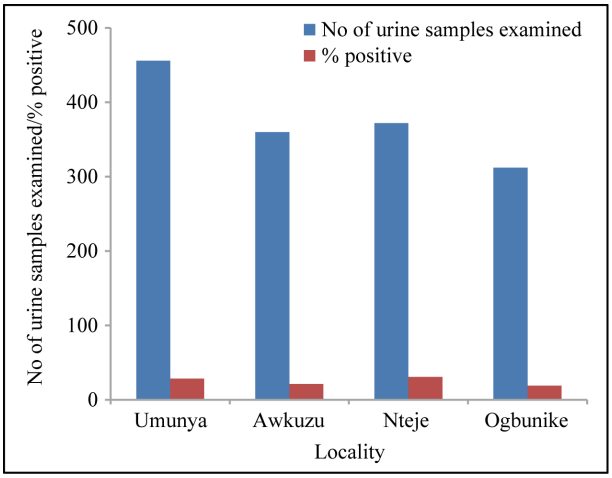
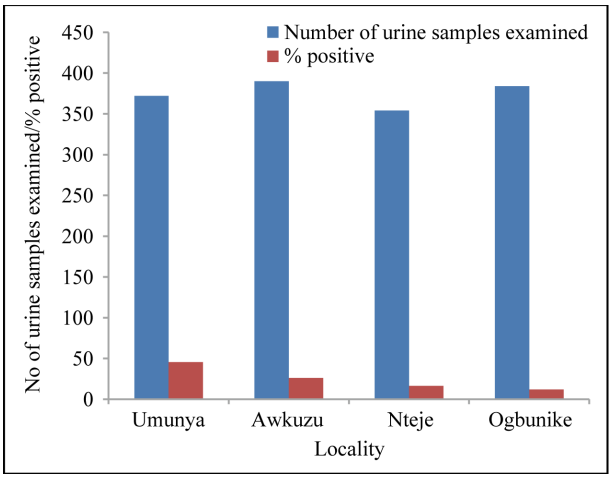


Figure 3. Gender related prevalence of the isolates among the patients.



(a)



(b)

Figure 4. (a) Cases of urinary tract infection in male patients in Oyi Local Government Area; (b) Cases of urinary tract infection in female patients in Oyi Local Government Area.

Table 1. (a) Susceptibility test of the isolates to cephalexin (cephalosporins); (b) Susceptibility test of the isolates to Penicillin V; (c) Susceptibility test of the isolates to Erythromycin; (d) Susceptibility test of the isolates to Gentamycin.

(a)

Organism	Dilution						MIC	MBC
	5.0	2.50	1.25	0.63	0.31	0.16		
Staphylococcus aureus	−	−	−	−	+	+	0.31	0.63
Pseudomonas aeruginosa	+	+	+	+	+	+		
Escherichia coli	+	+	+	+	+	+		
Citrobacter intermedius	+	+	+	+	+	+		
Klebsiella species	+	+	+	+	+	+		

Note: − Sensitive; + Resistant; MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration.

(b)

Organism	Dilution						MIC	MBC
	5.0	2.50	1.25	0.63	0.31	0.16		
Staphylococcus aureus	−	+	+	+	+	+	5.0	
Pseudomonas aeruginosa	+	+	+	+	+	+		
Escherichia coli	+	+	+	+	+	+		
Citrobacter intermedius	−	−	−	−	+	+	0.63	1.25
Klebsiella species	+	+	+	+	+	+		

(c)

Organism	Dilution						MIC	MBC
	5.0	2.50	1.25	0.63	0.31	0.16		
Staphylococcus aureus	−	−	−	+	+	+	1.25	2.50
Pseudomonas aeruginosa	+	+	+	+	+	+		
Escherichia coli	+	+	+	+	+	+		
Citrobacter intermedius	+	+	+	+	+	+		
Klebsiella species	+	+	+	+	+	+		

(d)

Organism	Dilution						MIC	MBC
	5.0	2.50	1.25	0.63	0.31	0.16		
Staphylococcus aureus	−	−	−	−	+	+	2.5	5.0
Pseudomonas aeruginosa	+	+	+	+	+	+		
Escherichia coli	−	−	−	+	+	+	5.0	10.0
Citrobacter intermedius	−	−	−	+	+	+	5.0	10.0
Klebsiella species	−	−	−	+	+	+	5.0	10.0

Government Area of Anambra State and the susceptibility of the isolates to some conventional antibiotics were carried out. Out of the 3000 processed samples, 528 (17.6%) midstream urine samples yielded growth. Two thousand four hundred and seventy-two (82.4%) samples recorded no bacterial growth after 24 - 48 hours incubation at 37˚C. The reason for the no bacterial growth among a good number of the patients may be due to the fact that some of the patients have been on antibiotic therapy before reporting to the hospital or laboratory. These antibiotics may have inhibited bacterial growth [1].

The use of midstream, urine was aimed at reducing and eliminating the influence of normal flora and other contaminants on expected results. The prevalence rate of 17.6% UTI obtained in this study is in line with the reports of [2] [17] [18], who recorded 16.5%, 22% and 22.3% respectively. [19] recorded a much lower rate of 11.9% while [20], had a high rate of 25.6%. However, it is important to note that much higher prevalence rates have been reported by some authors [1] [9] [21]-[24], with a range of 39.0% - 71.6%.

The most common pathogen isolated in this study was *Escherichia coli* (24.2%). This finding is similar to other reports which indicate that gram negative bacteria, particularly *E. coli* is the most implicating pathogen isolated in patients with UTIs [11] [23] [25]-[27]. [9] [22] [28] also noted in their studies that *E. coli* was the most commonly isolated pathogen in significant bacteriuria. In a similar study by [29], *E. coli* was found to be the most implicating pathogen.

The result in **Figure 1** showed that *E. coli* (24.2%) predominated over *Klebsiella* species (18.2%). This is contrary to the findings of [1] [2], who found *Klebsiella* species to be more prevalent than *E. coli* in UTIs. However, the result compares favourably with the findings of other workers [2] [8] [11] [30]-[34] who found *E. coli* more predominant over *Klebsiella* species. This investigation confirms the involvement of both pathogens in UTIs as earlier established by [9]. The 18.2% incidence rate observed for *Klebsiella* species (**Figure 1**) brings to light the fact that they are achieving more prominence as aetiological agents of UTI than previously reported [34]-[38].

That *Staphylococcus aureus* is implicated in UTI in many sexually active females, as reported by [28] [39] is in agreement with the findings in the present study. This observation however indicates that the organism is also gaining clinical prominence in the etiology of UTI (**Figure 1**) and it is not in agreement with the much higher incidence rates recorded by other authors [1] [38] [40]-[44] in similar UTI investigations.

The incidence of UTI was higher in females (52%) than in males (48%) (**Figure 2(a)** and **Figure 2(b)**). This observation is supported by the works of [45]. The higher incidence of UTI in females is also supported by [19]. Contrary to this observation, [1] noted a prevalence rate of 58.3% in males and 41.7 in females. The reason for this, however was not given. They attributed the higher incidence rate of urinary tract infection in female to the nature of the anatomical structure of their urinogenital tract; the urethra of females is much shorter and closer to the anus than in males, and it also lacks the bacteriostatic properties of prostatic secretions. Therefore, the higher incidence in females observed in this work, may be as a result of the unhygienic habits of not cleaning the vagina from front to the back after urination but rather from back to front, thus resulting in autoinfection. This does not agree with the findings of [1] who found a higher UTI prevalence in males (58.3%) than in the female outpatients (41.7%). The reason for this was not clear as it contradicts reports of studies on UTI by the following authors [38] [42] [44] [46]-[50].

Urinary Tract Infection occurred highest in the age group 26 - 38 years in both sexes (**Figure 2(a)** and **Figure 2(b)**). This may be as a result of increased sexual activity within this age group which predisposes them to UTI. This observation is supported by reports of other workers [40] [50] who found same.

In gender related prevalence of uropathogens among the patients (**Figure 3**), it was observed that gram negative rods were main cause of UTIs in both sexes. The occurrence of members of the enterobacteriaceae group in this work, especially the coliforms show that a high percentage of UTI in the outpatients may be due to feacal contamination arising from poor hygiene. The coliform organisms are indicators of poor safety habits, poor hygiene and poor sanitary life style [51], and are usually found in the perineum of the large intestines as commensals [51]-[53].

Geographically, the high incidence of UTI (**Figure 4(a)** and **Figure 4(b)**) observed in Umunya may probably be due to the high level of promiscuity among the females. This view is supported by the work of [54], who reported that sex is the cause of 75% - 90% of bladder infections, and that the risk of infection is invariably related to the frequency of sex. The variation in UTI incidence from one geographical location to another could be attributed to differences in UTI perception, mode of screening, compounding risk factors such as age, parity [55], pregnancy [33] and host behavioural factors [28].

Antibiotic sensitivity testing of the isolates and the resulting profile (**Tables 1(a)-(d)**) showed variable susceptibility reactions to Cephalexin, Penicillin V, Erythromycin and Gentamycin. *Staph aureus* was sensitive to all the antibiotics tested. This agrees with the work of [56], who reported that *Staph aureus* was 100% sensitive to gentamycin and cephalosporins, but disagrees with the work of [57] who observed the resistance of *Staph auerus* to penicillin and erythromycin. *Pseudomonas aeruginosa* was resistant to all the antibiotics tested (**Tables 1(a)-(d)**). This finding is in line with the work of [1] [58], who reported that *P. aeruginosa* was resistant

to most of the conventional antibiotics. An increase in resistance of *P. aeruginosa* to these conventional antibiotics has been found worldwide [59]. The sensitivity of *E. coli* to gentamycin is supported by works of [60] [61] but in contrast, [62] noted an increase in resistance of the organism to the antibiotics. *Citrobacter intermedius* was resistant to cephalexin and erythromycin but sensitive to penicillin V and gentamycin. *Klebsiella* species was resistant to cephalexin, penicillin and erythromycin but sensitive to gentamycin. This finding is in line with the work of [63], who noted the sensitivity of *Klebsiella* species to gentamycin but resistant to the other antibiotics. [64], in contrast found *Klebsiella* species to be highly resistant to gentamycin and cephalexin. The high prevalence of multiple antibiotic resistant strains is a possible indication that very large populations of bacterial isolates have been exposed to several antibiotics [40].

The prevalence rate of 17.6% reported in this study should be of great concern as not only do UTI pose a threat to health, but they also impose an economic and social burden. The findings of this study revealed that the important infecting organisms were found to be the commensals of perianal and vaginal regions. This calls for increase in personal hygiene. It is therefore recommended that routine microbiological analysis and antibiotic sensitivity test of mid-stream, urine samples of patients be carried out so as to enhance in the administration of drugs for the treatment and management of UTIs. Further studies will, therefore be directed towards carrying out the histopathological effects of the isolated organisms on the visceral organs of mice, and this, of course, will help to monitor the progression of infection and treatment.

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