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Microbial Isolates from Urinary Tract Infection and their Antibiotic Resistance Pattern in Dhaka city of Bangladesh

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Abstract

The present study was conducted to investigate the bacterial infections of urinary tract and their antibiotic resistance pattern in Dhaka city in the Microbiology Department in MH Samorita Hospital & Medical College, Dhaka, Bangladesh. The study was carried out from November 2016 until July 2017. Two hundred and thirty-five patients with urolithiasis were included in the present study whose ages ranged from 05 to 70 years. Seven different bacterial species with noticeable growth were found in the urine sample after bacterial cultures were performed. *E. coli* was the most frequent bacterium found in patients' urinary tract infections, accounting for 50% of cases. *Klebsiella* (12%), *Pseudomonas* (10%), *Proteus* (10%), *Streptococcus* (8%), *Citobacter* (6%) and *Staphylococcus* (4%), were the next most frequent bacteria. According to *E. coli*, women (72%) had more urinary tract infections than men (21.28%). Diverse bacterial pathogens were discovered to be extremely susceptible to cotrimoxazole, ciprofloxacin, and the most of them were also quite sensitive to nitrofurantoin and nalidixic acid. Furthermore, nalidixic acid, cotrimoxazole, nitrofurantoin, and imipenem showed considerable sensitivity to *E. coli*, *Klebsiella spp.*, *Pseudomonas spp.*, *Proteus spp.*, *Staphylococcus spp.*, *Streptococcus spp.*, and *Citrobacter spp.*

Keywords: UTI; Antibiotic Resistance; Sensitivity; Dhaka; Bangladesh.

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Introduction

The urinary system consists of the organs, tubes, muscles, and nerves that work together to produce, store, and transport pee (Bari et al., 2023). Two kidneys, two ureters, the bladder, two sphincter muscles, and the urethra make up the urinary system. An illness that affects the urinary tract is referred to as a urinary tract infection (UTI), often known as acute cystitis or a bladder infection. It is recognized as a simple cystitis (a bladder infection) when it affects the lower urinary tract and as pyelonephritis (a kidney infection) when it affects the upper urinary tract. Lower urinary tract symptoms include painful urination, frequent urination, or an overwhelming urge to urinate (or both), whereas pyelonephritis symptoms also include fever and flank pain. In certain circumstances, even while not urinating, a painful burning sensation in the urethra may exist. The very young and the elderly may have ambiguous or non-specific symptoms. *Escherichia coli* is the primary cause of both types, however occasionally other bacteria, viruses, or fungus may also be to blame.

Women experience urinary tract infections more frequently than males do; at some time in their lives, 50% of women experience at least one infection. Recurrences happen frequently. Female anatomy, sexual activity, and family history are risk factors (Chakma, et al., 2022; Sunny et al., 2021a). If pyelonephritis develops, it frequently does so after a bladder infection, though it can also come from a blood-borne illness. Young, healthy women can be diagnosed only on the basis of their symptoms. Since bacteria may be present without an illness, diagnosis can be challenging in patients with hazy symptoms (Hasan, et al., 2023; Tufael, et al., 2023). A urine culture may be helpful in complex diseases or in circumstances where treatment has failed. In those with frequent infections, low dose antibiotics may be taken as a preventative measure. When bacteria enter urine and ascends to the bladder, it is known as a UTI. More than 8.1 million people seek medical attention for UTIs every year. In the course of their lives, at least 1 UTI symptom will affect 10 out of every 25 women and 3 out of every 25 males. The most prevalent bacterial illness in humans, urinary tract infections (UTIs), affect people of all ages (Sunny, 2017; Medina and Castillo-Pino, 2019). Lack of an accurate diagnosis and prompt treatment can result in serious problems such urinary tract diseases, scar tissue still present in the kidney parenchyma, high blood pressure, and uremia, as well as premature birth and even miscarriage in pregnant women (Uwaezuoke et al., 2019; Sunny et al., 2021b; Kuddus, et al., 2022; Sazzad, et al., 2023). Infections in the urinary system, such as cystitis and pyelonephritis, are frequent in medical facilities. *E. coli* is the most common pathogen among those that cause urinary infections, accounting for about 80% of infections and infecting 8–10 million individuals annually in the USA (Abbo and Hooton, 2014; Foxman, 2003).

According to statistics from international organizations, between \$17 and \$29 billion is spent yearly on treating infections in hospitals, with urinary infections accounting for 39% of those expenses (Foxman, 2003). The most frequent cause of UTIs is some gram-negative bacilli, of which *E. coli* is responsible for more than 80% of acute urinary tract infections (Agency, 2011). 5–10% of urine infections are caused by *Staphylococcus saprophyticus*; additional bacterial causes of urinary infections include *Klebsiella*, *Proteus*, *Pseudomonas*, and *Enterobacter*. These cases are uncommon and typically brought on by catheters or anomalies of the urinary system (Agency, 2011; Behzadi et al., 2015). The threat posed by infectious illnesses to human health is constant (Islam, et al., 2018; Islam, et al., 2023). The number of people dying from infectious diseases has drastically dropped since the development of antibiotics (Kuddus, et al., 2022). However, due to the unchecked use of antibiotics and resistance to them, many diseases are reappearing. The world urgently has to alter its patterns of consumption and prescription of this priceless therapeutic supply due to the rise in antibiotic resistance (Organization, 2001; Flores-Mireles et al, 2015). Even the creation and development of new medications cannot stop the rise of antibiotic resistance if medication consumption follows the same pattern. Vaccinations, frequent hand washing, and attention to food hygiene are all required infection control strategies in addition to the absence of unchecked antibiotic usage (Mortazavi and Shahin, 2009). It should be emphasized that antibiotics are ineffective against viral diseases including colds, sore throats, and the flu and can only cure bacterial infections.

In other terms, it can be said that gene mutation causes antibiotic resistance, and new generations of resistant bacteria develop that cannot be stopped (Kibret and Abera, 2011). The unchecked and excessive use of antibiotics is one of the most significant causes of this sort of drug resistance. This phenomenon puts human society in jeopardy, which is why its threat has been compared to terrorism. One of the main problems threatening human health in the current period is the antibiotic resistance of these bacteria (Raj et al., 2019; Davies and Davies, 2010). Due to the rising usage of antibiotics and the resulting rise in antibiotic resistance, treating these sorts of illnesses now presents serious challenges. Choosing a highly effective antibiotic is the cornerstone of treating urinary infections (Asadpour Rahimabadi et al., 2016). Due to the emergence and spread of bacteria-resistant strains, population growth, travel, and the unchecked and excessive use of antibiotics (Raj et al., 2019; Davies and Davies, 2010; Hejazi et al., 2018), antibiotics that were once effective now have little effect on bacteria that cause urinary tract infections. Various studies indicate that antibiotic-resistance genes can spread throughout bacterial populations regardless of the pattern of antibiotic intake (Hejazi et al., 2018). Infections of the urinary tract affect women more frequently than men. Recurrences of infections are frequent and affect almost half of all females (Grigoryan et al., 2014; Momtaz et al., 2013; Niranjana and Malini, 2014).

It has become a severe issue since bacteria's sensitivity patterns to certain antibiotics change over time and in different geographic locations. As a result, the information gleaned from the antibiotic sensitivity and resistance pattern should be used to guide antibiotic therapy of illnesses. Recognizing the sensitivity pattern of this organism to antibiotics can be helpful in the treatment of the majority of patients suffering from a urinary tract infection due to the rising use of antibiotics and the resulting rise in antibiotic resistance, as well as differences in antibiotic sensitivity when dealing with different bacteria (VA et al, 2013; Meddings et al, 2012; Razine et al, 2012). The present study aims to isolates the microbes that causes Urinary Tract Infection and their Antibiotic Resistance Pattern in Dhaka city.

Materials and Methods

Source of Specimens

This study was conducted in the Microbiology Department in MH Samorita Hospital & Medical College, Dhaka. The number of the patients was 235 and they were recruited from November 2016 until July 2017.

Sample collections and culture procedure Midstream urine samples were taken using a calibrated loop (0.01 ml) and sterile containers for the final diagnosis of urinary tract infection. Under sterile circumstances and incubated at 37°C, the midstream urine sample was grown on EMB and blood agar media. After 18–24 hours, samples were evaluated for urine infection positivity if the number of growing colonies was equal to or greater than 100,000 CUF/ml. Gram- negative, Gram-positive bacteria were identified using sensitivity tests, whereas Gram-positive bacteria were identified using MacConkey Agar, Blood Agar, and Muller Hinton Agar, respectively.

Culture of Urine Specimens

In accordance with the manufacturer's instructions, the media were prepared and sterilized. The prepared media were solidified before being utilized for isolation, determining the viable count, identification, and susceptibility testing (Baker et al., 1993).

Composition of the culture media used

The Kerby Bauer technique (1996) was used to assess the antibiotic sensitivity of all isolates in accordance with the National Committee of Clinical Laboratory Standards' (NCCLS, 1999) criteria on MacConkey, Blood Agar, and Mueller Hinton Agar media. At 45°C, the medium was allowed to cool before being poured into Petri dishes t

Table 1. MacConkey agar Media

Ingredients	Gram/liter
BactoPeptone	17.0
Proteas Peptone	3.0
Lactose	10.0
Bill Salt	1.5
Agar	15.0
Neutral red	0.03
Crystal violet	0.001
Distilled water	953 ml

Table 2. Blood Agar Media

Ingredients	Gram/liter
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Tryptone	14.0
Peptone	4.5
Yeast extract	4.5
NaCl	5.0
Agar	15.0
Distilled water	957 ml

Table 3. Mueller Hinton Agar Media

Ingredients	Gram/liter
Beef infusion	2.0
Bactocasamino acid	17.5
Starch	1.5
Bacto agar	17.0
Distilled water	961 ml

Inoculation and Incubation

The plates were inoculated by dipping a sterile swab into the inoculum, removing the excess by pressing and rotating the swab firmly against the side wall of the tube above the level of fluid, and then wiping the medium's surface thoroughly while rotating the plate three times at an angle of 60 degrees after each application. The plate was given some time to cure at room temperature with the lid shut. To prevent moisture buildup on the agar surface, antibiotic discs were added after 15 minutes of inoculation, and the plates were then turned over for incubation (Forbes et al., 2007). Each plate had a maximum of 5 antibiotic discs applied to it using flamed forceps. To ensure even contact with the medium, each disc was gently pressed down onto the plate. The diameter of each zone, including the diameter of the inhibition zone, was measured and recorded in mm after an overnight incubation at 37°C, and it was compared to the typical inhibition zone. For species that move around, such as *Proteus* spp., the swarming haze was disregarded, and zones were measured where growth was clearly impeded (Forbes et al., 2007).

Table 4. Colony morphology of different type of micro-organism on different media

Micro-organism	Media	Culture characteristic
<i>Escherichia Coli</i>	MacConkey agar	Small, Pink colony
<i>Pseudomonas sp.</i>	MacConkey agar	Small transparent, greenish colony
<i>Streptococcus sp.</i>	Blood agar	Pin point with clear zone of haemolysis.
<i>Klebsiella sp.</i>	MacConkey agar	Mucoid, large pink colony
<i>Proteus sp.</i>	Blood agar MacConkey agar	Colorless, swarming growth over the surface, fishy odors.
<i>Staphylococcus sp.</i>	Blood agar	Golden yellow colonies.
<i>Streptococcus sp.</i>	Blood agar	Colonies are circular low convex and semitransparent.

<i>Citrobacter sp.</i>	MacConkey agar	White, small, foot like extension.
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Data analysis

A predesigned data collecting sheet was used to record all of the data. The unpaired Student's 't' test was used to compare continuous variables between research subject groups. Continuous variables were represented as mean SD. Chi-square test was used to compare categorical variables, and absolute frequencies and percentages were provided. To compare the association between various factors and psoriasis, the Spearman's rank correlation coefficient (r) test was used. With significance set at p 0.05 or higher at the level of the 95% confidence interval, all p values were two-tailed. The Windows SPSS version 22 program was used to conduct the analyses.

Aspects of ethics and the process for maintaining confidentiality:

The Dhaka Medical College's ethical review committee granted the study its ethical approval. The danger of physical, psychological, social, and legal harm during blood collection was minimal. A unique code was assigned to each patient, which was followed in each and every step of the procedure, and the name and address were recorded on a separate sheet to protect anonymity. The study's nature, procedure, goal, risks, and benefits were thoroughly discussed to the study subjects before obtaining their written informed consent. Here, neither a placebo nor an experimental new medicine was used. To protect their rights and health, the study participants' interests were not jeopardized.

Results and Discussions

Identifying the frequency of bacteria causing urinary infection

Seven different bacterial species with noticeable growth were found in the urine sample after bacterial cultures were performed. E. coli was the most frequent bacterium found in patients' urinary tract infections, accounting for 50% of cases. Klebsiella (12%), Pseudomonas (10%), Proteus (10%), Streptococcus (8%), Citobacter (6%) and Staphylococcus (4%), were the next most frequent bacteria. The least prevalent isolates in this community were Staphylococcus (4%), Citobacter (6%), and Streptococcus (8%), as seen in Fig. 1.

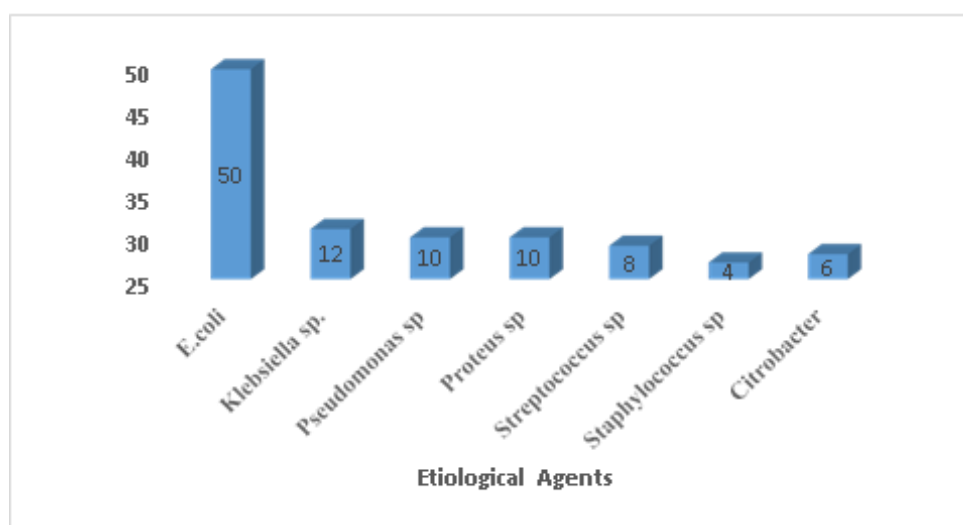


Figure 1: The percentage of bacteria found in urine samples.

Identifying the frequency of UTI-causing bacteria based on gender

The frequency of microorganisms that cause UTIs is shown by gender in Table 8. According to *E. coli*, women (72%) had more urinary tract infections than men (21.28%). In contrast, the percentage of UTIs brought on by the *Klebsiella* bacteria was 62% in women and 38% in men. In addition, *Pseudomonas*, *Streptococcus*, and *Citrobacter* caused 62.5%, 37.5%, 58%, and 42% of UTIs in men and women, respectively. Both sexes experienced the same level (50%) of UTI frequency from *Staphylococcus aureus* and *Proteus sp* bacteria.

Table 7. The frequency of sex and specific abnormal finding base distribution

Name of urine analytic	Total sample	Total no. of positive case	No. of positive case (Male)	No. of positive case (Female)
Pus cell (significant)	235	50	14	36

Table 8: The frequency of sex UTI-causing bacteria based on gender

Bacteria	Female (%)	Male (%)
<i>E.coli</i>	72	28
<i>Klebsiella sp.</i>	62	38
<i>Pseudomonas sp</i>	62.5	37.5
<i>Proteus sp</i>	50	50
<i>Streptococcus sp</i>	58	42
<i>Staphylococcus sp</i>	50	50
<i>Citrobacter</i>	50	50

Antibiotic resistance of UTI-causing bacteria

The largest levels of antibiotic resistance among bacteria causing UTI were shown to be associated to amoxicillin (82%), nalidixic acid (50%), and penicillin G (50%). In contrast, Table 9 shows that Cotrimoxazole (2%), Imipenem (5%), Gentamicin (10%), and Ciprofloxacin (1.5%) had the lowest antibiotic resistance of the bacteria causing urinary tract infections.

Table 9. Antibiotic sensitivity patterns of common isolation

Antibiotic	Sample	% of Sensitive	% of Resistance
Imipenem	50	95	5
Cephalexin	50	80	20
Cotrimoxazole	50	98	2
Gentamicin	50	90	10
Tetracycline	50	80	20
Nalidixic acid	50	50	50
Nitrofurantoin	50	70	30
Ciprofloxacin	50	90	10
Amoxicillin	50	18	82
Penicillin G	50	50	50

Identifying the frequency of antibiotic resistance and antimicrobial Sensitivity Testing of UTI-causing bacteria

All bacterial isolates were subjected to disc diffusion testing for antibiotic sensitivity and resistance to the most frequently prescribed antibiotics for the treatment of UTI, as indicated in Tables 10 through 14. Diverse bacterial pathogens were discovered to be extremely susceptible to Cotrimoxazole, Ciprofloxacin, and the most of them were also quite sensitive to nitrofurantoin and nalidixic acid. Cotrimoxazole exhibited the highest sensitivity to *E. coli* spp., followed by imipenem (80%), ciprofloxacin (80%), gentamicin (80%), and nitrofurantoin (70%) in that order (Table 10). Cotrimoxazole, followed by Gentamicin, Cephalexin, and Imipenem, showed the highest sensitivity to *Klebsiella* spp. (Table 11). Gentamicin, Tetracycline, Nalidixic Acid, Imipenem, Ciprofloxacin, Nitrofurantoin, and Cotrimoxazole all demonstrated sensitivity to *Pseudomonas* spp., with Cotrimoxazole showing the highest sensitivity (Table 12). Imipenem and Ciprofloxacin showed the maximum sensitivity in *Proteus* spp. Ciprofloxacin has the highest sensitivity to *Streptococcus* and *Staphylococcus* species (85%), followed by Imipenem (80%), Cotrimoxazole (80%), Gentamicin (75%) and Cephalexin (70%) (Table 13). Imipenem and Cotrimoxazole had the highest sensitivity for *Citrobacter* spp., followed by Ciprofloxacin and Gentamicin (Table 14).

Table 10: Antibiotic resistance pattern (%) of *E. coli*.

Antimicrobial agents	Percentage		
	Resistant (%)	Sensitive (%)	Intermediate (%)
Imipenem	10	80	
Cephalexin	20	50	
Cotrimoxazole	10	90	
Gentamicin	20	80	
Tetracycline	100		

Table 11: Antibiotic resistance pattern (%) of *Klebsiella* spp.

Antimicrobial agents	Percentage		
	Resistant (%)	Sensitive (%)	Intermediate (%)
Imipenem	20	80	
Cephalexin	30	70	
Cotrimoxazole	10	90	
Gentamicin	20	80	
Tetracycline	100		
Nalidixic acid	30	20	50
Nitrofurantoin	80	20	
Ciprofloxacin	80	20	
Amoxicillin	70	30	
Penicillin G	60	40	

Table 12: Antibiotic resistance pattern (%) of Pseudomonas spp.

Antimicrobial agents	Percentage		
	Resistant (%)	Sensitive (%)	Intermediate (%)
Imipemem	20	80	
Cephalexin		50	
Cotrimoxazole		25	75
Gentamicin		90	
Tetracycline		80	
Nalidixic acid		75	
Nitrofurantoin		70	
Ciprofloxacillin		70	20
Amoxicillin	90		10
Penicillin G	100		

Table 13: Antibiotic resistance pattern (%) of Proteus spp.

Antimicrobial agents	Percentage		
	Resistant (%)	Sensitive (%)	Intermediate (%)
Imipenem	25	75	
Cephalexin	100		
Cotrimoxazole	100		
Gentamicin	25	50	25
Tetracycline	100		
Nalidixic acid	100		
Nitrofurantoin			100
Ciprofloxacillin	25	75	
Amoxicillin		20	80
Penicillin G	100		

Table 14: Antibiotic resistance pattern (%) of Staphylococcus spp and Streptococcus spp.

Antimicrobial agents	Percentage		
	Resistant (%)	Sensitive (%)	Intermediate (%)
Imipenem	20	80	
Cephalexin	30	70	
Cotrimoxazole	20	80	
Gentamicin	25	75	25
Tetracycline	25	65	10
Nalidixic acid	100		
Nitrofurantoin	100		
Ciprofloxacillin	15	85	

Amoxicillin	85	15	
Penicillin G	100		

Table 15: Antibiotic resistance pattern (%) of *Citrobacter* spp

Antimicrobial agents	Percentage		
	Resistant (%)	Sensitive (%)	Intermediate (%)
Imipenem	20	80	
Cephalexin	60	40	
Cotrimoxazole	20	80	
Gentamicin	30	70	
Tetracycline	50		50
Nalidixic acid	100		
Nitrofurantoin	50		50
Ciprofloxacin	25	75	
Amoxicillin	100		
Penicillin G	100		

A urine culture for 235 individuals was performed, and 50 (21.27%) of those patients had positive results, as indicated in Table 6. That which was discovered elsewhere (Sutherland et al., 1985; Alpay et al., 2009) was essentially identical to this. The results from the current study, in contrast, were essentially different from those from Al-Jebouri (2006) and Al-Jebouri and Hasen (2012). However, among the patients tested, *E. coli* was the most common bacterium causing UTI, and this conclusion was reached elsewhere (Manikandan et al., 2011). Due to some virulence traits like hemolysin production and the presence of fimbriae, *E. coli* is one of the most frequent causes of UTI. Furthermore, *Staphylococcus* sp. was infrequently isolated (4% of the UTI cases examined here), and Manikandan et al. (2011) in India observed nearly the same pattern of isolation. Other organisms were identified in the current investigation that were linked to Iraqi patients who had UTIs; Al-Jebouri (2006) had previously reported on these species. In this investigation, gram negative bacteria accounted for the majority of the organisms that caused UTIs. These findings resembled those of Al-Jebouri (2006), Al-Rawi (1998), and Al-Naas et al. (2013) virtually exactly. Between infection-causing and non-infection-causing stones, there was statistically no discernible difference in the distribution of positive urine cultures (P value > 0.05). These findings were nearly identical to those made by Al-Jebouri (2006). In a ratio of 1.6:1, female patients had a higher prevalence of UTI than male patients, which is practically identical to AL-Bedri's (1987) findings. The urethra in women is shorter and closer to the anus than in men, and they lack the bacteriostatic qualities of prostatic secretions, making them more susceptible to UTIs (Al-Jebouri, 1989).

In comparison to earlier studies, the current study (Akortha and Ibadin, 2008) revealed a significant increase in antibiotic resistance among microorganisms isolated from Bangladeshi patients. This may be the result of antibiotic misuse, the use of antibiotics from uncontrolled sources of production, such as those that are unknown to the user, the use of inactivated antimicrobials, antibiotic selection pressure, and a lack of quality control on some sources of antibiotics entering Bangladesh, particularly by the private sector. The results of the current investigation, however, demonstrated that nearly all microorganisms that cause UTIs are resistant to amoxicillin, and the majority of them are also extremely resistant to tetracycline. Additionally, the study by Manikandan et al. (2011) found that almost 60% of the microorganisms causing UTI were amoxicillin-resistant. As opposed to this, Akortha and Ibadin discovered that the majority of their isolates were nalidixic acid sensitive (Hooton, 2003). However, a significant rise in pathogenic strain

resistance to ampicillin and cephalothin has been observed globally (Nwanze et al., 2007). However, some medications, such as gentamicin and cotrimoxazole, still exhibit a moderate level of efficacy against UTI pathogens due to their multiple mechanisms of action (Al-Jebouri, 2006). Furthermore, Nalidixic acid, Cotrimoxazole, Nitrofurantoin, and Imipenem remained very sensitive to *E. coli*, *Klebsiella* spp., *Pseudomonas* spp., *Proteus* spp., *Staphylococcus* spp., *Streptococcus* spp., and *Citrobacter* spp. Additionally, the most prevalent UTI germs' significant drug resistance highlights the necessity of using medicines sparingly.

Conclusion

According to the susceptibility and resistance profiles of all isolates in the current study, Cotrimoxazole and Imipenem have higher efficacy than Nitrofurantoin and Ciprofloxacin. All of the antibiotics employed in this study's isolates saw an overall rise in resistance patterns. According to the most recent research, bacterial resistance would be the biggest and scariest issue facing our nation. Locally, a new generation of antibiotics continues to work more quickly. More laboratory studies to explore the intrinsic and extrinsic parameters led to the conclusion that high rates of resistance occurred in the local pathogens that could be transmitted to other geographical areas. The use of antibiotics in our region of the world needs to be under health assessment and control.

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