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Original Research Article

BACTERIOLOGICAL ANALYSIS OF URINARY CULTURE ISOLATES: ANTIBIOTIC SUSCEPTIBILITY AND IDENTIFICATION

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Abstract

Background: Urinary tract infection are one of the most common bacterial infection prevalent globally in both community and healthcare settings. The uropathogens have shifted over the years becoming more resistant to antibiotics which is causing a decrease in the number of available treatment choices. Therefore, it is crucial to identify the uropathogens and their susceptibility to antimicrobial agents in order to ensure effective treatment of urinary tract infections and prevention of complications. This study aimed to analyze urine samples for the bacteriological profile and assess their antibiotic susceptibility. **Materials and Methods:** The research was carried out at the microbiology laboratory. A total of 330 individuals who were qualified for participation in the study had undergone a bacteriological analysis of urine and had not taken any antimicrobial medications in the two weeks before. This eligibility criterion was based on the requirement that the antibiotics should have hindered or eradicated the disease- causing microorganisms. **Result:** E. coli 191 being the most prevalent at 57.9%, followed by Klebsiella spp112 at 33.9%. The other bacteria include Proteus mirabilis 6 (1.8%), Proteus vulgaris 2 (0.6%), Enterococcus faecalis 1 (0.3%), Staphylococcus aureus 5 (1.5%), and Pseudomonas aeruginosa 13 (3.9%). Out of 330 individuals, 245 were female and 85 were male. The majority of the patients were aged 71 years and above (64) followed by the age group of 51-60 years (59), 61-70 years (56), 41-50 years (41), below 20 years (38), 21-30 years (37), and 31-40 years. For E.coli, the percentages are 112 (53.1%) and 79 (79%) respectively. For Klebsiella spp, the percentage was 91 (43.1%) and 21 (21%) respectively. As for Proteus mirabilis, the percentages are 6 (2.8%) and 0, and for Proteus vulgaris, the percentages are 2 (0.9%) and 0 respectively. The most effective antibiotics against E. coli was Meropenem (99.5%) and Imipenem (99.5%), followed by Fosfomycin, Cefoperazone/Sulbactam, and Piperacillin/Tazobactam. Klebsiella spp is highly susceptible to Meropenem (100%) and Imipenem (100%), followed by Piperacillin/Tazobactam (98.2%) and Amikacin (98.2%). Proteus mirabilis has 100% sensitivity to Fosfomycin, Cefoperazone/ Sulbactam, Piperacillin / Tazobactam, Meropenem, Imipenem, Ciprofloxacin, Norfloxacin, Gentamicin, Amikacin, and Cefepime. Proteus vulgaris is 100% susceptible to the following antibiotics: Cefoxitin, Cefixime, Ceftriaxone, Cefotaxime, Fosfomycin, Cefoperazone/Sulbactam, Piperacillin/Tazobactam, Meropenem, Imipenem, Ciprofloxacin, Norfloxacin, Gentamicin, Amikacin, and Cefepime. **Conclusion:** We concluded that the prevalence of Urinary Tract Infection was higher in females compared to men. Escherichia coli was the predominant bacteria found in urinary tract infections. This trend of developing resistance to routinely prescribed antibiotics for treating urinary tract infections serves as a warning against the indiscriminate use of antibiotics. The study's findings establish that E-coli is the primary causative agent of urinary tract infections (UTIs).

INTRODUCTION

Urinary tract infections (UTIs) are one of the most common infection worldwide with only respiratory and gastrointestinal infections being more frequent in ambulatory patients.[1-3] It can occur in both men and females. Approximately 20% or more of the female population is anticipated to get a urinary tract infection (UTI) at some point in their lives. The objective of this research was to analyze urine samples for the bacteriological profile and assess their antibiotic susceptibility. The study also attempted to identify multi- drug resistant bacteria isolated from patients with urinary tract infections. The predominant causative agents of urinary tract infections (UTIs) are Escherichia coli and Staphylococcus saprophyticus, whereas less often encountered pathogens include Proteus species, Klebsiella spp, Pseudomonas aeruginosa, Enterococci, and Candida albicans.[4,5] UTI patients are often treated empirically, with medication guided by the antibiotic resistance pattern of the urinary isolates. The widespread and improper use of antimicrobial drugs has inevitably led to the emergence of antibiotic resistance which has increasingly become a significant global issue in recent times.[6] Empirical antibiotic therapy is often initiated in individuals with suspected urinary tract infection (UTI) prior to the availability of urine culture susceptibility reports. It is essential to have information about the microorganisms responsible for urinary tract infections (UTIs) and their sensitivity to antibiotics in order to provide suitable therapy.[7] The objective of this research was to investigate the prevalent bacteria that cause urinary tract infections (UTIs) and to assess their susceptibility to antibiotics.[8-11]

MATERIALS AND METHODS

The research was carried out at the microbiology department. A total of 330 individuals who were qualified for participation in the study had undergone a bacteriological analysis of urine and had not taken any antimicrobial medications in the two weeks before. Non-compliant UTI patients were omitted from this research.

Methodology

Aseptic procedures were used to limit contamination of the urine sample during the collection process, using the clean catch mid-stream method. A grand total of 330 midstream urine samples were gathered from persons, including inpatients and outpatients, who had clinical symptoms of having urinary tract infections. The samples were collected using sterile screw-capped universal containers. The specimens were correctly labeled and promptly processed following collection.

Culturing and Identification of Isolates

The urine samples were inoculated over cysteine lactose electrolyte-deficient medium (CLED) using a calibrated loop method. The samples were then incubated aerobically at a temperature of 37°C for a duration of 24 hours. A significant bacteriuria was defined as a urine culture yielding >10^5 colony-forming units per milliliter (CFU/mL). Positive urine cultures were further identified based on their colony features, Gram- staining and biochemical reactions. Enterobacteriaceae were detected by the assessment of H2S generation and carbohydrate consumption on TSI agar, as well as through the motility test, urease test, oxidase test, and IMViC (indole, methyl red, Voges-Proskauer, and citrate utilization) tests. The Gram-positive bacteria were identified by the catalase, coagulase and bile esculin test.The quality control of the disc was tested by Escherichia coil ATCC 25922 and staphylococcus aureus ATCC 25923.

Antimicrobial Susceptibility Testing

Antimicrobial Susceptibility Testing was performed on Muller Hinton agar using Kirby-Bauer disk diffusion method and interpreted according to Clinical Laboratory Standards Institute(CLSI). The antibiotic discs used in this study were commercially available and included Ampicillin 10 µg, amoxicillin /clavulanic acid (AMC, 20/10 µg), cephalexin (CEPH, 30 µg), ceftriaxone (CRO, 30 µg), tetracycline (TE, 30 µg), gentamicin (CN, 10 µg), ciprofloxacin (CIP, 5 µg), nitrofurantoin (F, 300 µg), and norfloxacin (NOR, 10 µg). The discs were placed on the agar plates in a sterile manner, ensuring correct spacing. They were then firmly pushed into the agar using sterile forceps and incubated at a temperature of 35–37°C for a period of 18–24 hours. The width of the inhibitory zone surrounding the discs was measured with precision to the closest millimeter. The interpretations were categorized as sensitive (S), intermediate (I), or resistant (R). Isolates of bacteria that are resistant to three or more antimicrobials from distinct structural classes are classified as multidrug resistant (MDR).

Data Analysis

The data were inputted and analyzed using SPSS version 25.0 software. Discrete variables were represented using frequency and percentage values. The findings were shown using tables.

RESULTS

[Table 1] shows the distribution of bacteria, with E. coli 191 being the most prevalent at 57.9%, followed by Klebsiella spp 112 at 33.9%. The other bacteria include Proteus mirabilis 6 (1.8%), Proteus vulgaris 2 (0.6%), Enterococcus faecalis 1 (0.3%), Staphylococcus aureus 5 (1.5%), and Pseudomonas aeruginosa 13 (3.9%). [Table 2] displays the bacterial distribution categorized by gender. Among the total of 330 individuals, 245 were female and 85 were male. [Table 3] shows the distribution of m i c r o o r g a n i s m s according to the age of the patients. The majority of the patients were aged 71 years and above (64), followed by the age group of 51-60 years (59), 61-70 years (56), 41-50 years (41), below 20 years (38), 21-30 years (37), and 31-40 years. [Table 4] displays the percentages of ESBL Negative and Positive cases for different bacteria. For E.coli, the percentages are 112 (53.1%) and 79 (79%) respectively. For Klebsiella spp, the percentages are 91 (43.1%) and 21 (21%) respectively. As for Proteus mirabilis, the percentages are 6 (2.8%) and 0, and for Proteus vulgaris, the percentages are 2 (0.9%) and 0 respectively. [Table 5] demonstrates the susceptibility of E. coli to various antibiotics. The most effective antibiotics against E. coli are Meropenem (99.5%) and Imipenem (99.5%), followed by Fosfomycin, Cefoperazone / Sulbactam, and Piperacillin / Tazobactam. [Table 6] demonstrates that Klebsiella spp is highly susceptible to Meropenem (100%) and Imipenem (100%), followed by Piperacillin/Tazobactam (98.2%) and Amikacin (98.2%). [Table 7] demonstrates that Proteus mirabilis has 100% sensitivity to Fosfomycin, Cefoperazone/Sulbactam, Piperacillin/Tazobactam, Meropenem, Imipenem, Ciprofloxacin, Norfloxacin, Gentamicin, Amikacin, and Cefepime. [Table 8] demonstrates that Proteus vulgaris is 100% susceptible to the following antibiotics: Cefoxitin, Cefixime, Ceftriaxone, Cefotaxime, Fosfomycin, Cefoperazone/Sulbactam, Piperacillin/Tazobactam, Meropenem, Imipenem, Ciprofloxacin, Norfloxacin, Gentamicin, Amikacin, and Cefepime. [Table 9] show that all the antibiotics on Enterococci were 100% sensitive. [Table 10] demonstrates that Proteus vulgaris has 100% sensitivity to Nitrofurantion, Gentamicin, Fostomycin, Linezolid, and Vancomycin. [Table 11] demonstrates that Proteus vulgaris has 100% sensitivity to cefepime, ceftazidime, cefoperazone sulbactam, piperacillin, tazobactam, meropenem, imipenem, levofloxacin, and amikacin

Table 1: Isolated organism

Organism	Frequency	Percent
E.coli	191	57.9
Klebsiella spp	112	33.9
Proteus mirabilis	6	1.8
Proteus vulgaris	2	0.6
Enterococcus faecalis	1	0.3
Staphylococcus aureus	5	1.5
Pseudomonas aeruginosa	13	3.9
Total	330	100.0

Table 2: Distribution of bacteria based on the gender of the participants

Organism	Gender		Total
	Female	Male	
E.coli	147(60%)	44(51.8%)	191
Klebsiella spp	80(32.7%)	32(37.6%)	112
Proteus mirabilis	4(1.6%)	2(2.4%)	6
Proteus vulgaris	0	2(2.4%)	2
Enterococci	0	1(1.2%)	1
staphylococcus	4(1.6%)	1(1.2%)	5
Pseudomonas	10(4.1%)	3(3.5%)	13
Total	245	85	330

Table 3: Distribution of bacteria based on age of the patients

Age	Organism							Total
	E.coli	Klebsiella spp	Proteu s mirabilis	Proteus vulgaris	Enterococci	staphylococcus	Pseudomonas	
≤20	24(12.6%)	11(9.8%)	1(16.7%)	1(50%)	0	0	1(7.7%)	38
21-30	13(6.8%)	21(18.8%)	0	0	0	1(20%)	2(15.4%)	37
31-40	19(9.9%)	14(12.5%)	0	1(50%)	0	1(20%)	0	35
41-50	29(15.2%)	8(7.1%)	0	0	1(100%)	1(20%)	2(15.4%)	41
51-60	32(16.8%)	19(17%)	2(33.3%)	0	0	1(20%)	5(38.5%)	59
61-70	40(20.9%)	16(14.3%)	0	0	0	0	0	56
≥71	34(17.8%)	23(20.5%)	3(50%)	0	0	1(20%)	3(23.1%)	64
Total	191	112	6	2	1	5	13	330

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Table 4: ESBL positive and negative on isolated bacteria

Organism	ESBL		Total
	-	+	
E.coli	112(53.1%)	79(79%)	191
Klebsiella spp	91(43.1%)	21(21%)	112
Proteus mirabilis	6(2.8%)	0	6
Proteus vulgaris	2(0.9%)	0	2
Total	211	100	311

Table 5: Antibiotics pattern on E.coli

Antibiotics	Intermediate		Resistance		Sensitive	
	n	%	n	%	n	%
Ampicillin	2	1.0	149	78.0	40	20.9
Amoxicillin/Clavulanic acid	30	15.7	19	9.9	142	74.3
Cefazolin	2	1.0	119	62.3	70	36.6
Cefoxitin	2	1.0	59	30.9	130	68.1
Cefixime	3	1.6	100	52.4	88	46.1
Ceftriaxone	4	2.1	96	50.3	91	47.6
Cefotaxime	4	2.1	96	50.3	91	47.6
Fosfomycin	2	1.0	8	4.2	181	94.8
Cefoperazone /Sulbactam	0	0.0	3	1.6	188	98.4
Piperacillin /Tazobactam	0	0.0	3	1.6	188	98.4
Meropenem	0	0.0	1	0.5	190	99.5
Imipenem	0	0.0	1	0.5	190	99.5
Ciprofloxacin	5	2.6	73	38.2	113	59.2
Norfloxacin	5	2.6	67	35.1	119	62.3
Nitrofurantoin	14	7.3	21	11.0	156	81.7
Gentamicin	12	6.3	24	12.6	155	81.2
Amikacin	3	1.6	2	1.0	186	97.4
Cotrimoxazole	9	4.7	71	37.2	111	58.1
cefepime	3	1.6	56	29.3	132	69.1

Table 6: Antibiotics pattern on Klebsiella spp

Antibiotics	Intermediate		Resistance		Sensitive	
	n	%	n	%	n	%
Ampicillin	0	0.0	112	100.0	0	0.0
Amoxicillin/Clavulanic acid	19	17.0	30	26.8	63	56.3
Cefazolin	0	0.0	61	54.5	51	45.5
Cefoxitin	3	2.7	48	42.9	61	54.5
Cefixime	5	4.5	53	47.3	54	48.2
Ceftriaxone	4	3.6	50	44.6	58	51.8
Cefotaxime	4	3.6	51	45.5	57	50.9
Fosfomycin	0	0.0	32	28.6	80	71.4
Cefoperazone /Sulbactam	1	0.9	2	1.8	109	97.3
Piperacillin /Tazobactam	0	0.0	2	1.8	110	98.2
Meropenem	0	0.0	0	0.0	112	100.0
Imipenem	0	0.0	0	0.0	112	100.0
Ciprofloxacin	10	8.9	17	15.2	85	75.9
Norfloxacin	2	1.8	16	14.3	94	83.9
Nitrofurantoin	17	15.2	25	22.3	70	62.5
Gentamicin	3	2.7	11	9.8	98	87.5
Amikacin	0	0.0	2	1.8	110	98.2
Cotrimoxazole	3	2.7	18	16.1	91	81.3
cefepime	2	1.8	19	17.0	91	81.3

Table 7: Antibiotics pattern of Proteus mirabilis

Antibiotics	Intermediate		Resistance		Sensitive	
	n	%	n	%	n	%
Ampicillin	0	0.0	5	83.3	1	16.7
Amoxicillin/Clavulanic acid	0	0.0	5	83.3	1	16.7
Cefazolin	0	0.0	5	83.3	1	16.7
Cefoxitin	0	0.0	5	83.3	1	16.7
Cefixime	2	33.3	3	50.0	1	16.7
Ceftriaxone	2	33.3	2	33.3	2	33.3
Cefotaxime	2	33.3	2	33.3	2	33.3
Fosfomycin	0	0.0	0	0.0	6	100.0
Cefoperazone /Sulbactam	0	0.0	0	0.0	6	100.0
Piperacillin /Tazobactam	0	0.0	0	0.0	6	100.0
Meropenem	0	0.0	0	0.0	6	100.0
Imipenem	0	0.0	0	0.0	6	100.0
Ciprofloxacin	0	0.0	0	0.0	6	100.0

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Norfloxacin	0	0.0	0	0.0	6	100.0
Nitrofurantoin	0	0.0	4	66.7	2	33.3
Gentamicin	0	0.0	0	0.0	6	100.0
Amikacin	0	0.0	0	0.0	6	100.0
Cotrimoxazole	0	0.0	6	100.0	0	0.0
cefepime	0	0.0	0	0.0	6	100.0

Table 8: Antibiotics pattern on Proteus vulgaris

Antibiotics	Intermediate		Resistance		Sensitive	
	n	%	n	%	n	%
Ampicillin	0	0.0	2	100.0	0	0.0
Amoxicillin/Clavulanic acid	1	50.0	1	50.0	0	0.0
Cefazolin	1	50.0	1	50.0	0	0.0
Cefoxitin	0	0.0	0	0.0	2	100.0
Cefixime	0	0.0	0	0.0	2	100.0
Ceftriaxone	0	0.0	0	0.0	2	100.0
Cefotaxime	0	0.0	0	0.0	2	100.0
Fosfomycin	0	0.0	0	0.0	2	100.0
Cefoperazone /Sulbactam	0	0.0	0	0.0	2	100.0
Piperacillin /Tazobactam	0	0.0	0	0.0	2	100.0
Meropenem	0	0.0	0	0.0	2	100.0
Imipenem	0	0.0	0	0.0	2	100.0
Ciprofloxacin	0	0.0	0	0.0	2	100.0
Norfloxacin	0	0.0	0	0.0	2	100.0
Nitrofurantoin	0	0.0	2	100.0	0	0.0
Gentamicin	0	0.0	0	0.0	2	100.0
Amikacin	0	0.0	0	0.0	2	100.0
Cotrimoxazole	0	0.0	2	100.0	0	0.0
cefepime	0	0.0	0	0.0	2	100.0

Table 9: Antibiotics pattern of Enterococci

	Intermediate		Resistance		Sensitive	
	n	%	n	%	n	%
Pencillin	0	0.0	0	0.0	1	100.0
Ampicillin	0	0.0	0	0.0	1	100.0
amoxyclav	0	0.0	0	0.0	1	100.0
doxycycline	0	0.0	0	0.0	1	100.0
ciprofloxacin	1	100.0	0	0.0	0	0.0
norfloxacin	0	0.0	0	0.0	1	100.0
nitrofurantoin	0	0.0	0	0.0	1	100.0
gentamicin	0	0.0	0	0.0	1	100.0
Fostomycin	0	0.0	0	0.0	1	100.0
Rifampicin	0	0.0	0	0.0	1	100.0
Linezolid	0	0.0	0	0.0	1	100.0
Vancomycin	0	0.0	0	0.0	1	100.0

Table 10: Antibiotics pattern of Staphylococcus

	Intermediate		Resistance		Sensitive	
	n	%	n	%	n	%
Cefoxitin	0	0.0	1	20.0	4	80.0
Doxycycline	0	0.0	1	20.0	4	80.0
Ciprofloxacin	0	0.0	2	40.0	3	60.0
Norfloxacin	0	0.0	1	20.0	4	80.0
Nitrofurantion	0	0.0	0	0.0	5	100.0
Gentamicin	0	0.0	0	0.0	5	100.0
Cotrimoxazole	0	0.0	1	20.0	4	80.0
Fostomycin	0	0.0	0	0.0	5	100.0
Rifampicin	0	0.0	1	20.0	4	80.0
Linezolid	0	0.0	0	0.0	5	100.0
Vancomycin	0	0.0	0	0.0	5	100.0

Table 11: Antibiotics pattern of Pseudomonas

	Intermediate		Resistance		Sensitive	
	n	%	n	%	n	%
cefepime	0	0.0	0	0.0	13	100.0
ceftazidime	0	0.0	0	0.0	13	100.0
cefepime	0	0.0	0	0.0	13	100.0
cefoperazone sulbactam	0	0.0	0	0.0	13	100.0
piperacillin tazobactam	0	0.0	0	0.0	13	100.0
Meropenem	0	0.0	0	0.0	13	100.0
Imipenem	0	0.0	0	0.0	13	100.0

Ciprofloxain	1	7.7	1	7.7	11	84.6
Levofloxacin	0	0.0	0	0.0	13	100.0
Gentamicin	1	7.7	2	15.4	10	76.9
Amikacin	0	0.0	0	0.0	13	100.0
Aztreonam	0	0.0	2	15.4	11	84.6

DISCUSSION

Urinary tract infections (UTIs) may occur in both community and hospital settings. Urinary tract infections (UTIs) are often treated empirically with the choice of antimicrobial medication based on the prevalent bacteria and its anticipated resistance to antimicrobials. Furthermore, the treatment of urinary tract infections (UTIs) has been compromised due to the rise in the prevalence of antimicrobial resistance. Therefore, it is necessary to regularly monitor the bacteria responsible for urinary tract infections (UTIs) and their sensitivity to antimicrobial drugs in the specific area.[12] The causes of urinary tract infections (UTIs) and the susceptibility of bacteria that cause UTIs have been evolving in both community and hospital settings throughout time.[13,14] In the last ten years, the preferred therapy for urinary tract infections (UTIs) has shifted from co-trimoxazole to quinolones due to the increasing resistance to co- trimoxazole and its high risk of treatment failure.[15] Antimicrobial resistance has been linked to a higher likelihood of clinical treatment failure. Reports from Canada and the US suggest that the frequency of resistance to Cotrimoxazole is above 15% and may reach up to 25%. Fluoroquinolones can be used for treating simple urinary tract infections (UTIs) in locations where the rate of resistance to cotrimoxazole is more than 10%. They are also used to treat complex UTIs and acute pyelonephritis.[16] This finding is consistent with previous research conducted by Bashir MF et al,[17] Women have a higher susceptibility to urinary tract infections (UTIs) compared to men due to the fact that the female urethra is shorter in length and located in closer proximity to the anus.[18] E. coli was the most often found bacterium in urinary tract infections, accounting for 57.9% of cases. Klebsiella spp was the second making up 33.9% of cases. Other less common organisms were Proteus mirabilis (1.8%), Proteus vulgaris (0.6%), Enterococcus faecalis (0.3%), Staphylococcus aureus (1.5%), and Pseudomonas aeruginosa (3.9%). The number of bacterial species isolated was comparable to that reported in many prior research.[19-21] The research conducted by Bashir MF et al revealed comparable results, demonstrating that the organisms exhibited resistance to earlier urinary antimicrobial drugs like Ampicillin. This suggests that the increased use of a certain antibiotic might lead to the development of resistance. Antimicrobial resistance is an inherent biological reaction of microorganisms to antimicrobial medications. Resistance may be innate. The current investigation identified E.coli 191 (57.9%), Klebsiella spp112 (33.9%), and Pseudomonas aeruginosa 13 (3.9%) as the main species responsible for causing urinary tract infections (UTIs). The findings align with earlier studies conducted in Cameroon, Pakistan, Israel, and Turkey,[22,23] which identified E-coli and K. pneumoniae as the primary pathogens responsible for urinary tract infections (UTIs). ESBL bacteria are often characterized by their resistance to many drugs. The findings of our study indicated that 53.1% of E.coli samples tested negative for ESBL, while 79% tested positive. For Klebsiella spp 43.1% tested negative for ESBL, while 21% tested positive. As for Proteus mirabilis, 2.8% tested negative for ESBL and none tested positive. Finally, for Proteus vulgaris, 0.9% tested negative for ESBL and none tested positive. In our investigation, we found that Meropenem and Imipenem are the most effective antibiotics against E. coli, with a sensitivity rate of 99.5%. Following closely behind are Fosfomycin, Cefoperazone / Sulbactam, and Piperacillin/Tazobactam. The research found that Klebsiella spp is sensitive to Meropenem (100%) and Imipenem (100%), with somewhat lower sensitivity to Piperacillin / Tazobactam (98.2%) and Amikacin (98.2%). The research found that Proteus mirabilis had 100% sensitivity to Fosfomycin, Cefoperazone/Sulbactam, Piperacillin/Tazobactam, Meropenem, Imipenem, Ciprofloxacin, Norfloxacin, Gentamicin, Amikacin, and Cefepime medicines. Others have also conducted comparable studies.[19-23]

CONCLUSION

Our analysis revealed that the prevalence of Urinary Tract Infection was higher in females compared to men. Escherichia coli is the predominant bacteria found in urinary tract infections. The urinary bacteria showed resistance to routinely used antibiotics such as Ampicillin and Norfloxacin. This trend of developing resistance to routinely prescribed antibiotics for treating urinary tract infections serves as a warning against the indiscriminate use of antibiotics. The study's findings establish that E-coli is the primary causative agent of urinary tract infections (UTIs).

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