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Prevalence and Antimicrobial Resistance Patterns of Uropathogenic Bacteria Isolated at Tobruk Medical Center, Tobruk, Libya: A 4-Year Retrospective Study

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ABSTRACT

Background: The UTI is one of the most common bacterial diseases, and the emergence of antimicrobial resistance is now an acute risk to population health. The current study investigated the prevalence of Uropathogenic bacteria and their resistance trend in a sample of patients at the Tobruk Medical Centre. **Methods:** Between January 2021 and December 2024, 6,032 urine specimens were taken from patients suspected of UTI. Isolates were identified with standard microbiological and biochemical methods. Susceptibility was tested by Kirby-Bauer disk diffusion following CLSI (Clinical Laboratory Standards Institute) guidelines (20). **Results:** Significant bacterial growth was recovered from 1,264 specimens (20.95%). Gram-negative organisms dominated, with Escherichia coli as the leading isolate (49.36%), followed by Klebsiella spp. (16.85%) and Proteus spp. (11.07%). Staphylococcus aureus (14.79%) was the chief gram-positive isolate. Several isolates remained highly susceptible to nitrofurantoin and ciprofloxacin, but resistance increased against Augmentin, ceftazidime, Clarithromycin, and gentamicin. **Conclusion:** Gram-negative bacteria and *Escherichia coli*, in this case, were the main causative agents of UTIs. These findings highlight the need to have continuous surveillance and prudent use of antibiotics in an effort to strengthen the control of UTI in eastern Libya.

1. INTRODUCTION

The UTIs are among the most common types of bacterial infections that are contracted both in the community and in the healthcare facilities, and women are disproportionately affected. Untreated acute UTI can lead to pyelonephritis, chronic kidney disease as well as irreversible renal damage. UTIs currently constitute the number 1 cause of gram-negative bacteremia and urosepsis in all age groups and carry a huge mortality burden as well as a huge economic burden on the global health care system (Ullah et al., 2025). UTIs are a result of a Uropathogens spectrum that is highly predictable but constantly changing.

Gram-negative bacilli of the isolates are recovered most frequently and Uropathogenic *Escherichia coli* (UPEC) remains the most prevalent. Other organisms that are commonly seen are *Proteus* species, *Pseudomonas aeruginosa*, Acinetobacter species, *Klebsiella* species, Enterobacter species and Citrobacter species. On the gram-positive side, Staphylococcus saprophyticus, *Enterococcus* species and coagulase-negative Staphylococci (CoNS) are commonly detected, especially in complicated and catheter-related cases (Lee et al., 2019). Over the past several decades, the worldwide escalation of antimicrobial resistance (AMR) has greatly undermined how UTIs are managed. Patients are likely to have a poorer outcome when a non-sensitive strain is involved, spending more time in hospital and admitted to the intensive care unit (ICU) more often. The susceptibility and prevalence of such organisms also changes significantly between periods and areas; thus, the selection of empirical therapy has become increasingly difficult and more often leads to a treatment failure (Rojas et al., 2024). Increasing resistance observed in Uropathogens is due to complex molecular alterations. An example of beta-lactam resistance is largely mediated by enzymes produced by the bacteria, the most common of them being the Extended-Spectrum Beta-Lactamases (ESBLs) of the CTX-M, TEM, and SHV type (Hasan & Ibrahim, 2022). These enzymes make penicillin and cephalosporins useless by cleaving the beta-lactam ring (Hussain et al., 2021). Moreover, the emergence of Carbapenem-Resistant Enterobacteriaceae (CRE)-which depend on mechanisms such as *Klebsiella pneumoniae* carbapenemase (KPC) or New Delhi metallo-beta-lactamase (NDM-1)-has reduced the last-resort options available to clinicians (Lynch, Clark, & Zhanel, 2021). Besides enzymatic degradation of antibiotics, Uropathogens use efflux pump systems-such as the AcrAB-TolC system found in *E. coli*-to force drugs out of the cell and thus maintain their concentration inside the cell below the level that would be fatal (Halawa et al., 2024). Resistance to fluoroquinolones, which were previously the mainstay of UTI treatment, is also driven by mutations in the Quinolone Resistance-Determining Regions (QRDRs) of genes of DNA gyrase and topoisomerase IV (Bush, Diez-Santos, Abbott, & Maxwell, 2020). The ability of Uropathogens to form biofilms is one of the major factors that promote the development and persistence of UTIs, particularly among patients who have catheters. An organized community of bacterial cells that is surrounded by a self-created extracellular polymeric substance (EPS) matrix is called such a biofilm. As a physical barrier, this matrix restricts the range of penetration of antimicrobials and prevents the host phagocytosis of bacteria. Consequently, the organisms within the biofilm can withstand resistance as much as 1,000 times higher than the organism in a planktonic condition resulting in an emergence of chronic infections, which are notoriously difficult to eliminate (Harrell et al., 2021). Horizontal gene transfer is also encouraged by the biofilm environment, and it can accelerate the dissemination of the resistance genes in the crowded bacterial population (Fateh & Ali, 2025). These structural defenses are not the only ones; other subpopulations of bacteria enter into a dormant, tolerant state known as persistence, which is a non-genetic mechanism of antibiotic resistance (Murray et al., 2021).

2. METHOD

2.1 Study Population

This study was carried out retrospectively with 6,032 patients who are referred to the Tobruk Medical Centre and were suspected of having urinary tract infection (UTI). Within the cohort there were 3,294 females (54.6%) and 2,738 males (45.4%). The specimens were obtained in various hospital units and the sample varied across both inpatient wards, and the outpatient departments (OPD). All the urine samples collected between January 2021 and December 2024 were tested at the Microbiology Laboratory of the Tobruk Medical Centre.

2.2 Specimen Collection and Identification

Urine samples were stored in dry, clean, and sterile containers. Pediatric patients were collected using sterile bags, but straight catheterization was done whenever there was need to single-catheterize. Each sample was plated on Cysteine Lactose Electrolyte Deficient (CLED) agar, blood agar, MacConkey agar and nutrient agar using sterile calibrated disposable loops. Aerobic incubation at 37°C of the plates was then carried out over a period of 24 hours. Gram staining and the routine biochemical test, catalase and coagulase were used to define colonies with typical features (George, World Health Organization, & Paterson, 2021).

2.3 Antimicrobial Susceptibility Testing (AST)

Susceptibility testing was done using the Kirby-Bauer disk diffusion. Bacterial suspensions in 0.85% NaCl were made in short overnight cultures that were standardized to a 0.5 McFarland turbidity. The suspensions were then placed on the surface of Mueller-Hinton agar plates and antibiotic disks were then laid down (George, World Health Organization, & Paterson, 2021).

After the plates were incubated 24 hours at 37°C, the diameters of the inhibition zones were determined. In accordance with the typical clinical guidelines, every outcome was marked as Sensitive (S), Intermediate (I), or Resistant (R). The following antibiotic disks were tested:

Table 1. Panel of antimicrobial agents (disk in µg) used for susceptibility testing of isolated strains.

Cefoxitin (30µg)	**Gentamicin** (10µg)	**Ampicillin** (10µg)	**Trimethoprim Sulfamethoxazole** (25µg)	**Cephalothin** (30µg)	**Nitrofurantoin** (300µg)
Nalidixic acid (30µg)	**Augmentin** (30µg)	**Amikacin** (30µg)	**Tetracycline** (30µg)	**Cefotaxime** (30µg)	**Ciprofloxacin** (5µg)

3. ETHIC APPROVAL

The University of Tobruk's Faculty of Health Sciences granted ethical permission for the study. Anonymized laboratory records that were obtained from the Tobruk Medical Center laboratory in previous years were used for the study. During data collection, no patient names, personal identifiers, or private information were accessible or recorded. Every piece of information was treated with the strictest confidentially and utilized only for scientific studies. Individual informed permission was not required because the study only used already-existing laboratory information and did not include direct patient contact. All private information were maintained throughout the study with accepted ethical scientific research principles.

4. RESULT

4.1 Demographic and Sample Distribution

In the course of four years, 6,032 urine samples were collected both inpatient and community-based at Tobruk Medical Centre. Females made up 54.6% (n=3,294) of the cohort and males 45.4% (n=2,738), as shown in table 1.

Table 2. Distribution of included cases by gender at Tobruk Medical Center from 2021 to 2024.

Gender	Number of cases	Percentage (%)
Male	2738	45.4
Female	3294	54.6
Total	**6032**	**100**

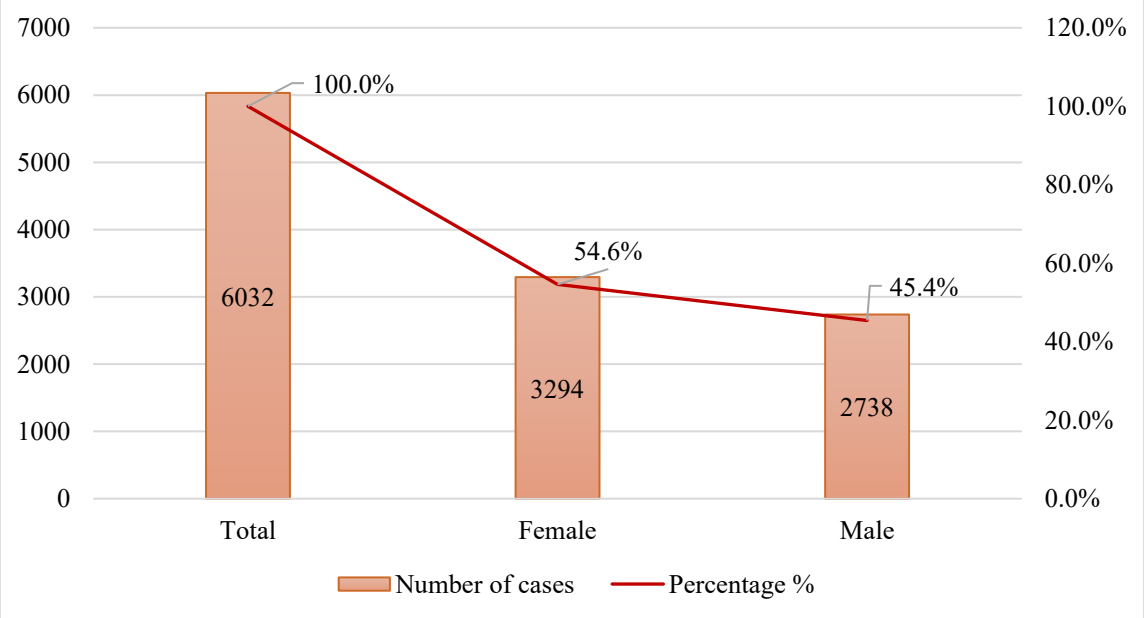

Fig. 1 Distribution of included cases by gender at Tobruk Medical Center from 2021 to 2024. A total of 6,032 cases were included, comprising 3,294 (54.6%) females and 2,738 (45.4%) males.

Among all of the sampled, 1,264 (20.95%) tests were positive due to urinary tract infection (UTI). Positivity fell steadily year on year, from 24.27% in 2021 down to a low of 17.57% in 2024, as shown in table 2.

Table 3. Yearly Distribution of Urine Culture Samples and Confirmed UTI Cases.

Year	Number of urine sample cultured	Number of UTI-Positive Cases	Percentage (%)
2021	1442	350	24.27
2022	886	192	21.67
2023	1866	399	21.38
2024	1838	323	17.57
Total	**6032**	**1264**	**20.95**

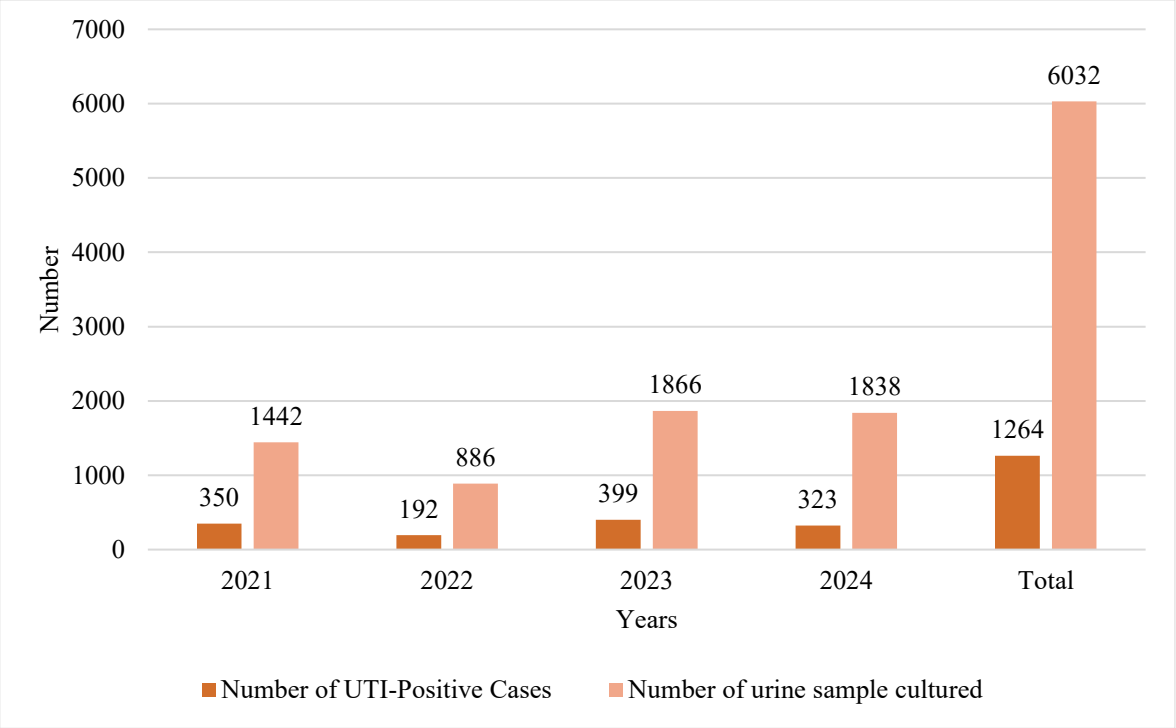

Fig. 2 Distribution of Total Urine Culture Samples and Confirmed UTI Cases Across the Study Period.

4.2 Prevalence of Uropathogenic Isolates:

Table 4 indicates that the top causative agents were gram-negative bacteria with a total of 81.80% (n=1,034) of all the isolates and gram-positive pathogens had the remainder 18.19% (n=230).

Escherichia coli had the highest recovery (49.36%, n=624), followed by *Klebsiella* spp. (16.85%, n=213), *Staphylococcus aureus* (14.79%, n=187) and *Proteus* spp. (11.07%, n=140). The other pathogens included *Pseudomonas* spp. (4.50%), *Staphylococcus epidermidis* (1.66%), *Enterococcus* spp. (1.18%), and *Streptococcus* spp. (0.55%).

Table 4. Distribution of Gram-Negative and Gram-Positive Uropathogenic Isolates

Name of Bacteria	Number of isolated bacteria	Percentage (%)
Gram Negative pathogens	**1034**	**81.81**
Escherichia coli	624	49.36
Klebsiella species	213	16.85
Proteus species	140	11.07
Pseudomonas species	57	4.50
Gram positive pathogens	**230**	**18.19**
Staphylococcus aureus	187	14.79
Staphylococcus epidermidis	21	1.66
Streptococcus species	7	0.55
Enterococcus species	15	1.18
Total	**1264**	**100**

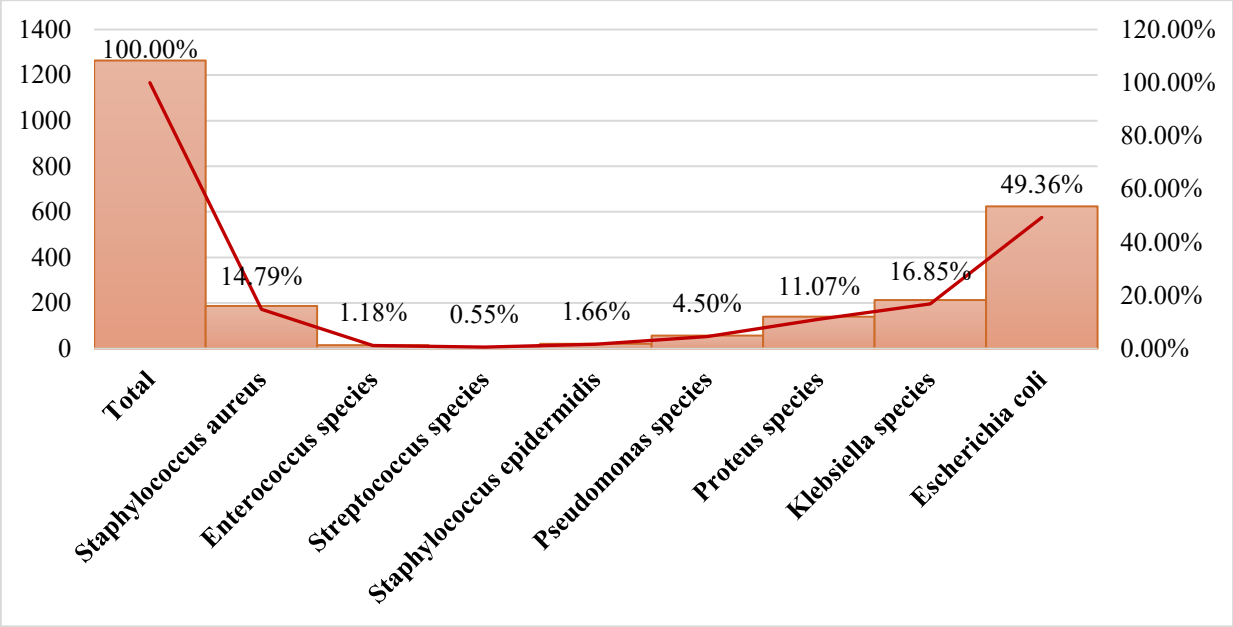

Fig. 3 Names, numbers and the percentage of Uropathogenic bacterial species.

4.3 Antimicrobial Susceptibility Trends (2021-2024)

The susceptibility profiles of the isolates exhibited significant temporal variations throughout the study period:

- Gram-Positive Isolates: In the initial phase (2021-2022), Nitrofurantoin showed high efficacy with approximately 60% sensitivity. However, sensitivity patterns shifted by 2023-2024, with Cefoxitin emerging as a relatively effective agent (sensitivity ranging from 13.04% to 16.67%). Cefoxitin resistance reached 68% in 2022; Augmentin and Gentamicin resistance both peaked at 34.85% in 2024.
- Gram-negative isolates: In 2021, Ciprofloxacin (35.33%) and Nitrofurantoin (52%) showed the highest sensitivity. In 2024, Nitrofurantoin (63.88%) and Augmentin (amoxicillin/clavulanic acid) (85.55%) ranked highest.
- Rising resistance: Clarithromycin resistance reached 72.89% in 2022, Augmentin 40.98% in 2023, and Nitrofurantoin 47.9% in 2024.

Table 5. Antibacterial agents versus urine isolates: sensitivity and resistance, 2021 (R = resistant, S = sensitive, - = not tested).

Antimicrobial Agents / Symbols 2021	Gram Positive pathogens (n = 47)		Gram Negative pathogens (n = 300)	
	R No. (%)	S No. (%)	R No. (%)	S No. (%)
Cefoxitin (FOX)	14 (29.79)	11 (23.40)	49 (16.33)	68 (22.66)
Ciprofloxacin (CIP)	6 (12.77)	21 (44.68)	63 (21.00)	106 (35.33)
Nitrofurantoin (F)	2 (4.26)	28 (59.57)	59 (19.67)	156 (52.00)
Clarithromycin (CL)	6 (12.77)	3 (6.38)	89 (29.67)	20 (6.66)
Gentamicin (CN)	15 (31.91)	8 (17.02)	78 (26.00)	44 (14.66)
Ceftazidime (CAZ)	17 (36.17)	4 (8.51)	89 (29.66)	97 (32.33)
Trimethoprim/Sulphamethoxazole (SXT)	-	-	91 (30.33)	53 (17.66)
Amoxicillin Clavulanic acid (AMC)	1 (2.13)	14 (29.79)	39 (13.00)	36 (12.00)
Ampicillin (AMP)	3 (6.38)	2 (4.26)	46 (15.33)	4 (1.33)
Tetracycline (TE)	2 (4.26)	1 (2.13)	71 (23.67)	47 (15.66)
Cephalothin (KF)	5 (10.64)	16 (34.04)	82 (27.33)	14 (4.66)

Table 6. Antibacterial agents versus urine isolates: sensitivity and resistance, 2022 (R = resistant, S = sensitive, - = not tested).

Antimicrobial Agents / Symbols 2022	Gram Positive pathogens (n = 25)		Gram Negative pathogens (n = 166)	
	R No. (%)	S No. (%)	R No. (%)	S No. (%)
Cefoxitin (FOX)	17 (68)	2 (8)	36 (21.68)	84 (50.60)
Ciprofloxacin (CIP)	8 (32)	0	21 (12.65)	19 (11.44)
Nalidixic acid (NA)	-	-	73 (43.98)	26 (15.66)
Nitrofurantoin (F)	4 (16)	15 (60)	39 (23.49)	62 (37.34)
Clarithromycin (CL)	18 (72)	3 (12)	121 (72.89)	6 (3.61)
Cefamandole (MA)	7 (28)	6 (24)	80 (48.19)	26 (15.66)
Ceftazidime (CAZ)	15 (60)	3 (12)	49 (29.52)	40 (24.09)
Pefloxacin (PEF)	9 (36)	8 (32)	49 (29.52)	58 (34.93)

Table 7. Antibacterial agents versus urine isolates: sensitivity and resistance, 2023 (R = resistant, S = sensitive, - = not tested).

Antimicrobial Agents / Symbols 2023	Gram Positive pathogens n = 92		Gram Negative pathogens n = 305	
	R No. (%)	S No. (%)	R No. (%)	S No. (%)
Cefoxitin (FOX)	48 (52.17)	12 (13.04)	49 (16.07)	71 (23.20)
Ciprofloxacin (CIP)	23 (25.00)	22 (23.91)	49 (16.07)	83 (27.20)
Nitrofurantoin (F)	28 (30.43)	32 (34.78)	57 (18.69)	88 (28.80)
Amikacin (AK)	2 (2.17)	0 (0.00)	23 (7.54)	0 (0.00)
Nalidixic acid (NA)	19 (20.65)	2 (2.17)	30 (9.84)	19 (6.22)
Ceftazidime (CAZ)	25 (27.17)	0 (0.00)	0 (0.00)	0 (0.00)
Trimethoprim/Sulphamethoxazole (SXT)	1 (1.09)	1 (1.09)	100 (32.79)	35 (11.40)
Amoxicillin Clavulanic acid (AMC)	35 (38.04)	9 (9.78)	125 (40.98)	14 (4.59)
Ampicillin (AMP)	15 (16.30)	5 (5.43)	40 (13.11)	15 (4.91)
Cephalothin (KF)	7 (7.61)	2 (2.17)	16 (5.25)	0 (0.00)

Table 8. Antibacterial agents versus urine isolates: sensitivity and resistance, 2024 (R = resistant, S = sensitive, - = not tested).

Antimicrobial Agents / Symbols 2024	Gram Positive pathogens n = 66		Gram Negative pathogens n = 263	
	R No. (%)	S No. (%)	R No. (%)	S No. (%)
Cefoxitin (FOX)	4 (6.06)	11 (16.67)	58 (22.05)	49 (18.63)
Ciprofloxacin (CIP)	3 (4.55)	6 (9.09)	44 (16.73)	39 (14.83)
Nalidixic acid (NA)	0 (0.00)	1 (1.52)	0 (0.00)	6 (2.28)
Nitrofurantoin (F)	12 (18.18)	11 (16.67)	126 (47.90)	168 (63.88)
Amoxicillin & Clavulanic acid (AMC)	23 (34.85)	6 (9.09)	47 (17.87)	225 (85.55)
Ampicillin (AMP)	9 (13.64)	1 (1.52)	4 (1.52)	92 (34.98)
Trimethoprim/Sulphamethoxazole (SXT)	14 (21.21)	14 (21.21)	73 (27.76)	148 (56.27)
Amikacin (AK)	-	-	38 (14.45)	27 (10.27)
Tetracycline (TE)	16 (24.24)	2 (3.03)	37 (14.07)	125 (47.53)
Cephalothin (KF)	-	-	18 (6.84)	47 (17.87)
Cefotaxime (CTX)	0 (0.00)	2 (3.03)	8 (3.04)	14 (5.32)
Gentamicin (CN)	23 (34.85)	5 (7.58)	83 (31.56)	114 (43.35)

5. DISCUSSION

This study reports prevalence and resistance patterns of Uropathogens at Tobruk Medical Centre from 2021 to 2024. Of 6,032 suspected cases, 1,264 (20.95%) were culture-positive for UTI. Female patients (54.6%) outnumbered males (45.4%), matching known epidemiology linked to the shorter female urethra and its proximity to the perianal region (Hamadalneel et al., 2024).

Gram-negative bacilli accounted for 81.80% of isolates, and Escherichia coli was the single most common organism (49.36%), in line with global data that put intestinal Enterobacteriaceae at the top of community and hospital UTI causes (Kala, Chakraverti, Kumar, & Kumar, 2026). Among gram-positive isolates, Staphylococcus aureus (14.79%) was the most frequent and remains a relevant opportunistic Uropathogens in this setting.

Antimicrobial susceptibility shifted over the four years. Resistance to commonly used antibiotics rose steadily. By 2024, Gentamicin resistance had reached 31.56%, and Ciprofloxacin sensitivity, once high enough for first-line use, had dropped sharply. Nitrofurantoin and Augmentin (amoxicillin/clavulanic acid) still retained relatively high sensitivity among gram-negative isolates in 2024 (63.88% and 85.55%), but the rise in multidrug-resistant (MDR) strains threatens empirical treatment protocols (Fakhralddin, Abdullah, Ibrahim, & Maarouf, 2026).

Resistance to Nalidixic acid and Ampicillin was high enough that neither drug is suitable for empirical use here. These phenotypes most likely reflect indiscriminate antibiotic use and treatment started without culture and sensitivity results (Anam, Mustafa, & Anjum, 2024).

6. CONCLUSION

Over four years at Tobruk Medical Centre, this retrospective study documents antimicrobial resistance (AMR) in Uropathogenic bacteria as a pressing local problem. Urinary tract infections remain common in the area and are more frequent in female patients (54.6%), matching patterns reported elsewhere.

On the microbiological side, *Escherichia coli* was once again the most common agent followed by *Klebsiella* species and *Staphylococcus aureus*. The most important message is the changing and worsening resistance patterns throughout the years. Older empirical drugs-Ampicillin, Nalidixic acid and Ceftazidime in particular-demonstrated high resistance and therefore they are not suitable as first-line options before susceptibility testing (Aballu, 2026).

Although therapeutic agents such as nitrofurantoin or Augmentin are not yet completely ineffective against gram-negative isolates, increasing numbers of multidrug-resistant (MDR) strains, which may be viewed as a consequence of uncontrolled use of these agents, are posing a serious threat to favorable clinical progress (Temesgen & Shiferaw, 2025). In addition to (Ibrahim, Abdelgyoum, Elfaki, Elbadawi, & Kashif, 2025).

To prevent the resistance spread and protect effective patient care, the CDC (Centers for Disease Control and Prevention) proposes the following ("CDC," 2026):

strongly recommend:

1. **Diagnostic Mandates:** Implementation of a policy requiring routine urine culture and Antibiotic Susceptibility Testing (AST) before the initiation of therapy.
2. **Antimicrobial Stewardship:** The development of localized hospital antibiograms to guide clinicians toward evidence-based, narrow-spectrum antibiotic selection.
3. **Continuous surveillance:** Keep monitoring local uropathogen trends so treatment protocols stay current and remaining effective agents are not wasted.

Containing AMR growth in Tobruk will need clinical laboratories, clinicians, and public-health managers working from the same culture-based treatment standards

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