Middle East Journal of Applied Science & Technology (MEJAST)
Volume 9, Issue 1, Pages 135-140, January-March 2026

A study on Isolation and Characterization of Uropathogenic Microorganisms and Their Antibiotic Sensitivity Profiles

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DOI: https://doi.org/10.46431/mejast.2026.9111

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Article Received: 23 January 2026 *Article Accepted: 24 March 2026* *Article Published: 27 March 2026*

ABSTRACT

Urinary tract infections (UTIs) are among the most common bacterial infections worldwide and represent a significant public health concern affecting millions of individuals each year. This study aimed to isolate and identify uropathogens from urine samples and determine their antibiotic susceptibility patterns for effective treatment. Fourteen urine samples were collected from patients and analyzed using standard cultural, morphological, microscopic, and biochemical identification methods in the microbiology laboratory. Five major bacterial species were identified, with *Escherichia coli* and *Staphylococcus aureus* being the most prevalent and frequently isolated pathogens in the samples studied. Antibiotic susceptibility testing using the Kirby–Bauer disk diffusion method revealed that Gentamicin and Amikacin were the most effective antibiotics against the isolated uropathogens. The findings emphasize the importance of culture-based antibiotic prescription and routine susceptibility testing to prevent multidrug resistance and ensure proper clinical management of urinary tract infections. A urinary tract infection (UTI) is an infection that occurs in any part of the urinary system and is commonly caused by bacterial invasion. The urinary system includes the kidneys, ureters, bladder, and urethra, which work together to produce and eliminate urine from the body. Most infections primarily involve the lower urinary tract, particularly the bladder and the urethra, leading to symptoms such as burning sensation during urination and increased frequency of urination. Women generally have a higher risk of developing UTIs than men due to anatomical and physiological differences in the urinary tract structure. A urinary tract infection that affects the bladder can be painful, uncomfortable, and irritating for the patient. However, if the infection spreads to the kidneys, the condition can become serious and may lead to severe complications requiring immediate medical treatment.

Keywords: Urinary Tract Infection; Uropathogens; Antibiotic Resistance; Gram-Positive Bacteria; Uropathogens; *Escherichia coli*; *Staphylococcus aureus*; Antibiotic Susceptibility; Antimicrobial Resistance; Kirby–Bauer Method; Urine Culture; Gram-Negative Bacteria.

1. Introduction

Urinary tract infection (UTI) is a common clinical condition caused by microbial invasion of the urinary tract. Women are more susceptible due to anatomical and physiological factors. The most common causative organisms include Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Enterococcus faecalis. Normally, urinary tract urine mostly dominated by Staphylococcus aureus followed by E. coli (70-80%), Klebsiella Pneumonia (40-50%) and Pseudomonas aureus and Enterococcus faecalis (10-20%). While Anatomy or physiology factors cause abnormality of urine tract and lead to lacalize infectious bacteria, such as different species of klebsiella, Proteus, Enterobactor, Enterococcus, staphylococcus and Pseudomonas aeruginosa. Those bacteria are more common in most of the cases and frequently cause to un- complicated cystitis and pyelonephiritis. Furthermore, pathooogenesis of urinary tract is more complicated and influenced by other factors, such as vaginal ecosystem especially Lactobacillus spp, Intestinal population, genetic and behavioral factors, virulence properties of uropathogens and host defense factors. The presence of factors will increase opportunity for uropathogens to colonize and invade urothelium. As UTI is one of the most prevalent problems among people of all ages and both genders the infection mostly associated with gram negative uropathogenic E. coli. For these reasons the Antibiotics which are prescribed by physicians mostly effective against gram negative uropathogen. In most cases of UTI associated with Bacterial infection many species of gram-positive bacterium are detected as infectious agent. In order maintaining the level of bacterial normal flora at the appropriate rate and

prevention from growth of Antibacterial resistant pathogen due to the usage of unnecessary and impracticable antibiotic.

2. Material and Methods

Midstream urine samples were collected in sterile containers. Samples were cultured on Nutrient Agar and MacConkey Agar and incubated at 37°C for 24 hours. Gram staining and biochemical tests (Indole, MR-VP, Catalase, Oxidase, Citrate) were performed for identification. Antibiotic susceptibility was tested using the Kirby–Bauer disc diffusion method. Chemicals and glassware Media used were nutrient agar, mackonkey agar, methyl red broth, tryptophan broth Chemicals and reagents used were crystals violets, gram iodine, safarnin, methyl reagent (MR), kovac oxidase reagent, hydrogen peroxide, absolute alcohol, 401KoH. All media and solution were prepared in distilled water and media were autoclaved at 121°C at 15p5i for 15minutes. All the glasswares including petriplates, conical flasks and test tubes were made of borosilicate. Glass wares were washed properly and oven dried before use. All the chemicals used were of analytical grade.

3. Collection of samples

Patients were asked to clean their external genitals with disinfectant and collect midstream urine in sterilized cap. Samples were kept in ice bag and directly transported to microbiology laboratory.

Physical examination

pH, color, volume, appearance parameters of collected urine specimens were analyzed.

Urine culturing

Urine samples were cultured on nutrient agar and mackonkey agar medium and incubated at 37° C for 24 hrs.

Isolation of microorganisms

Microorganism were isolated by using mackonkey agar and nutrient agar (NA) media. The urine and samples were spread on to nutrient mackonkey agar media plates. The plates were allowed to dried for 5min and were incubated at 37°C for overnight (24 hrs). The growth pattern was observed. The different morphological colonies were selected for further studies in term of their diversity.

Gram staining of Bacteria

The gram stain is a differential stain which was developed by Dr. Hans Christan Gram, a Danish physician, in 1884. It is done for differentiating between gram positive and gram-negative bacteria. In this process four different reagents are used upon the bacterial smear in the listed order, crystal violet (primary stain), iodine solution (mordent), alcohol (decolouring agent) and safranin (counter stain). The violet or dark blue stained smears indicates gram positive whereas pink or red stained smear is referred to as gram negative. These staining differences appear due the difference in their cell walls. The gram-negative bacterial cell wall is thin and high in lipid which gets readily dissolve by alcohol resulting in formation of large pores therefore decolorization take place and smear appears pink. In contrast the gram-positive bacteria contain thick cell walls so that crystals violets iodine complex remains intact and thus the smears appears violet.

Biochemical characterization

Selected colonies were identified and differentiate according to the culture characteristics, microscopically examination and microbiological analysis were tested biochemically for further confirmation of isolated bacteria such as Indole production test, catalase test, oxidase test, MRNP test (methyl D red), VP (vageb-proskauer test), citrate utilization test.

Table 1. Distribution of Isolated Uropathogens

Bacterial Species	Number of Isolates
Escherichia coli	4
Staphylococcus aureus	5
Klebsiella pneumonia	2
Pseudomonas aeruginosa	1
Enterococcus faecalis	1

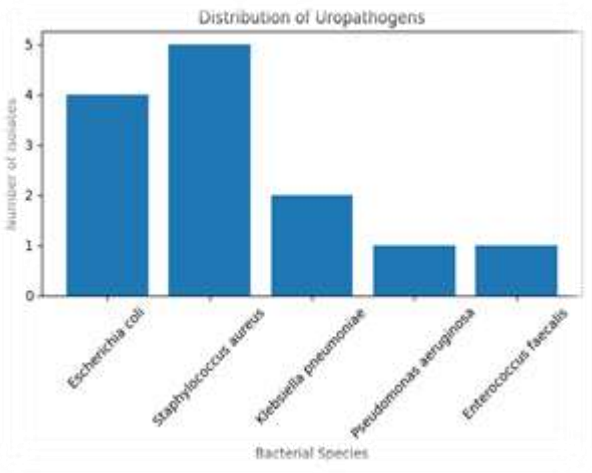
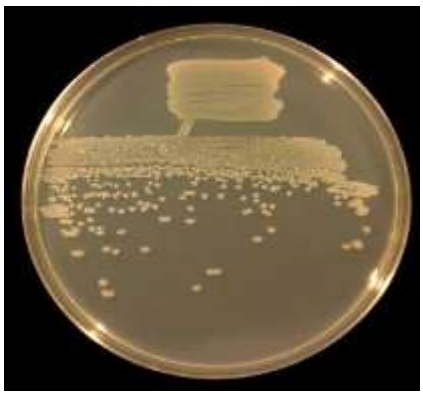


Figure 1. Distribution of Uropathogens

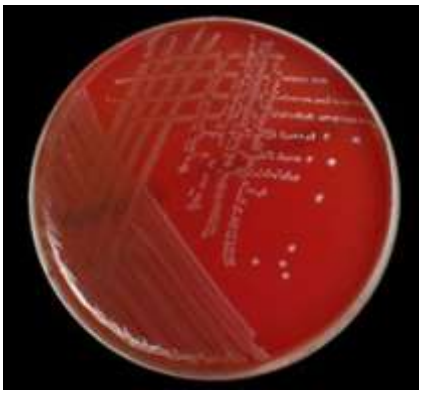
4. Results and Discussion

Five different bacterial species were isolated from fourteen urine samples. Staphylococcus aureus was the most frequently isolated organism, followed by Escherichia coli. Equal proportions of gram-positive and gram-negative bacteria were observed.

Escherichia coli



Pseudomonas aureus



Enterococcus faecalis

Klebsiella pneumonia

Staphylococcus aureus

Table 2. Antibiotic Sensitivity Pattern

Antibiotic	**Sensitivity (%)**
Gentamicin	80
Amikacin	70
Chloramphenicol	60
Nitrofurantoin	50
Ceftriaxone	40
Levofloxacin	30
Amoxicillin	20
Cefixime	10

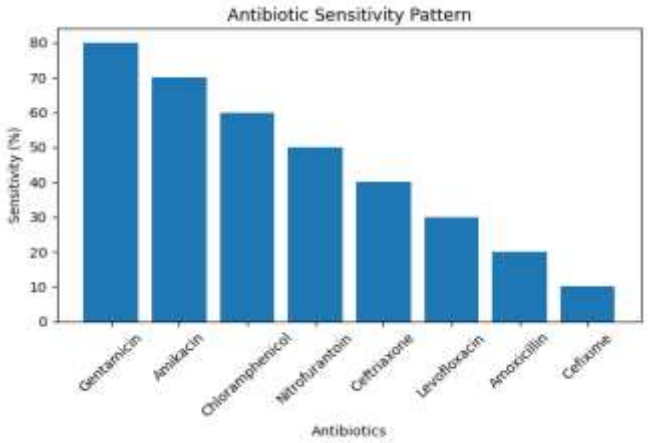


Figure 2. Antibiotic Sensitivity Pattern

5. Conclusion

The study concludes that Escherichia coli and Staphylococcus aureus were the predominant uropathogens isolated from patients with urinary tract infections. These findings are consistent with previous studies reporting E. coli as the most common causative agent of urinary tract infections worldwide. Other bacterial isolates identified during the study included organisms such as Klebsiella pneumonia, Proteus mirabilis, and Pseudomonas aeruginosa, although their prevalence was comparatively lower. The antimicrobial susceptibility pattern revealed that aminoglycoside antibiotics such as Gentamicin and Amikacin were the most effective drugs against the majority of isolated uropathogens. In contrast, higher levels of resistance were observed against commonly used antibiotics such as Ampicillin and Ciprofloxacin in several isolates, indicating the growing problem of antimicrobial resistance.

These findings highlight the importance of routine urine culture, proper identification of bacterial pathogens, and antibiotic susceptibility testing before initiating treatment. Such practices help clinicians select the most appropriate antibiotic therapy and prevent the misuse or overuse of antimicrobial agents. Furthermore, continuous surveillance of antimicrobial resistance patterns in urinary pathogens is essential for guiding empirical therapy and for developing effective infection control strategies. Overall, the study emphasizes the need for strict antibiotic stewardship programs, regular monitoring of resistance trends, and increased awareness among healthcare professionals to effectively manage urinary tract infections and reduce the spread of drug-resistant pathogens.

Declarations

Source of Funding

This study did not receive any grant from funding agencies in the public, commercial, or not–for–profit sectors.

Competing Interests Statement

Authors have declared no competing interests.

Consent for publication

The authors declare that they consented to the publication of this study.

Institutional Review Board Statement

Not applicable for this study.

Informed Consent

Not applicable for this study.

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