Journal of Pioneering Medical Sciences
Received: August 27, 2026 | Accepted: October 21, 2026 | Published: November 15, 2026
Volume 15, Issue S02, Pages 247-260

DOI https://doi.org/10.47310/jpms202615S0229



Antimicrobial Resistance Patterns and Biofilm Formation Among Urinary Microbial Isolates at a Tertiary-Care Hospital in Jeddah, Saudi Arabia: A Cross-Sectional Study

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Abstract **Background:** Antimicrobial resistance and biofilm formation are important laboratory characteristics of urinary isolates. This study described the microbial isolates recovered from urine cultures at King Fahad General Hospital, Jeddah, Saudi Arabia. **Methods:** In this single-centre, descriptive cross-sectional study, 464 urine specimens were submitted between June and August 2025. Identification and antimicrobial susceptibility testing were performed using the VITEK 2 system, as applicable to the organism. Selected resistant bacterial isolates underwent molecular testing with GeneXpert, while biofilm formation was assessed using three phenotypic methods in a subset of 50 bacterial isolates. **Results:** A total of 107 of 464 specimens were culture-positive (23.1% of submitted specimens). The 107 reported microbial isolates comprised 66 Gram-negative bacteria (61.7%), 20 Gram-positive bacteria (18.7%) and 21 yeasts or other fungi (19.6%). *Escherichia coli* (n = 29) and *Klebsiella pneumoniae* (n = 20) were the most frequently reported organisms. Antimicrobial resistance phenotypes and selected resistance genes were reported among the relevant tested bacterial isolates; their precise frequencies require confirmation of the testing denominators and classification rules. The biofilm results apply only to the selected 50-isolate bacterial subset and the three assays showed differing biofilm-category distributions. **Conclusions:** These findings provide local, descriptive laboratory observations from a single hospital over three months. They should not be interpreted as national prevalence estimates or evidence of treatment failure. Larger surveillance studies using consistent organism-specific methods and transparent denominators are warranted.

Key Words Urinary Isolates, Urinary Tract Infection, Antimicrobial Resistance, Extended-Spectrum Beta-Lactamases, Carbapenem Resistance, Biofilm Formation

INTRODUCTION

The World Health Organization (WHO) has identified antimicrobial resistance (AMR) as one of the most significant threats to global health, ranking it among the top ten issues in 2021 [1]. Earlier estimates projected substantial mortality attributable to antimicrobial resistance by 2050; such estimates are model-based scenarios, not observed future outcomes [2,3]. Additionally, Urinary Tract Infections (UTIs) are becoming increasingly challenging to treat due to the rising resistance of bacteria to last-line antibiotics, with some bacteria even becoming Pan-Drug-Resistant (PDR) and not responding to any commercially available antibiotics. Gram-negative organisms, particularly *Escherichia coli*, are common causes of UTIs; the distribution varies with patient population and healthcare setting [4,5]. According to a WHO report, the rising use of antibiotics has contributed to an increase in antibiotic resistance rates among UTI pathogens, which negatively impacts healthcare systems, escalates treatment costs and complicates patient management. Published UTI estimates differ across countries and studies because incidence, prevalence, study periods and sampling populations may not be comparable [6,7]. Specifically, in Saudi Arabia, the diverse ethnic population may contribute to variations in prevalence rates. A study Alanazi [8] has estimated the prevalence of UTIs in Saudi Arabia to be 24%. Additionally, Sula *et al.* [9] found that UTIs account for 10% of all infections and are ranked as the second most common cause of emergency department admissions. UTIs can affect individuals of all sexes and age groups, with a high incidence

rate of 50% among women [10]. Antimicrobial selection for UTI depends on the clinical syndrome, organism, susceptibility findings and applicable local guidance [11]. Extended-Spectrum β-Lactamase (ESBL) production, carbapenem resistance and multidrug resistance (MDR) are related but distinct characteristics and the categories can overlap. Additionally, Gram-positive bacteria, such as Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Vancomycin-Resistant Enterococci (VRE), are of concern. The emergence of MDR UTIs poses a significant public health threat, highlighting the urgent need for effective measures to prevent their spread and to manage affected patients [12,13].

Over the last decade, there has been an increase in the rate of carbapenem-resistant Gram negative bacilli in Saudi Arabia in comparison with rates recorded in the 1990s. The prevalence of extended-spectrum beta-lactamase-producing isolates from Saudi Arabia is 29% among *Escherichia coli* and 65% among *Klebsiella pneumoniae*. As a result, these increasing rates have been associated with many reported outbreaks with mortality rates ranging between 11 and 40% [14]. Similarly, CR in *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* spp., *Proteus* spp. and *Citrobacter* spp. has continually risen over the years and has become a public health problem, as carbapenems are considered last-resort antibiotics for treating severe infections [15-17]. Various studies have indicated a continuous rise in CR in healthcare facilities in Saudi Arabia, with the carbapenemase-encoding genes $bla_{\text{OXA-48-type1}}$ and $bla_{\text{NDM-1}}$ detected in some isolates [18,19]. Also, Vancomycin-Resistant Enterococci (VRE) are increasingly important nosocomial pathogens and Glycopeptide resistance in enterococci is mediated by the van genes, with *vanA* and *vanB* being the most prevalent [20]. The global spread of both ESBL and CR in the same bacterial isolates poses a huge challenge for global healthcare systems. Biofilm-associated organisms are clinically relevant in urinary and device-associated settings, although their contribution differs among infection types [21]. Biofilms can be found in the urothelium, prostate stones and implanted foreign bodies and are considered one of the most important mechanisms behind the high rates of recurrence and antimicrobial resistance of infections. Biofilms contain microorganisms attached to surfaces within an extracellular matrix. Such communities can show altered antimicrobial tolerance under laboratory conditions, especially on urinary catheters but the extent varies by organism and method [22]. Common CAUTI uropathogens include *E. coli*, *E. faecalis*, *P. aeruginosa*, *P. mirabilis*, *K. pneumoniae*, *S. aureus* and *Candida* spp. [23]. This study aimed to describe (1) The proportion of submitted urine specimens with positive cultures and the distribution of microbial isolates; (2) Antimicrobial susceptibility patterns of the relevant bacterial isolates; (3) Selected resistance genes among isolates that underwent molecular testing and (4) *In-vitro* biofilm formation among 50 selected bacterial isolates.

METHODS

Study Setting and Design

This descriptive cross-sectional study was conducted between June 2025 and August 2025 (a three-month, single-hospital collection period) at King Fahad General Hospital (KFGH) in Jeddah, Saudi Arabia, which is accredited by both CBAHI (Central Board for Accreditation of Healthcare Facilities) and CAP (College of American Pathologists). The patients were ≥18 years old and had positive urine cultures demonstrating mono-microbial growth with colony counts $\geq 10^5$ CFU/mL. The urine samples were from patients suspected of having Urinary Tract Infections (UTIs) and were obtained via midstream clean-catch (MS-CCU) or catheterized samples from both outpatient (ODP) and inpatient (IPD) settings. Urine samples were excluded from this study if they contained multiple types of bacteria (mixed growth), had negative culture results or belonged to patients younger than 18 years (Figure 1). The findings describe submitted specimens from this hospital rather than the population prevalence of symptomatic UTI.

Data Collection

All the protocols and procedures followed in this study were approved by the General Directorate of Health Affairs in Jeddah Province (IRB-A02209) on 5 May 2025 and were conducted in accordance with the ethical standards of the Helsinki Declaration and the specific regulations issued by

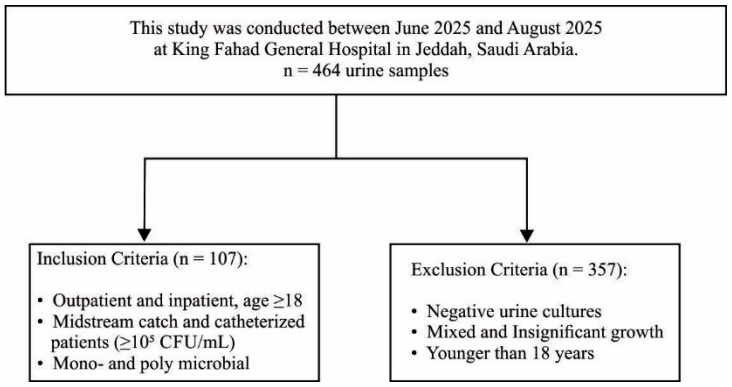


Figure 1: Flowchart of Inclusion and Exclusion Criteria for the Study
The letter n stands for the number of cases

the National Committee of Bioethics in Saudi Arabia (NCBE-KACST, KSA: H-02-J-002). Data were collected from patients with positive Urinary Tract Infection (UTI) cultures through the OASIS (Online Access to System Integrated Solutions). Data integrity and confidentiality were maintained and the collected information was only used for this research.

Urine Sample Collection and Analysis

The urine samples were collected in sterile containers from hospitalized patients and outpatients with suspected urinary tract infection. Specimens were collected by either the midstream clean-catch method or urinary catheterization into sterile containers. The samples were sent to the microbiology laboratory within 2 hours. Additionally, the automated system VITEK® 2 (bioMérieux, Marcy l'Etoile, France) was used for further identification and confirmation of organisms. The data were subjected to analysis and interpretation following the principles set out by the Clinical Laboratory Standards Institute (CLSI) [24].

The urine samples were cultured using a semiquantitative technique based on the WHO guidelines, utilizing standard culture media [25,26]. According to the Guideline for Urine Culture and Biochemical Identification of Bacterial Urinary Pathogens, urine was semiquantitatively cultured and then subjected to two levels of biochemical testing for appropriate phenotypic identification of the most common bacterial urinary pathogens [27].

Isolation, Identification and Antimicrobial Susceptibility of Uropathogens

For each urine sample, the end of a sterile 1 µL inoculating disposable plastic loop was dipped into the urine; then, a single streak was made across the center of a plate containing blood agar and Cysteine Lactose Electrolyte-Deficient (CLED) agar medium. Then, the inoculum was evenly distributed in a cross-zigzag arrangement next to the primary streak and the culture was then incubated at 37°C for 24 hour. Identification and antimicrobial susceptibility tests were performed using VITEK® 2 (bioMérieux, Marcy l'Etoile, France), and, as in most clinical microbiology laboratories, identification cards (ID-GN for Gram-Negative, ID-GP for Gram-positive, YST for yeast) and an AST card for antibiotic susceptibility testing (AST N417, P 580, P586) were used, depending on the clinical need. The Minimum Inhibitory Concentration (MIC) results, as well as categorical interpretation (susceptible, S; intermediate, I; or resistant, R) were based on the Clinical and Laboratory Standards Institute (CLSI) interpretive criteria. Institute M100, 35th edition (CLSI M100-Ed35, 2025) [24].

Enterococcus faecalis (ATCC29212), *Escherichia coli* (ATCC25922), *Proteus mirabilis* (ATCC29906), *Staphylococcus aureus* (ATCC29213), *Klebsiella pneumoniae* (ATCC700603), *Pseudomonas aeruginosa* (ATCC27853), *Acinetobacter baumannii* (ATCC 19606) and *Streptococcus agalactiae* (ATCC 12386) were used as the positive control strains to confirm the identification of bacterial isolates in VITEK system. Isolates resistant to three or more tested antibiotics were classified as multidrug-resistant (MDR) under the study's provisional operational definition; this should be validated against antimicrobial-category criteria. Selected resistant bacterial isolates were further processed using the GeneXpert® DX version 6.5 system (Cepheid, Sunnyvale, U.S) for molecular confirmation and detection of resistance genes, for example, *mecA* (for detection of MRSA and SA in skin and soft tissue infections), Carba-R (for detection and differentiation of *KPC*, *NDM*-1, *VIM*, *IMP* and *OXA-48-type1*) and *vanA*/*vanB* (for VANCOMYCIN-RESISTANT ENTEROCOCCI (VRE) screening).

Phenotypic Methods for the Detection of Biofilm Formation Among Selected Bacterial Urinary Isolates

Of the 107 culture-positive microbial isolates, 50 bacterial isolates were selected for biofilm assessment; results from this subset should not be generalized to all 107 isolates. They were as follows: *E. coli* (n = 17): 7 were ESBL producers, 9 were sensitive and 1 was classified as MDRO; *K. pneumoniae* (n = 17): 4 were ESBL producers, two were sensitive and 11 were MDRO. Also, *P. aeruginosa* (n = 4): 2 were sensitive and two were MDRO, while *E. faecium* (n = 5) isolates were classified as MDRO. Concerning *A. baumannii* (n = 2), all isolates were MDRO, while *P. mirabilis* (n = 1) was a sensitive isolate. Similarly, *S. aureus* and *E. faecalis* were represented by two sensitive isolates.

Christensen Test Tube Method

The Christensen Test Tube method (CTT), for qualitative assessment of biofilm formation, was used as previously described [28]. A loopful of bacterial culture from the agar plate was inoculated into a sterile glass test tube containing 5 mL of sterile Trypticase Soy Broth (TSB) (HiMedia Laboratories, Maharashtra, India) with 1% glucose. The tube was incubated aerobically at 36±1°C for 24 hour under stationary conditions (JEIO Tech, South Korea). After incubation, each tube was decanted and washed with Dulbecco's Phosphate-Buffered Saline (DPBS) (10×) without Ca and Mg (PAN-Biotech GmbH, Germany) at pH 7.3 and dried. Then, the dried tubes were stained with crystal violet (0.2%) (HiMedia Laboratories, Maharashtra, India) and gently rotated to ensure uniform staining. Excess stain was removed and tubes were washed with deionized water. The tubes were then placed upside-down to ensure draining and left at room temperature for 30 min. Biofilm formation was detected by the presence of a visible film on the wall and bottom of the tube. The absence of a film represented a negative result (−). The amount of biofilm formation was recorded as strong (+++), moderate (++) or weak (+). Experiments were performed in triplicate [29].

Congo Red Agar Method

The tube method is not always successful for detecting weak slime production and variations in media may affect its

results [30]. Congo Red Agar (CRA), described by [31], is an alternative qualitative method for detecting slime-associated colony characteristics. CRA medium (HiMedia Laboratories, Maharashtra, India), was prepared using solid medium Brain-Heart Infusion (BHI) broth (HiMedia Laboratories, Maharashtra, India) supplemented with 5% sucrose and Congo Red. The Congo Red stain was made in a concentrated aqueous solution and autoclaved at 121 °C for 15 min. (Labnet International, Inc, US) separately from the other medium constituents; then, it was added when the agar had cooled to 55°C. Plates were inoculated and incubated aerobically for 24-48 hour at 37 °C. Black colonies with a dry crystalline consistency indicated strong biofilm production, whereas moderate biofilm production (pink-colored with dark secretion) and weak/no-biofilm formation were identified as red or pink colonies.

Tissue Culture Plate (TCP) Stained with Crystal Violet Method

The tissue culture plate method is the most widely used and is a commonly used quantitative in-vitro method for assessing biofilm biomass [32]. Its aim is to quantify biofilms more objectively. For this method, the plates for biofilm cultivation should be sterile flat-bottomed 96-well polystyrene tissue culture-treated microtiter plates with a lid (Hawach Scientific Co., Ltd. (Xi'an, China).

Individual strains of the uropathogenic bacteria were grown overnight in 5 mL of Trypticase Soy Broth (TSB) supplemented with 1% glucose and incubated at 37°C for 24 hour. After, the cultures were diluted 1:100 with TSB with 1% glucose; then, 200 µL of the diluted cultures was transferred into the wells of 96-well polystyrene microtiter plates. Plates remained at 37°C for 24 hour under static incubation; afterwards, the content was gently removed from the wells, which was then washed 3 times with sterile Dulbecco's Phosphate-Buffered Saline solution (DPBS, pH 7.3) to remove free-floating "planktonic" bacteria. Biofilms formed by adherent "sessile" organisms on the plate were fixed with sodium acetate (2%) for 30 min. Then, 150 µL of 2% crystal violet was transferred to the wells and kept at room temperature for 10-15 min. Excess dye was washed off with deionized water and dried for 5 minute at room temperature. The modified Christensen method involves re-solubilization of the dye and measures the biofilm formed both on the bottom and walls of the well with the addition of 150 µL of 30% (v/v) acetic acid to the wells for 20 minute [33]. Optical densitometry (OD) was measured at 570 nm using an automated microplate reader (Figure 2). This OD value was considered an index of bacterial adherence/biofilm [34].

The negative control wells contained only broth, 200 µL of TSB supplemented with 1% glucose per well and 30% acetic acid in water as the blank. The experiment was performed in six replicates; the data generated were averaged and the standard deviation was calculated.

Biofilm formation was interpreted based on the optical density cut-off value (ODc), calculated as the average OD of the negative control + 3× standard deviation (SD) of the negative control as described in [33]. OD≤ODc mean no biofilm, ODc<OD≤2×ODc mean weak biofilm, 2×ODc<OD≤4×ODc mean moderate biofilm and 4×ODc<OD mean Strong biofilm.

Detection of MIC of Amoxicillin/Clavulanate for Three Selected Pathogens

The growth of *P. aeruginosa* CR, *E. coli* ESBLs and *K. pneumoniae* CR in the presence of the test antibiotic was assessed in a 96-well microtiter plate using the standard microbroth dilution method according to the National Committee for Clinical Laboratory Standards. Control tubes contained bacteria in growth medium without an antibiotic. Briefly, an overnight culture of tested bacteria was diluted in fresh TSB with 1% glucose broth to a final density of approximately 1×10^6 CFU/mL (OD_{600} ≈ 0.001) (Bibby Scientific Ltd, Staffordshire, U.K.). This bacterial suspension was aliquoted into a sterile 96-well microtiter plate. Different concentrations of Amoxicillin/Clavulanate (100 µg/mL) were added and the plate was incubated at 37°C and 180 rpm for 24 hours. At the end of the incubation period, the absorbance was read at 600 nm with an ELISA Microplate Reader (BioTek instruments, Winooski, Vermont, U.S.) [35]. Experiments were performed in duplicate. MIC was recorded at the lowest antibiotic concentration that completely inhibited bacterial growth. Then, all microplates were processed using the CV assay as described above to confirm biofilm formation at each concentration of antibiotic.

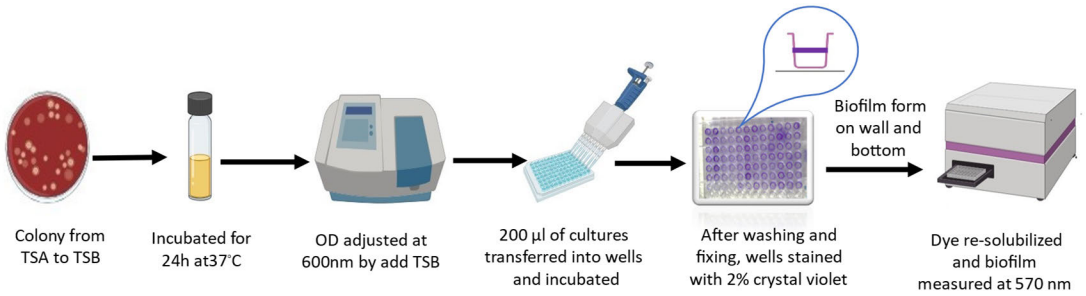


Figure 2: A Flowchart of the Tissue Culture Plate Method, in Which Crystal Violet Stain was Used to Determine Biofilm Formation
Created in BioRender by Suha AlGhamdi (2026) https://BioRender.com/

Also, ½ MIC was selected as a sub-inhibitory concentration to investigate the effect of amoxicillin/clavulanic acid on growth, biofilms and exopolysaccharide production of the tested bacteria [34]. Each bacterial culture was incubated with ½ MIC of Amoxicillin/Clavulanate in a 5 mL test tube at 37°C for 24 hour. After incubation, the growth was measured (A_{600nm}). The contents of the tube were transferred to a new test tube and the biofilm was quantified as described previously (CV method). Concerning the new tubes, the bacterial cells were separated through centrifugation at 4°C and 6000 rpm for 20 minute (Pro-Research, Centurion Scientific Ltd, U.K.) and the supernatants were filtered through a 0.22 µm syringe filter (BioSharp Life Sciences, China) to remove remaining cell debris. EPS was extracted according to the protocol in Hansen [36]. Two volumes of chilled absolute ethanol were added to the supernatant to precipitate EPS, followed by incubation at 4°C overnight. The next day, the precipitated EPS was centrifuged (6000 rpm, 15 min, 4°C) and the pellet containing EPS was dried at room temperature. The total quantification of the EPS pellet was estimated by Benedict's test [37] using a spectrophotometer at 490 nm. Concerning Benedict's test, 1 mL of Benedict's reagent ($CuSO_4$) (Loba Chemie Pvt. Ltd. India) was added to 1 mL of the prepared bacterial biopolymer, shaken well and heated for 5 min in a water bath at 100°C.

Statistical Analysis

The collected data were presented in tabular form and associations between variables were evaluated using Pearson's Chi-square test. When the expected cell counts were less than five, Fisher's Exact Test with Monte Carlo simulation was also considered to ensure the validity of the statistical analysis. The strength of associations was assessed using Cramer's V coefficient. Additionally, to compare two variables between a treatment group and a control group, the t-test was used, with statistical significance established at $p<0.05$.

RESULTS

During the study period, 107 of 464 submitted urine specimens yielded positive cultures (23.1%; Table 1). This is the culture-positive proportion among submitted specimens, not a population-based prevalence estimate. Among culture-positive specimens, a higher proportion originated from female than male patients. Among these positive isolates, 62 (58%) belonged to females. Patients were categorized into three age groups and the largest number of positive specimens occurred in patients older than 60 years, with fewer in those aged 18-39 years. Most urine samples were obtained via urinary catheterization (n = 77), compared with Midstream Clean-Catch (MSC) collection (n = 30). This finding reflects the fact that most of the patients were hospitalized, with 78 admitted to general wards, 16 in the Intensive Care Unit (ICU), 10 in the outpatient department (OPD) and 3 presenting from the emergency department (ER). Regarding antibiotic resistance patterns, the reported UTIs were classified as follows: MDRO accounted for 21% of cases; 77% of MDRO were carbapenem-resistant Enterobacteriaceae, while extended-spectrum beta-lactamase was detected in 23% of cases. Another group, called vancomycin-resistant *Enterococcus* was represent by 5%, while the rest group was sensitive group to the tested antibiotics. Of the 107 reported microbial isolates, 66 (61.7%) were Gram-negative bacteria, 20 (18.7%) were Gram-positive bacteria and 21 (19.6%) were yeasts or other fungi. Significant associations were observed between resistant type and hospital location, urine collection method and isolate type. The strongest association was identified between resistant type and isolate type (Cramer's V = 0.483, $p<0.001$), indicating a moderate-to-strong relationship. ESBL-producing isolates were exclusively associated with Gram-negative organisms, whereas VRE isolates were confined to Gram-positive bacteria. MDRO isolates were also predominantly identified among Gram-negative organisms. In addition, resistant isolates were more frequently observed among IPD and ICU patients and in catheter-collected urine specimens. Gender and age group demonstrated weaker or borderline associations with resistant type.

Table 2 shows the distribution of urinary microbial isolates: *E. coli* was the most frequent organism (n = 29), followed by *K. pneumoniae* (n = 20) and *P. aeruginosa* (n = 10). Other pathogens, including *Enterobacter cloacae, Streptococcus agalactiae, E. faecalis, Enterococcus faecium, S. aureus, S. haemolyticus, Proteus mirabilis* and *A. baumannii*, represented more minor cases. Of the 107 microbial isolates, 21 were yeasts or other fungi. Also, *Candida albicans, C. tropicalis, C. auris, C. glabrata* (previously known as *Nakaseomyces glabratus*) and *Cryptococcus laurentii* in addition to *Trichosporon asahii* were detected at lower cases.

Antibacterial Susceptibility Patterns

The most common ESBL-producing uropathogenic bacteria were *E. coli* and *K. pneumoniae*. ESBL-producing *E. coli* was isolated from 19 urine samples and they were resistant to Piperacillin/Tazobactam, cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime and ciprofloxacin. However, they were highly susceptible to gentamicin, amikacin, nitrofurantoin and fosfomycin while *K. pneumoniae* was isolated from 6 samples and showed the highest resistance rate to cefuroxime, ciprofloxacin and ceftazidime. The other isolates exhibited intermediate resistance to the tested antibiotics (Table 3).

Table 4 presents the susceptibility results of the pathogens to carbapenems. The *E. coli*, *A. baumannii* and *P. aeruginosa* isolates showed high resistance to meropenem, imipenem and ertapenem, while the *K. pneumoniae* isolates showed resistance to meropenem, imipenem and ertapenem. As shown in Table 5, *E. faecium* showed high resistance to all of the antibiotics tested, except for nitrofurantoin (60%) but it was reported as susceptible to tigecycline and linezolid (100%), as well as nitrofurantoin (40%). As displayed in Table 6, *S. aureus* showed a susceptibility pattern of high resistance to benzylpenicillin, Levofloxacin, Erythromycin (86%), oxacillin (71%) and fusidic acid (100%).

Table 1: Association Between Demographic, Clinical and Microbiological Variables and Resistant Type of the Included Data (n = 107)

Variable	Category	MDRO n (%)	ESBL n (%)	VRE n (%)	SN n (%)	p-value	Cramer's V
Gender	Female (n = 62)	11 (50.0)	19 (76.0)	1 (20.0)	31 (56.4)	0.071†	0.236
	Male (n = 45)	11 (50.0)	6 (24.0)	4 (80.0)	24 (43.6)		
Age group	18-39 (n = 11)	4 (18.2)	1 (4.0)	0 (0.0)	6 (10.9)	0.051†	0.236
	40-60 (n = 34)	6 (27.3)	3 (12.0)	2 (40.0)	23 (41.8)		
	>60 (n = 62)	12 (54.5)	21 (84.0)	3 (60.0)	26 (47.3)		
Location	IPD (n = 78)	15 (68.2)	16 (64.0)	4 (80.0)	43 (78.2)	0.007†*	0.246
	ICU (n = 16)	6 (27.3)	8 (32.0)	0 (0.0)	2 (3.6)		
	ER (n = 3)	1 (4.5)	0 (0.0)	0 (0.0)	2 (3.6)		
	OPD (n = 10)	0 (0.0)	1 (4.0)	1 (20.0)	8 (14.5)		
Urine collection method	Catheter (n = 77)	19 (86.4)	20 (80.0)	1 (20.0)	37 (67.3)	0.022†*	0.311
	MS-CCU (n = 30)	3 (13.6)	5 (20.0)	4 (80.0)	18 (32.7)		
Isolate type	Gram-positive (n = 20)	0 (0.0)	0 (0.0)	5 (100.0)	15 (27.3)	<0.001†*	0.483
	Gram-negative (n = 66)	17 (77.3)	25 (100.0)	0 (0.0)	24 (43.6)		
	Yeast (n = 21)	5 (22.7)	0 (0.0)	0 (0.0)	16 (29.1)		

†Fisher's Exact Test was reported due to expected cell counts less than 5, *Significant results, ER: Emergency room, ICU: Intensive care unit, ESBL: Extended-spectrum beta-lactamase, MDRO: Multidrug-resistant organism, VRE: Vancomycin-resistant Enterococci, OPD: Outpatient department, IPD: Inpatient department, MS-CCU: Midstream clean-catch urine, %: Percentage, SN: Sensitive

Table 2: Distribution of Urinary Microbial Isolates (n = 107) by the Resistance Groupings Reported in the Original Analysis; Fungal Isolate Categories Require Separate Laboratory Verification

Uropathogen	SN	ESBLs	MDROs	Total
E. coli	9	19	1	29
K. pneumonia spp. *pneumoniae*	3	6	11	20
Pseudomonas aeruginosa	8	-	2	10
Staphylococcus aureus	7	-	-	7
Enterococcus faecalis	4	-	-	4
Enterococcus faecium	-	-	5	5
Streptococcus agalactiae	3	-	-	3
Acinetobacter baumannii	-	-	3	3
Proteus mirabilis	1	-	-	1
Klebsiella aerogenes	1	-	-	1
Citrobacter freundii	1	-	-	1
Staph. haemolyticus	1	-	-	1
Enterobacter cloacae	1	-	-	1
Candida albicans	9	-	-	9
Candida tropicalis	5	-	-	5
Candida auris	-	-	3	3
Cryptococcus laurentii	-	-	2	2
Nakaseomyces glabratus	1	-	-	1
Trichosporon asahii	1	-	-	1
	55	25	27	107

ESBLs: Extended-Spectrum Beta-Lactamase, MDROs: Multidrug-Resistant Organisms, SN: Sensitive, -: Not Detected

Table 3: Results of Antimicrobial Susceptibility Test (AST) for ESBL Isolates

	Susceptibility (%)	Cephalosporins and Monobactams (%)							Aminoglycosides (%)		Fluoroquinolone, Nitrofuran, Sulfonamides, Phosphonic acid (%)			
		Amox/Clav	Pip/Taz	Cefaz	Cefur	Ceftaz	CTR	Cefep	Amik	Gent	Cipro	Nitro	TMP-SMX	Fos
E. coli (n = 19)	R.N.	26	84	100	100	74	79	79	0	16	79	5	63	5
	S.N.	58	11	0	0	16	0	21	100	84	10	90	37	95
	I.N.	16	0	0	0	10	21	0	0	0	1	5	0	0
K. pneumonia (n = 6)	R.N.	33	16	100	100	50	67	50	0	0	84	33	67	33
	S.N.	67	67	0	0	33	33	50	100	100	0	34	33	67
	I.N.	0	17	0	0	17	0	0	0	0	17	33	0	0

Amox/Clav: Amoxicillin/Clavulanate, Pip/Taz: Piperacillin/Tazobactam, Cefaz: Cefazolin, Cefur: Cefuroxime, Ceftaz: ceftazidime, CTR: Ceftriaxone, Cefep: Cefepime, Amik: Amikacin, Gent: Gentamicin, Cipro: Ciprofloxacin, Nitro: Nitrofurantoin, TMP-SMX: Trimethoprim and sulfamethoxazole, Fos: Fosfomycin; R.N.: Resistance number, S.N.: Sensitive number, I.N.: Intermediate number, %: Percentage, n: Number of samples

Table 4: Results of Antimicrobial Susceptibility Test (AST) for Carbapenem-Resistant Isolates

	Susceptibility (%)	Cephalosporins and Monobactams (%)							Aminoglycosides (%)		Fluoroquinolone, Nitrofuran, Sulfonamides, Phosphonic acid (%)				Carbapenems (%)		
		Amox/Clav	Pip/Taz	Cefaz	Cefur	Ceftaz	CTR	Cefep	Amik	Gent	Cipro	Nitro	TMP-SMX	Fos	ERT	IMP	MER
E. coli (n = 1)	R.N.	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	S.N.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	I.N.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K. pneumoniae (n = 11)	R.N.	100	91	100	100	91	100	100	40	45	91	82	27	64	100	82	100
	S.N.	0	9	0	0	9	0	0	60	55	0	18	73	36	0	0	0
	I.N.	0	0	0	0	0	0	0	0	0	9	0	0	0	0	18	0
A. baumannii (n = 3)	R.N.	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	S.N.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	I. N.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. aeruginosa (n = 2)	R.N.	50	50	100	100	50	100	100	50	50	50	100	100	100	100	100	100
	S.N.	50	50	0	0	50	0	0	50	50	50	0	0	0	0	0	0
	I.N.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Amox/Clav: Amoxicillin/Clavulanate, Pip/Taz: Piperacillin/Tazobactam, Cefaz: Cefazolin, Cefur: Cefuroxime, Ceftaz: Ceftazidime, CTR: Ceftriaxone, Cefep: Cefepime, Amik: Amikacin, Gent: Gentamicin, Cipro: Ciprofloxacin, Nitro: Nitrofurantoin, TMP-SMX: Trimethoprim and sulfamethoxazole, Fos: Fosfomycin, R.N.: Resistance number, S.N.: Sensitive number, I.N.: Intermediate number, %: Percentage, ERT: Ertapenem, IMP: Imipenem, MER: Meropenem, n: Number of samples

Table 5: The Susceptibility Pattern of Vancomycin-Resistant *Enterococcus faecium*

	Susceptibility (%)	Ben/Pen (%)	Amp (%)	Gentamicin (%)	Streptomycin (%)	Levo (%)	ERY (%)	Q/Dalf (%)	LZD (%)	TEI (%)	Vanco (%)	TET (%)	TGC (%)	Nitro (%)
E. faecium (n = 5)	R.N.	100	100	100	100	100	100	100	0	100	100	100	0	60
	S.N.	0	0	0	0	0	0	0	100	0	0	0	100	40

Ben/Pen: Benzylpenicillin, Amp: Ampicillin, Levo: Levofloxacin, ERY: Erythromycin, Q/Dalf: Quinupristin/dalfopristin, LZD: Linezolid, TEI: Teicoplanin, Vanco: Vancomycin, TET: Tetracycline, TGC: Tigecycline, Nitro: Nitrofurantoin, R.N.: Resistance number, S.N.: Sensitive number, %: Percentage, n: Number of samples

Table 6: The Susceptibility Pattern of *Staphylococcus aureus*

	Susceptibility (%)	Ben/Pen (%)	OXO (%)	Gent (%)	TOB (%)	Levo (%)	MXF (%)	ERY (%)	CLI (%)	LZD (%)	TEI (%)	Vanco (%)	TET (%)	TGC (%)	Nitro (%)	FA (%)	RIF (%)	TMP-SMX (%)
S. aureus (n = 7)	R.N.	86	71	0	0	86	0	86	0	0	0	0	0	0	0	100	0	0
	S.N.	14	29	100	100	14	14	14	100	100	100	100	100	100	100	0	100	100
	I.N.	0	0	0	0	0	86	0	0	0	0	0	0	0	0	0	0	0

Ben/Pen: Benzylpenicillin, OXO: Oxacillin, Gent: Gentamicin, TOB: Tobramycin, Levo: Levofloxacin, MXF: Moxifloxacin, ERY: Erythromycin, CLI: Clindamycin, LZD: Linezolid, TEI: Teicoplanin, Vanco: Vancomycin, TET: Tetracycline, TGC: Tigecycline, Nitro: Nitrofurantoin, FA: Fusidic acid, RIF: Rifampicin, TMP-SMX: Trimethoprim and sulfamethoxazole, R.N.: Resistance number, S.N.: Sensitive number, I.N.: Intermediate number, %: Percentage, n: Number of samples

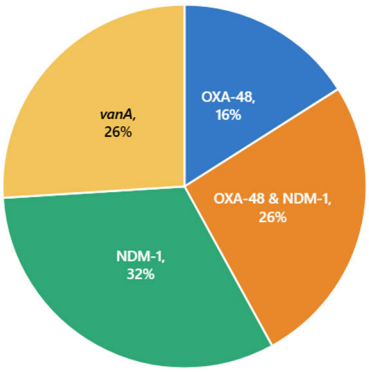


Figure 3: The Percentage Distribution of Resistance Genes in Uropathogenic Bacteria

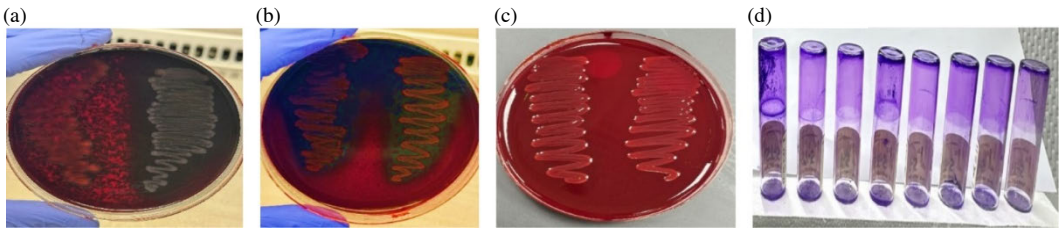


Figure 4(a-d): Biofilm Detection Using Congo Red Agar, (a) Strong, (b) Moderate and (c) Weak, (d) Biofilm Detection Using the Christensen Test Tube (CTT) Method

Table 7: Detection of Resistance Gene Types in Urinary Bacterial Isolates Using the Cepheid GeneXpert® System

Gen Resistance	Microorganism	Result	No. of Isolates
Xpert_Carba-R			
No Carba-R Gene	*Acinetobacter baumannii*	-	3
$bla_{\text{OXA-48-type1}}$	*K. pneumoniae* spp. *pneumoniae*	+	3
$bla_{\text{OXA-48-type1}}$ and $bla_{\text{NDM-1}}$	*K. pneumoniae* spp. *pneumoniae*	+	5
$bla_{\text{NDM-1}}$	*Escherichia coli*	+	1
	Pseudomonas aeruginosa	+	2
	K. pneumoniae spp. *pneumoniae*	+	3
Xpert *vanA* and *vanB* (VRE)			
vanA	*Enterococcus faecium*	+	5
Xpert MRSA/SA SSTI			
mecA gene	MRSA	-	7

Carba-R: Carbapenem resistance, VRE: Vancomycin-resistant Enterococci, MRSA: Methicillin-resistant *Staphylococcus aureus*, SA SSTI: Skin and soft tissue infection assay

Table 7 shows the different gene types detected in the urinary bacterial isolates by the Cepheid GeneXpert® System. In some of the urinary bacteria, certain strains carried two resistance genes, while others carried only one gene. For *K. pneumoniae*, the most prevalent carbapenemase genes were $bla_{\text{OXA-48-type1}}$ and $bla_{\text{NDM-1}}$, which were also harbored by *E. coli* and *P. aeruginosa*, while *A. baumannii* did not carry either of these genes. The *E. faecium* (n = 5) isolates carried the *vanA* gene. Notably, the *mecA* gene was not detected in the *Staphylococcus aureus* isolates (n = 7). The prevalence rate of the Carbapenem Resistance (CR)-encoding genes $bla_{\text{OXA-48-type1}}$ was 16% and bla_{NDM1} was 32% each, while that of both $bla_{\text{OXA-48-type1}}$ and $bla_{\text{NDM-1}}$ was 26%. Also, *vanA* gene was present in 26% of the urinary bacterial isolates, as shown in Figure 3. These percentages are reproduced from the original report and require verification of the number of isolates tested for each gene and the handling of co-detection.

Phenotypic Methods for the Detection of Biofilm Formation among Uropathogenic Bacteria

In this study, phenotypic methods, including the Congo Red Agar (CRA), Christensen Test Tube (CTT) and Tissue Culture Plate (TCP) methods, were applied to demonstrate biofilm formation for 50 different uropathogenic bacterial isolates which exhibiting different antibiotic-resistant patterns. According to Figure 4, the bacteria showed varying degrees of biofilm formation, as determined by the CRA and

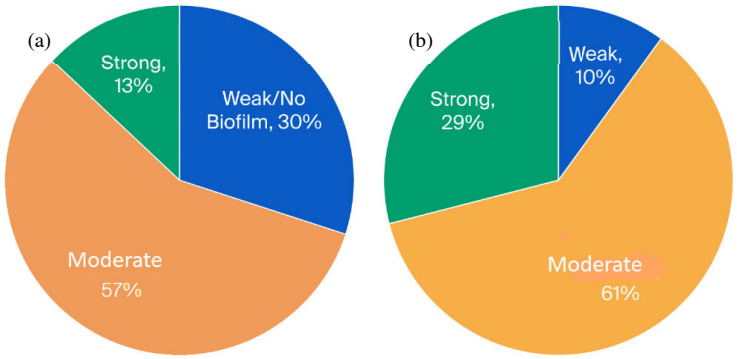


Figure 5(a-b): Distribution of Biofilm Formation Abilities Among Uropathogenic Bacteria According to (a) Congo Red Agar (CRA) and (b) Tissue Culture Plate (TCP) Methods

Table 8: Strength of Biofilm Formation Among the Tested Uropathogenic Bacteria (n = 50) Assessed by the Congo Red Agar (CRA), Christensen Test Tube (CTT) and Tissue Culture Plate (TCP) Methods

	Strength Of Biofilm Formation By the Tested Isolates (n= 50)			
Biofilm Detection Method	Strong (+++)	Moderate (++)	Weak (+)	No Biofilm
CRA	13%	57%	30%	0.0%
CTT	76%	18%	6%	0.0%
TCP	29%	61%	10%	0.0%

Table 9: Interpretation of the Quantitative Results of Biofilm of Uropathogenic Bacteria Based on Their Biofilm-Forming Capacity

Uropathogenic Bacteria	$OD_{570\ nm}$	Cut-Off Value (ODc)	Biofilm strength based on ODc
E. coli	0.673	≤0.652	Strong
E. coli (ESBL)	0.419	0.326≤OD≤0.652	Moderate
E. coli (MDROs)	0.547	0.326≤OD≤0.652	Moderate
P. aeruginosa	0.537	0.326≤OD≤0.652	Moderate
P. aeruginosa (MDROs)	0.537	0.326≤OD≤0.652	Moderate
K. pneumoniae	0.468	0.326≤OD≤0.652	Moderate
K. pneumoniae (ESBLs)	1.052	≤0.652	Strong
K. pneumonia (MDROs)	0.460	0.326≤OD≤0.652	Moderate
E. faecalis	0.320	0.163≤ OD≤0.326	Weak
E. faecium	0.317	0.163≤ OD≤0.326	Weak
S. aureus	0.481	0.326≤OD≤0.652	Moderate
A. baumannii	0.827	≤0.652	Strong
P. mirabilis	0.612	0.326≤OD≤0.652	Moderate
Control negative	0.134	0.163191	-

OD: Optical density, *ODc (optical density cut-off value) = Average OD of negative control+3× Standard Deviation (SD) of Negative Control

CTT methods. Concerning the CRA, the results showed that 57% of the isolates were moderate biofilm producers, 13% exhibited strong biofilm formation and 30% exhibited weak biofilm production; meanwhile, none of the isolates exhibited no biofilm formation (Figure 5a, Table 8). The assessment of biofilm formation using the CTT method was observed on both the walls and bottom of the tube. The majority of uropathogenic bacteria (76%) were strong biofilm producers, while 18% formed moderate biofilms and 6% formed weak biofilms, as shown in Table 8. According to the Tissue Culture Plate (TCP) method, a commonly used quantitative biofilm assay, there was a diverse range of biofilm formation rates recorded for the bacterial isolates: 61% exhibited moderate biofilm formation, while the remaining isolates showed strong or weak biofilm production, as shown in Figure 5b. Biofilm percentages are descriptive of the 50 selected bacterial isolates and should be checked against the original scoring sheets. The quantitative results of biofilm formation for some isolates are summarized in Table 8. The interpretation of the quantitative results of biofilm formation for some uropathogenic bacteria are summarized in Table 9.

Effect of Antibiotic on Growth, Biofilm Formation and Exopolysaccharide

Figure 6 and Table 10 show results for three selected bacteria: CR *K. pneumoniae* carried both the bla_{OXA-48} and bla_{NDM-1} genes, *P. aeruginosa* carried the bla_{NDM-1} gene and *E. coli* carried ESBL. They were grown in different concentrations of Amoxicillin/Clavulanate (Amox/Clav). The MIC was recorded for each bacterium and it was 3.12 for *E. coli*, 6.25 for *K. pneumoniae* and 12.5 for *P. aeruginosa.* The effect of the ½ MIC on growth, biofilm formation and EPS production was recorded. The highest growth and biofilm inhibition were recorded for *E. coli*, at 66 and 43%, respectively, followed by *K. pneumoniae*, at 64 and 31%. The lowest level of inhibition was recorded for growth and biofilm formation by *P. aeruginosa*. Exposure to

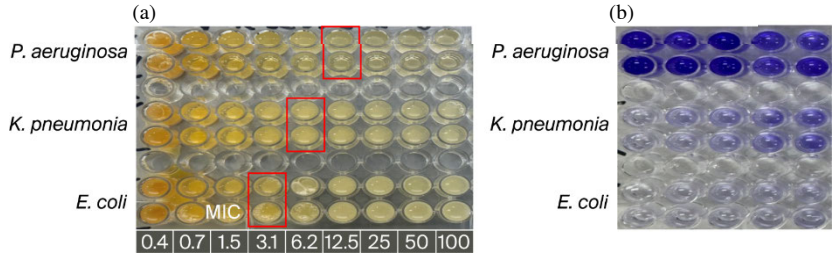


Figure 6(a-b): (a) Minimum Inhibitory Concentration and (b) Biofilm Formation of the Three Tested Uropathogenic Bacteria Treated with Different Concentrations of Antibiotics

Table 10: Effect of Amoxicillin/Clavulanate on Growth and Biofilm Formation for *E. coli*, *K. pneumoniae* and *P. aeruginosa.*

		Growth (A_{600nm})			Biofilm Formation (A_{570}nm)		
Uropathogen	MIC (µg/mL)	Control	Treated Amox/Clav	Inhibition (%)	Control	Treated Amox/Clav	Inhibition (%)
E. coli (ESBLs)	3.12	0.633	0.219 *	66	0.156	0.088 *	43
K. pneumoniae (CR)	6.25	1.451	1.007 *	64	0.16	0.1095 *	31
P. aeruginosa (CR)	12.5	1.211	1.0985 *	26	0.2395	0.175 *	26

ESBL: Extended-spectrum beta-lactamase, CR: Carbapenem resistance, *Significant results compared to control at p<0.05

Table 11: Effect of the Sub-Inhibitory Concentration (1/2 MIC) of Amoxicillin/Clavulanate on Exopolysaccharide (EPS) Production for *E. coli*, *K. pneumoniae* and *P. aeruginosa*

	Quantity of Exopolysaccharide (A_{490nm})		
Uropathogen	Control	Treatment	Percentage of Increase
K. pneumoniae (CR)	0.806±0.012	1.410±0.026 *	175
E. coli (ESBLs)	0.680±0.026	1.860±0.036 *	273
P. aeruginosa (CR)	0.684±0.017	1.270±0.002 *	185

ESBL: Extended-spectrum beta-lactamase, CR: Carbapenem resistance, *Significant results compared to control at p<0.05

sub-inhibitory concentrations (1/2 MIC) of Amoxicillin/ Clavulanate was associated with higher measured EPS-related optical-density signals than the untreated control in these three selected isolates. EPS levels were determined using Benedict's reagent by measuring optical density at 490 nm, as shown in Table 11.

DISCUSSION

The rise of antimicrobial-resistant pathogens represents a significant threat to global public health. Inappropriate antimicrobial use is one contributor to the emergence and spread of antimicrobial resistance [38]. This escalation in antibiotic use has contributed to the growing prevalence of common microbial infections, making UTIs increasingly difficult to treat. This challenge primarily stems from bacteria that are resistant to last-line antibiotics or, in some cases, are pan-drug resistant, meaning they do not respond to any commercially available antibiotics, especially those produced by ESBL and CR pathogens [14,39,40]. In Saudi Arabia, UTIs are a major health concern, placing considerable strain on the healthcare system. This issue is exacerbated by the high mortality rates associated with UTIs, alongside a rising prevalence of CR Enterobacteriales. The increasing number of CR isolates among patients underscores this growing problem. Additionally, the heightened use of carbapenem antibiotics to manage ESBL-related infections is likely to contribute to a further global increase in CR cases, including in Saudi Arabia [19].

In this hospital-based sample, 62 of the 107 culture-positive specimens (58%) were obtained from female patients. This finding aligns with previous research that reported a female prevalence rate of 63% [41]. Additionally, a short-term study conducted from March to June in the Qassim province [42] also indicated that females experienced more UTIs than males, primarily due to anatomical and physiological factors. Gram-negative bacteria, especially *E. coli*, *K. pneumoniae* and *P. aeruginosa*, accounted for the majority of the ESBL- and CR-encoding genes $bla_{\text{OXA-48-type1}}$ and $bla_{\text{NDM-1.}}$ Our findings are in concordance with previous research that also reported a higher number of UTIs caused by Gram-negative bacteria than by Gram-positive bacteria. In a similar study, bacteria were the most common causative organisms of UTI, which included *E. coli* (52%) followed by *K. pneumoniae* (15%), *P. aeruginosa* (8%), *S. agalactiae* (7%) and *E. faecalis* (5%) [43]. The most common isolated pathogens as causative agents of UTIs in Saudi Arabia are *E. coli*, *K. pneumoniae* and *P. aeruginosa* [44]. *E. coli* is the most common microbe, followed by *K. pneumoniae*, which are isolated as uropathogens from the Gulf Cooperation Council (GCC) area [45]. Furthermore, the distribution of UTIs among various healthcare settings has been reported as follows: 9% in the OPD, 3% in the ER, 15% in the ICU and 73% in the IPD. A higher proportion of UTIs was observed in the wards (IPD) compared to other settings, in contrast with previous studies reporting a high prevalence of UTIs in emergency departments [8].

Among the 107 culture-positive specimens, 62 (58%) were obtained from patients older than 60 years; this proportion does not estimate UTI prevalence among all older adults. In a similar study, patients were categorized into four different age groups and the UTI rate was higher in patients over 50 years old compared to those in other age groups [43,46].

Changes in both physiological and pathological characters of urine can significantly affect uropathogenic bacteria, it is relevant to study the effect of pH and biochemical changes on microorganisms [47]. Gram-negative bacteria were the most prevalent, consistent with previous studies. In the context of extended-spectrum beta-lactamase urinary tract infections (ESBL UTIs), the careful selection of antibiotics is critical. It is very important to use prudent discretion when prescribing them while considering the regional prevalence of bacterial species and susceptibility data. The present investigation has shown that strains of *E. coli* that produce Extended-Spectrum Beta-Lactamases (ESBLs) exhibit a significant level of resistance to a wide range of antibiotics. This explains why *E. coli* bacteria that produce extended-spectrum beta-lactamases (ESBLs) often exhibit resistance to other antimicrobial agents such as fluoroquinolones and aminoglycosides [48]. The tested antibiotics showing in-vitro susceptibility among the ESBL-producing *E. coli* isolates included amikacin, meropenem, imipenem, nitrofurantoin and fosfomycin. In contrast, *E. coli* with ESBL exhibited the highest levels of resistance against the cluster of cephalosporins and monobactems, ciprofloxacin and cotrimoxazole. This finding aligns with other previous studies that have shown that *E. coli* isolates containing ESBLs exhibit higher levels of resistance to TMP-SXT, ciprofloxacin, cotrimoxazole, gentamicin, augmentin and third-generation cephalosporins compared to *E. coli* isolates that do not produce ESBLs. In contrast, *K. pneumoniae* ESBLs exhibited the highest levels of resistance to cefuroxime, ciprofloxacin and ceftazidime, in accordance with this study's findings [14]. Nevertheless, this resistance did not impact the effectiveness of carbapenems [49]. The global rise and spread of CRE is alarming, highlighting the critical need for immediate actions to combat this growing threat. Carbapenem antibiotics, considered last-resort drugs in many hospitals, have encountered antimicrobial resistance [50]. The Enterobacteriales members most frequently investigated for carbapenem resistance are *E. coli* and *K. pneumoniae*. In this study, *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *A. baumannii* were carbapenem-resistant, with the CR-encoding genes $bla_{\text{OXA-48-type1}}$ and $bla_{\text{NDM-1}}$ prevalent. In a similar study, CR isolates of *K. pneumoniae*, *E. coli* and *P. aeruginosa* most often contained the carbapenemase genes $bla_{\text{OXA-48-type1}}$ (41.5%), $bl_{\text{NDM-1}}$ (2.5%) and both $bla_{\text{OXA-48-type1}}$ and bla_{NDM-1} (2.5%) [51]. The resistance to Carbapenem antibiotics showed a consistently higher prevalence in Al-Qassim (49%) and Al-Jouf (93%) provinces compared to other regions. In contrast, noticeably lower resistance rates (below 3%) were observed for imipenem (in the Eastern province and Madinah), meropenem (in Makkah, Madinah and Najran) and for ertapenem (in Makkah) [52].

The recent increase in nosocomial infections caused by vancomycin-resistant *E. faecium* (VREfm) presents a significant challenge in clinical settings, as reliable therapies for severe, invasive infections remain limited. In addition to vancomycin resistance, a large proportion of VRE strains also show high-level resistance to aminopenicillins and aminoglycosides, further limiting options for achieving synergistic bactericidal therapy. In this study, the five E. faecium isolates were reported resistant to vancomycin, whereas Table 5 reports susceptibility to linezolid and tigecycline (100%) and to nitrofurantoin (40%). According to Seevak *et al.* [43], the Gram-positive organism *Enterococcus faecium* showed a high level of resistance to ampicillin and ciprofloxacin. Also, Darraj [30] reported a high resistance to vancomycin, Ciprofloxacin, Levofloxacin, Penicillin and Tetracycline and an intermediate level of resistance to Quinupristin/dalfopristin.

Phenotypic methods, including the tube and Congo Red Agar (CRA) methods, are simple, inexpensive tools that can be used to screen clinical isolates for biofilm production, making them especially valuable in resource-limited laboratories [53]. Congo Red Agar, introduced by Rachid *et al.* [54], detects slime-producing colonies; however, multiple studies have reported variable sensitivity. In this study, three levels of biofilm formation among the uropathogenic bacteria were found, which could be used in routine clinical laboratories. The results were as follows: 76% had strong, 18% had moderate and 6% had weak biofilm abilities according to the tube method. The Congo Red Agar method results showed that most of the isolates (57%) exhibited moderate biofilm formation, with black secretion in the media.

Biofilms can be graded using different methods; for example, [55] reported that the Congo Red Agar method is easy to perform and interpret but the tube method is highly sensitive and specific. Another study did not recommend the CRA method for biofilm detection.

According to Lewis [55], the TCP method is considered ideal for detecting bacterial biofilm formation by uropathogenic bacteria and WHO [56] standardized the TCP assay, which led to excellent reproducibility and quantitative results. It is a commonly used quantitative phenotypic method; results depend on the assay protocol and organism.

Exopolysaccharides (EPS) are produced under antibiotic pressure or stressful circumstances; it is a mechanisms adapted by cells to protect them from other bacteria or antimicrobial agents [57]. Sub-minimal inhibitory concentrations (sub-MICs) can cause antibiotic resistance or tolerance by encouraging biofilm development and changing biofilm matrix formation. Many studies have shown that sub-MIC levels of antibiotics can increase biofilm formation and EPS synthesis as an adaptive stress response in some species [58,59]. In the current investigation, we observed that sub-MICs (1/2 MIC) of amoxicillin may operate as an adaptive stress signal, promoting EPS production. This is consistent with the hypothesis proposed by Christensen *et al.* [59], in which sub-lethal antibiotic doses alter bacterial gene expression and phenotypic behavior, including extracellular matrix production. Sub-inhibitory concentrations of fosfomycin caused a 1.82- to 4.27-fold increase in biofilm biomass in *S. aureus* compared to untreated controls [6], which is

consistent with our finding that EPS production in *E. coli* increased by about 2.88-fold higher in the treated group compared to the control. This implies that exposure to sub-MICs of antibiotics can reliably promote the synthesis of extracellular polysaccharides in a variety of bacterial species.

Our results are in agreement with those of [60], who demonstrated that sub-MIC concentrations of amoxicillin-clavulanic acid inhibited biofilm formation in chronic rhinosinusitis isolates. This suggests that the action of sub-MIC antibiotics varies by species and strain. The increase in EPS observed in our study could be attributed to the activation of regulatory mechanisms involved in polysaccharide production and cell adhesion. Another study found that sub-MICs of antibiotics, such as azithromycin, can promote EPS generation in *S. aureus* biofilms [61]. Several studies have reported increased biofilm formation following exposure to sub-MIC levels of antibiotics.

The sub-MICs of ampicillin promoted biofilm development in *Staphylococcus intermedius* biofilms through the autoinducer-2/LuxS signaling pathway [62]. Other researchers have reported that cephalexin can promote cell adhesion by increasing the hydrophobicity of the *S. aureus* cell surface. It was found that exposing minocycline-resistant *Klebsiella pneumoniae* to sub-MIC levels of tetracycline antibiotics significantly enhanced biofilm formation by modifying bacterial surface characteristics and regulating related genes [63,64]. Exposing *P. aeruginosa* to sub-inhibitory concentrations of antibiotics, such as half the MIC, was found to enhance EPS production, consistent with previous studies demonstrating that sub-MIC levels of aminoglycosides or β-lactams stimulate biofilm formation and upregulate EPS-related gene expression [65,66]. This implies that even non-lethal antibiotic exposure can trigger biofilm-associated adaptive responses. It has been reported that sub-MIC exposure significantly increases biofilm biomass in *S. aureus*, indicating enhanced extracellular matrix production, which includes exopolysaccharides [63]. Sub-MICs of antibiotics might also change quorum sensing and gene expression, resulting in increased EPS synthesis [62,67] or sub-MIC antibiotic stress signals could increase extracellular matrix production, which explains the phenotypic trends towards higher EPS output. Also, erythromycin, tetracycline and quinupristin-dalfopristin, when taken at sub-MIC levels, boosted *ica* gene expression in *S. epidermidis*, leading to increased EPS generation [68].

CONCLUSIONS

During June-August 2025 at King Fahad General Hospital in Jeddah, 107 of 464 submitted urine specimens were reported culture-positive (23.1%). The recovered organisms included Gram-negative and Gram-positive bacteria as well as yeasts and other fungi. Susceptibility testing and selected molecular assays provided descriptive information about resistance in the tested bacterial isolates, while three in-vitro methods were used to examine biofilm formation in a selected subset of 50 bacterial isolates. The study was conducted in one hospital over three months and the findings should not be generalized to national UTI prevalence or taken as evidence that biofilm formation caused antimicrobial resistance or clinical treatment failure. Further multicentre surveillance with clearly defined testing denominators and standardized laboratory methods is recommended.

Recommendations

The findings support continued local monitoring of urinary microbial isolates, organism-specific antimicrobial susceptibility testing and antimicrobial stewardship in accordance with current clinical guidelines. Any decisions about individual antibiotic treatment should take account of clinical symptoms, organism identification, relevant susceptibility results and patient factors. Future studies should use larger, multicentre samples with explicit bacterial resistance definitions, transparent molecular-testing denominators and standardized biofilm-assessment methods.

Limitations

This study has several limitations: it was conducted at a single hospital over a three-month period and several organism and resistance subgroups contained small numbers. Repeat specimen submission by the same patient was not explicitly addressed in the manuscript and clinical information may not distinguish symptomatic urinary infection from colonization, particularly for fungi recovered from urine. Only 50 of the 107 culture-positive microbial isolates were selected for biofilm testing, limiting the representativeness of these results. The resistance-category totals, gene-specific testing denominators and some antimicrobial susceptibility and biofilm percentages require verification against the original laboratory records. The study did not assess clinical treatment outcomes or strain-level transmission. These limitations restrict causal interpretation and generalizability beyond the study hospital.

Acknowledgement

We give special thanks to the Laboratory Department at King Fahd General Hospital, the Continuing Education and Training Unit for the laboratory, Mohammed Al-Qahtani and the Microbiology Lab, Ghadeer Nawfal, for their support with sample and data collection and King Abdulaziz University for providing access to its laboratory facilities for this research.

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