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Article

Isolation and Identification of Certain Resistant Bacterial Species Causing Bloodstream Infections Associated with Urinary Catheterization and Determination of the Role of Interleukins

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Abstract: This study was conducted on patients suffering from Urinary Catheterization infection in Salah El-Din General Hospital and Tikrit Emergency Hospital from March 2025 to the end of December 2026. The study systematically included 100 participants (30 healthy individuals and 70 patients). Age 20 to 60 years, The study involved the systematic collection of 100 blood and urine samples from all participants. Bacterial isolates, which had been previously cultured on agar media, were subjected to confirmatory identification using the VITEK 2 Compact system. This process strictly adhered to the manufacturer's instructions provided by the manufacturer. Immunoassays Measurement of Interleukin 6 and Interleukin 8 Concentrations in the enzyme-linked immunosorbent assay (ELISA) technique. Manufacturer: Bioassay Technology; Country of Origin: China. A thorough examination of the quantity and percentage of bacterial isolates found in patients with urinary tract infections, divided out by sex (male and female), is provided by the distribution of bacterial infections by sex in a Urinary Catheterization infection group. The most frequent bacterial isolate, according to the data, is Escherichia coli (14 (35%)). Proteus mirabilis (10 (25%) instances), Klebsiella pneumoniae (7 (17.5%), Pseudomonas aeuroginosa (6 (15%)), and Acinetobacter baumannii (3 (7.5%)). The majority of bacterial infections show gender group disparities (P-value > 0.4) that are not statistically significant. The results demonstrated that serum levels of Interleukin-6 (IL-6) and Interleukin-8 (IL-8) were considerably higher in patients with urinary catheterization infections compared to the healthy control group ($P < 0.001$). The mean blood level of IL-6 was 49.97 ± 11.67 pg/mL in infected patients and 19.65 ± 9.89 pg/mL in the control group. In a similar vein, the average blood level of IL-8 was 54.12 ± 13.58 pg/mL in the infected people and 22.12 ± 11.65 pg/mL in the controls. These findings demonstrate that pro-inflammatory cytokines are markedly increased in infections linked to urinary catheters, suggesting that the host immune response is triggered during infection.

Keywords: Catheter-Associated Urinary Tract Infections (CAUTIs), IL-6, IL-8, Bacterial Infection, Antibiotic Resistance

Introduction

Urinary tract infections (UTIs) are among the most prevalent infections in otherwise healthy people. Urinary tract infections are also prevalent in medical facilities, where patients frequently need urinary catheters to relieve urine retention. Catheter-associated urinary tract infections (CAUTIs), which are frequently brought on by a variety of opportunistic pathogens known as ESKAPE (*Enterococcus, Staphylococcus, Klebsiella, Acinetobacter, Pseudomonas,* and *Enterobacter*) pathogens, are frequently the result of urinary catheter placement [1]. The variations in pathogens, the initial microbial load, changes brought on by the length of catheterization, and the connection between infection (colonization) and clinical symptoms make it difficult to explain CAUTI [2]. In order to better comprehend CAUTI, *Pseudomonas aeruginosa*, which is distinct in that it is rarely linked to human microbiomes, is the cause of UTIs and CAUTIs. Because of this, catheterization is necessary for bacterial infection to enter the bladder and cause UTIs and CAUTIs [3]. Research on the virulence factors that bacterial infection uses in these infections once within the host is still continuing. The virulence factors linked to bacterial infection isolated from the urinary tract, animal studies to determine which bacterial factors are necessary for this infection, and studies that concentrate on P. aeruginosa UTI and CAUTI to better understand the infection dynamics and outcome in clinical settings [4].

Pseudomonas aeruginosa, which is distinct in that it is rarely linked to human microbiomes, causes UTIs and CAUTIs. Because of this, P. aeruginosa can only cause UTIs and CAUTIs when the germs are introduced to the bladder during catheterization [5]. The virulence factors that P. aeruginosa uses in these infections once within the host are still being studied. The virulence factors linked to P. aeruginosa isolated from the urinary tract, studies focusing on P. aeruginosa UTI and CAUTI to better understand the infection dynamics and outcome in clinical settings, and animal studies to determine which bacterial factors are necessary for this infection will all be covered [6].

A complex interaction of physiological, behavioral, and anatomical factors affects susceptibility to infection. Because of the urethra's small length, proximity to the anus, and hormonal fluctuations, especially during pregnancy and menopause, women are disproportionately impacted. Urinary tract abnormalities, diabetes, immunodeficiency, catheterization, sexual activity, and the use of spermicides are additional risk factors [7].

The anatomical location of the infection affects the clinical symptoms. Dysuria, urgency, frequent urination, suprapubic discomfort, and hematuria are common symptoms of lower urinary tract infections, often known as cystitis [8]. On the other hand, pyelonephritis, or upper urinary tract infections, exhibit more severe systemic symptoms that suggest renal tissue involvement and the possibility of bacteremia [9]. Urine culture is frequently used to definitively identify the pathogens and conduct antibiotic susceptibility testing. Urinalysis is the primary method of diagnosis, revealing the presence of pus, bacterial urine, and nitrite-positive urine [10]. A key component of treating urinary tract infections is comprehending antibiotic resistance. Antibiotic sensitivity varies among bacteria, and resistance varies even among populations that are identical [11]. Natural resistance can be generated, which frequently involves efflux pumps, or intrinsic, which is a general characteristic within a particular species. Chromosome mutations or horizontal gene transfer are the causes of acquired resistance. Plasmids are frequently used to transfer resistance genes. Acquired resistance is caused by mutations that impact drug targets, transporters, regulators, and enzymes that change antibiotics [12]. Kidney illness causes toxins to build up and impairs normal filtration function, which fosters the growth of germs in the urinary system. Urine stasis and tissue damage can result from kidney stones or urinary tract obstruction, two frequent side effects of renal illness that raise the risk of infection [13]. Urine leakage and recurrent UTIs can result from congenital abnormalities of the kidneys or urinary tract, which are frequently linked to chronic kidney disease. Due to altered urinary tract function and a compromised immune system, older adults—particularly those with chronic renal disease—are more vulnerable to UTIs. These infections can quickly worsen pre-existing renal disease by developing into dangerous side effects including sepsis or inflammation of the kidneys [14].

As a multifunctional cytokine, interleukin-6 is crucial for hematopoiesis, inflammatory processes, and immunological responses. Monocytes, macrophages, T cells, B cells, endothelium cells, and fibroblasts are the main cells that generate it in reaction to tissue damage or infection [15]. During bacterial infection, interleukin-6 functions as an acute-phase reactant, inducing the liver to produce acute-phase proteins such as serum amyloid A, fibrinogen, and C-reactive protein. Patients with end-stage kidney disease frequently have elevated IL-6 levels, which are linked to a higher death risk. The significance of IL-6 in chronic inflammation and its detrimental effects on immune system performance and general health are highlighted by this connection [16]. Its prolonged presence may cause immune cells to become less sensitive to acute stimuli, which could worsen immunodeficiency [17]. Chronically high levels of IL-8 may hinder neutrophil activity in those who are afflicted and contribute to the development of systemic inflammation. A possible dysregulation of the immune response is suggested by the fact that elevated levels of neutrophil chemotactic factors in the blood are not always linked to successful neutrophil responses. "Neutrophil exhaustion," a condition of chronic activation that eventually reduces their functional ability to respond to acute infections, may be exacerbated by the continuous presence of IL-8 [18].

The Aim of the Study

Bacterial species that cause urinary tract infections in kidney patients obtaining dialysis are isolated and identified.to evaluate the levels of inflammatory cytokines (IL-6, IL-8) and demographic traits across a group of healthy people, dialysis patients, and dialysis patients with confirmed UTIs.

Materials and Methods

Study Design

This study was designed as a comparative cross-sectional study, carefully conducted at a primary health care center and a hemodialysis unit at Tikrit Teaching Hospital in Tikrit over a specific time period from March 2025 to the end of December 2026

The study systematically included 100 participants (30 healthy individuals and 70 patients).

Inclusion criteria for all groups

- Age 20 to 60 years or older.
- For patient groups (hemodialysis and urinary tract infection), the urinary tract infection was laboratory-confirmed with significant bacterial growth in a urine culture at the time of sample collection.
- Availability of complete relevant demographic, biochemical, and immunological data.

Exclusion criteria for all included groups

- Presence of a systemic infection other than a urinary tract infection (for patient groups).
- Use of a recent antibiotic within the previous 72 hours (to minimize confounding effects on microbiological assessment).
- Known immunosuppressive therapy or a diagnosis of autoimmune diseases.
- Pregnancy.
- Any acute and severe illness unrelated to the primary health condition that could significantly alter biochemical or immunological parameters.

Study Design

The study involved the systematic collection of 100 blood and urine samples from all participants (both healthy individuals and hospitalized patients), with midstream urine samples carefully collected using sterile, single-use containers from all groups three days after discontinuation of antibiotic therapy. This specific timing was critical to minimize antibiotic interference with microbial growth, ensuring accurate bacterial isolation and identification for subsequent culture and antibiotic susceptibility testing. Concurrently, a maximum of 3 mL of venous blood was drawn at the same time from the same groups. Blood samples were collected in tubes containing anticoagulant gel, which effectively separated cellular

components from serum upon centrifugation. The isolated serum was then used for precise measurement of various immunological parameters, such as interleukin-6 and interleukin-8.

Isolation, Purification, and Culture of Bacteria

When the samples (swabs) arrived at the laboratory, they were divided into two groups: one for direct diagnosis of the specimen, and the other for culture on media for the initial isolation of bacterial pathogens. The media (blood agar, MacConkey agar, Nutrient agar and Nutrient broth) and samples were also inoculated onto MacConkey agar and blood agar; incubation was carried out aerobically at 37°C for 18–24 hours. The following day, the plates were examined, and the morphological characteristics as well as the gas requirements of the young, growing colonies were recorded; plates showing no growth were noted and reincubated for another 48 hours to confirm the results before being discarded [19]. The growing colonies were purified on blood agar and gelatin agar and, according to the plating method, were transferred to a new blood agar plate, taking into account the aeration conditions during incubation [20]. Daily working cultures were also used and stored at 4°C for use in subsequent diagnostic tests.

Bacterial isolates, which had been previously cultured on agar media, were subjected to confirmatory identification using the VITEK 2 Compact system. This process strictly adhered to the manufacturer's instructions provided by the manufacturer.

Antibiotic Resistance Testing of Bacterial Isolates

Antibiotic susceptibility testing, which is typically performed using the Kirby-Bauer diffusion method on Mueller-Hinton agar, is a crucial technique for determining the susceptibility or resistance of bacterial isolates to various antimicrobial agents

Immunoassays

Measurement of Interleukin 6 and Interleukin 8 Concentrations

Test Principle: This test, used to determine **Interleukin 6 and Interleukin 8 Concentrations** in the blood, is based on specific immune reactions inherent in the enzyme-linked immunosorbent assay (ELISA) technique. Manufacturer: Bioassay Technology; Country of Origin: China

Statistical analysis

Version 26 of the SPSS software was utilized to analyze the data using the ANOVA test. Mean ± SD was used for expressing every one of the research parameters. Pearson's correlation coefficient was used to assess the relationships between the two biomarkers. Figures done by Microsoft Excel program.

Results

Distribution of bacterial infection types according to sex among disease groups

Out of a total of 70 patients with urinary catheterization, 40 bacterial isolates were identified, distributed according to bacterial types as shown in Table 1.

The distribution of bacterial infections by sex in a Urinary Catheterization infection group provides a detailed analysis of the bacterial isolates (number and percentage) identified in patients with urinary tract infections, categorized by sex (male and female). The results indicate a clear predominance of Escherichia coli (14 (35%)) as the most common bacterial isolate, followed by Proteus mirabilis (10 (25%) cases), Klebsiella pneumoniae (7 (17.5%), Pseudomonas aeuroginosa (6 (15%)), and Acinetobacter baumannii (3 (7.5%)). P-value > 0.4 non-significant differences gender group's disparity is observed in most bacterial infections.

Table 1. Distribution of bacterial infection type according to gender groups in the Urinary Catheterization infection group

P-value	Total	Urinary Catheterization infection gender groups NO . (%)		bacterial infection type
		Male	Female	
0.4	3 (7.5%)	1(33%)	2(67%)	*Acinetobacter baumannii*
	14(35%)	6(43%)	(57%)8	*Escherichia coli*
	7(17.5%)	3(43%)	(57%)4	*Klebsiella pneumonia*
	10(25%)	(40%)4	(60%)6	*Proteus mirabilis*
	6(15%)	(50%)3	(50%)3	*Pseudomonas aeuroginosa*

Distribution of bacterial infection types according to age among disease groups

Table 2 shows the distribution of bacterial infections among patients with Urinary Catheterization infection according to age groups. Analysis of the results revealed that Escherichia coli was the predominant urinary tract pathogen, occurring in the 40-50 age group (29%), with a decrease in older age groups. Proteus mirabilis was the second most common causative agent (25%), showing a somewhat similar pattern with high percentages in the 31-40 age group (40%).

Table 2. Distribution of bacterial infection type according to age groups in the Urinary Catheterization infection group

P-value	Total	Urinary Catheterization infection Age groups NO . (%)				bacterial infection type
		>60	41-50	31-40	20-30	
0.4	3 (7.5%)	0(0%)	1(33%)	0(0%)	2(67%)	*Acinetobacter baumannii*
	14(35%)	2(14%)	4(29%)	5(36%)	3(21%)	*Escherichia coli*
	7(17.5%)	(14%)1	(29%)2	(43%)3	(14%)1	*Klebsiella pneumonia*
	10(25%)	(30%)3	2(20%)	(40%)4	1(10%)	*Proteus mirabilis*
	6(15%)	(33%)2	0(0%)	(50%)3	(17%)1	*Pseudomonas aeuroginosa*

The findings showed that patients with urinary catheterization infections had significantly higher serum levels of Interleukin-6 (IL-6) and Interleukin-8 (IL-8) than the healthy control group ($P < 0.001$). Infected individuals had a mean serum level of IL-6 of 49.97 ± 11.67 pg/mL, while the control group had a mean level of 19.65 ± 9.89 pg/mL. Similarly, the mean blood level of IL-8 was 22.12 ± 11.65 pg/mL in the controls and 54.12 ± 13.58 pg/mL in the infected individuals. These results show that pro-inflammatory cytokines are significantly elevated in urinary catheter-associated infections, indicating that the host immune response is activated during infection.

Table 3. Comparison of interleukins levels between study groups

P-value	Control Groups	Urinary Catheterization infection	Interleukin
	Mean ± SD		
<0.001	(19.65 ± 9.89 pg/mL)	(49.97 ± 11.67 pg /ml)	Interleukin-6
	(22.12 ± 11.65pg/ml)	(54.12 ± 13.58 pg/ml)	Interleukin-8

Antibiotic resistance patterns of bacterial isolates

The antibiotic resistance profile of bacterial isolates obtained from patients with illnesses linked to urinary catheterization is shown in Table 4. With 31 (77.5%) resistant isolates and just 9 (22.5%) sensitive isolates, gentamicin showed the highest resistance rate. Amikacin came next, revealing 10 (25%) susceptible and 30 (75%) resistant isolates. Azithromycin resistance was also high, with 15 (37%) susceptible and 25 (63%) resistant isolates. The isolates, on the other hand, had comparatively greater fluoroquinolone susceptibility. Ciprofloxacin had the highest susceptibility of all the studied medicines, with 24 (60%) sensitive isolates and 16 (40%) resistant isolates. Levofloxacin exhibited 55% sensitivity (22 isolates) and 45% resistance (18) isolates.

Table 4. Antibiotic resistance profile (frequency and percentage)

Urinary Catheterization infection	Type of antibiotic resistance	antibiotic
30 (75%)	R	**Amikacin**
10 (25%)	S	
(63%)25	R	
(37%)15	S	**Azithromycin**
(45%)18	R	**Levofloxacin**
(55%)22	S	
16(40%)	R	**Ciprofloxacin**
(60%)24	S	
(77.5%)31	R	
(22.5%)9	S	**Gentamycin**

R = resistance, S = sensitive

Discussion

These findings are consistent with numerous studies conducted in Iraq and worldwide, which have consistently identified Escherichia coli as the leading cause of Urinary Catheterization infection. For example, a study by Al-Zaidi et al. in Iraq reported that Escherichia coli is the most prevalent UTI causative agent [21]. Similarly, a study conducted in Basra, Iraq, corroborated these findings, highlighting the dominance of Escherichia coli as the causative agent in most Urinary Catheterization infection cases [22].

The high incidence of Urinary Catheterization infection in females, particularly those caused by Escherichia coli, is a well-established epidemiological pattern attributed to anatomical and physiological

differences. The presence of other Gram-negative bacteria, such as Klebsiella pneumoniae, Pseudomonas aeruginosa, and Proteus mirabilis, as significant contributors to Urinary Catheterization infection is also consistent with previous studies; these organisms are frequently associated with complicated Urinary Catheterization infection or healthcare-associated infections. The results of this study are consistent with a study conducted by Al-Hashimy and Al-Musawy [23] in Baghdad, Iraq, which observed an increasing prevalence of Klebsiella pneumoniae in cases of Urinary Catheterization infection. Although a single case of Acinetobacter baumannii may not be statistically significant in this sample, its presence in Urinary Catheterization infection, particularly among hospitalized or immunocompromised patients, is of increasing concern due to its multidrug resistance. This has been confirmed by previous studies, such as Lee et al. [24] on hospital-acquired infections. The observed gender distribution, with women disproportionately affected by Urinary Catheterization infection, is a globally recognized pattern, supported by numerous studies worldwide. This makes the results of this study comparable to broader local and international research on the epidemiology of Urinary Catheterization infection.

This study demonstrated a significant increase in IL-6 levels in both the dialysis and urinary tract infection (UTI) groups compared to the control group, consistent with the previously known inflammatory process in patients with chronic kidney disease (CKD), particularly those undergoing dialysis [25]. These findings align with several previous studies, including two separate studies in Iraq that found significantly elevated IL-6 levels in dialysis patients with UTIs compared to healthy individuals [26], [27]. IL-6 is a hallmark of chronic inflammation and plays a crucial role in Urinary Catheterization infection, initiating and amplifying the acute inflammatory response to bacterial invasion. It is a key component of host defense against infection, promoting both neutral utilization and acute protein synthesis [28].

Similarly, IL-8 levels were significantly elevated in the dialysis and urinary tract infection (UTI) groups compared to the control group, consistent with previous studies that demonstrated high IL-8 levels among patients undergoing dialysis and simultaneously suffering from UTIs [29], [30]. IL-8 is a chemokine primarily responsible for neutrophil chemotaxis and activation. In dialysis patients, chronic inflammation and oxidative stress contribute to the production of persistent IL-8, which can perpetuate immunodeficiency and contribute to atherosclerosis [31]. Regarding UTIs, the elevated IL-8 levels are a direct reflection of the robust inflammatory response demonstrated by the urinary tract epithelium in response to bacterial pathogens. IL-8 recruits neutrophils to the site of infection to clear the invading microorganisms.

Comparative analysis of antibiotic resistance patterns in Urinary Catheterization infection, patients and dialysis patients in this study reveals significantly high resistance rates in both groups. The current study found a high prevalence of fluoroquinolone resistance in Urinary Catheterization infection, particularly resistance to ciprofloxacin and levofloxacin in both UTI and dialysis groups. This high resistance to fluoroquinolones in urinary tract infections aligns with global trends attributed to their widespread and often inappropriate use, particularly in uncomplicated cases [32]-[34]. This study is consistent with previous research, particularly in Iraq, where all bacterial isolates were 100% resistant to fluoroquinolones [35]. While aminoglycoside resistance (amikacin and gentamicin) was also significant in both groups, the difference between the two groups was less pronounced, suggesting a broader distribution of resistance mechanisms to these agents. These findings are also consistent with previous Iraqi studies that found varying levels of resistance among bacterial isolates [36], [37].

Conversely, azithromycin exhibited the opposite pattern, with higher sensitivity observed in the hemodialysis group compared to the urinary tract infection group. This difference in sensitivity may reflect prior antibiotic exposures or different epidemiological pressures within these patient populations [38]. An Iraqi study conducted in Diyala found high sensitivity to azithromycin (14.04%) [39]. The widespread high rates of resistance, particularly the significant differences in fluoroquinolone resistance between groups, underscore the critical need for robust antimicrobial management programs, guided by local resistance surveillance data, to improve empirical treatment options and mitigate the emergence and spread of

resistance, especially in vulnerable populations such as those with urinary tract infections and those undergoing hemodialysis [40].

Conclusion

Elevated Inflammatory in patients with Urinary Catheterization infection showed a marked increase in pro-inflammatory cytokines (IL-6, IL-8). This confirms the presence of a systemic inflammatory response in these patients, contributing to disease progression and complications. As well as High Frequency of Infections and Variation in Bacterial Characteristics of, especially among dialysis patients was Escherichia coli was the most prevalent. Prevalence of Antibiotic Resistance with Group-Specific Patterns: High resistance to common antibiotics (such as Amikacin and Gentamicin) was observed in both groups. Patients with patients with Urinary Catheterization infection exhibited higher resistance rates, posing a public health challenge and necessitating tailored strategies to combat antibiotic resistance.

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