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Isolation and Identification of Bacterial Pathogens from Hemodialysis Patients at Zliten Dialysis Center and Evaluation of Their Antibiotic Susceptibility Patterns

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A B S T R A C T

Background: The most complication that Hemodialysis patient suffer from is get infected by microorganisms awhile they do the treatment these infections cause serious complications for dialysis patient especially catheter related bloodstream infection (CRBSIs).

Aims: This study amid to isolate isolation and identification of bacterial pathogens associated with bloodstream infections and bacterial colonization of venous catheter sites from hemodialysis patients in Zliten City Libya., Then determine the major bacterial species and test their antimicrobial susceptibility patterns.

Methods: A total of 124 clinical specimens were collected, comprising 62 catheter-site swab samples from peripheral venous catheter sites were conducted from hemodialysis patients and processed using standard microbiological procedures. The samples were cultured on appropriate selective and differential culture media and incubated under suitable conditions. The bacteria were examined based on colony morphology, microscopic characteristics, and Gram staining. Identification was performed using a series of conventional biochemical tests. Antimicrobial susceptibility testing was carried out to determine the susceptibility patterns of the isolated bacterial strains to commonly used antibiotics for hemodialysis patients.

Results: The results a total of 124 clinical specimens, comprising 62 catheter-site swab samples and 62 blood samples. 62 swab sample .93.55% (58 samples) showed bacterial growth, While 6.45% (4 sample) no bacterial growth. Among the 62 blood samples, showed result 22 bacterial growth. show the presence of several bacterial species associated with catheter sites and bloodstream infections. Antimicrobial susceptibility testing showed varying degrees of resistance among the bacterial isolates, with some strains exhibiting resistance to multiple classes of antibiotics. The results suggest the presence of multidrug-resistant bacterial strains and the potential risk of antimicrobial resistance within the hospital environment.

Conclusion: catheter-related infections are the most concern for hemodialysis patients. Infection prevention and control practices, particularly appropriate catheter insertion, aseptic techniques, sterilization, and catheter care, is essential to reduce the risk of infection. In addition, antimicrobial therapy should be done by laboratory-based antimicrobial susceptibility testing. Continuous microbiological surveillance and rational antibiotic use are recommended to reduce the emergence and dissemination of antimicrobial-resistant bacteria among hemodialysis.

Keywords: *Hemodialysis, catheter-related bloodstream infection, bacterial pathogens, antimicrobial susceptibility, multidrug resistance*

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1. Introduction

Renal failure is major health problems worldwide. It is a serious medical condition resulting from the inability of the kidneys to perform their vital functions adequately. This leads to the accumulation of waste products and toxins in the body and negatively affects various organs and the immune system, thereby reducing the body's ability to resist disease [1].

As a result of this impaired immune function, patients with chronic renal failure are more susceptible to bacterial infections, which represent a major challenge in their healthcare management. The danger of these infections is not limited to increasing patient suffering and worsening their health condition, they may also increase mortality rates and impose substantial healthcare **costs [**2]. To address this problem effectively, it is essential to accurately understand the types of bacteria responsible for infections among with chronic renal failure and determine their susceptibility and resistance to different antibiotic. This local knowledge is important for guiding physicians in selecting appropriate treatment quickly and effectively, particularly during the early stages before laboratory test results become available. It also contributes to developing strategies to prevent the spread of infections within hemodialysis centers [3]. The Zliten Dialysis Center is an important healthcare facility that provides services to large number of patients with chronic renal failure in the region. Since the environment of hemodialysis unite may provide favorable conditions for the transmission of certain types of infection, it is important to conduct a scientific study focusing on identifying the bacterial species responsible for infections among patients attending center **[**3,4].

Obtaining local date on these bacteria and their antibiotic resistance patterns will help made clearer treatment decisions and develop preventive measures specifically designed to meet the needs of patients in Zliten [5,6].

Aims:

This study aimed to isolate and identify the bacteria pathogens associated with bloodstream infections and bacterial colonization of venous catheter sits among hemodialysis patients in Zliten**,** Libya. The study also aimed to determine the most prevalent bacterial species and assess their antimicrobial susceptibility patterns.

2. Material and Methods

2.1 Sample Collection

This cross-sectional study was conducted from April to August 2025 in Zliten Dialysis Canter and its affiliated units in Ramaya and Dawao. A total of 124,62 swab samples and 62 blood culture were collected, comparing 50 males and 12 females, with ages ranging from 5 to 80 years.

2.2 Culture Media

Table 1: Several culture media were used for the isolation

No.	Culture Medium
1	MacConkey agar
2	Blood agar
3	Chocolate agar
4	Nutrient agar

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2.3 Antibiotics

Table 2: The following antimicrobial agents were used for antimicrobial susceptibility testing

No.	Antibiotic	Disc Code
1	Ampicillin	AM-10
2	Imipenem	IPM-10
3	Nitrofurantoin	F-300
4	Chloramphenicol	C-5
5	Sulfamethoxazole/Trimethoprim	SXT-25
6	Cefuroxime	CXM-30

2.4 Collection Procedure

2.4.1 Collection of Catheter-Site Swab Sample

Catheter-site swab samples were collected using sterile swabs containing appropriate transport media. Samples were obtained from the skin surrounding the vascular catheter insertion or exit site. Aseptic precautions were maintained throughout the sampling procedure to minimize the possibility of contamination. Appropriate personal protective equipment, including gloves facemasks, was used during sample **collection.** Then identification number were recorded and the swabs were transport to the laboratory for streaking on culture media. The petri **was** incubated at 37 °C for 24 hours. Following incubation, the plates were examined for bacterial growth. Individual colonies with distinct morphological characteristics were selected for further identification. Also, identification was performed using Gram staining and conventional biochemical tests.

2.4.2 Collection and Processing of Blood Samples

Blood samples were collected from the patients using stranded aseptic procedures to minimize contamination. The blood collection site was disinfected appropriately before sample collection, and sterile equipment and personal protective equipment were used. The required volume of blood was collected aseptically and immediately inoculated into an appropriate blood culture bottle. Each blood culture bottle was labeled with the relevant patient identification information and transport promptly to the laboratory. The blood culture bottles were incubated at 37°C according to the laboratory's standard procedures. Following incubation, bottles showing evidence of bacterial growth were processed for subculture. The external surface of the bottle was disinfected before sampling. Using sterile technique, a portion of the blood culture was removed and inoculated onto appropriate culture media. The inoculum was streaked using the streak-plate technique to obtain isolated bacterial colonies. The inoculated plates were incubated at 37°C for approximately 18-24 hours. After incubation, the plates were examined for bacteria growth, and representative colonies were selected for identification.

2.4.3 Identification and Antimicrobial Susceptibility Testing Using the Phoenix 100 System.

Selected pure bacterial colonies obtained from the blood culture plates were processed using the Phoenix 100 automated microbiology system for bacterial identification and antimicrobial susceptibility testing, The procedure was performed according to the manufacturer's instructions. Quality Control: To ensure the reliability and accuracy of the microbiological procedures, standard aseptic techniques were maintained throughout sample collection, transportation, culture, identification, and antimicrobial susceptibility testing. Culture media reagents, and

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laboratory materials were handled according to standard laboratory procedures. Where applicable, quality-control procedures for antimicrobial susceptibility testing were performed in accordance with the relevant CLSI guidelines and the manufacturer's instructions for the Phoenix 100 system.

3. Results

To assess the occurrence of bacterial growth among the study participants, the bacterial culture results of 62 clinical cases were analyzed. The findings demonstrated that bacterial growth was detected in 58 cases (93.5%), whereas 4 cases (6.4%) showed no bacterial growth. These findings indicate that bacterial growth was highly prevalent among the studied cases, with the vast majority of specimens yielding positive bacterial culture results. The distribution of the study sample according to bacterial growth is presented in Table (3).

Table 3- Distribution of the Study sample According to Bacterial Growth

Bacterial Growth	Result	Percentage (%)
No Growth	4	6.4
Growth	58	93.5
Total	62	100.0

3.1 Determination and Comparison of the Most Prevalent Bacterial Species Isolated from Swab Samples

The bacterial isolation results (Table4), revealed the presence of seven different bacterial species. The distribution of the isolated bacterial species differed significantly ($p< 0.001$). This finding indicates that the bacterial species were not equally distributed among the swab samples collected from patients with CKD, demonstrating considerable variation in their prevalence within the study population. Among the isolated bacteria, *Staphylococcus epidermidis* was the most prevalent species, accounting for 30 isolates (51.7%) of the total 58 samples. It was followed by *Staphylococcus aureus*, which was isolated from 13 samples (22.4%). *Pseudomonas aeruginosa* ranked third with 7 isolates (12.1%). The remaining bacterial species were detected at considerably lower frequencies. *Pseudomonas aeruginosa* was isolated from 3 samples (5.2%), Whereas *Klebsiella pneumoniae* and *Acinetobacter* were each identified in 2 samples (3.4%). *Escherichia coli* was the least prevalent bacterial species, with only 1 isolate (1.7%). Overall, the findings demonstrate that *S. epidermidis* was the predominant bacterial species isolated from the swab sample of patient with CKD, followed by *S. aureus* and *P. aeruginosa*. In contrast, *E. coli*, *Klebsiella pneumoniae*, and *Acinetobacter* were detected at relatively low frequencies. The statistically significant difference in the distribution of the isolated bacterial species ($p < 0.001$) indicates substantial variation in bacterial prevalence within the studied population.

Table 4- Distribution of bacteria species from swab sample of patients with chronic kidney

Bacterial species	Frequency	Percent (%)	p-value
S. aureus	13	22.4	<0.001
S. epidermidis	30	51.7	
Pseudomonas aeruginosa	7	12.1	
Klebsiella pneumoniae	2	3.4	
Acinetobacter	2	3.4	
E. coli	1	1.7	
Streptococcus pyogenes	3	5.2	
Total	58	100.0	

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3-2 comparison of Bacterial Prevalence among Healthcare Center

A comparison was conducted to assess the prevalence of the bacterial species isolated from samples of patients with Chronic Kidney Disease (CKD) across centers included in the study. The purpose of this comparison was to determine whether significant differences existed in the distribution of the bacterial species among the healthcare centers. As presented in Table (5), statistically significant differences were observed in the distribution of the bacterial species among the studies healthcare centres ($p< 0.001$). The Ramaya Health Center demonstrated the highest prevalence of most of the bacterial species compared with the other centers. Furthermore, all seven bacterial species investigated in the study were detected at the Ramya Health Center. In contrast, Dawao Health Centre demonstrated comparatively lower prevalence rates for most of the bacterial species. These finding indicate substantial variation in the distribution of bacterial species among the healthcare center. At the Ramaya Health Centre, *Escherichia coli* and *Streptococcus pyogenes* demonstrated the highest prevalence, with both species accounting for 100.0% of the isolates of their respective bacterial groups. This was followed by *Pseudomonas aeruginosa*, which accounted for 85.75%, and *Staphylococcus aureus*, which accounted for 61.5%. Both *Klebsiella pneumonia* and *Acinetobacter* showed a prevalence of 50.0%, whereas *Staphylococcus epidermidis* showed the lowest prevalence at 40.0% among the bacterial species detected at this center. At Zliten Central Hospital, four bacterial species were identified. *Acinetobacter* exhibited the highest prevalence at 50.0%, followed by *S. aureus* (30.8%) and *S. epidermidis* (30.0%). *Pseudomonas aeruginosa* was detected at a prevalence of 14.3%. No isolates of *Klebsiella pneumoniae*, *E. coli*, or *Streptococcus pyogenes* were detected at this healthcare center. At the Dawao Health canter, three bacterial species were identified. Klebsiella pneumonia showed the highest prevalence at 50.0%, followed by *S. epidermidis* at 30.0% and *S. aureus* at 7.7%. No isolates of *Pseudomonas aeruginosa*, *Acinetobacter, E. coli*, or *Streptococcus pyogenes* were detected at this center. Overall, the findings demonstrate marked differences in the distribution of bacterial species among the healthcare centers. The Ramaya Health Center exhibited the greatest diversity of bacterial isolates, with all seven bacterial species included in the study being detected. Zliten Center Hospital showed the presence of four bacterial species, whereas Dawao Health Center had three. The statistically significant differences observed among the healthcare centers ($p<0.001$) indicate that the distribution of bacterial species varied significantly according to the healthcare center.

Table 5: Distribution of bacterial specie isolated from swab samples of patients with chronic kidney disease

Bacterial species	Zliten Hospital	Ramaya Center	Dawao Center	p-value
S. aureus	4 (30.8)	8 (61.5)	1 (7.7)	<0.001
S. epidermidis	9 (30.0)	12 (40.0)	9 (30.0)	<0.001
Pseudomonas aeruginosa	1 (14.3)	6 (85.7)	0 (0.0)	<0.001
Klebsiella pneumoniae	0 (0.0)	1 (50.0)	1 (50.0)	<0.001
Acinetobacter	1 (50.0)	1 (50.0)	0 (0.0)	<0.001
E. coli	0 (0.0)	1 (100.0)	0 (0.0)	<0.001
Streptococcus pyogenes	0 (0.0)	3 (100.0)	0 (0.0)	<0.001

Table 6: Bacterial Species Isolated from Blood Samples of CKD

Bacterial species	Frequency	Percent (%)	p-value
S. aureus	6	54.5	0.000
Pseudomonas aeruginosa	2	18.2	0.000
Klebsiella pneumoniae	3	27.3	0.000
Total	11	100.0	

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Table 7: Distribution of Bacterial Species Isolated from Blood Samples of Patient CKD

Bacterial species	Zliten Hospital	Ramaya Center	Dawao Center	P-value
S. aureus	2 (33.3)	3(50.0)	1(16.7)	<0.001
Pseudomonas aeruginosa	0(0.0)	2(100.0)	0(0.0)	<0.001
Klebsiella pneumoniae	0(0.0)	2(66.7)	1(33.3)	<0.001

Table 8: Antibiotic Resistance rates of Bacterial Species to Antibiotics in Swab Samples

Bacterial species	Resistance rate (%)	p-value
S. aureus	66.7	<0.001
S. epidermidis	25.0	<0.001
Pseudomonas aeruginosa	66.7	<0.001
Klebsiella pneumoniae	66.7	<0.001
Acinetobacter	66.7	<0.001
E. coli	33.3	<0.001
Streptococcus pyogenes	54.2	<0.001

Table 9: Antibiotic Resistance Rate among Bacterial Species Isolated from Blood Samples

Bacterial species	Resistance rate (%)	p-value
S. aureus	61.1	<0.001
Pseudomonas aeruginosa	66.7	<0.001
Klebsiella pneumoniae	50.0	<0.001

4. Discussion

The present study investigated the distribution of bacterial special, antimicrobial susceptibility and bacteraemia among CKD patients. The findings were compared with those of previous studies to identify areas of agreement and discrepancy and to provide possible explanations for the observed patterns. Particular attention was given to the bacterial species isolated from swab and blood samples, their antimicrobial susceptibility profiles, variations among healthcare centers. The present study demonstrated that *Staphylococcus epidermidis* and *Staphylococcus aureus*. Several Gram-negative organisms, particularly *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, were also identified. The predominance of *Staphylococcus* species in the present study is consistent with the finding of Attia et al [7] .Who reported that *Staphylococcus* species accounted for approximately 88.9% of infections associated with CKD, with *S. epidermidis* being the predominant isolate .This finding is comparable with the study, in which *S.epidermidis* was the most prevalent bacteria recovered from swab sample. Similariy, Attia et al [7] reported that *S.epidermidis* was the most frequently isolated bacterial species, followed by *S. aureus*. This distribution closely corresponds to the pattern observed in the present study.

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The predominance of *staphylococci* in superficial sample may be related to their normal colonization of the skin and their ability to persist on catheter surfaces and medical devices. In contrast, Teshome Derese et al. [8] reported *K. pneumoniae* as the predominant bacterial pathogen among patents with bacteraemia. This observation is consistent with the present finding, in which *K. pneumonia* represented an important proportion of the bacterial isolates recovered from blood samples. Differences in the predominant bacterial species between swab and blood samples may reflect differences between superficial colonization and invasive infection. The present study revealed considerable variation in antimicrobial resistant among the bacteria species investigated. Relatively high resistance rates were observed *among P. aeruginosa, Acinetobacter, K. pneumoniae, and S. aureus*. These findings are consistent with the increasing concern regarding antimicrobial resistance among CKD, particularly those receiving long-term hemodialysis and frequently exposed to healthcare environments.

The findings are comparable with those reported by Teshome Derese et al. [8]. Who documented high levels of antimicrobial resistance, particularly among *K. pneumoniae* and *E. coli*, together with a substantial prevalence of multidrug-resistant MDR. The elevated resistance observed among *Klebsiella* and *Pseudomonas* in the present study follows a similar pattern. Dayana Fram also reported the presence of antimicrobial resistance determinants among bacterial isolates recovered from patients' haemodialysis. Their findings support the importance of antimicrobial resistance as a major challenge in this patient population. Repeated exposure to antimicrobial agents. Prolonged hospitalization, recurrent vascular access, and frequent contact with healthcare facilities may contribute to antimicrobial selection pressure and the persistence of resistant bacteria [9]. Furthermore, Attia et al. reported considerable resistance of *S. epidermidis* to several antimicrobial agents, while susceptibility to other agents was retained. The variable susceptibility pattern observed in that study is consistent with the present findings, which demonstrated substantial differences in resistance rates according to both bacterial species and antimicrobial agent [7].

Differences between healthcare centres may be associated with variations in infection-prevention and control practices, antimicrobial prescribing patterns, patient characteristics, environmental conditions, and management of vascular access devices. However, because these factors were not directly measured in the present study, their contribution should be interpreted as possible explanations rather than established causal relationships. The findings are consistent with those of Teshome Derese et al. who reported that the healthcare setting and patient-care environment may influence the risk of bacterial infection and bacteremia [8]. Similarly, Attia et al highlighted the potential influences in clinical practices, catheter care, and infection-control procedures on infection rates across healthcare unite [7]. The significant differences observed among the healthcare centers therefore, suggests that local factors may contribute to variations in bacterial prevalence and antimicrobial resistance. These findings emphasize the importance of strengthening infection-prevention, control programs and maintaining continuous microbiological surveillance at the local level. The present study found that bacteremia occurred in a relatively small proportion of the study population. With 11of the 58 patients (19.0%) showing positive blood cultures. Among the identified bloodstream isolates, *S. aureus* was the most frequently isolated organism, followed by *K. pneumoniae* and *P. aeruginosa*. Dayana Fram similarly reported the involvement of *S.aureus* and Gram-negative bacteria in catheter-related bloodstream infections CRBSTs among patients undergoing patients who repeated vascular access [9].

The lower frequency of bacteraemia compared with bacterial isolation from swab samples may indicate that bacterial colonization or localized infection occurs more frequently than invasive bloodstream infection. Nevertheless, bacteria remain clinically significant because patients with CKD, particularly those receiving hemodialysis, are exposed to repeated vascular access and consequently have an increased opportunity for bloodstream invasion. The findings of the present study highlight the importance of antimicrobial susceptibility testing in

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guiding antibiotic therapy among patients with CKD. The susceptibility results indicated that certain antimicrobial agents, particularly imipenem (IPM) and F300, demonstrated comparatively higher activity against some of the bacterial isolates. These findings are consistent with Dayana Fram [9], who emphasized the importance of performing antimicrobial susceptibility testing before initiating therapy because of the increasing prevalence of multidrug-resistant organisms. Similarly, Teshome Derese et al. reported substantial resistance to several antimicrobial agents among bacterial isolates recovered from patients with bacteria [8]. The observed variability in antimicrobial susceptibility further demonstrates that empirical antibiotic therapy may not reliably provide adequate coverage for all bacterial pathogens in this population. Therefore, culture-based identification and antimicrobial susceptibility testing should be incorporated into clinical decision-making whenever clinically feasible [10-11]. Attia HA, et al. also emphasized that antimicrobial selection should be based on the causative bacterial species as Gram-positive cocci and Gram-negative bacilli may exhibit substantially different susceptibility profiles(antibiograms) [7]. The present findings support this observation, as marked differences in antimicrobial agent with a relatively high susceptibility rate in the present study should not be interpreted as a recommendation for empirical use in all patient. Antibiotic selection should be individualized according to the identified pathogen, antimicrobial susceptibility profile, clinical condition, renal function, and relevant antimicrobial stewardship recommendations, patients with renal disease are considered particularly vulnerable to infection caused by antimicrobial-resistant bacteria, especially those undergoing haemodialysis or kidney transplantation. Among the major resistant bacterial pathogen of concern. These bacteria possess various virulent factors that enable them to survive and adapt within the host. They may also from biofilm, which can protect bacterial cells from antimicrobial agents and host immune responses, thereby increasing their persistence and making infections more difficult to eradicate [7.8.12].

5. Conclusion

The findings demonstrated the presence of several bacterial species associated with infection. These finding highlight the importance of strict adherence to appropriate sterilization and aseptic procedures during catheter insertion and subsequent catheter care. Furthermore, the appropriate selection of antimicrobial therapy based on microbiological culture and antimicrobial susceptibility testing is essential for reducing the incidence of bacterial infections and limiting the resistance.

ETHICAL STATEMENT:

This study was approved by Zliten Dialysis Center (Approval No. IRB/ 5/489) on April 22 ,2025.

CONFLICT OF INTEREST

The authors declare that they have no financial, professional, or personal relationships that could influence or appear to influence the work reported in this study.

AUTHORS' CONTRIBUTIONS

All authors contributed to study design, supervised experimental work, and contributed to manuscript drafting. Performed laboratory experiments, collected data, and conducted statistical analysis, prepared tables and figures, and contributed to interpretation of results.

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