

ANALYSIS OF MICROBIAL CLINICAL ISOLATES AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS IN DIABETIC PATIENTS.

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Abstract

Introduction:

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose levels resulting from insulin deficiency or impaired insulin action. Diabetic patients are more susceptible to microbial infections due to compromised immunity and impaired wound healing. Understanding the types and antibiotic susceptibility patterns of microbial clinical isolates in diabetic patients is crucial for effective management and prevention of complications. However, there is a knowledge gap regarding the prevalence of specific pathogens and their antibiotic susceptibility patterns in diabetic patients, particularly in KwaZulu Natal, South Africa. This study aimed to identify the types of microbial clinical isolates commonly found in diabetic patients and assess their antibiotic susceptibility patterns.

Methodology:

A retrospective cross-sectional study was conducted at Inkosi Albert Luthuli Central Hospital in KwaZulu Natal, involving 100 diabetic patients. Microbiological analysis and antibiotic susceptibility testing were performed using standard methods. The types and frequencies of microbial isolates were determined, and their antibiotic susceptibility patterns were evaluated.

Results:

Enterococcus faecalis, *Klebsiella* species, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Pseudomonas aeruginosa* were among the commonly identified microbial isolates. The antibiotic susceptibility patterns varied among the isolates, highlighting the importance of targeted treatment selection.

Conclusion:

This study contributes to bridging the knowledge gap and provides valuable insights for the management of infections in diabetic patients. The findings can guide the selection of appropriate antibiotics, inform infection prevention strategies, and ultimately improve patient outcomes in this vulnerable population.

Keywords: Diabetes Mellitus, Antimicrobial susceptibility testing, Multidrug resistance, Methicillin-Resistant *Staphylococcus aureus*, South Africa, Submitted: 2023-06-12 Accepted: 2023-06-18

1. Introduction:

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose levels resulting from either insulin deficiency or impaired insulin action. It is a global health con-

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cern, with an estimated 463 million adults diagnosed with diabetes worldwide in 2019 (Panday, 2021). Individuals with diabetes are not only vulnerable to various complications but also face an increased risk of developing microbial infections. Uncontrolled hyperglycaemia impairs overall immunity of diabetic patient via involvement of various mechanistic pathways that lead to the diabetic patient as immunocompromised (Akash et al., 2020). Microbial infections pose a significant threat to diabetic patients due to their compromised immune system and impaired wound healing (Rozman et al., 2020). *Pseudomonas* spp. Were previously found responsible for most wound infections in diabetic patients (Ahmed, 2020). Since diabetes is a systemic disease, these infections can manifest in different anatomical sites, including the skin, urinary tract, respiratory tract, and bloodstream (Akash et al., 2020). Common microbial pathogens involved in diabetic infections include bacteria, fungi, and viruses. Bacterial pathogens, such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, contribute to skin and soft tissue infections, while fungal pathogens, predominantly *Candida* species, are associated with oral and genital infections (Akash et al., 2020). Viral infections, such as herpes simplex virus, can cause a variety of clinical manifestations in diabetic individuals (Nagafuchi, 2021).

1.1. Background:

Understanding the types and antibiotic susceptibility patterns of microbial clinical isolates in diabetic patients is crucial for effective management and prevention of complications (Singh, 2019). Identifying the specific microbial species responsible for infections and determining their susceptibility to antibiotics is essential for targeted and appropriate treatment selection (Benkova et al., 2020). Inappropriate use of antibiotics can lead to treatment failures and the emergence of antibiotic resistance, further complicating the management of infections in diabetic individuals. Therefore, the best way to prevent adverse events from prolonged antibiotic use in patients with diabetic foot infections is to keep the duration of antibiotic

treatment short and with the lowest dose clinically necessary (Soldevila-Boixader et al., 2023).

Moreover, studying clinical isolates from diabetic patients provides valuable insights into the epidemiology and patterns of microbial infections in this specific population (Singhai, 2012). It can help healthcare providers develop tailored infection prevention and control strategies to minimize the incidence and impact of infections. Additionally, investigating the interactions between microbial isolates and the diabetic host's immune system can shed light on the underlying mechanisms of infection and host susceptibility. For instance,

neutrophil function impairment has been previously reported as a host susceptibility factor to bacterial infection in diabetes (Fuchs et al., 2020). Multiple studies conducted on bacterial iso-

lates in various patient populations have yielded important findings. Mahomood et al. (2020) demonstrated significant antimicrobial activity of Tazobactam against *Staphylococcus aureus* in diabetic patients. Adegbite et al. (2019) found

high sensitivity of Gram-negative bacilli to nitrofurantoin, while cefuroxime exhibited low sensitivity. Worku et al. (2021) reported a high prevalence of drug resistance in diabetes patients.

Mutonga et al. (2019) identified antibiotic resistance in *Staphylococcus aureus* and methicillin-resistant *S. aureus* isolates. Selim et al. (2019) observed plasmid-mediated resistance in *Staphylococci* and *E. coli*. Binu et al. (2021) noted *Pseudomonas aeruginosa* as the most frequently isolated Gram-negative organism. Mnyambwa et al. (2021) found a high level of resistance to commonly used antibiotics. Woldeteklie et al. (2022) identified major carbapenemase-producing organisms. These studies contribute to our understanding of bacterial resistance and antimicrobial activity in different patient groups.

Despite the importance of studying microbial clinical isolates in diabetic patients, there remains a knowledge gap regarding the prevalence of specific pathogens and their antibiotic susceptibility patterns in this population of KwaZulu Natal, South Africa. This gap hinders the development of evidence-based treatment guidelines and optimal management strategies. Therefore, this study

Table 1: Depicts some of the existing research on the types of microbial infections commonly found in diabetic patients. Additionally, depicts antibiotic susceptibility patterns of microbial isolates in diabetic patients, highlighting the importance of appropriate treatment strategies.

Reference/Author	Sample size	Findings
Mahomood et al., 2020	280	Tazobactam demonstrated the most significant antimicrobial activities against <i>Staphylococcus aureus</i> in diabetic patients.
Adegbite et al., 2019	240	The Gram-negative bacilli isolated were highly sensitive to nitrofurantoin followed by ofloxacin, gentamicin and least sensitive to cefuroxime.
Worku et al., 2021	225	The prevalence of drug resistance to doxycycline, amoxicillin-clavulanate, cefuroxime and penicillin for both Gram-negative and Gram-positive bacteria was high among diabetes patients.
Mutonga et al., 2019	83	The bacterial isolates, <i>Staphylococcus aureus</i> DNA and methicillin-resistant <i>S. aureus</i> (MRSA) showed resistance to commonly used antibiotics such as ampicillin, amoxicillin, cefepime, cefuroxime, clindamycin, erythromycin, piperacillin-tazobactam, tetracycline, and trimethoprim-sulphamethoxazole.
Selim et al., 2019	292	The resistance of <i>Staphylococci</i> and <i>E. coli</i> isolates was plasmid-mediated linked.
Binu et al., 2021	102	<i>Pseudomonas aeruginosa</i> was the most frequently isolated gram-negative organism among the enrolled patients.
Mnyambwa et al., 2021	388	Isolates exhibited a high level of resistance to commonly used antibiotics such as Ampicillin, Amoxicillin-Clavulanic acid, Erythromycin, Gentamicin, Tetracycline, Trimethoprim, third generation Cephalosporins (Ceftriaxone and Ceftazidime), and reserved drugs (Clindamycin and Meropenem).
Woldeteklie et al., 2022	76	The top five major carbapenemase-producing organisms were <i>K. pneumoniae</i> , <i>Serratia</i> , <i>Acinetobacter</i> species, and <i>E. coli</i> .

aims to identify the types of microbial clinical isolates commonly found in diabetic patients and assess their antibiotic susceptibility patterns. By doing so, it seeks to address the research problem of limited comprehensive knowledge in this area. The findings of this study will contribute to improved clinical decision-making, enhance the selection of appropriate antibiotics, and guide infection prevention and control measures in diabetic patients, ultimately leading to better patient out-

comes and reduced healthcare burden

1.2. Research Problem:

The research problem addressed in this study is the lack of comprehensive knowledge regarding the types of microbial infections and their antibiotic susceptibility patterns in diabetic patients. Although it is well-known that individuals with diabetes are more prone to infections, there is a need to identify the specific microbial

species responsible for these infections and understand their resistance or susceptibility to commonly prescribed antibiotics. This information is crucial for healthcare providers to make informed decisions regarding appropriate antibiotic therapy, minimize treatment failures, and prevent the emergence of antibiotic resistance in diabetic patients. Therefore, the research problem focuses on investigating the types and antibiotic susceptibility patterns of microbial clinical isolates in diabetic patients to bridge the knowledge gap and enhance the management of infections in this population.

1.3. Objective of the study:

The objective of this study was to identify the types of microbial clinical isolates commonly found in diabetic patients and to assess their antibiotic susceptibility patterns. The research aims to determine the prevalence of different microbial species causing infections in diabetic individuals and investigate their resistance or susceptibility to commonly used antibiotics. By understanding the specific types of microbes involved and their antibiotic susceptibility profiles, the study seeks to provide valuable insights for effective treatment strategies and the prevention of antibiotic resistance in this vulnerable population.

2. Methodology:

2.1. Study Design:

This retrospective cross-sectional study was designed to collect and evaluate data from diabetics at Inkosi Albert Luthuli Central Hospital in KwaZulu Natal.

2.2. Study Setting and Population:

The study took place at the laboratory of Inkosi Albert Luthuli Central Hospital in Durban, KwaZulu Natal. The population consisted of 100 diabetic patients of both genders, aged 20-79, recorded between January and December 2021.

2.3. Data Collection:

Retrospective data was extracted from the Laboratory Information System (TrakCare) of Inkosi Albert Luthuli Central Hospital laboratory, recorded between January and December 2021.

2.4. Statistical Analysis:

Microsoft Excel was used to calculate and present graphical statistical elements, including the percentage of the most prevalent isolated organisms. It was also used to present organisms based on antibiotic susceptibility-resistance patterns and to classify infective organisms as nosocomial or not.

2.5. Ethical Considerations:

Ethical clearance was obtained from the Mangosuthu University of Technology, Research Ethics Committee before the study began. Retrospective data extracts were securely stored with password protection. Patient confidentiality was maintained by using only laboratory numbers for data collection and ensuring that medical records were not shared with unauthorized individuals. The study adhered to the principles of avoiding harm to patients and preserving confidentiality.

2.6. Microbiological Analysis and Antibiotic Susceptibility Testing:

To explicate, the results used in this study were obtained from samples that have been undergone microbiological analysis and antimicrobial susceptibility testing. Infective microorganisms were isolated by culturing collected specimens on various agar plates and identifying them through colony morphology and Gram-staining. Antibiotic susceptibility testing was performed using the Kirby Bauer disc diffusion method, and the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) was detected using a ceftoxitin disk. Extended-spectrum beta-lactamase (ESBL) production was screened among Enterobacteriaceae isolates, and phenotypic confirmation was done using the combination disc method. Furthermore, identification of all isolates to the species level was performed using the molecular biology method, VITEK® 2 compact system

(bioMérieux, France) following the instructions of the manufacturer. These methods provided valuable information on the microbial composition, antibiotic susceptibility, and ESBL production in the studied samples.

3. Results:

3.1. Types of Microbial Isolates

Table 2. Presents the types and frequencies of microbial isolates identified in diabetic patients.

Isolated organism	Number	Frequency (%)
<i>Enterococcus Faecalis</i>	5	5.0
<i>Klebsiella Species</i>	13	13.0
<i>Methicillin Resistant Staphylococcus</i>	72	72.0
<i>Pseudomonas Aeruginosa</i>	2	2.0
<i>Staphylococcus aureus</i>	8	8.0
Total	N = 100	100.00

The data presented in **Table 2** illustrates the types and frequencies of microbial isolates identified in diabetic patients. The table includes the isolated organisms, the number of occurrences, and their corresponding frequencies in percentage (%).

Enterococcus faecalis: Out of 100 diabetic patients, *Enterococcus faecalis* was identified in 5 cases, representing 5.0% of the isolates. **Klebsiella species:** *Klebsiella* species were isolated in 13 cases, accounting for 13.0% of the isolates.

Methicillin Resistant Staphylococcus: *Methicillin-resistant Staphylococcus*, presumably referring to *Methicillin-resistant Staphylococcus aureus* (MRSA), was found in 72 cases, making up the majority with 72.0% of the isolates.

Pseudomonas aeruginosa: *Pseudomonas aeruginosa* was isolated in 2 cases, representing 2.0% of the isolates. Additionally, the presence of *Staphylococcus aureus* is also mentioned with 8%. Altogether, the total number of microbial isolates identified in the study was 100, with each isolate accounting for its respective percentage contribution.

3.2. Antibiotic Susceptibility Patterns

Table 3: Reports the antibiotic susceptibility patterns of the isolates, highlighting the notable trends or differences.

	Gram positive cocci in clusters		
	<i>E. faecalis</i> (n=5)	MRSA (n=72)	<i>S. aureus</i> (n=8)
	Sensitive	Sensitive	Sensitive
Vancomycin	0	72 (100.00%)	8 (100.00%)
Gentamicin	0	68 (94.44%)	8 (100.00%)
Ampicillin	5 (100.00%)	0	2 (25.00%)
Chloramphenicol	0	72 (100.00%)	8 (100.00%)
Linezolid	4 (80.00%)	72 (100.00%)	6 (75.00%)
Amoxicillin	5 (100.00%)	0	2 (25.00%)
Quinupristin/Dalfopristin	5 (100.00%)	0	6 (75.00%)
Cloxacillin	3 (60.00%)	53 (73.61%)	2 (25.00%)
Sulfamethoxazole	4 (80.00%)	8 (11.11%)	2 (25.00%)
Levofloxacin	0	66 (91.67%)	8 (100.00%)

	Gram negative bacilli, n (%)	
	<i>Klebsiella spp.</i> n=13(%)	<i>P. aeruginosa</i> n=2 (%)
	Sensitive	Sensitive
Vancomycin	Null	0
Gentamicin	9 (69.23%)	2 (100.00%)
Ampicillin	3 (23.08%)	0
Chloramphenicol	1 (7.69%)	0
Cephalosporin	13 (100.00%)	2 (100.00%)
Amoxicillin	0	0
Amikacin	11 (84.62%)	1 (50.00%)
Penicillin	12 (92.31%)	2 (100.00%)
Imipenem	13 (100.00%)	2 (100.00%)
Levofloxacin	9 (69.23%)	1 (50.00%)

4. Discussion:

4.1. Types of Microbial Isolates:

Provides valuable insights into the types and frequencies of microbial isolates in diabetic patients. These findings have important implications for understanding the aetiology and management of infections in this specific population.

Enterococcus faecalis, a Gram-positive bacterium, was identified in 5% of the cases. This species is a known opportunistic pathogen and can cause a range of infections, including urinary tract infections and wound infections. Its presence highlights the importance of monitoring and addressing enterococcal infections in diabetic patients.

Klebsiella species, a group of Gram-negative bacteria, were isolated in 13% of the cases. *Klebsiella* infections are often associated with health-care settings and can cause severe respiratory and urinary tract infections. The higher frequency of *Klebsiella* isolates suggests the need for effective infection control measures and targeted treatment strategies in diabetic patients.

The most prevalent microbial isolate identified in this study was methicillin-resistant *Staphylococcus*, which accounted for 72% of the cases. This finding is of particular concern as methicillin-resistant *Staphylococcus*, commonly referred to as MRSA, is notorious for its resistance to multiple antibiotics. MRSA infections can be challenging to treat, and their presence in diabetic patients underscores the need for appropriate antibiotic stewardship and infection prevention protocols to mitigate the spread of these resistant strains.

Pseudomonas aeruginosa, a Gram-negative bacterium, was isolated in 2% of the cases. *Pseudomonas aeruginosa* is known for its ability to cause severe infections, especially in immunocompromised individuals. Its identification highlights the potential risk of *Pseudomonas*-associated infections in diabetic patients and the importance of early detection and appropriate antibiotic therapy.

It is worth noting that *Staphylococcus aureus* is a common pathogen associated with various infections, including skin and soft tissue infections, and its presence should not be overlooked in diabetic patients. The data presented in Table 2 emphasize the diverse microbial profile observed in diabetic patients, with a notable prevalence of methicillin-resistant *Staphylococcus*. This highlights the importance of implementing robust infection control measures, judicious use of antibiotics, and targeted treatment strategies tailored to the specific microbial isolates and their antibiotic susceptibility patterns. Continuous surveillance of microbial isolates in diabetic patients is crucial for understanding the local epidemiology, guiding treatment decisions, and implementing preventive measures to improve patient outcomes.

4.2. Antibiotic Susceptibility Patterns:

4.2.1. Gram positive cocci:

The data presented in Table 3 provides information on the antibiotic susceptibility patterns of the isolated microbial strains, specifically *Enterococcus faecalis*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Staphylococcus aureus*. These patterns are crucial in guiding appropriate

antibiotic selection for the treatment of infections in diabetic patients.

For *Enterococcus faecalis*, all isolates (100%) were sensitive to vancomycin, ampicillin, and linezolid. However, none of the isolates showed sensitivity to gentamicin or chloramphenicol. This suggests that vancomycin, ampicillin, and linezolid are viable treatment options for *Enterococcus faecalis* infections in diabetic patients, while alternative antibiotics should be considered in cases where gentamicin or chloramphenicol may be ineffective.

Regarding MRSA, all isolates (100%) were sensitive to vancomycin, indicating its effectiveness as a treatment option. However, there was a significant decrease in sensitivity to other antibiotics, such as ampicillin (94.44% sensitivity), gentamicin (80% sensitivity), and linezolid (75% sensitivity). This highlights the importance of appropriate antibiotic selection to ensure effective treatment of MRSA infections in diabetic patients, with vancomycin being a reliable choice.

For *Staphylococcus aureus*, all isolates (100%) were sensitive to vancomycin, cloxacillin, and levofloxacin. However, there was lower sensitivity to amoxicillin (25% sensitivity), quinupristin/dalfopristin (25% sensitivity), and sulfamethoxazole (75% sensitivity). This implies that vancomycin, cloxacillin, and levofloxacin are effective treatment options for *Staphylococcus aureus* infections in diabetic patients, while caution should be exercised when considering amoxicillin, quinupristin/dalfopristin, and sulfamethoxazole.

It is important to note that these results reflect the antibiotic susceptibility patterns observed in the specific isolates from the studied diabetic patients. The findings highlight the need for tailored antibiotic therapy based on the specific microbial isolates and their susceptibility patterns. It is crucial to monitor antibiotic resistance trends and regularly update treatment guidelines to ensure optimal management of infections in diabetic patients.

The data in **Table 3** indicate that vancomycin shows consistent effectiveness against *Enterococcus faecalis*, MRSA, and *Staphylococcus aureus*. However, there are variations in sensitivity to

other antibiotics, emphasizing the importance of considering the specific susceptibility patterns when selecting appropriate antibiotics for the treatment of microbial infections in diabetic patients.

4.3. Gram negative bacilli:

The data presented for Gram-negative bacilli, specifically *Klebsiella* spp. and *Pseudomonas aeruginosa*, in **Table 3** provides insights into their antibiotic susceptibility patterns. These patterns are crucial in guiding appropriate antibiotic selection for the treatment of infections caused by these organisms.

For *Klebsiella* spp., the majority of isolates (69.23%) were sensitive to ampicillin, while a smaller proportion showed sensitivity to gentamicin (23.08%), chloramphenicol (7.69%), and cephalosporin (100%). No sensitivity was observed for vancomycin. This suggests that cephalosporin may be an effective choice for treating *Klebsiella* spp. infections in diabetic patients, while alternative antibiotics should be considered for cases where ampicillin, gentamicin, or chloramphenicol may not be effective.

In the case of *Pseudomonas aeruginosa*, all isolates (100%) were sensitive to vancomycin, amikacin, penicillin, and imipenem. No sensitivity was observed for gentamicin, amoxicillin, or levofloxacin. This indicates that vancomycin, amikacin, penicillin, and imipenem are potential treatment options for *Pseudomonas aeruginosa* infections in diabetic patients, while alternative antibiotics should be considered for cases where gentamicin, amoxicillin, or levofloxacin may not be effective.

These findings highlight the importance of selecting appropriate antibiotics based on the specific susceptibility patterns of Gram-negative bacilli. It is crucial to consider the susceptibility of each organism to ensure effective treatment and to prevent the development of antibiotic resistance. Regular monitoring of antibiotic resistance patterns and adherence to appropriate antibiotic stewardship practices are essential in managing infections caused by Gram-negative bacilli in diabetic patients.

In summary, the data in Table 3 indicate that cephalosporin shows acceptable sensitivity against *Klebsiella* spp., while vancomycin, amikacin, penicillin, and imipenem are effective options for treating *Pseudomonas aeruginosa* infections. However, caution should be exercised when considering other antibiotics, as their effectiveness may vary. Proper antibiotic selection based on the specific susceptibility patterns of Gram-negative bacilli is crucial for successful treatment outcomes in diabetic patients.

4.4. Interpretation of Findings:

The results of the microbial isolates and their antibiotic susceptibility patterns in diabetic patients have important implications in the context of existing literature. The findings provide insights into the types of microbial infections commonly observed in diabetic patients and shed light on the effectiveness of various antibiotics for treating these infections.

One notable finding is the high frequency of Methicillin Resistant *Staphylococcus aureus* (MRSA) isolates, accounting for 72% of the identified microbial isolates. This aligns with previous studies that have highlighted the increased risk of MRSA infections in diabetic patients (Stacey et al., 2019). MRSA is known for its resistance to multiple antibiotics, making treatment challenging (Turner et al., 2019). The finding of high sensitivity to vancomycin, a commonly used antibiotic for MRSA infections, reaffirms its importance as an effective treatment option in diabetic patients (Talaie et al., 2019).

The presence of *Enterococcus faecalis* as a significant isolate is consistent with previous literature, which has identified *Enterococcus* species as common pathogens in diabetic individuals (Suryaletha et al., 2018). The high sensitivity of *Enterococcus faecalis* isolates to vancomycin and ampicillin is in line with established treatment guidelines (Talaie et al., 2019). This emphasizes the importance of appropriate antibiotic selection based on the specific susceptibility patterns of the isolated organisms to ensure successful treatment outcomes.

Regarding Gram-negative bacilli, the identification of *Klebsiella* spp. and *Pseudomonas aeruginosa* highlights their clinical relevance in diabetic patients. These organisms are known for their intrinsic resistance and ability to acquire antibiotic resistance mechanisms (Omer et al., 2020). The observed susceptibility patterns indicate that cephalosporin and imipenem are potential treatment options for *Klebsiella* spp. and *Pseudomonas aeruginosa* infections, respectively. However, the reduced sensitivity to other commonly used antibiotics, such as ampicillin, gentamicin, and levofloxacin, emphasizes the need for careful antibiotic selection and consideration of alternative agents in diabetic patients.

The identified antibiotic susceptibility patterns align with the principles of antibiotic stewardship, which emphasize the importance of appropriate antibiotic use to prevent the development of resistance (Giacomini et al., 2021). In fact, the widespread inappropriate use of antibiotics emerged as one of the main causes of antibiotic resistance (Giacomini et al., 2021). These findings highlight the need for ongoing surveillance of antibiotic resistance in diabetic patients and the importance of tailoring treatment based on the specific susceptibility patterns of microbial isolates.

5. Limitations of the study:

It is worth noting that the study's retrospective nature and the limited sample size may influence the generalizability of the findings. Future research with larger and more diverse patient populations would provide a more comprehensive understanding of microbial infections and their antibiotic susceptibility patterns in diabetic patients.

Overall, the results of this study contribute to the existing literature by providing valuable insights into the prevalence of microbial infections and the antibiotic susceptibility patterns in diabetic patients. These findings have implications for clinical practice, emphasizing the importance of individualized treatment approaches and regular monitoring of antibiotic resistance in diabetic individuals to optimize patient outcomes

and minimize the development of antibiotic resistance.

6. Future studies:

Future research should focus on larger-scale studies, investigating the mechanisms of antibiotic resistance, monitoring changes in microbial prevalence over time, and assessing the impact of microbial infections on clinical outcomes. Overall, this study provides valuable insights into microbial infections and antibiotic susceptibility patterns in diabetic patients, informing optimal treatment strategies and antibiotic stewardship practices.

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9. Conflict of Interest:

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