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Research Article

Isolation and Characterization of Bacteria Isolates Associated with Diabetic Foot Infection among Patients Attending General Hospital, Wushishi, Niger State

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ABSTRACT
Diabetic foot wounds are frequently polymicrobial and increasingly drug-resistant. Chronic diabetic wounds are colonized by biofilm producing microorganism, which could lead to soft tissue infection, bone infection, and subsequently lower limb amputation. This study aims to characterize and determine the antimicrobial susceptibility patterns of bacteria isolated from diabetic foot wounds of patients attending General Hospital, Wushishi, Niger State. Fifteen adult diabetic patients with foot wounds were selected. Wound swab samples were cultured on blood agar, MacConkey agar, and chocolate agar (aerobic with 5–10% CO). Isolates were identified using standard biochemical tests. This was followed by susceptibility testing using the disk diffusion method. Seventeen bacterial isolates were identified, including *Staphylococcus aureus* (6; 35.3%), *Escherichia coli* (5; 29.4%), *Pseudomonas aeruginosa* (4; 23.5%), and *Klebsiella spp*. (2; 11.8%). High resistance was observed for amoxicillin and amoxicillin–clavulanate, while high susceptibility was observed for fluoroquinolones and aminoglycosides for Gram-negatives. The study revealed that diabetic foot wounds at the facility studied were commonly infected with *S. aureus* and Enterobacter ales, with resistance observed to β-lactams antibiotics. There is a need for controlled empirical treatment therapy for antibiotic-resistant bacterial infections in diabetic wound patients.

Keywords: Antibiotic resistance; Diabetic wounds; Gangrene; Infection; Wushishi

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INTRODUCTION

Diabetes mellitus (DM) is a serious public health problem worldwide. The prevalence of DM has been estimated to be 589 million adults and expected to rise to 853 million by 2050 (IDF, 2025). More than 4 million adults died from direct and associated complications of diabetes in 2019. As a consequence of hyperglycemia, diabetic patients are at increased risk for comorbid conditions affecting several organs (Maffi & Secchi, 2017). One of the main consequences of diabetes is the impairment of self-repairing abilities of tissues (Dinh *et al*., 2011). Diabetic patients develop wounds characterized by impaired healing, prolonged inflammation, reduced epithelization kinetics and chronic infection. Hyperglycemia contributes to a variety of complications, leading to pathologies manifesting within the wound microenvironment, including dysregulated inflammation and angiogenesis, oxidative stress, neuropathy, advanced glycation end-products, impaired neuropeptide signaling and infection (Baltzis *et al*., 2014; Al-Rawaf *et al*., 2019).

Various microorganisms colonize the diabetic wound and in some patients one or more species of organisms proliferate in the wound, which may lead to tissue damage, host response accompanied by

inflammation, that is, clinical infection (Baron *et al.,* 2021). Diabetic foot infections are mixed bacterial infection which may include Gram positive organisms such as *Staphylococcus aureus* and *Staphylococcus epidermidis*, while the Gram-negative organisms such as *Pseudomonas aeruginosa*, *Escherichia coli, Proteus* species and *Klebsiella* species are rare (Macdonald *et al.,* 2021).

These wounds are highly susceptible to bacterial infections due to the compromised immune response associated with diabetes, delayed healing, and poor blood circulation. The presence of bacterial infections not only impedes wound healing but also increases the risk of more severe complications such as sepsis, gangrene, and the need for limb amputation (Falcone *et al*., 2021). Despite advancements in medical care, the identification and treatment of bacterial infections in diabetic wounds remain a significant challenge. This is largely due to the polymicrobial nature of these infections, where multiple bacterial species, including resistant strains, are involved. Moreover, the growing prevalence of antibiotic-resistant bacteria has further complicated the management of these infections, limiting the effectiveness of conventional treatments (Atlaw *et al*., 2022). The isolation and characterization of bacteria associated with diabetic wounds are crucial for effective treatment strategies. The aim of this research is to isolate and characterize bacteria associated with diabetic wound among patient attending General Hospital Wusushi.

MATERIALS AND METHODS

Study Design and Sample Collection

All patients (≥18 years old) with diabetes and clinically infected wound hospitalized at General hospitals Wushishi, Niger State, between February and March, 2025 and consented were enrolled. Excluding patients on antibiotic within 72 hours of the collection of samples without recent debridement and residual infection. A total of 15 Patients consent was also gotten in written before samples were collected. The wound area was swabbed gently using a sterile cotton swab and the samples were transported to the laboratory within 2 hours of sample collection.

Bactria culture and identification

The collected specimen was streaked independently on the surface of MacConkey agar, Chocolate agar, and Blood agar. The MacConkey agar and Blood agar plates were incubated aerobically while the Chocolate agar plates were incubated anaerobically (5 - 10% CO_2) for 24 hrs at 37°C. Bacteria isolates were identified based on colony morphological characterization on culture media and other typical growth characteristics on non-selective, selective, and differential culture media and complemented with gram staining as well as biochemical tests to confirm their identity/purity (Bauer *et al*., 1966).

Gram staining

This method of differential staining is beneficial for bacteriology. It classifies microorganisms into the two clearly identifiable Gram-positive and Gram-negative groups. Briefly, on a dry, transparent glass slide, the swab was applied. Dried by air and fixing with low heat. After that, the area was flooded for 1 min with Gram's Crystal Violet, rinsed with distilled water, and then flooded for 1 min with Gram's iodine. Then, acetone was poured (decolorization step). The sample was stained with Safranine after being rinsed for 1 min. The stains were washed with tap water, *then* dried before being viewed using an oil immersion objective (Amsel *et al.*, 1983).

Antimicrobial Susceptibility Testing

All identified pure bacterial isolates were subjected to invitro antibiotic disc susceptibility testing as previously described by Bauer et al., 1966 and modified by Maglorakos et al., 2012. Isolates were tested against Streplomycine, Oflocine, Ciprofloxacin, Vancomycin, Amoxacine, Augumentin. The bacteria suspension was swabbed evenly over the entire surface of Muller Hinton Agar (MHA) plate using a sterile swab stick. The discs containing the antibiotic were placed on inoculated plate no closer than 15 mm from the edge and 24 mm from center of discs and incubated at 37^{O}C for 24 hours. Diameter of the zone of inhibition around the disc was measured and the isolate was classified as sensitive, intermediate, and resistant according to CLSI 2019.

RESULTS

Bacterial Isolate from Patient and Their Biochemical Test

Out of the 15 wound swab samples collected, 9 yielded bacterial growth while 6 showed no growth. The predominant bacterial isolates were *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella* spp. Mixed

infections were observed in two cases: *P. aeruginosa* with *E. coli*, and *E. coli* with *S. aureus*.

DISCUSSION

Bacterial wound contamination is a serious problem in the hospital and the treatment of wound infections remains a significant concern for surgeons. The risk of developing wound infection depends on the number of bacteria colonies on the wound. The problem has been magnified due to the unrestrained and rapidly spreading resistance to the available array of antimicrobial agents (Farrag *et al.,* 2016).

The current result revealed that all the wound swabs were positive for bacterial growth while. Few of the swabs gave multiple organism similar to result reported by Atlaw *et al*. (2022) who observed that 100% of diabetic wound specimens were showed bacterial growth. The infection in diabetic wounds is due to hyperglycemia which weaken the immune system and allow bacteria to thrive (Rodríguez-Rodríguez *et al*., 2021). Most of the isolated organism are known biofilm producer which hinders penetration of tropical antimicrobial agents contributing to chronic inflammation, promoting further tissue damage and delayed angiogenesis (Huang *et al*., 2025).

The present work revealed that the age group of 40-70 years had the higher rate of wound infection while the lowest rate was observed among the age group less than 30-40 years. A similar study by Mama *et al*. (2014) observed that the age group of 45-59 years has the highest rate of wound infection. Age increases the risk of infection due to age related immune system decline and physiological changes such as thinner skin allowing easier infection (Alghalibi *et al*., 2011; Nakajo & Nishiura, 2023)

The incidence of wound infection in this study was more common in males than in females. This is in agreement with studies done in Sana'a (Alghalibi *et al*., 2011) and Ethiopia (Mama *et al*., 2014).

The most bacteria isolated in this study from wound infection were *Staph. aureus* (followed by *P. aeruginosa*, *E. coli Klebsiella spp* in a similar study by Alghalibi *et al*. (2011) recorded that the *S. aureus* was most frequently bacteria isolated (47.8%), followed by *P. aeruginosa* (23%), *E. coli* (5.3%), and *Klebsiella* sp, (0.96% for each). The *E. coli* bacterium normally lives in the human's colon and often causes infections of wounds contaminated with urine. Most the contaminated wounds with hospital-acquired infections such as bacteria are known due to poor hospital hygiene (Samuel *et al.*, 2010).

The findings obtained revealed that the isolated bacteria varied in their susceptibility to all the antibacterial used. It was found that the *S. aureus* showed high sensitivity to imipenem and erythromycin and was resistant to ceftriaxone. A study by Yakha *et al*. (2014) showed the isolated *S. aureus* of wound sensitive to erythromycin at 66.4% and resistant to penicillin and amoxicillin. Also, Mama *et al*. (2014) revealed that *S. aureus* was highly sensitive to amikacin, vancomycin, gentamicin, and ciprofloxacin.

CONCLUSION

This study highlights the wide variety of pathogenic bacteria and emerging antibiotic resistance among bacterial isolates from diabetic foot wounds in General Hospital, Wushishi, Niger State. The predominance of *Staphylococcus aureus* and Gram-negative Enterobacterales, coupled with high resistance to β-lactam antibiotics, highlights the importance of conducting routine microbial sensitivity testing before initiating therapy. Empirical treatment protocols should be guided by local antimicrobial susceptibility patterns, with emphasis on responsible use of antibiotics to prevent resistance development. Enhancing infection prevention and control measures are essential to improve clinical outcomes and reduce the risk of chronic infection and lower limb amputation.

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Table 1: Patient and Bacterial Isolate Characteristics

S/N	Hospital No	Sex	Age	Isolation	Motility	Gram stain	Catalase	Coagulase	Vogar P.	Indole	CITRATE
1	001	F	50yrs	*Pseudomonas aeruginosa*	Motile	Gram Negative bacilli	+ve	-	+ve	-	-
				Escherichia coli	Motile	Gram Negative bacilli	-	-	-	+ve	-
2	002	F	30yrs	*Pseudomonas aeruginosa*	Motile	Gram Negaive bacilli	+ve	-ve	+ve	+ve	-
3	003	M	65yrs	*Escherichia coli*	Motile	Gram Negative bacilli	-	-	-	+ve	-
4	004	F	33yrs	*Staphylococcus aureus*	Smooth	Gram Positive cocci	+ve	+ve	-	+ve	-
5	005	M	60yrs	*Escherichia coli*	Smooth	Gram Negative bacilli	+ve	-	-	+ve	-
				Staphylococcus aureus	Smooth	Gram Positive cocci	+ve	+ve	-	+ve	-
6	006	M	74yrs	*Staphylococcus aureus*	Smooth	Gram Positive cocci	+ve	+ve	-	+ve	-
7	007	F	65yes	*Pseudomonas aeruginosa*	Motile	Gram Negative baccili	+ve	-	+ve	-	-
8	008	M	78yrs	*Staphylococcus aureus*	Smooth	Gram Positive cocci	+ve	+ve	-	+ve	-
9	009	M	38yrs	*Pseudomonas aeruginosa*	Motile	Gram Negative baccili	+ve	-	+ve	-	-
10	010	M	32yrs	*Staphylococcus aureus*	Smooth	Gram Positive cocci	+ve	+ve	-	+ve	-
11	011	F	52yrs	*Klebsella species*	Motile	Gram Negative bacilli	+ve	-ve	-	+ve	+ve
12	012	M	65yrs	*Escherichia coli*	Smooth	Gram Negative bacilli	+ve	-	-	+ve	-
13	012	F	48yrs	*Staphylococcus aureus*	+ve colli	Gram Positive	+ve	-	+ve	-	-
14	014	F	60yrs	*Escherichia coli*	Smooth	Gram Negative bacilli	+ve	-	-	+ve	-
15	015	M	54yrs	*Klebsella species*	Motile	Gram Negative bacilli	+ve	-ve	-	+ve	+ve

Table 2. Bacterial Colony Morphology

S/N	Isolate	Shape	Size	Texture	Elevation	Pigment/color	Fermentation	Gram stain
1	*Pseudomonas aeruginosa*	Irregular	3mm	Soft	Flat	Brownish	NLF	-ve baccili
	Escherichia coli	Circular	2mm	Soft and shining surface	Raised	Polished	LF	-ve baccili
2	*Pseudomonas aeruginosa*	Irregular	3mm	Soft and shining surface	Flat	Brownish	NLF	-ve baccili
3	*Escherichia coli*	Circular	2mm	Soft and shining surface	Raised	Polished	LF	-ve baccili
4	*Staphylococcus aureus*	Round	1mm	Golden, yellow	Raised	Brownish	NLF	+ve cocci
5	*Escherichia coli*	Circular	2mm	Mucous	Raised	Pinkish	LF	-ve baccili
	Staphylococcus aureus	Round	1mm	Golden yellow	Raised	Brownish	NLF	+ve cocci
6	*Staphylococcus aureus*	Round	1mm	Golden, yellow	Raised	Brownish	NLF	+ve cocci
7	*Pseudomonas aeruginosa*	Irregular	3mm	Soft	Flat	Brownish	NLF	-ve baccili
8	*Staphylococcus aureus*	Round	1mm	Golden, yellow	Raised	Brownish	NLF	+ve cocci
9	*Pseudomonas aeruginosa*	Irregular	3mm	Soft	Flat	Brownish	NLF	-ve baccili
10	*Staphylococcus aureus*	Round	1mm	Golden, yellow	Raised	Brownish	NLF	+ve cocci
11	*Pseudomonas aeruginosa*	Circular	2mm	Mucoid	Raised	Pinkish	LF	-ve baccili
12	*Escherichia coli*	Circular	2mm	Soft and shining surface	Raised	Polished	NLF	+ve cocci
13	*Staphylococcus aureus*	Round	1mm	Golden, yellow shining soft	Raised	Brownish	NLF	+ve cocci
14	*Escherichia coli*	Circular	2mm	Mucoid	Raised	Pinkish	LF	-ve baccili
15	*Klebsella species*	Circular	2mm	Mucoid	Raised	Pinkish	LF	-ve baccili

Table 3. Occurrence of bacterial isolated from wound samples

S/N	Organism	Occurrence	Percentage occurrence
1.	*Staphylococcus aureus*	6	35.29
2.	*Escherichia coli*	5	29.41
3.	*Pseudomonas aeruginosa*	4	23.53
4.	*Klebsiella species*	2	11.77
5.	Total	17	100

Table 4. Antibiotic Resistance Profile of Bacteria Isolated from Wound Samples

S/N	Isolate	S	OFX	PEF	CPX	VA	AMX	AUG	CN	LEV
1.	*Pseudomonas aeruginosa*	**+**	**3+**	**2+**	**2+**	**3+**	**3+**	**R**	**1+**	**R**
2.	*E coli*	**R**	**3+**	**R**	**2+**	**3+**	**R**	**R**	**+**	**R**
3.	*Staphylococcus aureus*	**+**	**2+**	**+**	**3+**	**3+**	**R**	**+**	**R**	**3+**
4.	*Pseudomonas aeruginosa*	**R**	**+**	**R**	**2+**	**3+**	**R**	**3+**	**R**	**R**
5.	*Pseudomonas aeruginosa*	**R**	**2+**	**2+**	**3+**	**+**	**R**	**R**	**R**	**R**
6.	*Klebsella species*	**R**	**3+**	**3+**	**+**	**R**	**+**	**R**	**R**	**R**

KEYS= S - streplomycine, OFX - Oflocine, CPX - Ciprofloxacin, VA - Vancomycin, AMX - Amoxacine, AUG - Augumentin, CN - Aentamycin, LEV - Levofloxacin, R - Resistance and + positive (sensitiv