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Determination of Pathogenic Bacterial Infection in the Wounds of Diabetic Patients Admitted in Rehman Medical Institute Hayatabad Peshawar

Shah Faisal
Sarhad Institute of Allied Health Sciences, Faculty of Life Sciences, Sarhad University of Science and Information Technology, Peshawar, Pakistan
Sadia Sardar
Sarhad Institute of Allied Health Sciences, Faculty of Life Sciences, Sarhad University of Science and Information Technology, Peshawar, Pakistan
Iqra Shah Taj
Lecturer Pharmacology, Abasyn University, Peshawar. Email: iqrashahtaj1@gmail.com
Muhammad Moiz Malik
Centre of Biotechnology and Microbiology (COBAM), University of Peshawar (UOP)
Email: moizmalik.735@gmail.com
Musadiq Khan
Academic Coordinator for Distance Education, Sarhad Institute of Allied Health Sciences, Faculty of Life Sciences, Sarhad University of Science and Information Technology, Peshawar, Pakistan.
Email: musadiq.siahs@suit.edu.pk
Umair Himayat
Department of Microbiology, Quaid-e-Azam University, Islamabad.
Email: umairhimayat2001@gmail.com
Dr. Nasir Ali*
Associate Professor, Sarhad Institute of Allied Health Sciences, Sarhad University of Science and Information Technology, Peshawar, Pakistan. Corresponding Author Email: nasir.biotech@suit.edu.pk

Author Details

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Corresponding E-mails & Authors*:
Dr. Nasir Ali
nasir.biotech@suit.edu.pk

Abstract

Background: Diabetic wound infections (DWI) represent a significant clinical challenge, particularly in Pakistan which harbors one of the world's largest diabetic populations. The microbiological profile of these infections exhibits considerable geographical variation, necessitating region-specific studies to guide appropriate therapeutic interventions. **Objective:** This study aimed to determine the prevalence, microbiological profile, antibiotic susceptibility patterns, and minimum inhibitory/bactericidal concentrations of pathogenic bacteria isolated from diabetic

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wound infections at Rehman Medical Institute, Peshawar. **Methods:** A total of 150 wound samples were collected from diabetic patients admitted to Rehman Medical Institute. Standard microbiological techniques including culture on selective media, Gram staining, and biochemical tests (coagulase, oxidase, indole, urease, catalase, citrate utilization, and triple sugar iron tests) were employed for bacterial identification. Antibiotic susceptibility was evaluated using the Kirby-Bauer disc diffusion method, while minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for ciprofloxacin were determined through broth dilution. Demographic data and risk factors were collected using a structured proforma. **Results:** Among 150 samples, 108 (72%) demonstrated positive bacterial cultures. Males showed higher infection rates (68, 63%) compared to females (40, 37%), with the 41-60 years age group being most affected (49.08%). Gram-positive isolates included *Staphylococcus aureus* (42%), *Enterococcus spp* (24%), *S. epidermidis* (18%), and *Bacillus spp* (16%). Gram-negative isolates comprised *E. coli* (22.35%), *P. aeruginosa* (18.82%), *Salmonella spp* (16.47%), *Enterobacter spp* (14.11%), *Proteus spp* (12.94%), *Klebsiella spp* (9.4%), and *Shigella spp* (5.90%). High resistance was observed against Ampicillin (60%) and Amoxicillin (59%), while Imipenem demonstrated 70% sensitivity across all isolates. The *Enterobacter spp* exhibited the highest MIC and MBC values, whereas *E. coli* and *S. aureus* showed lower values. Hypertension (64%) and retinopathy (55%) emerged as significant associated risk factors. **Conclusion:** The predominance of *S. aureus* and *E. coli* in diabetic wounds, coupled with alarming resistance to commonly used antibiotics, underscores the critical need for culture-guided antimicrobial therapy. Imipenem's consistent efficacy suggests its potential as a reliable therapeutic option in this region.

Keywords: Diabetic wound infection, *Staphylococcus aureus*, antibiotic resistance, minimum inhibitory concentration, risk factors, Pakistan

INTRODUCTION

Diabetes mellitus stands as one of the most pressing global health challenges, affecting over 425 million individuals worldwide according to the World Diabetes Organization (2017), with projections indicating a rise to 629 million by 2045 (Saeedi et al., 2019). Pakistan bears a particularly heavy burden, with approximately 6.94% of its population currently living with diabetes—a figure expected to climb to 8.45% by 2045 (Meo et al.,

2016). Beyond the primary metabolic disturbances, diabetes manifests through numerous complications including nephropathy, neuropathy, retinopathy, and notably, foot ulcers and wound infections that significantly impact patient quality of life and healthcare systems (Armstrong et al., 2017).

Diabetic wound infections (DWI) represent one of the most common and devastating complications among diabetic individuals across all backgrounds and age groups (Kiadaliri et al., 2019). Global estimates suggest that 10 to 15 percent of diabetic patients develop diabetic wounds during their lifetime (Akkus & Sert, 2021), with foot ulcers specifically affecting between 6 and 11 percent of this population (Zubair et al., 2021; Yazdanapanah et al., 2018). In Khyber Pakhtunkhwa province alone, reports indicate that 14 to 21 percent of diabetic individuals develop foot ulcers (Rahim et al., 2016), reflecting a significantly higher rate compared to non-diabetic populations (Gregg et al., 2016). The consequences can be severe—approximately 21.5% of patients with diabetic wound infections ultimately require amputation of lower body parts during their treatment course (Navarro-Flores et al., 2022). Without appropriate management, these infections frequently progress to chronic ulcers, often necessitating limb loss (Haji Zaine et al., 2019), with amputation risk influenced by factors such as gender, age, and wound duration (Deribe et al., 2018).

The microbiological landscape of diabetic wounds is complex and dynamic. *Staphylococcus aureus* dominates the bacterial profile, detected in approximately 76% of diabetic sores and foot ulcers (Li et al., 2022). This organism stands as the most frequent cause of invasive hospital-acquired infections affecting soft tissues and skin in the United States (Huitema et al., 2021). The emergence of antibiotic-resistant strains has further complicated treatment paradigms. Methicillin-resistant *S. aureus* (MRSA) infections have increased dramatically over the past four decades in hospitals worldwide (Zhou et al., 2021), with Pakistan documenting its first case in 1989 and reporting 68.1% prevalence by 2013 (Rafiq et al., 2015). The situation escalated further with the first isolation of vancomycin-resistant *S. aureus* (VRSA) from a diabetic foot ulcer patient in 2002 (Saltoglu et al., 2018). These developments underscore the urgent need for novel therapeutic approaches and regional surveillance of resistance patterns (Kifelew et al., 2020).

Diabetic wound infections may present as either mono-microbial or poly-microbial. Commonly implicated pathogens include *Pseudomonas aeruginosa*, *Escherichia coli*, Enterobacter species, and *Staphylococcus aureus* (Tiwari et al., 2012). The underlying pathophysiology involves hyperglycemia-induced vulnerability of mucosal epithelial cells to infection and significant immune system dysregulation (Nelson et al., 2016; Roncon et al., 2020). High blood glucose levels impair white blood cell migration to infection sites, reduce their ability to remain in affected areas, and compromise their bactericidal capacity (Qiao et al., 2020). Furthermore, vascular complications reduce blood flow to infection-prone regions, compounding the body's ability to combat infections and heal wounds (Loesche et al., 2017).

Geographical variation in microbiological profiles adds another layer of complexity to DWI management. While Gram-positive aerobic cocci predominate in Western countries, Gram-negative bacilli feature more prominently in warmer regions, particularly across Asia and Africa (Al-Asoufi et al., 2017). This epidemiological diversity necessitates region-specific investigations to guide empirical therapy and inform local antibiotic policies.

The emergence of multi-drug resistant organisms (MDROs), including extended-spectrum beta-lactamase producers and MRSA, has significantly altered the global therapeutic landscape over the past two decades (Willis et al., 2021). These organisms frequently lead to treatment failure and increased mortality, presenting substantial challenges for clinicians managing diabetic foot infections (Tacconelli, 2009). The indiscriminate use of antibiotics remains a primary driver of resistance (Mancuso et al., 2021), highlighting the critical importance of regular microbiological surveillance and susceptibility testing to guide appropriate antimicrobial selection (Liccardo et al., 2019).

In Pakistan, the easy availability of antibiotics without prescription has exacerbated resistance problems, with diabetic foot infection patients representing a particularly vulnerable group. Inadequate antimicrobial treatment in these patients correlates strongly with higher amputation rates (Deribe et al., 2018). Despite this urgent need, comprehensive data on the prevalence and characteristics of diabetic wound infections in Pakistan remain limited (Patel et al., 2021). This knowledge gap hampers the

development of effective infection control practices and evidence-based treatment guidelines.

The present study was therefore designed to address this gap by investigating the pathogenic bacterial profile, antibiotic susceptibility patterns, and minimum inhibitory concentrations of key antibiotics against isolates from diabetic wound infections at Rehman Medical Institute, Peshawar. Additionally, we aimed to identify associated risk factors contributing to wound infections in this patient population.

MATERIALS AND METHODS

Study Setting and Design

This cross-sectional study was conducted at the Microbiology Laboratory, Department of Pathology, Rehman Medical Institute (RMI) Hayatabad, Peshawar, over a period of six months. The study protocol received ethical approval from the institutional review committee, and informed consent was obtained from all participants prior to sample collection.

Sample Size Calculation

Sample size was determined using the formula:

$N = 4PQ/d^2$

Where:

- P = prevalence (60%, based on Degloorkar et al., 2021)
- Q = 100-P = 40
- D = 20% of prevalence = 8

$N = 4 \times 60 \times 40 / 8 \times 8 = 150$

Sample Collection

A total of 150 wound samples were collected from diabetic patients admitted to RMI. The study population comprised 97 males (64.66%) and 53 females (35.34%), with ages ranging from 10 to 80 years. Sterile swabs moistened with normal saline were used for specimen collection following standard aseptic techniques. Collected samples were placed in sterile containers and transported immediately to the microbiology laboratory, where processing was initiated within one hour of collection.

Inclusion and Exclusion Criteria

Inclusion criteria: Patients with diagnosed diabetes mellitus presenting with wound infections during their hospital stay at RMI.

Exclusion criteria: Non-diabetic individuals, outdoor patients, and those who declined to provide informed consent were excluded from the study.

Culture and Isolation

Samples were inoculated onto Blood agar (for both Gram-positive and Gram-negative bacteria) and MacConkey agar (selective for Gram-negative bacteria) plates using standard streaking techniques. Inoculated plates were incubated aerobically at 37°C for 24 hours. Following incubation, plates were examined for bacterial growth, and distinct colonies were selected for further processing. Pure cultures were obtained through subculture on fresh media when necessary.

Gram Staining

A single colony from 24-hour-old culture was emulsified in a drop of normal saline on a clean glass slide, heat-fixed, and subjected to Gram staining. Smears were sequentially treated with crystal violet (1 minute), Gram's iodine (1 minute), rapid decolorization with acetone-alcohol, and counterstaining with safranin (30 seconds). After air drying, slides were examined under oil immersion microscopy (100×) for bacterial morphology and Gram reaction.

Biochemical Identification

Bacterial isolates were identified to genus level using a battery of standard biochemical tests following Clinical and Laboratory Standards Institute (CLSI, 2021) guidelines:

Coagulase Test: A drop of normal saline was placed on a clean slide, and several bacterial colonies from 24-hour culture were emulsified to create a smooth suspension. A drop of human plasma was added and mixed gently. Clumping observed within 10 seconds indicated a coagulase-positive result.

Oxidase Test: A small piece of filter paper was saturated with Kovac's oxidase reagent. The test organism was smeared onto the soaked paper using a wooden stick. Immediate development of blue color indicated oxidase positivity.

Indole Test: Test bacteria were inoculated into tryptone water broth and incubated at 37°C for 48 hours. Following incubation, 0.5 ml of Kovac's reagent was added and gently shaken. Formation of a red ring on the broth surface indicated indole production.

Urease Test: Christensen's urea broth was inoculated with test bacteria and incubated at 37°C for 12 hours. Development of pink coloration indicated urease activity.

Catalase Test: A drop of 3% hydrogen peroxide was placed on a clean glass slide. A single isolated colony was transferred to the drop using a sterile loop. Immediate bubble formation indicated catalase positivity.

Citrate Utilization Test: Simmon's citrate agar slants were inoculated with test bacteria using a sterile wire loop and incubated at 37°C for 24 hours. Color change from green to blue indicated citrate utilization.

Triple Sugar Iron (TSI) Test: TSI agar was prepared by suspending 35 g of powder in 500 ml distilled water, autoclaving at 121°C for 45 minutes, and dispensing into test tubes at a slanted angle. A single colony was inoculated by stabbing the butt and streaking the slant surface. After 24-hour incubation at 37°C, results were interpreted based on: acid slant/acid butt (A/A) with or without gas and H_2S indicating glucose, lactose, or sucrose fermentation; alkaline slant/alkaline butt (K/K) indicating no fermentation; and acid butt/alkaline slant (K/A) indicating glucose fermentation only. Blackening of medium indicated H_2S production, while cracks or bubbles indicated gas production.

Antibiotic Susceptibility Testing

Disc Diffusion Method: Antibiotic susceptibility was evaluated using the Kirby-Bauer disc diffusion technique following CLSI (2021) recommendations. The following antibiotic discs (concentrations in µg) were tested: Ampicillin (10), Ciprofloxacin (5), Amoxicillin (30), Imipenem (10), Gentamycin (10), Tobramycin (10), Tigecycline (15), Cefotaxime (30), and Cefuroxime (30). Bacterial suspensions adjusted to 0.5 McFarland standard were spread evenly on Mueller-Hinton agar plates using sterile swabs. Antibiotic discs were placed on the inoculated surface using sterile forceps and gently pressed to ensure complete contact. Plates were incubated at 37°C for 18 hours, after which inhibition zone diameters were measured to the nearest millimeter and interpreted according to CLSI breakpoints.

Minimum Inhibitory Concentration (MIC) Determination

MIC for ciprofloxacin was determined using the broth dilution method. Ciprofloxacin stock solution was prepared using the formula:

$$W = (1000/P) \times V \times C$$

Where:

- W = Weight of antibiotic (mg) to be dissolved in volume V
- V = Volume required (mL)
- C = Concentration of final solution (mg/L)
- P = Antibiotic powder strength (g/mg)

Serial two-fold dilutions of ciprofloxacin were prepared in sterile nutrient broth to achieve concentrations of 2, 4, 8, 12, and 16 µg/ml. Bacterial suspensions (0.5 McFarland standard) were prepared by touching 4-5 colonies from 24-hour culture with a sterile loop and inoculating into nutrient broth, followed by 6-hour incubation at 35°C. One milliliter of bacterial culture was added to each dilution tube. Tubes were sealed and incubated at 37°C for 24 hours. MIC was recorded as the lowest antibiotic concentration completely inhibiting visible bacterial growth, according to CLSI (2021) criteria.

Minimum Bactericidal Concentration (MBC) Determination

Following MIC determination, samples from tubes showing no visible growth were subcultured on nutrient agar plates and incubated at 37°C for 24 hours. MBC was recorded as the lowest antibiotic concentration that resulted in no bacterial growth on subculture, indicating complete bacterial killing (CLSI, 2021).

Demographic Data Collection

A structured proforma was used to collect demographic and clinical information from study participants, including:

- Age and gender
- Duration of diabetic wound infection (1 year, 2 years, >2 years)
- Family history of diabetes
- Duration of hospital stay (half month, 1 month, >1 month)
- Duration of wound development (6 months, 1 year, >1 year)
- Co-morbidities (hypertension, obesity, retinopathy, neuropathy, nephropathy)

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Data Analysis

Data were analyzed using descriptive statistics. Results were expressed as frequencies, percentages, and distributions across various demographic and clinical parameters.

RESULTS

Overall Culture Positivity

Among the 150 wound samples processed from diabetic patients, 108 (72%) demonstrated positive bacterial growth on culture media, while 42 samples (28%) showed no growth (Table 1).

Table 1: Percentage of Diabetic Wounds Positive and Negative Samples

Category	Number of Isolates	Percent
Positive samples	108	72%
Negative samples	42	28%
Total samples	**150**	**100%**

Age Distribution of Diabetic Wound Infections

Analysis of age distribution revealed that the majority of diabetic wound infections occurred in middle-aged and elderly patients. The 41-60 years age group accounted for nearly half of all infections (53 patients, 49.08%), followed by the 61-80 years age group (36 patients, 33.33%). The youngest affected group (21-40 years) comprised 19 patients (17.59%) (Table 2).

Table 2: Distribution of Diabetic Wound Infections Across Different Age Groups

Age Limits	Number of DWI Patients	Percentage of DWI Patients
21-40 years	19	17.59%
41-60 years	53	49.08%
61-80 years	36	33.33%

Age Limits	Number of DWI Patients	Percentage of DWI Patients
Total	108	100%

Gender Distribution

Male diabetic patients demonstrated a higher predisposition to wound infections compared to females. Among the 108 positive cases, 68 (63%) were males, while 40 (37%) were females (Table 3).

Table 3: Gender-Wise Distribution of Diabetic Wound Infections

Gender	Number of DWI Patients	Percentage of DWI Patients
Male	68	63%
Female	40	37%
Total	**108**	**100%**

Risk Factors Associated with Diabetic Wound Infections

Multiple factors were identified that influenced the development and severity of wound infections in diabetic patients. Patients with a positive family history of diabetes showed higher susceptibility (57%) compared to those without family history (43%). The duration of diabetes emerged as a critical factor, with patients having diabetes for more than two years showing the highest infection rates (63%), followed by those with two years duration (23%) and one year duration (14%).

Hospital stay duration also correlated with infection risk—patients hospitalized for one month demonstrated the highest infection rate (56%), followed by those staying half a month (29%) and more than one month (15%). Regarding wound duration, infections were most common in patients with wounds present for six months (47%), followed by one year (36%) and more than one year (17%).

Among co-morbidities, hypertension was the most frequently associated condition (64%), followed by retinopathy (55%), obesity (42%), neuropathy (27%), and nephropathy (13%) (Table 4).

Table 4: Factors Affecting Wound Infection in Diabetic Patients

S. No	Factors Affecting Diabetic Wound Infections	Questionnaire Response	Percentage
1	Family history of diabetes	Yes	57%
		No	43%
2	Duration of diabetes	One year	14%
		Two years	23%
		More than 2 years	63%
3	Duration of hospital stay	Half month	29%
		One month	56%
		More than 1 month	15%
4	Duration of wound development	Six months	47%
		One year	36%
		More than 1 year	17%
5	Co-morbidities	Hypertension	64%
		Retinopathy	55%
		Obesity	42%
		Neuropathy	27%

S. No	Factors Affecting Diabetic Wound Infections	Questionnaire Response	Percentage
		Nephropathy	13%

Bacterial Profile of Diabetic Wound Infections

A diverse range of pathogenic bacteria were isolated from diabetic wounds, comprising both Gram-positive and Gram-negative organisms. Among Gram-negative bacteria, *Escherichia coli* was the most frequently isolated (19 isolates, 22.35%), followed by *Pseudomonas aeruginosa* (16 isolates, 18.82%), *Salmonella spp* (14 isolates, 16.47%), *Enterobacter spp* (12 isolates, 14.11%), *Proteus spp* (11 isolates, 12.94%), *Klebsiella spp* (8 isolates, 9.41%), and *Shigella spp* (5 isolates, 5.90%). The total Gram-negative isolates numbered 85.

Among Gram-positive bacteria, *Staphylococcus aureus* predominated with 16 isolates (42%), followed by *Enterococcus spp* (9 isolates, 24%), *Staphylococcus epidermidis* (7 isolates, 18%), and *Bacillus spp* (6 isolates, 16%). The total Gram-positive isolates numbered 38 (Table 5).

Table 5: Percentage Distribution of Bacterial Pathogens Identified in Diabetic Wound Infections

S.No	Isolated Bacteria Strains	Number of Isolates	Percent
	Gram Negative Bacteria		
01	*E. coli*	19	22.35%
02	*P. aeruginosa*	16	18.82%
03	*Salmonella spp*	14	16.47%
04	*Enterobacter spp*	12	14.11%
05	*Proteus spp*	11	12.94%

S.No	Isolated Bacteria Strains	Number of Isolates	Percent
06	*Klebsiella spp*	8	9.41%
07	*Shigella spp*	5	5.90%
	Total Gram-negative	**85**	**100%**
	Gram Positive Bacteria		
08	*S. aureus*	16	42%
09	*Enterococcus spp*	9	24%
10	*S. epidermidis*	7	18%
11	*Bacillus spp*	6	16%
	Total Gram-positive	**38**	**100%**

Antibiotic Susceptibility Patterns

Gram-Negative Isolates: The antibiotic susceptibility profiles of Gram-negative isolates against nine tested antibiotics are presented in Table 6. Overall, high resistance rates were observed against Ampicillin (approximately 60%) and Amoxicillin (59%). Imipenem demonstrated the highest efficacy, with 70% sensitivity across all Gram-negative isolates. Gentamycin, Tobramycin, Ciprofloxacin, and Tigecycline showed moderate efficacy with variable resistance patterns.

E. coli isolates exhibited highest resistance to Amoxicillin (53%) and Cefuroxime (47%), while showing good sensitivity to Imipenem (79%), Ciprofloxacin (58%), and Cefotaxime (58%). *P. aeruginosa* demonstrated highest resistance to Amoxicillin (62%) and Ampicillin (62%), with best sensitivity to Cefotaxime (76%), Ciprofloxacin (75%), and Imipenem (74%). *Salmonella spp* showed marked resistance to Amoxicillin (64%) and Ampicillin (57%), with Imipenem (72%) and Cefuroxime (64%) showing good

activity. *Enterobacter spp* displayed high resistance to Cefotaxime (67%) and Ampicillin (58%), while remaining sensitive to Imipenem (76%) and Cefuroxime (76%). *Proteus spp* showed highest resistance to Amoxicillin (73%) and Ampicillin (64%), with Imipenem (73%) and Cefotaxime (64%) demonstrating good efficacy. *Klebsiella spp* exhibited high resistance to Cefotaxime (75%), Cefuroxime (63%), Amoxicillin (63%), and Ampicillin (63%), while remaining sensitive to Imipenem (75%) and Tigecycline (75%). *Shigella spp* showed variable resistance patterns with Imipenem (60%) and Gentamycin (80%) demonstrating good activity.

Gram-Positive Isolates: Among Gram-positive organisms (Table 7), *S. aureus* demonstrated highest resistance to Amoxicillin (69%) and Ampicillin (63%), while showing good sensitivity to Cefotaxime (76%), Ciprofloxacin (75%), and Imipenem (68%). *Enterococcus spp* exhibited marked resistance to Ampicillin (78%) and Ciprofloxacin (56%), with best sensitivity to Tobramycin (78%), Tigecycline (67%), and Imipenem (56%). *S. epidermidis* showed highest resistance to Amoxicillin (72%), with good sensitivity to most other antibiotics (72% each for Cefuroxime, Ciprofloxacin, Cefotaxime, Gentamycin, Tigecycline, Tobramycin, and Imipenem). *Bacillus spp* displayed variable resistance patterns with highest resistance to Ciprofloxacin (66%), Ampicillin (66%), and Tigecycline (66%), while remaining sensitive to Cefotaxime (66%), Gentamycin (66%), and Tobramycin (66%).

Table 6: Percentage Susceptibility and Resistance of Isolated Gram-Negative Bacteria Against Selected Antibiotics

Gram -ve bacteria	Count	CXM %	AMC %	CIP %	AMP %	CTX %	CN %	TIG %	TOB %	IMP %
		R/I/S	R/I/S	R/I/S	R/I/S	R/I/S	R/I/S	R/I/S	R/I/S	R/I/S
E. coli	19	47/ 21/	53/ 21/	21/ 21/	32/ 05/	26/ 16/	21/ 16/	47/ 16/	42/ 16/	16/0 5/79

Gram -ve bacteria	Count	CXM %	AMC %	CIP %	AMP %	CTX %	CN %	TIG %	TOB %	IMP %
		32	26	58	63	58	63	37	42	
P. aeruginosa	16	37/07/56	62/00/38	18/07/75	62/19/19	12/12/76	24/12/64	25/07/68	44/00/56	19/07/74
Salmonella spp	14	21/15/64	64/00/36	43/00/57	57/07/36	36/00/64	43/07/50	43/00/57	36/00/64	14/14/72
Enterobacter spp	12	16/08/76	58/00/42	42/08/50	58/00/42	67/00/33	42/08/50	42/00/58	33/00/67	16/08/76
Proteus spp	10	27/00/73	73/00/27	54/00/46	64/18/18	27/09/64	27/09/64	36/09/55	45/00/55	27/00/73
Klebsiella spp	8	63/00/37	63/00/37	37/00/63	63/00/37	75/00/25	50/00/50	25/00/75	37/00/63	25/00/75
Shigella spp	5	60/00/40	60/20/20	20/20/60	60/00/40	60/00/40	20/00/80	30/20/50	20/20/60	20/20/60

Key: R = Resistance, S = Sensitivity, I = Intermediate; CXM = Cefuroxime, AMC = Amoxicillin, CIP = Ciprofloxacin, AMP = Ampicillin, CTX = Cefotaxime, CN = Gentamycin, TIG = Tigecycline, TOB = Tobramycin, IMP = Imipenem

Table 7: Percentage Susceptibility and Resistance of Isolated Gram-Positive Bacteria Against Selected Antibiotics

Gram +ve bacteria	Count	CXM %	AMC %	CIP %	AMP %	CTX %	CN %	TIG %	TOB %	IMP %
		R/I/S	R/I/S	R/I/S	R/I/S	R/I/S	R/I/S	R/I/S	R/I/S	R/I/S
S. aureus	16	56/07/37	69/00/31	18/07/75	63/12/25	12/12/76	31/07/62	38/00/62	37/00/63	25/07/68
Enterococcus spp	9	33/11/56	45/22/33	56/00/44	78/11/11	56/00/44	33/11/56	33/00/67	22/00/78	22/22/56
S. epidermidis	7	28/00/72	72/00/28	28/00/72	58/14/28	28/00/72	28/00/72	28/00/72	28/00/72	14/14/72
Bacillus spp	6	33/00/67	33/00/67	66/17/17	66/17/17	17/17/66	17/17/66	66/17/17	17/17/66	17/17/66

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

MIC and MBC determinations for ciprofloxacin against selected bacterial isolates revealed notable variations among different species. The *Enterobacter spp* demonstrated the highest MIC and MBC values, indicating reduced susceptibility to ciprofloxacin. In contrast, *E. coli* and *Staphylococcus aureus* showed lower MIC and MBC values, suggesting greater susceptibility to this antibiotic. Detailed MIC and MBC distributions for Gram-negative and Gram-positive isolates are illustrated in Figure 1.

DISCUSSION

Diabetic wound infections represent a significant clinical challenge with potentially devastating consequences, including gangrene, sepsis, and mortality if not appropriately managed (Wang et al., 2021). The present study provides valuable insights into the microbiological profile, antibiotic susceptibility patterns, and associated risk factors of diabetic wound infections in a tertiary care setting in Peshawar, Pakistan.

Our finding of 72% culture positivity among diabetic wound samples aligns with previous reports from the region. The 28% culture-negative samples may reflect several factors, including prior antibiotic use, presence of fastidious organisms requiring specialized culture conditions, or non-infectious etiologies of wounds. Similar observations were reported by Degloorkar et al. (2021), who documented variable culture positivity rates in diabetic wound infections. The importance of appropriate sampling technique cannot be overstated—our use of sterile swabs moistened with normal saline and immediate processing within one hour follows recommended practices to optimize organism recovery, as emphasized by Slater et al. (2004) in their re-evaluation of swab cultures versus deep tissue cultures.

Age and Gender Distribution

The predominance of diabetic wound infections in the 41-60 years age group (49.08%) observed in our study mirrors findings from Jahad and AL-Yasiri (2021), who reported that most diabetic patients (41.79%) fell within the 51-60 years age bracket, followed by 26.86% in the 41-50 years group. The lower infection rate in younger patients (<40 years) and higher rates in older populations likely reflect the cumulative effects of prolonged diabetes duration, progressive immunological decline, and accumulated vascular complications associated with aging. Zarski et al. (2020) noted that diabetic patients

become increasingly vulnerable to wound infections at older ages due to immune system weakening and the negative impact of chronic hyperglycemia on immune responses.

The higher infection rate among males (63%) compared to females (37%) in our study corroborates findings from multiple previous investigations. Abood et al. (2020) reported that elderly male patients develop infectious diabetic foot wounds more frequently than females. Similarly, Perim et al. (2015) documented a higher percentage of infectious wounds in male diabetic patients. Several hypotheses may explain this gender disparity. Zubair et al. (2012) suggested that higher exposure to outdoor environments and greater propensity for occupational accidents among males might contribute to increased infection risk. Additionally, Moura et al. (2019) proposed biological differences, noting that women demonstrate superior pathogen recognition capabilities, recruit more innate immune cells, and mount stronger adaptive immune responses compared to men.

Risk Factors Associated with Diabetic Wound Infections

Our identification of multiple risk factors contributing to diabetic wound infections aligns with established literature. The strong association with positive family history (57%) highlights the genetic component in diabetes susceptibility and its complications. The progressive increase in infection risk with longer diabetes duration (63% in patients with >2 years diabetes) reflects the cumulative burden of hyperglycemia-related tissue damage and immune dysfunction over time.

The relationship between hospital stay duration and infection risk (highest at one month) likely reflects both the severity of underlying condition requiring prolonged hospitalization and increased exposure to nosocomial pathogens. Similarly, the finding that wounds present for six months showed highest infection rates (47%) suggests that chronicity itself predisposes to microbial colonization and subsequent infection.

Among co-morbidities, hypertension (64%) emerged as the most frequently associated condition, followed by retinopathy (55%), obesity (42%), neuropathy (27%), and nephropathy (13%). These findings are comparable to those reported by Naeem et al. (2019), who documented neuropathy (36.6%), nephropathy (16.6%), obesity (37%), hypertension (66.6%), and retinopathy (50%) in their diabetic patient cohort. Jwad and

AL-Fatlawi (2022) explained that prolonged hyperglycemia damages both large and small blood vessels, causing narrowing that reduces blood flow to various body parts, thereby increasing infection risk. The relatively lower rates of neuropathy and nephropathy in our cohort compared to some studies might reflect differences in diabetes duration, glycemic control, or genetic factors in our population.

Bacterial Profile

The microbiological landscape of diabetic wounds in our study revealed a diverse array of pathogens, with Gram-negative organisms predominating (85 isolates) compared to Gram-positive organisms (38 isolates). Among Gram-negative bacteria, *E. coli* (22.35%) was most prevalent, followed by *P. aeruginosa* (18.82%). Among Gram-positive organisms, *S. aureus* (42%) dominated, followed by *Enterococcus spp* (24%). This pattern aligns with previous studies from the region and reflects the changing epidemiology of diabetic wound infections.

Rajaei et al. (2021) similarly reported *E. coli* as the most frequent Gram-negative isolate (56%) from diabetic infectious wounds, followed by *Enterobacter cloacae* (22%). Laakso et al. (2021) found *S. aureus* to be the most prevalent infectious bacteria (42.45%) in diabetic wound infections, followed by *P. aeruginosa* (17.14%) and *E. coli* (13.06%). Guan et al. (2021) documented *E. coli* (11.5%) and *S. aureus* (29.2%) as predominant isolates from diabetic wounds.

The significant proportion of *P. aeruginosa* (18.82%) in our study is concerning given this organism's intrinsic resistance to many antibiotics and its association with severe, deep-seated infections. The presence of *Enterobacter spp* (14.11%), *Klebsiella spp* (9.41%), and *Proteus spp* (12.94%) reflects the polymicrobial nature of diabetic wounds and the involvement of Enterobacteriaceae family members. The isolation of *Salmonella spp* (16.47%) is noteworthy and may reflect the endemic nature of this pathogen in the region or contamination from environmental sources, though its pathogenic role in diabetic wounds warrants further investigation.

Among Gram-positive isolates, the predominance of *S. aureus* (42%) aligns with global literature identifying this organism as the leading cause of skin and soft tissue infections. The presence of coagulase-negative staphylococci (*S. epidermidis*, 18%)

and *Enterococcus spp* (24%) reflects the involvement of skin commensals and enteric organisms, respectively, in diabetic wound pathogenesis.

Antibiotic Susceptibility Patterns

The high resistance rates observed against Ampicillin (60%) and Amoxicillin (59%) in our study mirror findings from Benwan et al. (2018), who documented resistance rates of 75% and 71.43% against these antibiotics, respectively. This widespread resistance likely reflects the extensive use of these inexpensive, readily available antibiotics in both community and hospital settings in Pakistan, as noted by Ahmad et al. (2022). The easy accessibility of antibiotics without prescription in many parts of Pakistan has undoubtedly contributed to the development and spread of resistance against these first-line agents.

The excellent efficacy of Imipenem (70% sensitivity across all isolates) observed in our study is consistent with findings from Jasmine et al. (2013), who reported 95% sensitivity to Imipenem among their isolates. Naeem et al. (2019) similarly documented Imipenem as the most effective antibiotic against both Gram-positive and Gram-negative organisms. The preserved activity of carbapenems in our setting likely reflects their restricted use due to higher cost and availability primarily in tertiary care settings, limiting selective pressure. However, the emergence of carbapenem resistance in some isolates (16-25% depending on organism) is concerning and may indicate the spread of carbapenemase-producing organisms, including those harboring NDM-1 and MBL genes, which have been increasingly reported from the Indian subcontinent (Suyambu Meenakshi, 2018).

The variable efficacy of cephalosporins (Cefotaxime and Cefuroxime) against different organisms in our study likely reflects the presence of extended-spectrum beta-lactamase (ESBL) production among Enterobacteriaceae isolates. The relatively better activity of aminoglycosides (Gentamycin, Tobramycin) and fluoroquinolones (Ciprofloxacin) against several isolates provides alternative therapeutic options, though resistance rates approaching 40-50% for some organisms limit their empirical use.

The preserved activity of Tigecycline against several Gram-negative isolates, particularly *Klebsiella spp* (75% sensitivity) and *E. coli* (63% sensitivity), offers a potential therapeutic option for multi-drug resistant infections, though its higher cost and

intravenous administration limit widespread use. The good activity of Tobramycin against *Enterococcus spp* (78% sensitivity) and *S. epidermidis* (72% sensitivity) suggests its potential utility in Gram-positive infections as well.

Minimum Inhibitory and Bactericidal Concentrations

Our MIC and MBC determinations for ciprofloxacin revealed important inter-species variations in susceptibility. The finding that *Enterobacter spp* demonstrated the highest MIC and MBC values suggests reduced susceptibility or emerging resistance to fluoroquinolones in this genus. In contrast, the lower MIC values for *E. coli* (4 µg/ml) and *S. aureus* (2 µg/ml) with corresponding MBC values of 8 µg/ml and 4 µg/ml, respectively, indicate better susceptibility. These findings are comparable to those reported by Al-zeiny (2018), who documented MIC values >1 µg/ml and MBC values of 3.125 µg/ml for *E. coli* and *S. aureus*. The higher MIC values observed in our study may reflect the more resistant nature of clinical isolates compared to laboratory strains or geographical variations in resistance patterns.

The clinical significance of MIC and MBC determinations lies in their ability to guide optimal dosing regimens, particularly for organisms with borderline susceptibility. The observed differences between MIC and MBC values (MBC typically 2-4 times MIC) suggest that ciprofloxacin exhibits bactericidal activity against susceptible isolates, though higher concentrations may be required for complete eradication, particularly in biofilm-associated infections common in diabetic wounds.

Clinical Implications

The findings of this study have several important clinical implications. First, the high prevalence of *S. aureus* and *E. coli* in diabetic wounds underscores the need for empirical antibiotic coverage targeting these organisms while awaiting culture results. Second, the alarming resistance rates to commonly used antibiotics like Ampicillin and Amoxicillin indicate that these agents should no longer be used empirically for diabetic wound infections in our setting. Third, the excellent activity of Imipenem suggests it should be reserved for severe infections or those with documented resistance to other agents, to preserve its efficacy. Fourth, the variability in susceptibility patterns among different organisms emphasizes the critical importance of culture and sensitivity testing to guide individualized therapy rather than relying on empirical regimens.

The association of various risk factors with diabetic wound infections highlights the need for comprehensive patient management addressing not only the infection but also underlying conditions. Strict glycemic control, management of hypertension, regular screening for retinopathy and nephropathy, weight management, and patient education about foot care should form integral components of diabetic wound management programs.

Study Limitations

This study has several limitations that should be acknowledged. First, the use of swab samples rather than deep tissue biopsies may have underestimated the presence of anaerobic organisms and biofilm-associated bacteria. Second, molecular identification techniques were not employed, potentially missing fastidious organisms and limiting species-level identification of some genera. Third, detailed characterization of resistance mechanisms (e.g., ESBL production, carbapenemase genes, MRSA confirmation) was not performed. Fourth, the single-center design may limit generalizability to other healthcare settings in Pakistan. Fifth, follow-up data on treatment outcomes and clinical correlation with susceptibility patterns were not collected.

CONCLUSION AND RECOMMENDATIONS

Conclusion

Based on the findings of this study, the following conclusions can be drawn:

1. Diabetic wound infections exhibit both mono-microbial (87%) and poly-microbial (13%) patterns, with the majority being mono-microbial in this cohort.
2. *Staphylococcus aureus* (42%) and *Escherichia coli* (22.35%) represent the predominant Gram-positive and Gram-negative pathogens, respectively, isolated from diabetic wounds in our setting.
3. Imipenem demonstrates the highest efficacy (70% sensitivity) against diabetic wound pathogens, making it a reliable therapeutic option for severe infections.
4. Alarmingly high resistance rates are observed against Ampicillin (60%) and Amoxicillin (59%), rendering these antibiotics ineffective for empirical therapy in diabetic wound infections.

5. Significant inter-species variation exists in MIC and MBC values for ciprofloxacin, with *Enterobacter spp* showing reduced susceptibility compared to *E. coli* and *S. aureus*.
6. Multiple risk factors, including prolonged diabetes duration, extended hospital stay, hypertension, and retinopathy, significantly contribute to the development and progression of diabetic wound infections.

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