Bacteriological Profile and Antibiotic Susceptibility Patterns of Chronic Non-healing Wound Infections in a Tertiary Care Hospital from North India

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ABSTRACT

Introduction: Chronic wounds are becoming increasingly common as a result of an increase in the ageing population and a rising population with diabetes and obesity. These have been associated with significant financial burdens on society, directly (medical and healthcare expenditures) and indirectly. Factors like infection by multi-drug resistant microorganisms, biofilm formation, inflammatory mediators, hypoxia, poor nutrition, and recurrent trauma may make the wounds chronic. **Aims:** To assess the bacteriological profile of chronic wound infections and their antibiotic susceptibility pattern. **Methods:** Two swabs were collected to isolate aerobic and facultative anaerobic bacteria. The isolates were then subjected to antimicrobial testing by a modified Kirby Bauer disc diffusion method, and the multi-drug resistant strain was identified to be resistant to at least three different antibiotic classes. The strain of *Staphylococcus aureus* was screened for methicillin resistance using a cefoxitin disc (30 µg). **Results:** 100% of the samples collected from chronic wounds yielded bacterial growth. *S. aureus* was the most prevalent isolate at 29.2%, followed by *Klebsiella pneumoniae* at 22.5% and *Pseudomonas aeruginosa* at 17.5%. Monobacterial infection was the most frequent in 51.7% of wounds. 88.6% of the strains of *S. aureus* were methicillin-resistant (MRSA). MDR bacteria accounted for 95.83% of all bacterial isolates. **Conclusion:** High levels of resistance to one or more antibiotic agents have been reported, and a substantial number of them exhibited multidrug resistance , indicating limited therapeutic options for managing chronic non-healing wounds. Therefore, to contain antibiotic resistance, periodic surveillance of the bacteriological profile and antibiotic sensitivity pattern in the study region is of the utmost importance in ensuring successful wound infection care with appropriate antiobiotics.

***Keywords**:* Antibiogram, bacteriological profile, chronic wound, MDR

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INTRODUCTION

Chronic wounds (hard-to-heal or difficult-to-heal ulcers) do not heal within a normal healing timeframe, i.e., longer than six weeks, even after the best possible treatment.[1,2] Chronic wounds are becoming increasingly common as a result of an increase in the ageing population and a rising population with diabetes and obesity. These ailments prevent wound healing and retain them in a low-level inflammatory state.[3] These have been associated with significant financial burdens on society, directly (medical and healthcare expenditures) and indirectly (productivity losses, such as daily wages and often early retirement.[2] It is estimated that cost may fall between 1% and 3% of all healthcare expenditures in developed countries.[4] Factors like infection by multi-drug resistance (MDR) microorganisms, biofilm formation, inflammatory mediators, hypoxia, poor nutrition, and also recurrent trauma because of neurological deficiency or anatomical location may make the wounds to become chronic.[5]

Wound infections are typically caused by various microorganisms, including facultative, anaerobic, aerobic bacteria, fungi, and viruses.[6] The most frequent causes of infection include methicillin-resistant *Staphylococcus aureus,* which accounts for 20–40% of all infections. *Escherichia coli, Pseudomonas aeruginosa, Enterococcus sp., Proteus sp.,* and *Klebsiella* species are other common bacteria implicated in the infection.[7]

Considering the concerns regarding wound infections caused by antibiotic-resistant bacteria, it is paramount to adhere to the protocol of promptly testing microorganisms for culture and sensitivity to offer appropriate treatment. To fill such a gap, this study was carried out to assess the bacteriological profile of chronic wound infections and their antibiotic susceptibility.

METHODS

This single-centered, prospective study was conducted in the Wound Clinic of the Departments of General Surgery and Microbiology, Institute of Medical Sciences, Banaras Hindu University, from December 2021 to July 2023. All the eligible patients in the study received thorough information about the study and its procedures before obtaining written informed consent. The institutional review board and ethics committee of BHU approved the study protocol [Dean/2022/EC/3329]. A total of 60 cases were included from the patients visiting the wound clinic of the university hospital. The selection criteria were >12 years of age and had at least one eligible full-thickness chronic wound not healing for a 6-week with conventional therapy. Patients suffering from systemic diseases, burns, malignancy, dermatological disorders, and ulcers with leprosy or tuberculosis, vascular diseases (like venous and arterial ulcers) were excluded.

Isolation of bacterial pathogen

The wound surface was cleaned gently with sterile gauze soaked in sterile saline (0.9% NaCl) thrice. Two swabs/tissue biopsies were collected to isolate aerobic and facultative anaerobic bacteria. All these swabs were put in a tube containing 1 mL of sterile normal saline. The tube with the swab was vortexed for 5 min to release entrapped bacteria in the swab. A hundred microliters of the above suspension were inoculated on MacConkey (MA) and blood agar (BA) plates and incubated overnight at 37^0C. The next day, the plates were examined for bacterial colonies. If colonies did not appear, broth subcultures were made on the fresh solid media, and the old plates were further incubated for 24 h. The colonies were purified and identified by colony morphology, Gram's staining characteristics, and performing a battery of different biochemical tests. The isolates were then subjected to antimicrobial testing by a modified Kirby Bauer disc diffusion method following the guideline of CLSI 2019(8), and the MDR strain has been identified to be resistant to at least three different antibiotic classes. Using a cefoxitin disc (30 µg), the strain of *S. aureus* was screened for methicillin resistance following the guidelines of CLSI (2019).[8] A zone of inhibition ≤21 mm is considered a positive result for MRSA strain. The lawn culture on MHA was prepared after matching the turbidity of the test inoculum with 0.5 Mac Farland standard.[9] Software SPSS Windows version 25.0 was used for the statistical analysis.

RESULTS

The study population (60) ranged in age from 20 to 65 years, with a mean of 48.9 (SD ±13.13) years. There were 75% male, and 25% were female. The incidence of chronic wound infections was higher in the age group 41-60 y (48.3%), followed by the age group 60 years old or more (26.7%) and 21-40 (23.3%). The patient demography (age, T2DM, HTN, gender, distribution by age group, and comorbidities are summarized in Table I.

Characteristics		Total
Age (Years)	**Mean**	**48.9**
	SD	13.1
Gender	Male	45
	Female	15
T2DM		30
HTN		18
Age groups (years)	<20	1
	21-40	14
	41-60	29
	>60	16

Table I: Representation of patient's demographic data and comorbidities

Among 120 bacterial isolates, gram-negative bacteria were predominant, with 80 (66.7%) isolates, while gram-positive bacteria contributed to 40 (33.3%) of total isolates. *S. aureus* was the most prevalent isolate 35 (29.2%), followed by *K. pneumoniae* 27 (22.5%), *P. aeruginosa* 21 (17.5%), *E. coli* 13 (10.8%), Proteus species 11 (9.2%), *Citrobacter* species 5 (4.2%) Coagulase negative Staphylococci 3 (2.5%), *A. baumannii* and *E. faecalis*, each with 2 (1.67%). *M. morganii* 1(0.8%) was the least isolated bacteria (Figure 1).

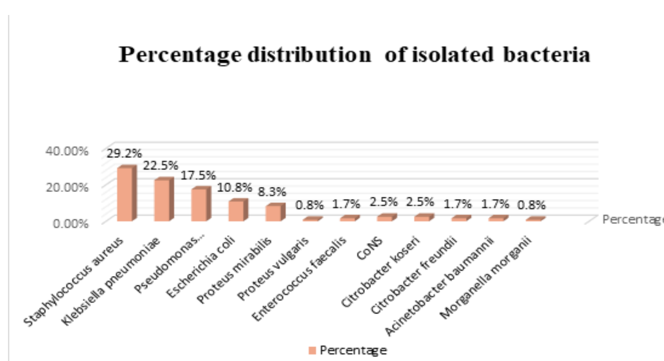

Figure 1: Distribution of bacterial species isolated from wound

Monobacterial infection was the most frequent in 31 (51.7%) wounds. Bi-bacterial infection was found in 24 (40%) cases, whereas 5 (5.3 %) had poly-bacterial infection. *P. aeruginosa, P. mirabilis,* and *S. aureus* were the most common species detected in poly-bacterial infections (Figure 2).

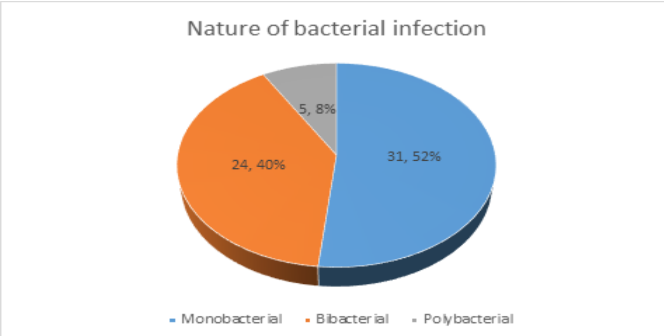

Figure 2: Status of mono/mixed bacterial infections of chronic wound

Antimicrobial profile:

Analysis of species-specific resistance rates indicated that most *S. aureus* isolates were resistant to penicillin G (100%), followed by ampicillin and erythromycin, each with 34 (97.1%) resistance. On the other hand, *S. aureus* was susceptible to vancomycin, linezolid, ciprofloxacin, and clindamycin, with resistance rates of 5.7%, 34.3%, 54.3%, and 85.7%, respectively. Of the 35 *S. aureus* isolates, 31(88.6%) were methicillin-resistant. Overall, 29 (72.5%) gram-positive isolates were resistant to both Penicillin G and cefixime, 27 (67.5%) to ampicillin sulbactam, 26 (65%) to amikacin, 23 (57.5%) to ciprofloxacin and14 (35%) to linezolid respectively. With 2 (5%) resistance, vancomycin was most effective in all the gram-positive isolates (Table II).

Bacteria	Number and percentage of resistance to various antibiotics, N (%)										
	VA	LZ	E	CD	AMP	COT	CX	GEN	AK	CIP	P
Staphylococcus aureus (n=35)	2 (5.7)	12 (34.3)	34 (97.1)	30 (85.7)	34 (97.1)	26 (74.3)	31 (88.6)	25 (71.4)	28 (80)	19 (54.3)	35 (100)
Enterococcus faecalis (n=2)	0 (0)	0 (0)	1 (50)	1 (50)	1 (50)	1 (50)	Nt	1 (50)	1 (50)	2 (100)	1 (50)
Coagulase-negative Staphylococci (n= 3)	0 (0)	2 (66.7)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	1 (33.3)	2 (66.7)	3 (100)
Total (n=40)	2 (5)	14 (35)	38 (95)	34 (85)	38 (95)	30 (75)	34 (85)	29 (72.5)	30 (75)	23 (57.5)	39 (97.5)

VA- Vancomycin, LZ- Linezolid, E- Erythromycin, CD- Clindamycin, AMP- Ampicillin, COT- Co-Trimoxazole (Trimethoprim/Suphamethoxazole), CX-Cefoxitin, GEN- Gentamicin, AK- Amikacin, CFM-Cefixime, CIP- Ciprofloxacin, P- Penicillin-G, NT- Not Tested

Table II: Antibiotic resistance pattern of gram-positive bacteria

Likewise, the least effective antibiotics against gram-negative isolates were ampicillin (100%), ceftazidime (96.2%), cefepime (95%), piperacillin 90%, and Co-Trimoxazole (Trimethoprim/Suphamethoxazole) 83.7%. In contrast, among all the gram-negative strains, low levels of resistance were found to Polymyxin B with 7 (8.7%) resistance, followed by Piperacillin-tazobactam with 24 (30%) resistance and meropenem with 35 (43.7%) resistance. *K. pneumoniae* showed the highest resistance to ampicillin (100%), piperacillin and ceftazidime, each with 96.3% resistance, while polymyxin B and piperacillin-tazobactam were relatively effective antibiotics with a resistance rate of 14.8% and 33.3%, respectively. *P. aeruginosa, E. coli*, and *Proteus* species showed the highest resistance rates to ampicillin (100%), cefepime (93.3%), co-trimoxazole and amoxicillin/clavulanic acid, each with 84.44% resistance (Table III).

Bacterial isolates resistant to more than one antimicrobial agent of three or more antimicrobial classes are known as multidrug-resistant isolates. The overall MDR rate among gram-positive isolates was 99%. Out of 35 *S. aureus* isolates tested, 34 (97.1%) were resistant to 3 or more classes of antibiotics, while all isolates (100%) of CoNS and *Enterococcus* species were resistant to 3 or more classes of antibiotics. The overall MDR rates among gram-negative bacteria were 91.4%. High rates of MDR were seen among *K. pneumoniae* and P. *aeruginosa* (100%) each. In contrast, *Proteus* species, *E. coli, Citrobacter* species, and *A. baumannii* showed overall MDR rates of 92.6%, 95.67%, and 66.5%, respectively (Table IV).

Bacteria	Number and percentage of resistance to various antibiotics, N (%)														
	MRP	IPM	ERT	GEN	AK	COT	PI	PIT	AMC	CPM	CAZ	PB	AMP	AS	CIP
***Klebsiella pneumoniae* (n=27**	15 (55.6)	19 (70.4)	21 (77.8)	18 (66.7)	19 (70.4)	22 (81.5)	26 (96.3)	9 (33.3)	25 (92.6)	26 (96.3)	26 (96.3)	4 (14.8)	27 (100)	19 (70.4)	23 (85.2)
***Pseudomonas aeruginosa* (n=21)**	9 (42.8)	11 (52.4)	12 (57.1)	13 (38.1)	14 (61.9)	20 (95.2)	19 (90.5)	5 (23.8)	21 (100)	20 (95.2)	21 (100)	2 (9.5)	21 (100)	14 (66.7)	17 (80.9)
***Escherichia coli* (n=13)**	4 (30.8)	6 (46.1)	7 (53.8)	5 (38.5)	6 (46.1)	8 (61.5)	13 (100)	4 (30.8)	10 (76.9)	12 (57.1)	12 (92.3)	0 (0)	13 (100)	8 (61.5)	8 (61.5)

Total (n=80)	M. morganii (n=1)	*A. baumannii* (n=2)	*Citrobacter freundii* (n=2)	*Citrobacter koseri* (n=3)	*Proteus vulgaris* (n=1)	Proteus mirabilis (n=10)
35 (43.8)	0 (0)	1 (50)	1 (50)	0 (0)	1 (100)	4 (40)
46 (57.5)	0 (0)	2 (100)	1 (50)	1 (33.3)	1 (100)	5 (50)
49 (61.2)	0 (0)	1 (50)	1 (50)	1 (33.3)	0 (0)	6 (60)
45 (56.2)	0 (0)	2 (100)	1 (50)	0 (0)	0 (0)	6 (60)
51 (63.7)	0 (0)	2 (100)	2 (100)	1 (33.3)	1 (100)	6 (60)
67 (83.7)	1 (100)	2 (100)	2 (100)	2 (66.7)	1 (100)	9 (90)
72 (90)	1 (100)	2 (100)	0 (0)	3 (100)	1 (100)	7 (70)
24 (30)	1 (100)	0 (0)	1 (50)	2 (66.7)	1 (100)	1 (10)
59 (73.7)	1 (100)	1 (50)	2 (100)	2 (66.7)	1 (100)	6 (60)
76 (95)	1 (100)	2 (100)	2 (100)	3 (100)	1 (100)	9 (90)
77 (96.2)	1 (100)	2 (100)	2 (100)	3 (100)	1 (100)	9 (90)
7 (8.7)	0 (0)	0 (0)	0 (0)	1 (33.3)	0 (0)	0 (0)
80 (100)	1 (100)	2 (100)	2 (100)	3 (100)	1 (100)	10 (100)
54 (67.5)	1 (100)	2 (100)	1 (50)	3 (100)	1 (100)	5 (50)
62 (77.5)	1 (100)	2 (100)	1 (50)	1 (33.3)	1 (100)	8 (80)

MRP- Meropenem, IPM- Imipenem, ERT- Ertapenem, GEN- Gentamicin, AK-Amikacin, COT- Co-Trimoxazole (Trimethoprim/Suphamethoxazole), PI- Piperacillin, PIT- Piperacillin/ Tazobactam, AMC- Amoxyclav (Amoxicillin/Clavulanic acid), CPM-Cefepime, CAZ- Ceftazidime, PB- Polymyxin B, AMP- Ampicillin, A/S- Ampicillin/Sulbactam, CIP-Ciprofloxacin

Table III: Antibiotic resistance pattern of gram-negative bacteria

Organism	No. of isolates	MDR isolates	
		Frequency	%
Staphylococcus aureus	35	34	97.1
Klebsiella pneumoniae	27	27	100
Pseudomonas aeruginosa	21	21	100
Escherichia coli	13	12	92.3
Proteus mirabilis	10	8	80
Proteus vulgaris	1	1	100
Enterococcus faecalis	2	2	100
CoNS	3	3	100
Citrobacter koseri	3	3	100
Citrobacter freundii	2	1	50
Acinetobacter baumannii	2	2	100
Morganella morganii	1	1	100
Total	**120**	**115**	**95.8**

Table IV: Distribution of MDR pathogens among total isolates

DISCUSSION

Wound care and antibiotic therapy are the two fundamental pillars in the clinical management of wounds. The typical method of administering antibiotics is empirical, which may induce the emergence of antimicrobial-resistant pathogens to multiple classes of antibiotics.[10] Culture techniques are primarily used in routine microbiology laboratories, and isolation and identification of all the possible pathogens from wounds and antibiotic sensitivities serve as a guide for efficient treatment. All the samples collected from 60 patients yielded bacterial growth, indicating that infection is a universal phenomenon for all chronic wounds. Out of the 60 patients included in the present study, 51.7% had mono-bacterial growth, 40% had bi-bacterial, and 8.3% had polybacterial infections. Numerous studies on wound infection have demonstrated similar findings that mono-bacterial infections were more common than polybacterial infections.[11,12] It seems that only a few bacterial species colonization may be compatible with the wound.

In this study, the frequency of isolation of gram-negative bacteria was higher than that of gram-positive bacteria. Gram-negative bacteria were more frequently isolated during this study due to their prevalence as facultative anaerobes and aerobes in abscesses and skin wounds. These bacteria are more common in open and closed wound types because of their recognized properties to cause abscesses in visceral infections and open wounds. The finding is in concordance with previous studies showing a higher isolation rate of gram-negative bacteria.[13-15] *S. aureus* 35 (29.2%), *K. pneumoniae* 27 (22.5%), *P. aeruginosa* 21 (17.5%), *E. coli* 13 (10.8%), and *Proteus* species 11 (9.2%) were the most frequently isolated bacteria in this study.

Owing to the presence of *S. aureus* in nasal cavities as normal flora in most people, its isolation rates in wound infections were the highest among all the bacteria. Unhygienic behaviour, such as touching a wound with a hand contaminated with nasal discharge, maybe a possible reason. This argument is further supported by the finding that the risk of infection is two to nine times higher for *S. aureus* carriers than non-carriers.[16] Wounds can also become contaminated with gram-positive bacteria directly from the skin's surfaces.[17] Furthermore, *S. aureus* is studied more than most gram-negative bacteria.[18]

In the present study, *K. pneumoniae* was the second most commonly isolated bacteria with an isolation rate of 22.5%, which is in good agreement with the earlier report by Wangoye et al[19] with a 29% *K. pneumoniae* isolation rate.

Similarly, the fact that these bacteria, particularly *P. aeruginosa*, are part of the normal flora in healthy individuals and are also prevalent in the environment may cause the high frequency. As shown by Khanal et al in 2010 in Nepal[20] and Flammm et al in the United States[21], these bacteria are easily spread when they break through the skin and soft tissue in mechanical cases or burns. Additionally, these bacteria can survive in many antiseptics and disinfectants.[15]

Of the 35 isolates of *S. aureus*, 31 (88.6%) were resistant to the antibiotic methicillin (MRSA). A range of 20% to 56.5% has been recorded for the prevalence of MRSA in other investigations.[22-25] The difference in the rates of MRSA isolation between studies may be caused by variations in the degree of inappropriate antibiotic use, the standard of hygienic practices followed in various places, different time frames of these studies and the degree to which hand hygiene programs are implemented. Gram-positive isolates, including *S. aureus*, exhibited the highest sensitivity to vancomycin (95%), linezolid (65%), ciprofloxacin (34.28%), and co-trimoxazole (25%) in their antibiograms. However, they were highly resistant to penicillin (97.5%), ampicillin (95%) and erythromycin (95%). Other studies also support increased resistance to penicillin and cephalosporins, as observed in the present study.[22,26]

The antibiogram of the Gram-negative isolates revealed mild susceptibility to aminoglycoside and fluoroquinolones, good susceptibility to carbapenems, and resistance to cephalosporins, ampicillin, piperacillin, co-trimoxazole, and amoxicillin-clavulanic acid. Rising resistance to these antibiotics may be caused by the alarmingly widespread prevalence of organisms that produce extended-spectrum beta-lactamases (ESBLs) and overuse of penicillin along the cephalosporin group of antibiotics. Other studies corroborate the steady rise in antibiotic-resistant bacteria among wound patients.[25,27] In particular, *K. pneumoniae* demonstrated the highest resistance towards ampicillin, followed by piperacillin, piperacillin-tazobactam, cefepime (96.3% each) and Amoxycillin-clavulanic acid (92.6%). This outcome is contrary to that of Azzah S et al.[28] who reported a high sensitivity of *K. pneumoniae* to meropenem, imipenem (92.3% each), and ciprofloxacin (68%). However, this study was done five years ago.

Presently, MDR bacteria account for 95.83% of all bacterial isolates. This aligns with the study by Mohammed A. et al from Ethiopia, which found that MDR bacteria account for 95.55% of wound infections.[29] This is still significantly higher than the previously reported rates from Saudi Arabia (22%)[28] and Bangladesh (66–69%).[30] This may be due to the extensive use of antimicrobials by physicians in this area as an empirical treatment alternative or it may be because prolonged antibiotic usage for chronic wounds has caused the emergence of pathogen strains that are becoming more and more resistant. *K. pneumoniae, P. aeruginosa, P. vulgaris, E. faecalis*, CoNS, *C. koseri, A. baumannii* and *M. morganii*, each with 100 % MDR next most common species with 100% MDR rates, followed by *S. aureus* (97.1%), E. coli (92.3%) and *P. mirabilis* (80%). Compared to other developing countries' reports, this species-specific MDR profile trend was higher.[28-30] In the present study, there were more male patients (45 or 75%) than female patients of chronic wound infections, considering men are more likely engaged in outdoor work and thus more susceptible to injuries.

Although it is still a complicated issue, the effective treatment of bacteria in a wound is of the utmost importance. As a result, our study evaluates the current situation in a particular area, which is mainly helpful to the clinicians and microbiologists involved by helping them become aware of the real-world circumstances they have to deal with.

CONCLUSION

In the present study, *S. aureus, K. pneumoniae, P. aeruginosa*, and *E. coli* were the most frequently isolated microorganisms. Compared to Gram-positive wound infections, isolation rates for Gram-negative pathogens were higher. High levels of resistance to one or more antibiotic agents have been reported, and a substantial number of them exhibited multidrug resistance (MDR), indicating limited therapeutic options for managing chronic non-healing wounds. The results of this study will assist healthcare professionals in choosing empirical antibiotic therapy and implementing infection control measures, which are essential for minimizing the rate at which antibiotic resistance (MDR) emerges.

LIMITATIONS

Only aerobic/ facultative bacteria were examined, which is a significant limitation of this study. However, the prevalence of anaerobes in open wound infection is rare. However, it will be better to look for anaerobes also. An additional limitation was that the investigation was conducted only on a limited sample size for a brief period. A multicenter study with a good number of participants would have yielded a better understanding of chronic wound infection.

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