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Original Article

Prevalence and antibiotic susceptibility of bacteria from acute and chronic wounds in Malaysian subjects

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Abstract

Introduction: Chronic wounds represent a major health burden worldwide. It has been hypothesized that the polymicrobial nature of wounds plays an important role in their healing process. Thus, a review of pathogen frequency and susceptibility patterns in wounds is necessary to provide appropriate guidelines for antimicrobial usage.

Methodology: In this study, microbiota and antimicrobial resistance in both acute and chronic wound patients treated at the University of Malaya Medical Centre, Malaysia, were compared. Wound swabs from 84 patients with acute wounds and 84 patients with chronic wounds were collected. The specimens were cultured using standard microbiological techniques. Isolates were then tested for antibiotic sensitivity with the broth microdilution method.

Results: Of 210 pathogenic bacteria isolates, *Staphylococcus aureus* (49; 23.3%) and *Pseudomonas aeruginosa* (31; 14.8%) were the most prevalent bacteria found in wounds. *Staphylococcus aureus* was found significantly more often in patients with chronic wounds (41; 48.8%) than in patients with acute wounds (8; 9.5%), while *Staphylococcus epidermidis* was found predominantly in acute wounds (15; 17.9%). At the time of study, patients with chronic wounds (58.3%) had received more antibiotic treatments in the past previous 12 months compared with patients with acute wounds (16.7%). In the antibiotic susceptibility test, *Staphylococcus* spp. revealed highest resistance towards penicillin and ampicillin. Isolates showed no decrease in susceptibility against a number of newly developed antibiotics (linezolid, daptomycin, and tigecycline).

Conclusions: Our finding showed that bacteria diversity and antimicrobial-resistant strains are more frequently found in chronic wounds than in acute wounds.

Key words: antibiotic resistance; wound; microbiota.

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Introduction

Wounds are a significant cause of morbidity worldwide. Studies show that for every million wound patients, at least 10,000 die from microbial infections [1]. The skin is a vital organ that serves as a protective barrier between the human body and its external environment [2]. Breaks in the skin, such as ulcers or traumatic wounds, expose the subcutaneous tissue, providing appropriate moisture, temperature, and nutritive conditions for microbial colonization [3].

Wound healing is a highly dynamic process involving four consecutive and overlapping phases: coagulation, inflammation, cell proliferation, and tissue remodelling, which leads to rapid closure of the wound within 3–14 days [4]. Wounds can be classified into two categories: acute and chronic. Acute wounds heal in a well-organized process within a predictable time frame, resulting in constant recovery of anatomical and functional integrity, while chronic wounds fail to progress through these stages [5]. In developed countries, approximately one to two percent of the population will experience a chronic wound during their lifetime [6]. The socioeconomic consequences of having a chronic wound include severe patient suffering, restricted mobility, loss of employment, and decreased quality of life [7]. Chronic wounds may be sub-classified into vascular ulcers (*e.g.*, venous and arterial ulcers), diabetic ulcers, and pressure ulcers [8]. Most chronic wounds are arrested in a chronic inflammatory state [6]. Studies have shown that the presence of microbial colonization and proliferation in chronic wounds induces a continuous influx of polymorphonuclear leucocytes, which leads to release of cytotoxic enzymes, oxygen free radicals, inflammatory mediators, and matrix metalloproteases that provoke extensive local tissue damage in the host [9-11].

Bacterial colonization is present on virtually all wounds. Recent studies using molecular techniques have revealed the complex microbial ecology of these wounds [12]. *Staphylococcus aureus* and coagulase-negative staphylococci are by far the most common species isolated in both prospective and retrospective studies worldwide. Other microorganisms commonly associated with skin infections are β-haemolytic *Streptococci, Pseudomonas aeruginosa*, *Escherichia coli*, and *Acinetobacter* spp. [13-15].

The antibiotics selected for the management of wound infections are based on the results of culture and susceptibility tests. However, initial antimicrobial therapy usually remains empirical [16]. Bacteria also have many ways to adapt to antibiotic treatment [17]. The inappropriate use of antimicrobials may place patients at risk for significant adverse side effects and promote the development and spread of antimicrobial resistance [18]. Prolonged hospitalization, increases in cost of management, and increases in the rate of morbidity and mortality were observed in patients infected with multidrug-resistant (MDR) organisms [19]. Thus, surveillance of antimicrobial resistance in wounds is necessary to monitor the effect of treatment and to provide information about resistance trends.

Methodology

Sample collection isolation of bacteria

This was a prospective study performed at the University Malaya Medical Centre (UMMC) Wound Clinic in accordance with the protocol approved by ethics committee of UMMC (ethics number: 962.13). A total of 84 patients with chronic wounds and 84 patients with acute wounds were included in this study (Figure 1). Classification of the acute wounds and chronic wounds was based on the patient's history and on clinical assessment of the wound. A chronic wound was defined as an ulcer that showed no reduction of size or continued increase in size over three months. An acute wound was characterized as a wound that progressed through the normal healing process within three months [18].

Using sterile technique, a cotton swab was rotated over the wound for five seconds, and the tip of the swab was broken off into a sterile transport tube containing phosphate-buffered saline. Patient information, including clinical history, duration of wound, and associated diseases were recorded. Next, 100 μL of suspension was inoculated on chromogenic agar (Oxoid, Basingstoke, UK). The plates were incubated aerobically and anaerobically at 37°C for four days. Pure cultures were obtained after several plates were streaked.

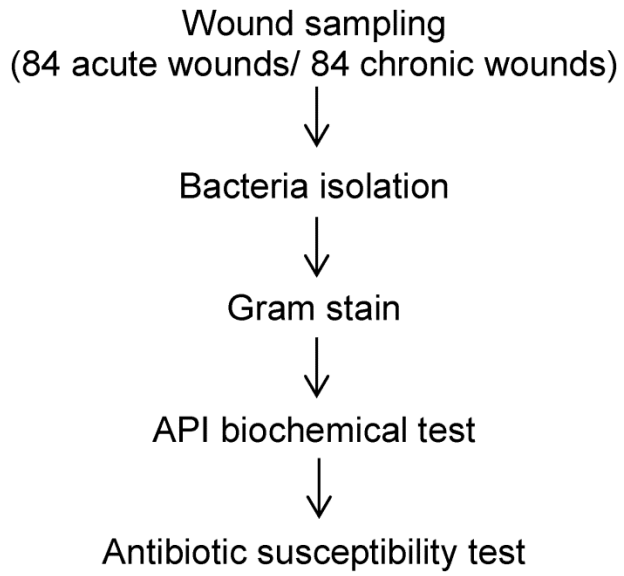
Biochemical methods

Isolates were phenotypically identified using Gram stain and API biochemical test (Biomérieux, Marcy l'Etoile, France). In the API test, which consists of a series of 20 dehydrated substrates within the cupules, pure colonies were picked up and re-suspended with sterile saline. The bacterial suspension was then inoculated into the cupules according to the manufacturer's instructions. After 24–48 hours of incubation, the strip was examined by referring to the reading table, and the interpretation of the data was performed using the identification software.

Antibiotic susceptibility test

Antimicrobial susceptibility testing was performed using dry-form broth microdilution panels prepared by the TREK diagnostic system (Thermo Fisher Scientific, Massachusetts) and processed according to the methods described by the Clinical and Laboratory Standards Institute (CLSI). Antibiotics studied are listed in Table 1 and 2. Three to four pure colonies of the bacteria isolated were inoculated into sterile saline. The turbidity of the inoculums was adjusted to match 0.5 McFarland standards. Next, 10 μL of the bacterial suspension was transferred to 10 mL of cation-adjusted Mueller-Hinton broth, and the microplates were inoculated according to the manufacturer's instruction.

Figure 1. Flow chart of the experiment work for this study.



Plates were incubated at 37°C for 18–24 hours. The breakpoint interpretive criteria used were those recommended by the CLSI (2013) [20]. Quality control was routinely performed using the following test organisms: *Escherichia coli* ATCC 25922 and 35218, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Streptococcus pneumoniae* ATCC 49619, *Enterococcus faecalis* ATCC 29212, and *Haemophilus influenzae* ATCC 49427.

Statistical analysis

Patient demographics, the prevalence of bacterial species, and the bacteria's antimicrobial susceptibility profiles between chronic and acute wounds were compared by Fisher's exact test. Cramer's V-test was used to calculate the correlation between wound healing duration and co-morbidities. P values of < 0.05 were considered significant.

Results

The clinical demographics of the 168 studied subjects are shown in Table 3. In general, the ages of chronic wound patients (56.19 ± 16.12) were significantly higher than those of acute wound patients (45.67 ±20.17) (p = 3.12E-03). In the patients with chronic wounds, 82.1% of the wounds were associated with systemic disease (diabetes mellitus, hypertension, and chronic venous disease). The co-morbidities were strongly associated with the duration of wound healing (r = 0.754, p = 7.10E-15). Penicillin group antibiotics and chloramphenicol group antibiotics were the most commonly prescribed antibiotics. Of 168 patients, 66.1% had received penicillin antibiotic treatment, while 42.9% had received chloramphenicol antibiotic treatment. In the preceding 12 months, 58.3% of chronic wound patients had been exposed to at least three courses of antibiotic treatment.

Table 1. Frequency of occurrence for 210 bacterial pathogens isolated from 168 wound samples

Bacteria	Acute wounds	Chronic wounds	Total
Staphylococcus aureus	8 (12.5%)	41 (28.1%)	49 (23.3%)
Pseudomonas aeruginosa	7 (10.9%)	24 (16.4%)	31 (14.8%)
Staphylococcus epidermidis	15 (23.4%)	2 (1.4%)	17 (8.1%)
Staphylococcus haemolyticus	7 (10.9%)	8 (5.5%)	15 (7.1%)
Escherichia coli	3 (4.7%)	7 (4.8%)	10 (4.8%)
Proteus mirabilis	0 (0.0%)	9 (6.2%)	9 (4.3%)
Enterococcus faecalis	4 (6.3%)	5 (3.4%)	9 (4.3%)
Enterobacter clocae	2 (3.1%)	7 (4.8%)	9 (4.3%)
Klebsiella pneumoniae	2 (3.1%)	5 (3.4%)	7 (3.3%)
Corynebacterium striatum	1 (1.6%)	6 (4.1%)	7 (3.3%)
Citrobacter koseri	1 (1.6%)	5 (3.4%)	6 (2.9%)
Acinetobacter baumannii	1 (1.6%)	3 (2.1%)	4 (1.9%)
Staphylococcus caprae	0 (0.0%)	4 (2.7%)	4 (1.9%)
Staphylococcus capitis	3 (4.7%)	1 (0.7%)	4 (1.9%)
Staphylococcus hominis	3 (4.7%)	1 (0.7%)	4 (1.9%)
Streptococcus agalactiae	1 (1.6%)	2 (1.4%)	3 (1.4%)
Staphylococcus lugdunensis	1 (1.6%)	1 (0.7%)	2 (1.0%)
Staphylococcus sciuri	0 (0.0%)	2 (1.4%)	2 (1.0%)
Staphylococcus warneri	1 (1.6%)	1 (0.7%)	2 (1.0%)
Bacillus cereus	2 (3.1%)	0 (0.0%)	2 (1.0%)
Klebsiella oxytoca	1 (1.6%)	1 (0.7%)	2 (1.0%)
Providencia stuartii	0 (0.0%)	2 (1.4%)	2 (1.0%)
Enterobacter aerogenes	1 (1.6%)	1 (0.7%)	2 (1.0%)
Staphylococcus cohnii	0 (0.0%)	1 (0.7%)	1 (0.5%)
Serratia marcescens	0 (0.0%)	1 (0.7%)	1 (0.5%)
Morganella morganii	0 (0.0%)	1 (0.7%)	1 (0.5%)
Proteus vulgaris	0 (0.0%)	1 (0.7%)	1 (0.5%)
Pseudomonas putida	0 (0.0%)	1 (0.7%)	1 (0.5%)
Enterobacter gergoviae	0 (0.0%)	1 (0.7%)	1 (0.5%)
Arthrobacter cumminsii	0 (0.0%)	1 (0.7%)	1 (0.5%)
Comamonas kerstersii	0 (0.0%)	1 (0.7%)	1 (0.5%)
Total	**64 (100%)**	**146(100%)**	**210 (100%)**

%: Refers to number of isolates with given bacteria divided by total number of isolates.

Table 2. Antimicrobial activity and spectrum for 19 antibiotics tested against the Gram-positive pathogens isolated from 84 chronic and 84 acute wounds

Antibiotic class	Bacterial species											
	Staphylococcus spp.				*Enterococcus* spp.				*Streptococcus* spp.			
	Resistance (µg/mL)	AW (%R)	CW (%R)	P value	Resistance (µg/mL)	AW (%R)	CW (%R)	P value	Resistance (µg/ml)	AW (%R)	CW (%R)	P value
Penicillins												
Penicillin	≥ 0.25	33.3	57.1	***0.003***	≥ 16	0.0	2.4	0.497	≤ 0.12[C]	0.0	0.0	-
Ampicillin	≥ 0.50	31.0	51.2	***0.012***	≥ 16	0.0	2.4	0.497	≤ 0.25[C]	0.0	0.0	-
Oxacillin	≥ 4[A]/ ≥ 0.5[B]	3.6/ 21.4	13.1/ 13.1	***0.047*** / 0.786	-	-	-	-	-	-	-	-
MLS												
Erythromycin	≥ 8	11.9	21.4	0.146	≥ 8	2.4	3.6	1.000	≥ 1	0.0	2.0	1.000
Clindamycin	≥ 4	4.8	9.5	0.370	-	-	-	-	≥ 1	0.0	2.0	1.000
SYN	≥ 4	1.2	3.6	0.620	≥ 4	3.6	6.0	0.720	≥ 4	0.0	2.0	1.000
Quinolones												
Ciprofloxacin	≥ 4	7.1	20.2	***0.023***	≥ 4	1.2	1.2	1.000	-	-	-	-
Levofloxacin	≥ 8	4.8	15.5	***0.038***	≥ 8	0.0	1.2	1.000	≥ 8	0.0	2.0	1.000
Moxifloxacin	≥ 8[A]	0.0	6.0	0.059	-	-	-	-	-	-	-	-
Others												
Chloramphenicol	≥ 32	4.8	11.9	0.161	≥ 32	2.4	1.2	1.000	≥ 16	0.0	4.0	0.495
Daptomycin	≤ 1[C]	0.0	0.0	-	≤ 4[C]	0.0	0.0	-	≤ 1[C]	0.0	0.0	-
Gentamicin	≥ 16	9.5	11.9	0.804	-	-	-	-	-	-	-	-
Linezolid	≤ 4[C]	0.0	0.0	-	≥ 8	1.2	0.0	1.000	≤ 2[C]	0.0	0.0	-
Rifampin	≥ 4	4.8	4.8	1.000	≥ 4	2.4	4.8	0.682	-	-	-	-
SXT	≥ 4/78	11.9	14.3	0.820	-	-	-	-	-	-	-	-
Tetracycline	≥ 16	8.3	15.7	0.160	≥ 16	2.4	6.0	0.443	≥ 8	0.0	4.0	0.495
Vancomycin	≥ 16	0.0	0.0	-	≥ 32	0.0	0.0	-	-	-	-	-
Tigecyclines	≤ 0.5[C]	0.0	0.0	-	≤ 0.25[C]	0.0	0.0	-	-	-	-	-
Nitrofurantoin	≥ 128	1.2	7.1	0.117	≥ 128	0.0	0.0	-	-	-	-	-

AW: acute wound; CW: chronic wound; %R: percentage of resistance; MLS: macrolide/lincosamide/streptogramin; SYN: quinupristin/dalfopristin; SXT: trimethoprim/sulfamethoxazole; [A] Minimun inhibitory concentration for *Staphylococcus aureus;* [B] Minimum inhibitory concentration for coagulase-negative *Staphylococcus* spp.; [C] Performed for verification of antimicrobial susceptibility test results and confirmation of organism identification; * P value < 0.05.

Table 3. Demographic data of study subjects

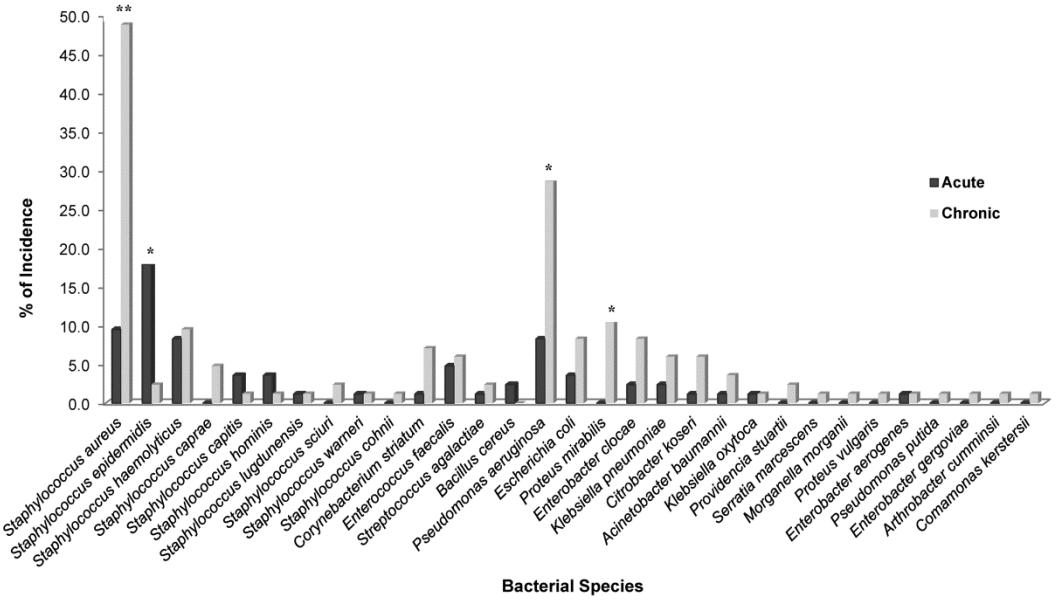
Characteristics	No. of subjects			P value
	Acute wounds (n = 84)	Chronic wounds (n = 84)	Total (n = 168)	
Age (mean ± SD)	45.67 ± 20.17	56.19 ± 16.12	50.93 ± 18.95	3.12E-03
Sex				
Male	54 (64.3%)	49 (58.3%)	103 (61.3%)	0.526
Female	30 (35.7%)	35 (41.7%)	65 (38.7%)	
Race				
Malay	34 (40.5%)	33 (39.3%)	67 (39.9%)	0.676
Chinese	22 (26.2%)	20 (23.8%)	42 (25.0%)	
Indian	19 (22.6%)	25 (29.8%)	44 (26.2%)	
Others	9 (10.7%)	6 (7.1%)	15 (8.9%)	
Primary diagnosis				
Diabetes mellitus ulcer	0 (0.0%)	58 (69.0%)	58 (34.5%)	1.29E-30
Vascular ulcer	0 (0.0%)	10 (11.9%)	10 (6.0)	
Post-surgical ulcer	84 (100.0%)	0 (0.0%)	84 (50.0)	
Others	0 (0.0%)	16 (19.1%)	16 (9.5%)	
Antibiotic treatment				
Received ≥ 3 systemic antibiotics in the last 12 months	14 (16.7%)	49 (58.3%)	63 (37.5%)	3.29E-08

Table 4. Antimicrobial activity and spectrum for 21 antibiotics tested against the Gram-negative pathogens isolated from 84 chronic and 84 acute wounds

Antibiotic class	Bacterial species											
	Enterobacteriaceae				*Pseudomonas aeruginosa*				*Acinetobacter* spp.			
	Resistance (µg/mL)	AW (%R)	CW (%R)	P value	Resistance (µg/mL)	AW (%R)	CW (%R)	P value	Resistance (µg/mL)	AW (%R)	CW (%R)	P value
Penicillins												
Ampicillin	≥ 32	9.5	25.0	***0.013**	-	-	-	-	-	-	-	-
Cephems												
Cefazolin	≥ 8	6.0	15.5	0.078	-	-	-	-	-	-	-	-
Cephalothin	≥ 32	6.0	20.2	***0.011**	-	-	-	-	-	-	-	-
Cefuroxime	≥ 32	3.6	14.3	***0.027**	-	-	-	-	-	-	-	-
Ceftazidime	≥ 16	0.0	4.8	0.121	≥ 32	0.0	2.4	0.497	≥ 32	0.0	2.4	0.497
Ceftriaxone	≥ 4	2.4	8.3	0.168	-	-	-	-	≥ 64	0.0	2.4	0.497
Cefepime	≥ 32	1.2	2.4	1.000	≥ 32	0.0	4.8	0.121	≥ 32	0.0	2.4	0.497
Cefpodoxime	≥ 8	3.6	7.1	0.496	-	-	-	-	-	-	-	-
Cefoxitin	≥ 32	3.6	13.1	***0.047**	-	-	-	-	-	-	-	-
Beta-lactamase inhibitors												
Ampicillin/sulbactam	≥ 32/16	3.6	10.7	0.131	-	-	-	-	≥ 32/16	0.0	2.4	0.497
Piperacillin/tazobactam	≥ 128/4	1.2	6.0	0.210	≥ 128/4	0.0	7.1	***0.028**	≥ 128/4	0.0	2.4	0.497
Ticarcillin/clavulunic acid	≥ 128/2	2.4	6.0	0.443	≥ 128/2	1.2	8.3	0.064	≥ 128/2	0.0	2.4	0.497
Penems												
Ertapenem	≥ 4	0.0	1.2	1.000	-	-	-	-	-	-	-	-
Meropenem	≥ 4	0.0	0.0	-	≥ 8	0.0	3.6	0.246	≥ 16	0.0	1.2	1.000
Monobactams												
Aztreonam	≥ 16	2.4	4.8	0.682	≥ 32	0.0	8.3	***0.014**	-	-	-	-
Aminoglycosides												
Amikacin	≥ 64	0.0	0.0	-	≥ 16	0.0	2.4	0.497	≥ 64	0.0	2.4	0.497
Gentamicin	≥ 16	1.2	8.3	0.064	≥ 16	0.0	4.8	0.121	≥ 16	0.0	2.4	0.497
Tobramycin	≥ 16	1.2	4.8	0.367	≥ 16	0.0	4.8	0.121	≥ 16	0.0	2.4	0.497
Others												
Ciprofloxacin	≥ 4	1.2	7.1	0.117	≥ 4	0.0	4.8	0.121	≥ 4	0.0	1.2	1.000
Tetracycline	≥ 16	2.4	14.3	***0.010**	-	-	-	-	≥ 16	0.0	1.2	1.000
SXT	≥ 4/76	2.4	7.1	0.277	-	-	-	-	≥ 4/76	0.0	0.0	-

AW: acute wound; CW: chronic wound; SXT: trimethoprim/sulfamethoxazole; %R: percentage of resistance; * P value < 0.05

Figure 2. Prevalence of bacteria isolates in 84 chronic wounds and 84 acute wounds.



** p-value < 0.001; * 0.001 < p ≤ 0.05.

Compared wth subjects with acute wounds (16.7%), this exposure to treatment was statistically significant (p = 3.29E-08).

A total of 210 bacteria isolates were obtained from the subjects. A total of 94% of the chronic wounds had a positive culture, whereas only 50% of the acute wounds showed positive culture. The distribution of the pathogens isolated from chronic and acute wounds is presented in Table 4. Aerobic Gram-positive bacteria represented 57.6% of the isolates, while aerobic Gram-negative bacteria represented 42.4% of the isolates. No anaerobic bacteria were isolated in this study. The following organisms were isolated: *S. aureus* (49; 23.3%), *P. aeruginosa* (31; 14.8%), *S. epidermidis* (17; 8.1%), and *S. haemolyticus* (15; 7.1%). The prevalence of bacteria isolated from 84 chronic wounds and 84 acute wounds is shown in Figure 2. *S. aureus* (48.8%) and *P. aeruginosa* (28.6%) were the most prevalent pathogens found in chronic wounds. The prevalence of *S. aureus* (p = 2.14E-08), *P. aeruginosa* (p = 1.19E-03), and *Proteus mirabilis* (p = 3.12E-03) were found significantly more frequently in chronic wounds than in acute wounds, whereas *S. epidermidis* was predominantly detected in acute wounds rather than in chronic wounds (p = 1.40E-03).

Antimicrobial susceptibility of Gram-positive and Gram-negative isolates is summarized in Tables 3 and 4. In chronic wounds, *Staphylococcus* spp. showed highest resistance to penicillin and ampicillin. Penicillin- and ampicillin-resistant *Staphylococcus* spp. were isolated from 57.1% and 51.2% of chronic wounds, respectively. This was followed by erythromycin, ciprofloxacin, and tetracycline. Methicillin-resistant *S. aureus* (MRSA) was present in 13.1% of the chronic wounds. *Staphylococcus* spp. in acute wounds demonstrated a similar antibiotic resistance pattern to that in chronic wounds, showing highest resistant to penicillin (33.3%) and ampicillin (31.0%). A significantly higher resistance to penicillin, ampicillin, ciprofloxacin, and levofloxacin in staphylococci was observed in chronic wounds than in acute wounds. MRSA was also found to be more prevalent in chronic wounds (13.1%) than in acute wounds (3.6%) (p = 0.047). *Enterococcus* spp. isolated showed the highest resistance rate to quinupristin/dalfopristin and tetracycline. No decreased in susceptibility to daptomycin, tigecycline, and vancomycin was detected in the Gram-positive bacteria isolated.

In Enterobacteriaceae, high resistance towards ampicillin and cephalothin was detected in both chronic and acute wounds, followed by ampicillin/sulbactam, cefazolin, and cefuroxime. Ampicillin-, cephalothin-, cefuroxime-, cefoxitin-, and tetracycline-resistant Enterobacteriaceae were found significantly more frequently in chronic wounds than in acute wounds. For *P. aeruginosa*, piperacillin/tazobactam- and aztreonam-resistant strains were identified predominantly in chronic wounds. Amikacin and meropenem were the most effective antibiotics against all the Gram-negative bacteria isolated. Overall, a higher occurrence of resistant strains was observed in chronic wounds than in acute wounds.

Discussion

Wound healing is a complex and dynamic process comprising a series of events: hemostasis, inflammation, proliferation, and tissue remodeling [21]. Acute wounds normally progress through an orderly and timely healing pathway that results in the closure of the wound within 30 days [22,23]. In normal wound healing, the transition from inflammatory phase to proliferation stage lasts approximately 48 hours after injury [24]. Chronic wounds, however, are thought to persist in the inflammatory stage of wound healing [25]. Studies have shown that the presence of bacterial components in chronic wounds may stimulate excessive levels of cytokines and reduced levels of beneficial growth factors [9], thereby preventing the wound from progressing into the proliferative stage [10].

Our findings indicate that the ages of patients with chronic wounds were significantly higher than those of patients with acute wounds. Chronic wounds were found to predominantly affect elderly patients. Studies in Europe have shown that the prevalence of chronic wounds increases with advancing age from 1.48/1,000 to 36/1,000 population in those over 65 years of age [26-28]. Co-morbidities (diabetes mellitus, hypertension, and chronic venous disease) were found to be strongly associated with the duration of wound healing. Guo and DiPietro [29] found that systemic factors such as endocrine disorder (diabetes mellitus), age, obesity, and nutrition deficiency may lead to impaired wound healing.

In the present study, the most prevalent bacteria found in wounds were *S. aureus* (23.3%) and *P. aeruginosa* (14.8%). This is in consistent with various reports that *S. aureus* and *P. aeruginosa* were among the most common bacteria isolated from wounds of different aetiologies [13,30,31]. In this study, higher occurrence rates of *S. aureus* and *P. aeruginosa* were

observed in chronic wounds than in acute wounds. Madsen *et al*. [32] and Zhao *et al*. [33] reported that wounds infected with *S. aureus* and *P. aeruginosa* were generally slower to heal. Athanasopoulos *et al*. [34] and Edwards *et al*. [35] postulated that the extracellular adherence protein (Eap) of *S. aureus* may play a pivotal role in impaired wound healing by impeding the inflammatory state and inhibiting angiogenesis in the proliferative stage. Virulence factors, including lipopolysaccharide (LPS), enterotoxin A, and ADP ribosylating enzymes excreted by *P. aeruginosa* have proven to be cytotoxic and have potent inhibitory effects on the proliferation of human granulocytes and macrophage progenitor cells during the wound healing process [36,37]. *In vivo* studies of mice and rabbits have further confirmed that *S. aureus* and *P. aeruginosa* evade host immunity and establish persistent infections via biofilm formation [38-40]. When compared to planktonic cells, bacteria in biofilm promote higher resistance towards antibiotics and other antimicrobial agents [6,41].

Overall, among isolates, staphylococci show highest resistance to penicillin and ampicillin. Chronic wounds demonstrated significantly higher occurrence of penicillin-resistant staphylococci than did acute wounds. The first penicillinase-producing *S. aureus* was described by Kirby *et al*. [42] as early as 1944. In 1969, a study conducted by Jensen *et al*. [43] further revealed a high occurrence of penicillin-resistant strains from hospital isolates (85%–90%) and community isolates (65%–70%). Since then, penicillinase-producing *S. aureus* has been reported worldwide. In our study, a higher prevalence rate of MRSA was found in chronic wounds (13.1%) than in acute wounds (3.6%). The incidence of *S. aureus* resistant to methicillin has risen dramatically worldwide. Goldstein *et al*. [44] revealed that 20% of *S. aureus* isolates from diabetic foot ulcers in California were methicillin resistant, while Tentolouris *et al*. [45] found that as much as 40% of *S. aureus* isolates from leg ulcers in the United Kingdom were MRSA. In this study, MDR *P. aeruginosa* was found in 9.5% of the chronic wounds. MDR *P. aeruginosa* has been frequently reported from many Asian countries [46], and a study in India showed that almost one-half of the *P. aeruginosa* isolates were resistant to multiple drug classes [47].

At the time of study, 58.3% of chronic wound patients had been exposed to at least three courses of antibiotic treatments in the preceding 12 months. Penicillin group antibiotics and chloramphenicol group antibiotics were the most commonly used antibiotics in wound management. Linezolid, daptomycin, tigecycline, and new glycopeptides are the principal antibacterial agents that are currently being used. Chronic wounds that fail to progress to healing after two to four weeks are frequently treated with either systemic or topical antimicrobial therapy. Tamelin *et al*. [48] showed that > 60% of patients with chronic wounds had received prolonged durations of antibiotic therapy in the previous 6 to 12 months. Alinovi *et al*. [49] and O'Meara *et al*. [50] evaluated the efficiency of systemic antibiotics in chronic wound treatment. Their results showed no significant differences between chronic wound patients treated with systemic antibiotics and standard care. The polymicrobial nature of chronic wounds provides a favorable environment for genetic exchange between bacteria, and the longer-term prospect of antibiotic use makes comorbidities more difficult to treat [51]. Hence, it is necessary to fully understand the type of bacteria and their antibiotic susceptibility to suppress the emergence of antibiotic-resistant strains.

In our study, we did not detect anaerobic bacteria, but several studies have reported the occurrence of anaerobic bacteria in wounds [14,15,52]. However, only 2% of anaerobic bacteria are cultivatable, thus molecular techniques such as 16S ribosomal DNA sequencing, denaturing gradient gel electrophoresis, and real-time polymerase chain reaction (PCR) may be applied in future studies for the identification of anaerobic bacteria.

Conclusions

Our findings show that antibiotic-resistant strains were more commonly isolated from chronic wounds than from acute wounds. This may largely be due to the frequent usage of systemic antibiotics in the management of chronic wounds. Bacteria became more tolerant of antibiotics and ROS may be involved in this situation [53]. Systemic antibiotics should be avoided if clinical signs of infections are absent. In the event of critical colonization, a topical therapeutic approach using antiseptics or topical antibiotics should be adopted. The oral or parenteral systemic antibiotic approach should only be prescribed when the deeper wound compartments are involved [54]. Hence, accurate wound assessment and good clinical practice are the keys to success in wound management.

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