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ORIGINAL RESEARCH

Distribution and Antibiotic Resistance Characteristics of Pathogenic Bacteria in Chronic Wound Infections: A Five-Year Retrospective Study at a Tertiary Hospital

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Objective: This study aimed to characterize the microbial profiles and antibiotic resistance patterns of predominant pathogens in chronic wounds, with the ultimate goal of optimizing evidence-based antimicrobial therapy to improve wound healing outcomes.

Methods: Clinical data and microbial specimens (wound secretions, blood and urine) were collected from patients admitted to the Burns Department of the Second Affiliated Hospital of Kunming Medical University (January 2019–December 2023). Bacterial identification and antibiotic susceptibility testing were performed via standard microbiological methods and interpreted per CLSI guidelines. Statistical analyses were conducted via SPSS 27.0 and WHONET 5.6.

Results: Among the 784 pathogenic isolates, gram-negative bacteria predominated (53.32%), followed by gram-positive bacteria (43.62%) and fungi (3.06%). The most prevalent pathogens were *Staphylococcus aureus* (52.05%), *Escherichia coli* (23.68%), *Enterobacter cloacae* (14.83%), and *Pseudomonas aeruginosa* (13.64%). Multidrug-resistant (MDR) strains accounted for 16.8%, with methicillin-resistant *S. aureus* (MRSA) representing 66.67% of MDR isolates. Resistance to ciprofloxacin, gentamicin, and levofloxacin increased significantly over time ($P < 0.05$).

Conclusion: *Staphylococcus aureus* is the most prevalent pathogen in chronic wounds, and the presence of multidrug-resistant bacteria complicates treatment, increasing resistance to empiric antibiotics. Tailored antimicrobial therapy on the basis of local resistance patterns is critical for effective management.

Keywords: chronic wound infections, antimicrobial resistance, microbial sensitivity tests, multidrug-resistant bacteria, retrospective study

Background

Chronic wounds are defined as wounds that fail to achieve anatomical and functional integrity through the normal, orderly process of tissue repair and remain difficult to heal after more than one month of treatment.[1,2] Owing to the reduced blood supply and the formation of abundant exudates, a large number of bacteria proliferate on chronic wounds, leading to infection and hindering wound healing.[3] The prevalence of chronic wounds has been increasing annually, driven by population aging, obesity, and lifestyle changes.[4,5]

Professor Fu Xiaobing's team analyzed 2513 inpatients from 17 hospitals across China between 2007 and 2008, and found that the proportion of patients with chronic non-healing wounds was 1.7‰ among hospitalized individuals.[6] This finding is largely consistent with subsequent research—a national multicenter retrospective study by Cheng et al in 2020,

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based on 3300 patients, also reported an incidence of 1.7‰ in the inpatient population.[7] Other studies have documented that the prevalence of chronic wounds among surgical inpatients in China ranges from 1.5‰ to 3‰.[8] Nationwide, the annual volume of patients receiving wound repair interventions has reached the scale of hundreds of millions, with chronic non-healing wounds accounting for approximately 30%.[9]

The microbiological profile of chronic wounds differs considerably across regions. In Western countries, *Staphylococcus aureus* (30–50%) and *Pseudomonas aeruginosa* (15–25%) are the most common isolates,[10–12] while studies from Asia—including China—report a higher proportion of gram-negative bacteria such as *Escherichia coli, Klebsiella pneumoniae*, and *Enterobacter cloacae* alongside *S. aureus*.[6,13]

National surveillance data from China (CHINET) reveal that MRSA prevalence declined from 69.0% in 2005 to 35.3% in 2017,[14] though this remains higher than in Western countries (15–25%).[15] Fluoroquinolone resistance in *E. coli* has increased from approximately 40% to over 70% over the same period,[14] and carbapenem resistance in *K. pneumoniae* has emerged as a growing concern.[16]

Tertiary care hospitals manage more complex chronic wound cases and typically report higher rates of multidrug-resistant organisms (MDROs) compared to primary or secondary care settings. Studies from Chinese tertiary hospitals have documented MDRO rates of 15–25% among chronic wound isolates, with MRSA being the most prevalent.[13,14]

The emergence of antimicrobial resistance (AMR) further complicates the management of chronic wound infections. With the widespread and often indiscriminate use of antimicrobial agents, resistant bacterial strains have become increasingly prevalent, leading to a rise in difficult-to-treat chronic wound infections. Consequently, the judicious and evidence-based application of antimicrobial therapy remains a critical yet unresolved clinical dilemma.

This study aims to provide insights into the bacterial characteristics and antibiotic resistance profiles of chronic wounds to guide the rational use of antibiotics in clinical practice.

Materials and Methods

Subjects

This retrospective study included inpatients diagnosed with chronic wounds in the Burns Department of the Second Affiliated Hospital of Kunming Medical University between January 2019 and December 2023. Clinical samples—including wound exudates, and tissue biopsies—were aseptically collected and subjected to standard microbiological analysis. Clinical data, pathogen detection results, and antibiotic susceptibility testing results from patients with positive pathogen detection results were retrospectively analyzed.

Microbiological Methods

Wound specimens were collected following a standardized protocol——Levine technique for chronic wound swab sampling.[17,18] After the wound surface was cleansed with sterile 0.9% saline solution (1–2 applications) to remove superficial contaminants, exudates were collected using sterile rayon-tipped swabs by rotating the swab over clean granulation tissue with sufficient pressure to express the wound fluid. The swabs were immediately placed in a Thermo CO_2 incubator and delivered to the microbiology laboratory within 30 minutes under controlled temperature conditions (20–25°C). Bacterial isolation and identification were performed via standardized microbiological procedures. The samples were inoculated onto 5% sheep blood agar, MacConkey agar, chocolate agar, and then incubated at 35±1°C under appropriate atmospheric conditions for 24–48 hours. Isolates were identified via the VITEK 2 automated system (bioMérieux, France) with GN and GP identification cards.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed via determination and the Kirby-Bauer disk diffusion method. Antibiotic disks (Oxoid, UK; distributed by Guangzhou Nat Biological Technology Co., Ltd.) were applied according to the manufacturer's specifications. The zone diameters were measured via calibrated Vernier calipers (Mitutoyo, Japan) and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) M100 guidelines (2016–2020 editions), applying the specific breakpoints corresponding to the isolation year.

Quality Control Strains

Routine quality control was performed using reference strains: *Escherichia coli* (ATCC 25922, ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923, ATCC 29213), and *Enterococcus faecalis* (ATCC 29212). The isolates were categorized as susceptible (S), intermediate (I), or resistant (R), with reduced sensitivity defined as intermediate or resistant,[19] in this article, the analysis of the bacterial isolates was limited to a descriptive count of their antibiotic resistance profiles, without statistical analysis. To avoid duplication bias, subsequent isolates of the same species with identical antimicrobial profiles from the same patient during a single hospitalization were excluded from analysis. All the incubations were performed at 37°C in a Thermo Scientific incubator (Thermo Fisher, USA).

Statistical Analysis Software

Count data are expressed as frequencies (percentages), and continuous data with a normal distribution are presented as mean ± standard deviation. For data that did not follow a normal distribution, the median and interquartile range were used. WHONET 5.6 software was used to analyze the infection sites, and detection rates of gram-negative bacteria, gram-positive bacteria, fungi, and multidrug-resistant bacteria. SPSS 27.0 statistical software was used to conduct a logistic regression analysis on the resistance data. Antibiotic resistance (resistant/nonresistant) was used as the dependent variable, with the year as the independent variable. A P-value less than 0.05 was considered statistically significant.

Results

Clinical Data of Patients

During the five-year study period (2019–2023), our institution admitted 1909 consecutive inpatients meeting the diagnostic criteria for chronic wounds. The cohort demographics were as follows:

Age distribution: The patient population spanned all age groups from pediatric (1 year) to geriatric (92 years), with a mean age of 39.47 ± 22.46 years. Patients aged 18–64 years accounted for62.44% of the cohort, followed by elderly (≥65 years, 14.04%) and children (<18 years, 23.52%).

Gender distribution: Of the 1909 patients, 1216 (63.7%) were male and 693 (36.3%) were female.

Hospitalization duration: The length of stay varied considerably (range: 3–93 days; mean: 17.39 ± 10.69 days).

Healthcare utilization: Total medical expenses demonstrated substantial variability (range: 838.20–324,877.35 CNY; mean: 18,709.36 ± 17,943.34 CNY).

Microbiological analysis yielded 813 clinically significant pathogenic isolates (isolation rate: 42.6%) from chronic wound specimens. Wound infections can be categorized into several distinct etiological types (Table 1):

Distribution of Pathogenic Bacteria

Our analysis of 1909 chronic wound cases identified 813 pathogenic isolates, with 784 nonduplicate strains representing a 41.07% detection rate (784/1909). All results presented below are derived exclusively from wound exudate and tissue biopsy specimens. The microbial distribution demonstrated bacterial predominance (96.94%, 760/784), comprising gram-

Table 1 Annual Distribution of Chronic Wound Etiologies (2019–2023)

	2019	2020	2021	2022	2023	Total
Pressure ulcers	9	4	26	51	83	173
Diabetic foot ulcers	17	12	20	46	50	145
Scar-associated ulcers	17	28	18	11	51	125
Trauma/surgical ulcers	20	15	6	13	26	80
Radiation-induced ulcers	0	1	0	0	1	2
Chronic skin ulcers	75	48	44	53	68	288
Total	138	108	114	174	279	813

Table 2 Distribution and Composition of Bacterial and Fungal Isolates from Clinical Samples (2019–2023)

	2019	2020	2021	2022	2023	Total	%
Gram-positive	59	42	54	60	127	342	43.62%
Staphylococcus aureus	24	26	27	34	67	178	52.05%
Staphylococcus epidermidis	8	5	8	1	12	34	9.94%
Enterococcus faecalis	8		6	8	10	32	9.36%
Gram-negative	76	62	58	95	127	418	53.32%
Escherichia coli	12	11	14	33	29	99	23.68%
Proteus mirabilis	14	8	7	13	20	62	14.83%
Pseudomonas aeruginosa	8	12	6	13	18	57	13.64%
Klebsiella pneumoniae	9	12	9	10	9	49	11.72%
Enterobacter cloacae	4	3	3	7	12	29	6.94%
Fungal	2	2	1	9	10	24	3.06%
Candida albicans	–	–	–	5	5	10	41.67%
Candida parapsilosis	–	–	–	1	3	4	16.67%
Candida tropicalis	–	1	–	1	1	3	12.50%
Candida krusei	–	–	1	1	–	2	8.33%
Total	137	106	113	164	264	784	100.00%

negative (53.32%, 418/784) and gram-positive (43.62%, 342/784) organisms. The gram-negative spectrum was dominated by *Escherichia coli* (23.68%), *Enterobacter cloacae* (14.83%), *Pseudomonas aeruginosa* (13.64%), and *Klebsiella pneumoniae* (11.72%), whereas *Staphylococcus aureus* constituted the majority of gram-positive isolates (52.05%, 178/342). Fungal infections, primarily *Candida albicans* were infrequent (3.06%, 24/784). Longitudinal analysis revealed *Staphylococcus aureus, Escherichia coli, Enterobacter cloacae, Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* as the five predominant pathogens (Table 2), with *S. aureus* demonstrating a clinically significant annual increase in detection rates (Figure 1), highlighting its growing epidemiological importance in chronic wound infections. These findings underscore the necessity for ongoing microbial surveillance and tailored antimicrobial strategies in wound management.

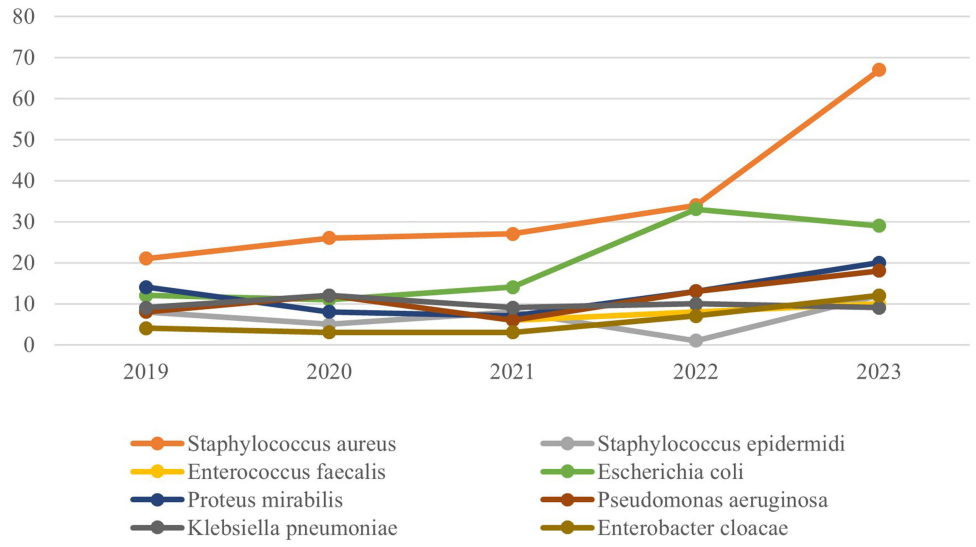

Figure 1 Annual distribution of major bacterial isolates (2019–2023).

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Table 3 Annual Distribution and Proportion of Multidrug-Resistant (MDR) Bacterial Strains (2019–2023)

	2019	2020	2021	2022	2023	Total	%
MRSA	7	9	12	17	43	88	66.67%
CRAB	6	2	0	2	4	14	10.61%
CRKP	2	6	2	2	2	14	10.61%
CRPA	2	2	2	3	3	12	9.09%
CRE	1	1	0	1	1	4	3.03%
Total	18	20	16	25	53	132	100.00%

Distribution of Multidrug-Resistant Bacteria

Among the 784 pathogenic strains isolated, 132 (16.8%) exhibited multidrug-resistant (MDR) phenotypes (Table 3). *Methicillin-resistant Staphylococcus aureus* (MRSA) was the predominant MDR pathogen, representing 49.4% (88/178) of all *S. aureus* isolates (Table 2). Other clinically significant MDR strains included *carbapenem-resistant Acinetobacter baumannii* (CRAB), *carbapenem-resistant Klebsiella pneumoniae* (CRKP), and *carbapenem-resistant Enterobacteriaceae* (CRE). Notably, no *vancomycin-resistant enterococci* (VREs) were identified during the study period.

Antibiotic Susceptibility Testing

Logistic regression analysis was performed with year (2019–2023) as a continuous independent variable. An odds ratio (OR) > 1 indicates a significant annual increase in resistance rate, whereas OR < 1 indicates a significant annual decrease. The results of the antibiotic susceptibility tests, as shown in Table 4, revealed statistically significant changes in the following bacterial strains: *Staphylococcus aureus* exhibited increased resistance to ciprofloxacin (OR 1.316), gentamicin (OR 1.37), levofloxacin (OR 1.261), moxifloxacin (OR 1.272), rifampin (OR 1.622), minocycline (OR 1.37), and chloramphenicol (OR 1.622). *Escherichia coli* presented increased resistance to trimethoprim-sulfamethoxazole (OR 1.512). *Enterobacter cloacae* showed increased resistance to ceftriaxone (OR 1.544) and tigecycline (OR 2.041). *Pseudomonas aeruginosa* exhibited increased resistance to tobramycin (OR 1.65) and ticarcillin-clavulanic acid (OR

Table 4 Antimicrobial Resistance Profiles of Bacterial Isolates

	Antimicrobial Agent	n	%	OR	CI	Trend (P)
Staphylococcus aureus (n=178)	Ciprofloxacin	77	43.26%	1.316	1.062–1.632	0.275
	Gentamicin	37	20.79%	1.37	1.101–1.706	0.315
	Levofloxacin	67	37.64%	1.261	1.013–1.570	0.232
	Moxifloxacin	6	3.37%	1.272	1.020–1.585	0.24
	Penicillin	172	96.63%	1.494	0.859–2.602	
	Rifampin	47	26.40%	1.622	1.227–2.145	0.484
	Nitrofurantoin	6	3.37%	0.669	0.384–1.165	
	Oxacillin	105	58.99%	1.187	0.965–1.459	
	Minocycline	61	34.27%	1.616	1.258–2.078	0.48
	Vancomycin	0	0.00%			
	Linezolid	0	0.00%			
	CHL	66	37.08%	1.538	1.212–1.951	0.43
	ERY	110	61.80%	1.022	0.83–1.258	
	Q-D	2	1.12%	1.974	0.421–9.257	
	Clindamycin	107	60.11%	0.994	0.808–1.223	0.414

(*Continued*)

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Table 4 (Continued).

	Antimicrobial Agent	n	%	OR	CI	Trend (P)
Escherichia coli (n=99)	SXT	70	70.71%	1.512	1.090–2.098	0.414
	Levofloxacin	86	86.87%	1.12	0.733–1.711	
	Ciprofloxacin	73	73.74%	0.851	0.598–1.209	
	Ampicillin	90	90.91%	0.938	0.554–1.589	
	Amikacin	3	3.03%	0.628	0.280–1.408	
	Ertapenem	3	3.03%	1.337	0.487–3.675	
	Imipenem	3	3.03%	0.880	0.386–2.004	
	Tigecyclin	11	11.11%	0.179	0.073–0.438	
	Cefazolin	82	82.83%	0.753	0.484–1.172	
Proteus mirabilis (n=62)	Ceftriaxone	28	45.16%	1.544	1.086–2.196	0.435
	Tigecycline	47	75.81%	2.041	1.305–3.190	0.713
	Amikacin	0	0.00%			
	CFS	0	0.00%			
	TZP	4	6.45%	0.368	0.126–1.076	
	Aztreonam	4	6.45%	1.106	0.568–2.153	
	Ceftazidime	1	1.61%	0.895	0.258–3.10	
	Meropenem	1	1.61%	0.895	0.258–3.1	
	Cefazolin	45	72.58%	1.244	0.871–1.778	
	Ampicillin	48	77.42%	1.032	0.707–1.507	
Pseudomonas aeruginosa (n=53)	Colistin	30	56.60%	1.227	0.766–1.965	0.204
	Imipenem	13	24.53%	0.877	0.515–1.494	−1.31
	Tigecycline	47	88.68%	0.285	0.058–1.408	−1.25
	Tobramycin	27	50.94%	1.65	1.004–2.714	0.501
	SXT	44	83.02%	0.202	0.038–1.057	
	TIM	36	67.92%	1.882	1.114–3.179	0.632
Staphylococcus epidermidis (n=34)	Vancomycin	11	32.35%	5.354	1.501–19.095	1.678
	Oxacillin	16	47.06%	0.32	0.148–0.692	−1.14
	Tigecycline	0	0.00%			
	Linezolid	0	0.00%			

1.882). *Staphylococcus epidermidis* showed increased resistance to vancomycin (OR 5.354). Additionally, *Staphylococcus aureus* displayed universal susceptibility to vancomycin, linezolid, nitrofurantoin, moxifloxacin, and quinupristin-dalfopristin, with 100% sensitivity to vancomycin and linezolid. The resistance to penicillin was high at 96.63%. *Escherichia coli* exhibited high resistance to ampicillin (90.91%), levofloxacin (86.87%), and cefazolin (82.83%), whereas it presented increased sensitivity to gentamicin, ertapenem, imipenem, and tigecycline. *Enterobacter cloacae* had universal susceptibility to amikacin, cefoperazone/sulbactam, piperacillin/tazobactam, meropenem, and cefotaxime, with 100% sensitivity to amikacin and cefoperazone/sulbactam. *Pseudomonas aeruginosa* presented increased resistance to tigecycline, trimethoprim-sulfamethoxazole, and ticarcillin-clavulanic acid. *Staphylococcus epidermidis* showed 100% sensitivity to tigecycline and linezolid.

Discussion

In recent years, the global incidence of chronic wounds, arising from diverse etiologies, has increased significantly. As established in the literature, a hallmark of chronic nonhealing wounds is bacterial colonization, which often progresses to infection and may involve biofilm formation.[20] Biofilm-associated infections are known to be persistent and recalcitrant to treatment, substantially impairing wound healing, prolonging morbidity, and severely compromising patients' quality of life.[3] While the present study did not directly assess biofilm formation in the isolated organisms, it provides valuable

insights into the microbial profiles and antibiotic resistance patterns that underlie these challenging infections. To advance the understanding of chronic nonhealing wounds and guide the judicious use of antimicrobial agents for improved wound management, this study performed a retrospective analysis of microbial profiles and antibiotic susceptibility patterns in patients with chronic wounds.

Our findings indicate that chronic skin ulcers were the predominant wound type, with infection being the most frequent complication, followed by pressure ulcers, diabetic ulcers, trauma-related wounds, and postoperative ulcers. In contrast, Western populations exhibit a distinct etiology, with diabetes mellitus, venous insufficiency, pressure injuries, and surgical wounds cited as primary contributors to chronic wounds.[13,21–24] These disparities may reflect variations in socioeconomic development, healthcare infrastructure, and lifestyle or dietary practices across regions, particularly in developing countries such as China.

Chronic wounds result from persistent impairment of the skin's barrier function, with multiple factors contributing to delayed or impaired healing. Among these factors, microbial colonization and infection play critical roles in the pathogenesis of chronic nonhealing wounds.[25,26] In this study, the pathogen culture positivity rate (41.07%) was significantly lower than the rates reported in Western studies, such as those reported by Howell-Jones (82%) and Kassam (91.4%).[27,28] This discrepancy may be attributed to several factors. First, increased patient awareness of self-care and earlier medical consultation in China have been documented in recent years.[29] Second, preemptive wound disinfection and antibiotic use before clinical evaluation are common practices in Chinese healthcare settings.[14] Third, heterogeneity in study populations, as well as variations in sample size and sampling techniques, may also influence microbial detection rates.[18]

In our study, the most commonly isolated bacteria were *Staphylococcus aureus, Escherichia coli, Enterobacter cloacae, Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. These findings are largely consistent with previous reports by Calina et al (Romania),[24,30] Guan et al (China),[13] and others, with the notable exception that *Enterobacter cloacae* was more prevalent than *Pseudomonas aeruginosa* in our cohort. Notably, *Staphylococcus aureus* emerged as the predominant pathogen, accounting for 52.05% of the isolates. This observation aligns with epidemiological data from North America,[31–33] Europe[34–38] and Asia,[32,39–41] including multiple centers in China.[13,39] Furthermore, gram-negative bacteria predominated over gram-positive organisms in chronic wound infections, a pattern corroborated by prior studies.[42] However, contrasting reports have identified gram-positive bacteria as the most common isolates. Specifically, Dong et al (China)[26] reported a predominance of gram-positive organisms in their cohort, while Gayathri et al (India)[43] and Mendes et al (Portugal)[44] similarly found gram-positive bacteria to be most prevalent. These discrepancies may stem from regional variations, differences in patient demographics, climatic influences, wound etiologies, and methodological approaches to microbial culture. Despite these variations, our data support the prevailing trend that gram-negative bacteria are more prevalent in chronic wounds overall. Consequently, empirical antibiotic therapy for chronic wound infections should prioritize agents with enhanced activity against gram-negative pathogens.

Indeed, the antibiotic susceptibility of bacteria isolated from infected wounds varies depending on the clinical department's use of antibiotics. The study results revealed that among gram-positive bacteria, *Staphylococcus aureus* demonstrated universal susceptibility to vancomycin, linezolid, nitrofurantoin, moxifloxacin, and quinupristin-dalfopristin, with 100% sensitivity to vancomycin and linezolid. However, resistance to ciprofloxacin, gentamicin, levofloxacin, moxifloxacin, rifampin, minocycline, and chloramphenicol significantly increased, which may be attributed to the large proportion of methicillin-resistant *Staphylococcus aureus* (MRSA) (66.67%) isolated in this study. While *Staphylococcus aureus* is highly sensitive to vancomycin, prolonged use of vancomycin can induce bacterial resistance. Therefore, vancomycin should not be the first-choice drug for prophylaxis and routine treatment. As shown in Table 4, the resistance of *Staphylococcus epidermidis* to vancomycin significantly increased. Once *Staphylococcus aureus* becomes resistant to vancomycin, it poses a significant challenge in clinical treatment. Among gram-negative bacteria, *Escherichia coli* presented a significant increase in resistance to trimethoprim-sulfamethoxazole, and *Enterobacter cloacae* presented increased resistance to ceftriaxone and tigecycline. *Pseudomonas aeruginosa* displayed a significant increase in resistance to tobramycin and ticarcillin-clavulanic acid. Therefore, antibiotics with high resistance rates—such as penicillin for *S. aureus* (96.63%) and ampicillin for *E. coli* (90.91%)—should be avoided in empirical therapy for chronic wound infections. An epidemiological study conducted in 2008 indicated that the proportion of chronic skin

wound patients receiving antimicrobial therapy in China (78%)[29] was significantly greater than that in Western countries.[45,46] Undoubtedly, the overuse and misuse of antibiotics is a global issue, that directly contributes to the spread of antibiotic resistance, especially in China.

Longitudinal analysis of our five-year data revealed several clinically significant temporal patterns. First, the detection rate of *Staphylococcus aureus* increased progressively from 24 isolates in 2019 to 67 isolates in 2023, representing a nearly threefold increase over the study period (Figure 1 and Table 2). This trend aligns with national surveillance data showing stable or increasing *S. aureus* prevalence in wound infections,[13,14] and underscores the growing clinical importance of this pathogen in chronic wound management. Second, the proportion of methicillin-resistant *S. aureus* (MRSA) among all *S. aureus* isolates increased substantially from 29.2% (7/24) in 2019 to 64.2% (43/67) in 2023 (Table 3). This rising MRSA trend contrasts with national CHINET surveillance data, which reported a decline in MRSA prevalence from 69.0% in 2005 to 35.3% in 2017.[14] The divergence may reflect regional differences in antibiotic prescribing practices, infection control measures, or patient characteristics in our tertiary care setting in southwestern China. Third, resistance to several antibiotics showed significant annual increases, as indicated by the odds ratios (ORs) presented in Table 4. Notably, *S. aureus* resistance to rifampin (OR 1.622, 95% CI 1.227–2.145) and chloramphenicol (OR 1.538, 95% CI 1.212–1.951) increased significantly over time, while *Enterobacter cloacae* resistance to tigecycline showed the most pronounced increase among all tested pathogen-antibiotic combinations (OR 2.041, 95% CI 1.305–3.190). These emerging resistance patterns highlight the dynamic nature of antimicrobial resistance and the need for continuous local surveillance to guide empirical therapy.

Among the 784 pathogenic isolates, 132 (16.8%) were classified as multidrug-resistant (MDR). The detection rate of methicillin-resistant *Staphylococcus aureus* (MRSA) was substantially higher than that reported in other studies, representing 66.67% of all MDR strains and 49.44% of *Staphylococcus aureus*. These findings were higher than those reported by N. Mohamed[47] and Haonan Gua.[13] The epidemiology of multidrug-resistant organisms (MDROs) is highly complex and multifactorial, changing over time and across regions, and depending on patient characteristics.[42] While national surveillance data (CHINET) revealed a decline in the prevalence of MRSA from 69.0% (2005) to 35.3% (2017),[14] our study revealed an increasing trend. In addition to the inherent resistance mechanisms of the bacteria, this result could also be attributed to the study being conducted in a remote southwestern region, where economic and medical conditions are less favorable, hand hygiene awareness among healthcare workers and patients is lacking, and antibiotic use is not always well–regulated. Therefore, from a clinical perspective, it is crucial for healthcare professionals to rigorously implement hand hygiene practices and follow strict guidelines for antibiotic use. In clinical practice, antibiotics are necessary for managing chronic wounds to control infections and prevent the formation of bacterial biofilms. However, the choice of antibiotic regimen should be based on several factors, including suspected pathogens, local antimicrobial resistance patterns, wound type, and infection severity. The selection of initial antibiotics is often based on empirical decisions. Thus, microbial cultures and susceptibility testing should be performed before starting antibiotic therapy for infected wounds, as this provides a more accurate foundation for rational antibiotic use in clinical practice. Whenever possible, oral antibiotics should be preferred over intravenous antibiotics, and topical antibiotics should be considered over systemic therapy. Topical treatments such as polymyxin B, silver sulfadiazine, and mupirocin are often effective for localized wound infections and may reduce systemic antibiotic exposure.[48,49] However, the comparative effectiveness of topical versus systemic antibiotics requires further investigation, and the choice should be guided by infection severity and wound characteristics. Once the wound is adequately cleaned, antibiotic therapy should be discontinued immediately to avoid unnecessary prolonged use.[48,49]

Tertiary care hospitals differ fundamentally from primary or secondary care facilities in several aspects that influence the interpretation of our findings. First, patients treated at tertiary centers typically present with more complex comorbidities, longer disease duration, and prior antibiotic exposure—all of which are risk factors for colonization and infection with multidrug-resistant organisms (MDROs). The MDRO rate of 16.8% observed in our study is comparable to the 15–25% range reported from other Chinese tertiary hospitals,[13,26] but considerably higher than the <10% typically reported from community settings.[45] This confirms that tertiary care patients represent a high-risk population requiring enhanced infection control measures. Second, the high prevalence of MRSA (49.4% of *S. aureus* isolates) in our tertiary center has direct implications for empirical antibiotic selection. Unlike primary care settings

where beta-lactams remain effective for most staphylococcal infections, empirical therapy for suspected staphylococcal wound infections at tertiary centers should consider MRSA coverage until susceptibility results are available. However, the universal susceptibility of *S. aureus* to vancomycin and linezolid (100%) provides reassurance that these agents remain reliable options for severe infections. Third, the predominance of gram-negative bacteria (53.32%) in our tertiary care cohort suggests that empirical regimens should also provide adequate coverage against *Escherichia coli, Enterobacter cloacae*, and *Pseudomonas aeruginosa*. The high resistance rates observed for commonly used agents—such as ampicillin (90.91% in *E. coli*) and tigecycline (88.68% in *P. aeruginosa*)—indicate that these antibiotics are not appropriate for empirical use in our setting. Conversely, the universal susceptibility of *E. cloacae* to amikacin and cefoperazone-sulbactam (100%) suggests these agents may be valuable options.

While this study provides valuable insights into microbial profiles and antimicrobial resistance patterns in chronic wounds, several limitations should be acknowledged. First, although the use of sterile cotton swabs is a noninvasive and widely used method, improper handling or iatrogenic contamination may affect the accuracy of the results, making it difficult to determine whether the bacteria are infecting the wound or simply colonizing it. Second, as a retrospective study, we were unable to determine the source of infection (community-acquired or hospital-acquired). The lack of sufficient information in our database may have directly impacted the antimicrobial resistance rates. Third, as a single-center investigation, our findings may not be broadly applicable because of unique regional antimicrobial prescribing practices, specific patient demographics at our institution and local variations in wound etiology and management protocols. Future studies should consider these factors and conduct multicenter studies with comparative analyses to ensure the accuracy and reliability of the results. Fourth, due to the retrospective nature of this study, complete data on patient comorbidities and clinical outcomes were not available for all cases, which may limit the generalizability of our findings.

Conclusion

This five-year retrospective study from a tertiary care hospital in southwestern China reveals a complex and evolving microbial landscape in chronic wound infections. Gram-negative bacteria predominate (53.32%), with *Escherichia coli* (23.68%), *Enterobacter cloacae* (14.83%), and *Pseudomonas aeruginosa* (13.64%) being the most prevalent, while *Staphylococcus aureus* remains the single most common gram-positive pathogen (52.05% of gram-positive isolates).

Key resistance patterns include near-universal resistance to penicillin in *S. aureus* (96.63%) and ampicillin in *E. coli* (90.91%), as well as high resistance to tigecycline in *P. aeruginosa* (88.68%). Encouragingly, *S. aureus* remains universally susceptible to vancomycin and linezolid (100%), and *E. cloacae* shows 100% susceptibility to amikacin and cefoperazone-sulbactam.

Over the five-year period, significant annual increases in resistance were observed for multiple antibiotic-pathogen combinations, most notably *S. epidermidis* to vancomycin (OR 5.354), *E. cloacae* to tigecycline (OR 2.041), and *S. aureus* to rifampin (OR 1.622). MRSA prevalence increased from 29.2% in 2019 to 64.2% in 2023, contrasting with national declining trends and highlighting the unique resistance ecology of our tertiary setting.

In the tertiary hospital context, where patients present with greater comorbidity burden and prior antibiotic exposure, the MDRO rate of 16.8% (with MRSA accounting for 66.67% of MDR isolates) supports the need for setting-specific empirical guidelines. Empirical therapy should provide coverage for both MRSA and predominant gram-negative pathogens, while avoiding antibiotics with high local resistance rates. Continuous surveillance and antimicrobial stewardship programs are essential to address the dynamic resistance patterns documented in this study.

Abbreviations

CLSI, Clinical and Laboratory Standards Institute; AMR, Antimicrobial resistance; MIs, Minimum inhibitory concentrations; MRSA, Methicillin-resistant *Staphylococcus aureus*; CRPA, CarbaPenem Resistant *Pseudomonas aeruginosa*; CRAB, CarbaPenem Resistant *Acinetobacter baumann*; CRKP, Carbapenem-resistant *Klebsiella pneumoniae*; SXT, Trimethoprim-sulfamethoxazole; TIM, Ticarcillin-clavulanat; TZP, Piperacillin-tazobactam; CFS, Cefoperazone-sulbactam; Q-D, Quinupristin-dalfopristin; CHL, Chloramphenicol; ERY, Erythromycin.

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Data Sharing Statement

The data set supporting the conclusions of this article is available from the corresponding author (Wu-Quan Li) on reasonable request. Data used in this paper is included in the paper.

Ethics Approval and Informed Consent

This study was approved by the ethics committee of the Second Affiliated Hospital of Kunming Medical University (Review -PJ- Section −2024-154). The ethics committee abandoned the requirement for participants to give formal informed permission because of the retrospective nature of this study. Patients' anonymous information was provided from the microbiology hospital laboratory, which isolated the strains. The study completely followed the guiding principles in the Declaration of Helsinki.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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