

Antibiotic Resistance Profile of Bacterial Isolates from Soft Tissue Infections in a Teaching Hospital in South India

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Abstract:

Background: Infected wounds, especially those involving antimicrobial-resistant microorganisms, impose a substantial physical, psychological, and economic burden on patients and society. The considerable resources required to treat such wounds. The objective of the current study was to determine the rate of soft tissue infections and perform antibiotic susceptibility testing on bacterial pathogens isolated from soft tissue-infected patients at a Teaching Hospital in South India.

Methods: A total of 190 wound specimens (open and closed), including pus and wound swabs, were processed in the laboratory of Teaching Medical College. The specimens were cultured on Blood Agar and MacConkey agar and incubated at 37°C for 24 hours. Antibiotic Susceptibility Testing was performed using the modified Kirby-Bauer disc diffusion method, identifying multidrug-resistant (MDR) bacteria and methicillin-resistant *Staphylococcus aureus* (MRSA).

Results: A total of 190 samples were collected for the study and 100 samples showed growth of microorganisms. Age-related variations in the incidence of soft tissue infections were observed, with a higher prevalence among the 31- 40 age group. The predominant strain isolated was *Staphylococcus aureus*, followed by Gram-negative bacteria like *E. coli* and *Pseudomonas aeruginosa*. There was a high prevalence of antibiotic resistance among key pathogens, such as *E. coli*, *K. pneumoniae*, and *Acinetobacter spp.* These infections are increasingly difficult to treat due to rising antibiotic resistance, particularly among Gram-negative organisms. While newer antibiotics are effective, prudent use is crucial to prevent further resistance development.

Conclusion: The antibiotic susceptibility pattern indicated that Gentamicin, Amikacin, Levofloxacin, Piperacillin/Tazobactam, and Doxycycline were effective against Gram-negative bacteria. For Gram-positive organisms, Amikacin, Teicoplanin, Linezolid, Doxycycline, Gentamicin, and Azithromycin were most effective. Thus, these antibiotics may be recommended for empirically treating soft tissue infections.

Keywords: Antibiotic Susceptibility, Bacteria, Soft Tissue Infections.

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Introduction

The skin, the largest organ of the human body, covers a surface area of 1.5 - 2.0 square meters in an average adult. It is resilient and flexible, serving as a protective barrier against invasions [1], and comprises a layered epidermis and a connective tissue-rich dermis [2]. The skin plays a crucial role in maintaining electrolyte balance, regulating water, controlling temperature, and acting as a shield against microorganisms and harmful external agents [3]. The development of bacterial infections involves three main steps: bacteria adhering to host cells, evading host defense mechanisms, and releasing toxins and virulence factors [4].

When the skin's integrity is compromised, the exposed subcutaneous tissue creates a warm, moist, and nutrient-rich environment that fosters microbial colonization. These microbes can originate from

the environment, the surrounding skin, and internal sources such as the gastrointestinal tract, genitourinary system, and oropharyngeal mucosa [5]. Due to their frequency and severity, bacterial skin infections present a significant clinical challenge. An epidemiological study in the United States in 2005 indicated that bacterial skin diseases are among the most common conditions in clinical practice, accounting for roughly 14.2 million outpatient visits [6].

In developing countries like India, many skin diseases are transmissible, preventable, and controllable [7]. Skin infections often lead to extended hospital stays, higher hospitalization costs, increased morbidity and mortality, and contribute significantly to the development of antimicrobial resistance [8]. Studies have shown

that patients with skin infections typically remain in the hospital 6-10 days longer than those whose wounds heal without infection, effectively doubling treatment costs. In individuals with compromised immune systems, such as those with AIDS or diabetes mellitus, even mild infections can rapidly progress to life-threatening conditions. The selection of antimicrobials for treating bacterial skin infections is based on culture and sensitivity tests, although initial treatment is often empirical [9]. Bacteria have developed mechanisms to resist antimicrobial therapy. Over the past twenty years, there has been a rise in infections caused by organisms resistant to commonly used antibiotics [8]. The increasing prevalence of *methicillin-resistant Staphylococcus* and extended-spectrum beta-lactamase (ESBL) producing gram-negative pathogens in both hospital and community settings poses a significant challenge for clinicians initiating empirical antimicrobial therapy [9].

Material and Methods

This cross-sectional study was carried out in the Department of Microbiology at Government Medical College, Mahabubabad, Telangana. During the study period, a total of 190 specimens, including pus and swabs, were processed from patients with soft tissue infections. The study population consisted of patients of all ages and genders who had been referred by physicians for routine clinical care.

Isolation and Identification: Wound swabs were collected and inoculated on Blood agar plates and Mac-Conkey agar plates. Blood agar plates were incubated at 37°C for 24 hours in a CO₂-enriched environment, while Mac-Conkey agar plates were incubated aerobically at 37°C for 24 hours. Blood agar was examined for hemolysis of the medium, colonial characteristics, and Gram staining. Mac-

Conkey agar plates were assessed for Gram-negative organisms, lactose fermenters, non-lactose fermenters, and the colonial characteristics of the organisms. Isolates were identified using standard microbiological techniques as described by Cheesbrough, including colony morphology, Gram staining, and various biochemical tests such as the catalase production test, coagulase production test, oxidase test, IMViC tests, and Triple Sugar Iron Agar tests.

Antibiotic Susceptibility Testing: The antibiotic susceptibility of each isolate was determined using the modified Kirby-Bauer disc diffusion method following CLSI guidelines with Mueller-Hinton Agar (MHA). The antibiotics used in this study were Ampicillin (10 µg), Ceftriaxone (30 µg), Ciprofloxacin (5 µg), Cloxacillin (5 µg), Cotrimoxazole, Erythromycin (15 µg), Gentamicin (10 µg), Aztreonam (30 µg), Amoxicillin (30 µg), Ofloxacin (5 µg), Cefepime (30 µg), Amikacin (30 µg), Amoxiclav (20/10 µg), Clindamycin (2 µg), Levofloxacin (5 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Doxycycline (30 µg), Azithromycin (15 µg), Piperacillin (100 µg), Piperacillin+Tazobactam (PTZ/100/10 µg), Teicoplanin (30 µg), Polymyxin B (300 units), and Linezolid (30 µg). Organisms resistant to more than three different classes of antibiotics were classified as multi-drug-resistant isolates. Screening for methicillin resistance was conducted using the cefoxitin disc diffusion method and interpreted according to CLSI guidelines [9].

Results

Out of the 190 samples collected and analyzed during the duration of the study, we found 100 (53%) exhibited bacterial growth, while 90 (47%) showed no growth (Figure 1).

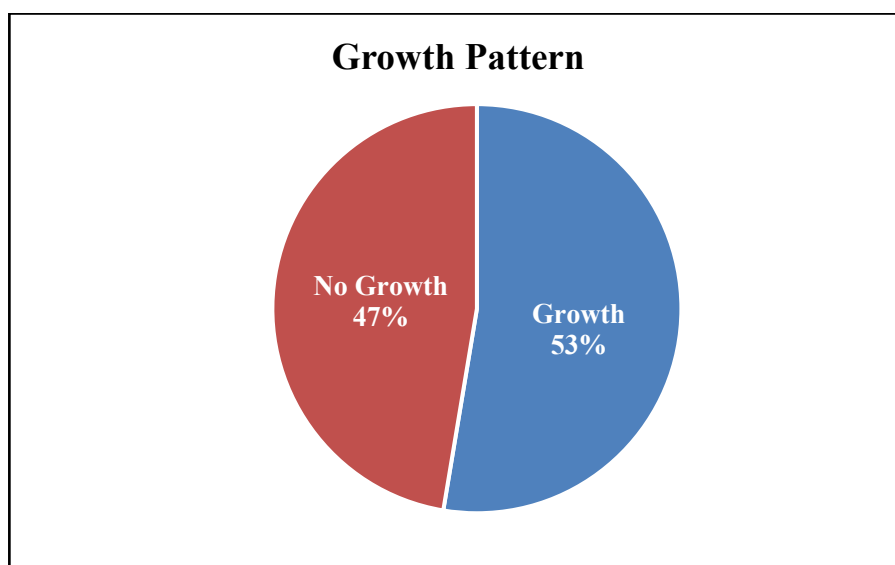


Figure 1: Growth pattern in the specimen samples collected during the study

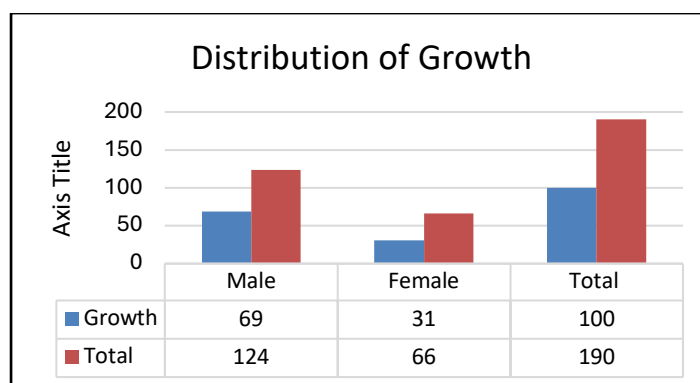


Figure 2: Growth pattern in male and female cases recorded in this study

The distribution of growth was found to be present in 69 males and 31 females depicted in Figure 2.

Table 1: Age-wise distribution of cases included in the study

Age in years	Growth	Percentage	Total	Percentage
<15	7	3.68	11	5.79
16- 20	10	5.26	20	10.53
21 - 30	11	5.79	34	17.89
31 - 40	22	11.58	40	21.05
41 - 50	25	13.16	30	15.79
51 - 60	18	9.47	34	17.89
61 - 70	4	2.10	15	7.89
> 71	3	1.58	6	3.16
Total	100	52.63	190	100.0

Table 1 shows the age distribution of cases of soft tissue infections included in the study. The age group with the highest percentage of cases is 31- 40 years old, accounting for 21.05% of the cases. The age groups with the lowest percentages are <15 years old and >71 years old, each accounting for 5.79% and 3.16% of the cases, respectively. This suggests that soft tissue infections are most common among individuals between 31 and 40 years old.

Out of the 190 samples collected, bacterial growth occurred in 100(52.63%) samples. Among these, 44 samples contained Gram-positive bacteria, and

66 contained Gram-negative bacteria. The most predominant isolate was *Staphylococcus aureus*, found in 40% of cases, followed by *Escherichia coli* in 17%, and *Pseudomonas aeruginosa* in 15%. *Klebsiella pneumoniae* was found in 9% of cases, *Acinetobacter spp.* in 6%, and *Enterobacter spp.* in 3%. The least frequently isolated bacteria were *Coagulase-Negative Staphylococci* (CoNS) in 2%, and *Proteus mirabilis*, *Citrobacter freundii*, *Serratia marcescens*, *Citrobacter koseri*, and *Klebsiella oxytoca* in 1% of cases each, as depicted in Table 2.

Table 2: Distribution patterns of Gram-positive and Gram-negative bacteria among growth

Organism	Frequency	Percentage
<i>Staphylococcus aureus</i>	40	40
<i>E. Coli</i>	17	17
<i>P. aeruginosa</i>	15	15
<i>K. pneumoniae</i>	9	9
<i>Acinetobacter spp</i>	6	6
<i>Enterobacter spp</i>	3	3
CoNS	2	2
<i>Proteus Mirabilis</i>	1	1
<i>Citrobacter freundii</i>	1	1
<i>Citrobacter kaserii</i>	1	1
<i>Serratia marcescens</i>	1	1
<i>Klebsiella oxytoca</i>	1	1
<i>Enterococcus spp</i>	1	1
<i>Streptococcus spp</i>	1	1
Total	100	100

Table 3: Antibiotic susceptibility profiles of *E. coli* and *K. pneumoniae* isolates

Isolates	<i>E.coli</i> (17)				<i>K. pneumoniae</i> (9)			
	Sensitive	(%)	Resistant	(%)	Sensitive	(%)	Resistant	(%)
Amoxicillin	2	11.7	15	88.2	0	0.0	9	100.0
Amoxiclav	4	23.5	13	76.4	1	11.1	8	88.9
Ceftriaxone	3	17.6	14	82.3	2	22.2	7	77.8
Cefotaxime	2	11.7	15	88.2	1	11.1	8	88.9
Cotrimoxazole	6	35.3	11	64.7	2	22.2	7	77.8
Gentamycin	14	82.3	3	17.6	3	33.3	6	66.7
Amikacin	8	47.0	9	52.9	4	44.4	5	55.6
Ciprofloxacin	3	17.6	14	82.3	2	22.2	7	77.8
Ofloxacin	4	23.5	13	76.4	3	33.3	6	66.7
Levofloxacin	8	47.0	9	52.9	3	33.3	6	66.7
Piperacillin	2	11.7	15	88.2	0	0.0	9	100
PTZ	6	35.3	11	64.7	1	11.1	8	88.9
Ampicillin	4	23.5	13	76.4	0	0.0	9	100
Doxycycline	9	52.9	8	47.0	4	44.4	5	55.6

Table 3 presents the antibiotic susceptibility profiles of *E. coli* and *K. pneumoniae* isolates. Both *E. coli* and *K. pneumoniae* demonstrate high resistance rates to several antibiotics, particularly amoxicillin, amoxiclav, and cephalosporins (ceftriaxone, cefotaxime). The susceptibility patterns vary among different antibiotic classes. For, aminoglycosides (gentamicin, amikacin) exhibit relatively higher sensitivity rates compared to other antibiotics. *E. coli* and *K. pneumoniae*

show comparable resistance patterns to most antibiotics, with similar percentages of sensitive and resistant isolates. The data highlights a concerning level of antibiotic resistance among *E. coli* and *K. pneumoniae*, two common causative agents of infections. The similarity in resistance patterns between the two organisms suggests a potential for cross-resistance and challenges in therapeutic management.

Table 4: antibiotic sensitivity pattern of *Acinetobacter spp* and *Enterobacter spp*

Isolates	<i>Acinetobacter spp</i> (6)				<i>Enterobacter spp</i> (3)			
	Sensitive	(%)	Resistant	(%)	Sensitive	(%)	Resistant	(%)
Amoxicillin	0	0.0	6	100.0	0	0.0	3	100.0
Amoxiclav	0	0.0	6	100.0	0	0.0	3	100.0
Ceftriaxone	0	0.0	6	100.0	1	33.3	2	66.7
Cefotaxime	0	0.0	6	100.0	1	33.3	2	66.7
Cotrimoxazole	2	33.3	4	66.7	2	66.7	1	33.3
Gentamycin	1	16.7	5	83.3	2	66.7	1	33.3
Amikacin	0	0.0	6	100.0	1	33.3	2	66.7
Ciprofloxacin	0	0.0	6	100.0	0	0.0	3	100.0
Ofloxacin	0	0.0	6	100.0	2	66.7	1	33.3
Levofloxacin	1	16.7	5	83.3	2	66.7	1	33.3
Piperacillin	0	0.0	6	100.0	1	33.3	2	66.7
PTZ	0	0.0	6	100.0	1	33.3	2	66.7
Ampicillin	0	0.0	6	100.0	0	0.0	3	100.0
Doxycycline	0	0.0	6	100.0	0	0.0	3	100.0

Table 4 presents the antibiotic susceptibility patterns of *Acinetobacter spp.* and *Enterobacter spp.* Both *Acinetobacter spp.* and *Enterobacter spp.* exhibit high resistance rates to most of the antibiotics tested, particularly the beta-lactam antibiotics (amoxicillin, amoxiclav, cephalosporins). *Acinetobacter spp.* showed no sensitivity to any of the tested antibiotics, indicating a critical level of resistance. The limited number of antibiotics with some degree of sensitivity (gentamicin, amikacin, levofloxacin)

highlights the challenge in treating infections caused by these organisms.

The isolates of *Proteus mirabilis* were tested for antibiotic susceptibility against 14 antibiotics, with all isolates showing resistance to Amoxicillin (100%) and Cefotaxime (100%). Among the two isolates of *Citrobacter* species, one was identified as *Citrobacter freundii*. These isolates were tested against 14 antibiotics, and all (100%) were resistant to Amoxicillin, Amoxiclav, Ceftriaxone, Piperacillin, and Cefotaxime. The single isolate of

Serratia marcescens was tested against 14 antibiotics and found to be resistant to Amoxyclav and Doxycycline. Of the 15 isolates of *Pseudomonas* species, all were tested against nine antibiotics. The highest sensitivity was observed with Polymyxin B, with 14 isolates (93.33%) showing susceptibility, followed by Gentamicin (12 isolates, 80%), Amikacin (11 isolates, 73.33%),

Piperacillin/Tazobactam (11 isolates, 73.33%), and Aztreonam (11 isolates, 73.33%). The lowest sensitivity was towards Cefepime, with only six isolates (40%) showing susceptibility. Among the 100 positive isolates, 66 were Gram-negative organisms. Out of these Gram-negative isolates, 31 (46.96%) were multi-drug resistant (MDR), while 35 (53.03%) were not MDR.

Table 5: Antibiotic sensitivity of Gram positive cocci

Isolates	<i>Staphylococcus aureus</i> (40)				<i>Coagulase Negative Staphylococcus aureus</i> (2)			
	Sensitive	(%)	Resistant	(%)	Sensitive	(%)	Resistant	(%)
Cotrimoxazole	15	37.5	25	62.5	2	100.0	0	0.0
Gentamicin	33	82.5	7	17.5	1	50.0	1	50.0
Amikacin	39	97.5	1	2.5	0	0.0	2	100.0
Ofloxacin	15	37.5	25	62.5	0	0.0	2	100.0
Cloxacillin	20	50.0	20	50.0	2	100.0	0	0.0
Erythromycin	6	15.0	34	85.0	2	100.0	0	0.0
Azithromycin	25	62.5	15	37.5	2	100.0	0	0.0
Clindamycin	23	57.5	17	42.5	1	50.0	1	50.0
Teicoplanin	32	80.0	8	20.0	0	0.0	1	50.0
Doxycycline	34	85.0	6	15.0	1	50.0	1	50.0
Linezolid	36	90.0	4	10.0	0	0.0	2	100.0
Ampicillin	2	5.0	38	95.0	2	100.0	0	0.0

Table 5 presents the antibiotic susceptibility patterns of *Staphylococcus aureus* and *Coagulase-Negative Staphylococcus* (CoNS). *Staphylococcus aureus* and CoNS demonstrate high sensitivity rates to newer antibiotics like amikacin, linezolid, teicoplanin, and doxycycline. Both *Staphylococcus aureus* and CoNS exhibit varying degrees of resistance to older antibiotics such as cotrimoxazole, ofloxacin, erythromycin, and clindamycin. While both *Staphylococcus aureus* and CoNS show similar sensitivity patterns to some antibiotics (e.g., amikacin, linezolid), there are notable differences, such as the higher resistance of *Staphylococcus aureus* to cloxacillin compared to CoNS.

Table 6 presents the antibiotic susceptibility patterns of *Enterococcus spp.* and *Streptococcus spp.* It includes the number and percentage of sensitive and resistant isolates for each antibiotic tested. Both *Enterococcus spp.* and *Streptococcus spp.* demonstrate high resistance rates to most of the antibiotics tested, particularly the beta-lactam antibiotics and cotrimoxazole. *Enterococcus spp.* showed no sensitivity to any of the tested antibiotics, indicating a critical level of resistance. The limited number of antibiotics with some degree of sensitivity (Teicoplanin, Linezolid, and Doxycycline) highlights the challenge in treating infections caused by these organisms.

Table 6: Antibiotic sensitivity of Gram-Positive cocci

Isolates	<i>Enterococcus spp</i> (1)				<i>Streptococcus spp</i> (1)			
	Sensitive	(%)	Resistant	(%)	Sensitive	(%)	Resistant	(%)
Cotrimoxazole	0	0.0	1	100	1	100	0	0.0
Gentamicin	0	0.0	1	100	1	100	0	0.0
Amikacin	0	0.0	1	100	0	0.0	1	100
Ofloxacin	0	0.0	1	100	0	0.0	1	100
Cloxacillin	0	0.0	1	100	1	100	0	0.0
Erythromycin	0	0.0	1	100	1	100	0	0.0
Azithromycin	1	100	0	0.0	1	100	0	0.0
Clindamycin	1	100	0	0.0	0	0.0	1	100
Teicoplanin	1	100	0	0.0	0	0.0	1	100
Doxycycline	1	100	0	0.0	1	100	0	0.0
Linezolid	0	0.0	1	100	0	0.0	1	100
Ampicillin	1	100	0	0.0	1	100	0	0.0

Discussion

In this study, the overall rate of bacterial soft tissue infections (STIs) among the study population was found to be 100 (53%). This result aligns with the findings of Sah et al. [11], who reported a 62% growth rate which is close to the 50.7% reported by Acharya et al. [12] Male patients were predominant in this study, with a male-to-female ratio of 6:3, similar to other studies where a higher number of male patients were reported [13, 14]. Patients over the age of 30 had a significantly higher incidence of STIs (35%) compared to a (17%) incidence among patients 21-30 years old. Similarly, a study by Murphy et al. [15] reported a higher incidence of STIs (89.41%) in the age group over 30 years. Advancing age is a significant factor in the development of STIs, as older patients tend to have lower healing rates, decreased immunity, increased catabolic processes, and co-morbid conditions such as diabetes and hypertension [16].

In this study, Gram-negative bacteria were more frequently observed than Gram-positive bacteria. In contrast, a study by Surucuoglu et al. [17] reported a higher prevalence of Gram-positive bacteria (69%) compared to Gram-negative bacteria (29%). Similarly, Kaftandzieva et al. [18] also found a higher prevalence of Gram-positive bacteria. Specifically, *Staphylococcus aureus* was identified as the major pathogenic Gram-positive organism, while *Escherichia coli* was the predominant Gram-negative organism for soft tissue infections (STIs). This is consistent with the findings of Bess et al. [19] showed that the most common bacterial species detected were *Staphylococcus aureus* (37.50%) and *E. coli* (25%). Karkee [20] reported similar results, indicating that the most common bacteria were *S. aureus* (46.58%), followed by *E. coli* (12.38%), *Coagulase-negative Staphylococci* (CoNS) (11.40%), and *Pseudomonas aeruginosa* (7.49%). The least common bacteria isolated were *Citrobacter freundii* (0.65%). In the antibiotic susceptibility pattern of Gram-negative organisms, gentamicin was the most sensitive (57.14%), followed by amikacin (45.43%), levofloxacin (40.92%), doxycycline (37.14%), PTZ (22.85%).

In our study, among Gram-positive isolates, the most effective antibiotic was amikacin (88.63%), followed by teicoplanin (85.66%), linezolid (82.31%), doxycycline (79.54%), gentamicin (79.54%), azithromycin (65.9%), clindamycin (56.81%), cloxacillin (52.27%), cotrimoxazole (40.9%), ofloxacin (34.09%), erythromycin (20.45%), and ampicillin (13.63%). In contrast, Tuladhar (1999) reported that gentamicin was the most effective (89.53%) drug, followed by ciprofloxacin (83.72%), while only 16.27% of Gram-positive cocci were sensitive to ampicillin. Abussaud et al. [21] identified *S. aureus* (35%), *P. aeruginosa* (25%), and *Klebsiella spp.* (10%) as

major causative agents of infections. However, several studies have indicated that *P. aeruginosa* is a leading cause of wound infections. Mousa et al. [22] found that 19.1% of wound infections were caused by *P. aeruginosa*. A similar study on wound infections by Nasser et al. [23] showed that *P. aeruginosa* (21.6%) was the most common isolate. In our study, the rate of infection by *P. aeruginosa* was found to be 15.07%, which aligns with these findings.

The patterns of multidrug resistance (MDR) among Gram-negative bacterial isolates were as follows: 100% in *Acinetobacter spp.*, 83.33% in *Enterobacter spp.*, 77.77% in *Klebsiella pneumoniae*, 64.86% in *Escherichia coli*, 50% in *Proteus mirabilis*, 50% in *Citrobacter freundii*, 50% in *Citrobacter koserii*, and 10% in *Pseudomonas spp.* There were no MDR isolates found in *Klebsiella oxytoca* and *Serratia marcescens*. Kadivarian S et al. [24] reported by 10 (16.1%) *Klebsiella pneumoniae*, 9 (14.5%) *Escherichia coli*, 58 (93.6%) *Acinetobacter baumannii*, and 8 (12.9%) *Pseudomonas aeruginosa* isolates were Carbapenemase-producing isolates were resistant to at least four classes of antimicrobials (MDR). Among Gram-positive cocci (GPC), 36 out of 40 (90%) were *Staphylococcus aureus*, with 19 (52.78%) identified as methicillin-resistant *Staphylococcus aureus* (MRSA) and 17 (47.22%) as methicillin-susceptible *Staphylococcus aureus* (MSSA). This finding is similar to a study by Khanal et al. [25] reported 68% MRSA and 32% MSSA but differs from Edelsberg et al. [26], who reported 35.9% MRSA. Although a wide range of bacteria can be involved in wound infections, we were able to identify only a limited number of pathogens due to inadequate laboratory facilities and time constraints.

Conclusion

This study found that a wide range of bacterial species, including both Gram-positive and Gram-negative organisms, were implicated in soft tissue infections. *Staphylococcus* species emerged as a predominant pathogen. The high prevalence of antibiotic resistance among key pathogens, such as *E. coli*, *K. pneumoniae*, and *Acinetobacter spp.*, poses a significant challenge in the management of soft tissue infections. Age-related variations in the incidence of soft tissue infections were observed, with a higher prevalence among the 31 - 40 age group. The data underscores the need for judicious antibiotic use, rapid diagnostic methods, and the development of novel antimicrobial agents to address the increasing challenge of antibiotic resistance in soft tissue infections.

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