

# Clinical-Microbial Synergy: Mapping Microbiological Profiles to Clinical Attributes in Skin and Soft Tissue Infections

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## ABSTRACT

**Objective:** To determine the clinical microbial synergy in skin and soft tissue infections (SSTIs) based on bacterial groups and explore the likelihood ratios of clinical parameters.

**Study Design:** Descriptive cross-sectional study.

**Place and Duration of the Study:** The study was conducted at the Department of Microbiology, University of Karachi in collaboration with Jinnah Postgraduate Medical Centre, and Jinnah Sindh Medical University, Karachi, Pakistan, from June 2023 to May 2024.

**Methodology:** A total of 304 pus samples from clinically diagnosed cases of SSTIs were included in the study and were processed for microbiological work-up. Isolates were cultured on blood and MacCokney's agar media. Staphylococcal species were identified via the Rapid-ID staph plus system. Continuous data was represented as mean and standard deviation and categorical data were expressed as frequencies (percentages) and were further analysed by using the Chi-square test and multinomial regression model.

**Results:** The study revealed substantial associations between bacterial types and factors such as clinical unit, ethnicity, skin-barrier disruptions, infection site, and wound classification ( $p$ -value  $<0.05$ ) in SSTIs. Metabolic and endocrine disorders increased the odds ratio of gram-negative rod infections (OR = 3.25). Accidents and trauma were associated with higher odds ratio of gram-positive cocci infections (OR = 3.288). Bacterial types varied across wound classes, with gram-positive cocci more common in classes I, II, and III (OR = 3.29, 2.00).

**Conclusion:** This study identifies key predictors of bacterial aetiology in skin SSTIs, revealing increased associations between gram-negative rods and metabolic and endocrine disorders, gram-positive cocci and trauma-related SSTIs, and gram-negative rods in surgical site infections.

**Key Words:** Bacterial infections, Infection control, Soft tissue infections, Surgical site infections.

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## INTRODUCTION

The skin, along with its underlying subcutaneous layers, constitutes the body's largest organ and acts as a mechanical barrier against pathogens. The stratum corneum of the epidermis provides major protection against the pathogens. Skin and soft tissue infections (SSTIs) develop when microorganisms breach various skin layers and overpower the body's immune system. These infections are the major cause of morbidity and mortality worldwide. The global occurrence rates of SSTIs are estimated at 21.35 to 34.10%.<sup>1</sup>

Based on the epidemiological evidence, the emergency room receives 7 - 10% of severe cases of SSTIs.<sup>2</sup> In this regard, SSTIs can be classified into four types based on severity, presence of comorbidities and nature of management.<sup>3</sup> Class I includes milder SSTIs without comorbid and is typically treatable in outpatient services, class II involves moderate infection with systemic signs or symptoms indicating spread or with stable comorbidities or infection without systemic spread but with uncontrolled comorbidities, this may require inpatient management or parenteral antibiotics. Class III indicates infection with signs or symptoms of systemic spread or uncontrolled comorbidities and requires inpatient management with parenteral antibiotics. Class IV denotes the SSTIs with signs of potentially fatal systemic sepsis and needs parenteral antibiotics; inpatient management or surgery may be indicated.<sup>4</sup>

The degree of severity of SSTIs can be affected by the multiple predisposing factors including the type of underlying pathogen and their clinical-microbial synergy. Various parameters such

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as age, gender, body mass indices, comorbidities, immune status, risk of skin trauma, and healthcare and community-related risks can be aligned to the development and progression of SSTIs.<sup>5</sup> SSTIs can exhibit either mono-microbial or poly-microbial infections. The most common pathogens that cause these infections are gram-positive cocci (GPC) including, *Staphylococcus aureus*, *Streptococcus pyogenes*, and coagulase-negative staphylococcal species (CONS). Another group of bacteria that can cause SSTIs include gram-negative rods (GNR) *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter baumannii*, and other antibiotic-resistant bacteria.<sup>6</sup> Among these infections, methicillin-resistant *Staphylococcus aureus* (MRSA) has been identified as the culprit in a substantial 59% of cases.<sup>7</sup> It is worth noting that GNRs, which are frequently underestimated as contributors to SSTIs, have been suggested by several studies to be the predominant causative agents.<sup>8,9</sup> The majority of mixed-species or poly-microbial SSTIs are commonly associated with chronic infections such as diabetic foot infections, pressure ulcers, and burn infections.<sup>10</sup>

There are notable differences in the skin and soft tissue infections caused by GPC and GNR. GPCs are more commonly involved in SSTIs, but GNRs are usually observed in complicated cases and among patients with specific risk factors.<sup>11</sup> However, other parameters which can be linked with these major bacterial groups is still a research query, as both groups of bacteria exhibit considerable antibiotic resistance and necessitate careful selection of the empiric therapy to improve patient outcomes. Several parameters can sway to clinical-microbial synergy; therefore, this study was conducted to determine the association between bacterial types and clinical attributes among the patients of SSTIs. The data from Pakistan with this approach is scarce, hence the study was designed to get insight from the local data. Given the limited data available, the objective of the current research was to explore the clinical microbial synergy in SSTIs based on bacterial groups and connotes the likelihood ratios of clinical variables.

## METHODOLOGY

This cross-sectional study was conducted at the Department of Microbiology, University of Karachi in collaboration with Jinnah Postgraduate Medical Centre, and Jinnah Sindh Medical University Karachi, Pakistan, from May 2023 to April 2024 after getting Institutional Review Board (IRB) approval. The sample size was calculated by the open-epi version - 3 by taking 16% as the anticipated frequency at the confidence interval of 95% with a 5% bound of error, the minimum required sample size was calculated to be 207.<sup>7</sup> A cohort of 400 patients irrespective of age and gender with clinical diagnoses of skin SSTIs were initially enrolled from various departments of Jinnah Postgraduate Medical Centre and University of Karachi using a convenient sampling technique. Of the 400 specimens collected, 304 yielded positive cultures, constituting the final sample size for analysis. Surgical site infections were also included in SSTIs due to anatomical similarity, microbiological overlap, and treatment principles. Those receiving systemic antibiotics for more

than two weeks or those who did not provide consent were excluded. Patients were briefed on the pus collection procedure before samples were taken. Pus was collected from superficial wounds by wiping the area with normal saline and swabbing along the leading edge of the wound. A sterile needle and syringe were used to collect 5 - 10 ml of aspirated material after appropriate surface decontamination from deeper wounds and abscesses. The samples were transported to the microbiology laboratory for culture and sensitivity testing. The specimens were cultured on blood and MacConkey's agar and identification was made by the relevant biochemical tests. The staphylococcal species were identified by the Rapid-ID staph-plus system (Rimel, UK).

The data were collected on a predesigned questionnaire and gathered information was recorded on SPSS (version 24). Descriptive statistics calculated the mean and standard deviation or frequencies (percentages). The Chi-square test determined the association between categorical variables. A multinomial regression model for maximum likelihood estimation further analysed the explanatory variables which showed p-value <0.05 by the Chi-square test.

## RESULTS

The present study elucidated various clinical and microbiological factors associated with skin and soft tissue infections (SSTIs). The demographic characteristics of the study cohort are described in Table I. The 304 pus specimens obtained from SSTIs revealed a predominance of gram-positive cocci (GPC) ( $n = 162, 53.3\%$ ), while gram-negative rods (GNRs), accounting for 118 (38.8%) cases and mixed bacteria (gram-positive and gram-negative both) were identified in 24 (7.9%) specimens. The species breakdown is illustrated in Figure 1. The highest number of specimens were collected from the Dermatology unit ( $p < 0.001, n = 91, 29.9\%$ ) followed by general medicine ( $n = 65, 21.4\%$ ), orthopaedics ( $n = 63, 20.7\%$ ), general surgery ( $n = 32, 10.5\%$ ), gynaecology and obstetrics ( $n = 32, 10.5\%$ ). Notably, there was no statistically significant difference in bacterial distribution between genders ( $p = 0.45$ ) or age groups ( $p = 0.35$ ). However, there was a higher preponderance of SSTIs in the age group between 40-60 years. There was no significant difference between urban and rural populations regarding bacterial distribution ( $p = 0.86$ ). A substantial variation in micro-organisms was observed across different ethnic groups ( $p = 0.004$ ).

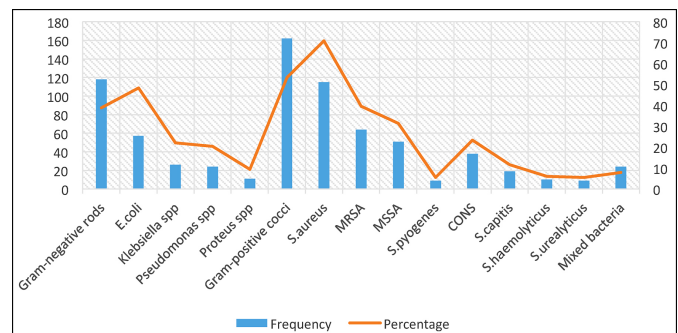


Figure 1: Bacterial species breakdown of the clinical specimens.

**Table I: Demographic profile of the study participants (N = 304).**

Study variables	Gram-negative rods N (%)	Gram-positive cocci N (%)	Polymicrobial infections (Mixed bacteria N (%))	Total N (%)	p-value
Gender					
Male	53 (44.9)	84 (51.9)	13 (54.2)	150 (49.3)	
Female	65 (55.1)	78 (48.1)	11 (45.8)	154 (50.7)	0.45
Age; Mean ± SD	41.5 ± 14.9	38.6 ± 17.6	41.6 ± 16.5	40 ± 16.5	0.61
Age range					
Under 20 Years	16 (13.6)	35 (21.6)	3 (12.5)	54 (17.8)	
Between 20.1 to 40.00 years	35 (29.7)	50 (30.9)	9 (37.5)	94 (30.9)	
Between 40.1 to 60.00 years	61 (51.6)	64 (39.5)	11 (45.8)	136 (44.7)	0.35
Above 60 Years	6 (5.1)	13 (8)	1 (4.2)	20 (6.6)	
Residence					
Urban	79 (66.9)	113 (69.8)	17 (70.8)	209 (68.8)	0.86
Rural	39 (33.1)	49 (30.2)	7 (29.2)	95 (31.2)	
Ethnicity					
Baloch	6 (5.1)	5 (3.1)	1 (4.2)	12 (3.9)	
Hazara	8 (6.8)	1 (0.6)	2 (8.3)	11 (3.6)	
Pashtoon	19 (16.1)	39 (24.1)	6 (25)	64 (21.1)	
Punjabi	10 (8.5)	30 (18.5)	5 (20.8)	45 (14.8)	0.04
Sindhi	33 (28)	54 (33.3)	6 (25)	93 (30.6)	
Siraiki	7 (5.9)	11 (6.8)	2 (8.3)	20 (6.6)	
Urdu	35 (29.7)	22 (13.6)	2 (24)	59 (19.4)	
Occupation					
Skilled workers	22 (18.6)	34 (21)	4 (16.7)	60 (19.7)	
Unskilled workers	79 (66.9)	103 (63.6)	15 (62.5)	197 (64.8)	0.91
Unemployed	17 (14.4)	25 (15.4)	5 (20.8)	47 (15.5)	

\*p-value is calculated by Chi-square test.

**Table II: Relationship between clinical parameters and bacterial aetiology in skin and soft tissue infections.**

Clinical parameters	Monomicrobial infections		Polymicrobial infections (Mixed bacteria) N (%)	Total N (%)	p-value
	Gram-negative rods N (%)	Gram-positive cocci N (%)			
Body mass index (BMI)					<0.43
Underweight	9 (7.6)	24 (14.8)	2 (8.3)	35 (11.5)	
Normal	54 (45.8)	69 (42.6)	9 (37.5)	132 (43.4)	
Overweight	25 (21.2)	37 (22.8)	5 (20.8)	67 (22)	
Obese	30 (25.4)	32 (19.8)	8 (33.3)	70 (23.1)	
Comorbid					<0.01
Metabolic and endocrine diseases	47 (39.8)	33 (20.4)	4 (16.7)	84 (27.6)	
Cardiovascular diseases	12 (10.2)	14 (8.6)	4 (16.7)	30 (9.9)	
Chronic inflammatory diseases	12 (10.2)	21 (13)	3 (12.5)	36 (11.8%)	
No comorbid	47 (39.8)	94 (58)	14 (54.2)	154 (50.7)	
Predisposing factor					<0.01
Accidents and trauma	6 (5.1)	30 (18.5)	1 (4.2)	37 (12.2)	
Surgical intervention	85 ( 72)	59 (36.4)	15 (62.5)	159 (52.3)	
Skin barrier disruptions	27 (22.9)	73 (45.1)	8 (33.3)	108 (35.5)	
Previous antibiotic use					<0.76
Beta-lactam drugs	35 (29.7)	49 (30.2)	8 (33.3)	92 (30.3)	
Fluoroquinolones	13 (11)	24 (14.8)	3 (12.5)	40 (13.2)	
Topical antibiotics	11 (9.3)	20 (12.4)	1 (4.2)	140 (46.1)	
No antibiotics	59 (50)	69 (42.6)	15 (62.5)	32 (10.5)	
Site					<0.001
Head, neck and torso	4 (3.4)	19 (11.7)	6 (25)	29 (9.5)	
Upper and lower extremities	58 (49.2)	100 (61.8)	9 (37.5)	167 (54.9)	
Perianal and genital region	56 ( 47.4)	43 (26.5)	9 (37.5)	108 (35.5)	
Diagnosis					<0.001
Pyoderma	19 (16.1)	90 (55.6)	3 (12.5)	112 (36.8)	
Infected wounds and ulcers	8 (6.8)	11 (6.8)	6 (25)	25 (8.3)	
Bone and deep tissue infections	91 (77.1)	61 (37.6)	15 (62.5)	167 (54.9)	
Class					<0.001
I	13 (11)	49 (30.2)	2 (8.3)	64 (21.1)	
II	44 (37.3)	61 (37.7)	8(33.3)	113 (37.2)	
III	53 (44.9)	48 (29.6)	12(50)	113 (37.2)	
IV	9 (6.8)	4 (2.5)	2(8.4)	14 (4.6)	
Association of infection					<0.001
Community-associated	31 (26.3)	98 (60.5)	6(25)	135 (44.4)	
Hospital-associated	87 (73.7)	64 (39.5)	18(75)	169 (55.6)	

**Table III: Multinomial logistic regression analysis: Clinical factors associated with bacterial types.**

Clinical attribute	GNR vs. MB OR 95% CI	GPC vs. MB OR 95% CI
Comorbidity		
No comorbidity	Reference	Reference
Metabolic and endocrine disorders	3.250 (0.987-10.698)	1.141 (0.348-3.746)
Cardiovascular disorders	0.830 (0.229-3.007)	0.484 (0.138-1.695)
Chronic inflammatory diseases	1.106 (0.271-4.515)	0.968 (0.253-3.703)
Predisposing factors		
Skin barrier disruptions	Reference	Reference
Accident and trauma	1.778 (0.186-17.024)	3.288 (0.394-27.441)
Surgical intervention	1.679 (0.642-4.390)	0.431 (0.171-1.086)
Association of infection		
Hospital associated	Reference	Reference
Community-associated	1.069 (0.389-2.937)	4.594 (1.731-12.193)
Site		
Pelvic region	Reference	Reference
Head, neck, and torso	0.107 (0.025-0.456)	0.663 (0.207-2.799)
Upper and lower limbs	1.036 (0.383-2.799)	2.326 (0.864-6.263)
Diagnosis		
Infected wounds and ulcers	Reference	Reference
Pyoderma	1.044 (0.275-3.965)	7.377 (2.048-26.572)
Bone and deep tissue infections	0.220 (0.067-0.723)	0.451 (0.144-1.415)
Class		
IV	Reference	Reference
I	1.625 (0.190-13.933)	12.250 (1.345-111.570)
II	1.375 (0.246-7.701)	3.813 (0.599-24.258)
III	1.104 (0.208-5.874)	2.000 (0.327-12.238)

Table II shows the clinical attributes of collected specimens. Regarding body mass index (BMI), the majority of the patients had normal weight ( $n = 132$ , 43.4%). Among those with coexisting conditions, metabolic and endocrine disorders were more prevalent than other types of diseases ( $n = 84$ , 27.6%). There was an association of skin barrier disruptions owing to burns, blisters, cuts, abrasions, and insect bites with the bacterial type ( $n = 108$ , 35.5%,  $p < 0.001$ ). No specific group of antibiotics was found to be statistically related to the types of bacteria ( $p = 0.76$ ). However, a large group of individuals have previously used topical anti-biotics such as fusidic acid, polymixin B, and mupirocin with or without local steroids ( $n = 143$ , 47%). A higher number of SSTIs were observed in the upper and lower extremities in comparison with the head, neck, torso, and pelvic regions ( $n = 167$ , 54.9%,  $p < 0.001$ ). Bone and deep tissue infections displayed the highest frequency and also depicted a statistically significant relation with the bacterial types ( $p < 0.001$ ). Also, the Class III and IV were highly related to the bacterial subgroups ( $n = 113$ , 37.2%,  $p < 0.001$ ).

Regarding comorbidities, patients with metabolic and endocrine disorders had 3.25 times higher odds ratio of having a GNR infection than a mixed bacterial infection. For both GNR and GPC, the odds were lower compared to mixed bacterial infections in patients with cardiovascular disorders (Table III). For chronic inflammatory diseases, the odds for GNR are slightly higher and for GPC slightly lower compared to mixed infections (OR = 1.106). GPC presented 1.141 times higher odds with patients with metabolic and endocrine disorders in comparison with other comorbid diseases. Accidents and trauma indicated a potential risk for GPC (OR = 3.288) in comparison to GNR. Surgical interventions showed higher odds with GNR (OR = 1.679) infection.

GPC had an increased likelihood of causing upper and lower extremities in comparison with mixed bacteria (OR = 2.236). GPC depicted a higher probability with pyoderma with OR = 7.377. There was a significantly lower probability of GNR infection compared to MB infection in the bone and deep tissue infection category relative to infected wounds and ulcers. There was a trend towards higher odds of GNR infection compared to MB infection in Class I, while the odds of GPC infection were about 12.25 times higher than MB infection. Class II and III showed trends towards increased odds of GPC with OR = 3.813 and 2.000, respectively compared to mixed bacterial (MB) infections, *vis-a-vis* the reference category IV. The GNRs were found to be 37.4% and 10.4% more related to Classes II and III, respectively when compared with the MB subgroup.

## DISCUSSION

The findings of this study spotlight the potential of harnessing patient-specific clinical data to relate bacterial isolates, a crucial step towards personalised antimicrobial strategies. In the consent study, GPCs were the most isolated organisms followed by the GNRs, other researchers have stipulated similar observations.<sup>12,13</sup> A previous study from Pakistan has predominantly marked GPC as the top offender in SSTIs. According to this, 54.1% of cases of SSTIs were associated with MRSA, however, the role of GNRs in SSTIs cannot be overlooked. The present work showed that GNRs were involved in 38.8% of cases, the findings are in concordance with a study from Turkiye, suggesting the higher rates of GNR in SSTIs.<sup>13</sup> The results presented by Shuaib *et al.* from Pakistan indicated a higher prevalence of GNRs (50.45%) in SSTIs than in GPC.<sup>14</sup> Multinomial regression model results have been considered to determine the association between bacterial types and clinical attributes. This

study showed higher odds of GNRs with metabolic and endocrine diseases than GPC. Recent studies have illustrated gram-negative bacteria (GNB) as an increasing cause of SSTIs, especially in patients with diabetes mellitus.<sup>15</sup> This rising trend in the diabetic population is supposed to be associated with shifts in the microbial ecology of community and healthcare settings.<sup>16</sup> These ecosystems can change over time due to irrational antibiotic use, cleaning protocols, and diverse patient populations. Diabetics, who often require frequent hospital visits or prolonged stays, have increased exposure to these altered microbial environments. As Pakistan is facing a growing threat of anti-microbial resistance, the microbiome shift in an environmental niche is increasingly predictable.

In this study, traumatic injuries and accidents were identified as important predisposing factors associated with an elevated risk of GPC infection. This finding is in complete congruence with Patel *et al.* and Ferreria *et al.* who have elucidated the highest rates of GPC infections in accidents and trauma.<sup>17,18</sup> Contradictory results asserted by Suwal *et al.* indicated GNR as the leading pathogens of traumatic wounds, the current results indicate otherwise.<sup>19</sup>

GPC are prevalent in infections related to accidents and traumatic injuries due to their dominance in normal skin flora and their ubiquity in the environment. When trauma breaches the skin barrier, these organisms get direct access to the underlying tissues. GPC have microbial surface components recognising adhesive matrix molecules (MSCRAMM) that facilitate adherence to host tissues and medical devices, enhancing their ability to develop infections in traumatic wounds.<sup>20</sup>

According to the current results, the OR of 1.679 for gram-negative rods elucidated that the odds of a surgical site infection being caused by gram-negative rods alone were 1.679 times higher than the odds of it being caused by polymicrobial infections. This finding is in line with Sabir *et al.*'s study, which reported the predilection of GNR with surgical site infections.<sup>21</sup> In contrast, Qamar *et al.* identified GPCs as the primary contributors to the surgical site infections in their study.<sup>22</sup> Gram-negative rods, such as *Escherichia coli* and *Klebsiella* species, are common inhabitants of the gut microbiome and can contaminate surgical sites, particularly during procedures involving the gastrointestinal tract. These bacteria are increasingly developing resistance to commonly used antibiotics such as ampicillin and cefasolin.

The rising antibiotic resistance makes these infections harder to treat, emphasising the importance of considering the type of surgery and surgical field cleanliness to understand the predominant pathogens, and guide appropriate treatment. Time and resource constraints prevented a comprehensive analysis of the relationship between pathogen antibiotic susceptibility patterns and associated risk factors. This limitation restricted the authors' ability to

draw more detailed conclusions about these important connections.

## CONCLUSION

There is an increasing association of GNR among patients with underlying metabolic and endocrine disorders, a trend that warrants close attention and further investigation. There was a strong association between GPC and SSTIs arising from traumatic and accidental injuries, highlighting the need for targeted preventive measures and treatment strategies in the local context. Furthermore, the current analysis underscores the predominance of GNR in surgical site infections emphasising the importance of stringent infection-control practises and judicious antibiotic use.

### ETHICAL APPROVAL:

The study was conducted after getting approved by the Institutional Review Board of the Jinnah Postgraduate Medical Centre, Karachi, Pakistan (F.2-81/2023-GENL/66/JPMC).

### COMPETING INTEREST:

The authors declared no conflict of interest.

### AUTHORS' CONTRIBUTION:

FZ: Study conceptualising, sample collection, and manuscript writing.

AN: Drafting of the work and revising it critically for the important intellectual content.

FU: Data analysis and critical review of the manuscript.

BKK: Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved.

BAS: Sample collection and data recording.

All authors approved the final version of the manuscript to be published.

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