RESEARCH ARTICLE

The bacterial profile and antibiotic susceptibility in skin and soft tissue infections at a tertiary care hospital of Quetta, Pakistan

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Abstract

Objective: To determine the bacterial profile and antibiotic susceptibility in skin and soft tissue infections among patients in a tertiary care setting.

Method: The cross-sectional cohort study was conducted at the Centre for Advanced Studies in Vaccinology and Biotechnology, University of Balochistan, Quetta, Pakistan, from June 2021 to May 2022, and comprised bacteria-infected skin samples that were collected from the Bolan Medical Complex Hospital, Quetta, and the Sandeman Provincial Hospital, Quetta. The swab samples were immediately cultured, and positive samples were evaluated for biochemical tests, antibiotic susceptibility test and polymerase chain reaction. Data was analysed using SPSS 22.

Results: Of the 800 samples, 598(74.7%) tested positive for pathogenic bacteria. *Staphylococcus aureus* accounted for 316(39.5%) infections, followed by *clostridium perfringens* 18.96(2.37%), *escherichia coli* 120(15.12%), *pseudomonas aeruginosa* 98(12.25%) and *klebsiella pneumoniae* 44(5.5%). Among all the infected samples, 380(47.5%) belonged to males, 218(27.25%) to patients aged 5-20 years, 448(56%) to the uneducated subjects, and 462(57.87%) to patients having lower socioeconomic status. *Pseudomonas aeruginosa* showed the highest level of resistance against all antibiotics.

Conclusion: Regular surveillance and proper use of antibiotics should be encouraged in hospitals to limit the spread of antibiotic resistance against pathogenic bacteria.

Keywords: Antibiotics, Bacteria, Surveillance, Frequency, Skin. (JPMA 74: 1249; 2024)

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Introduction

Skin and soft tissue infections (SSTIs), also known as *pyodermas,* are common purulent skin infections that are typically caused by staphylococcal or streptococcal pathogens. About 80% of babies in endemic areas are affected by pyodermas that range from a superficial skin infection, like impetigo, to a deeper skin infection, like cellulitis or abscess, and, occasionally, even a necrotising soft tissue infection.[1] SSTIs can be divided into primary (induced by direct infection of healthy skin and subcutaneous tissue) and secondary (coming from an earlier dermatosis like eczema, pediculosis or scabies). Follicular (folliculitis, furuncles, carbuncles and abscess) and non-follicular (impetigo, erysipelas and cellulitis) pyodermas can also be distinguished from one another.[2]

Up to 14 million persons in the United States present with SSTIs annually, and the number is rising. SSTIs are more prevalent in people aged <50 years.[3] There were 49 occurrences of SSTIs per 1,000 people in the US in 2009, the year with the most incidence data (4.9 episodes per 1000 people for *pneumonia* and 17.3 episodes per 1,000 for urinary tract infections [UTIs]).[4] The rising prevalence of metabolic disorders, particularly cardiovascular diseases and type 2 diabetes, as well as an ageing population are the main causes of the rising incidence of SSTIs.[5]

The use of injectable drugs (where 64% of bacterial infections among injecting drug users are caused by bacterial infections) and human immunodeficiency virus (HIV) positivity are other at-risk groups for SSTIs. This is particularly crucial in sub-Saharan Africa, where the prevalence of HIV is high, including the spread among inpatients.[6] As a result of increased incidence rates, SSTIs are currently among the most common causes of hospitalisation for antibiotic therapy worldwide.4 SSTI-related costs are also increasing, and it was estimated that they would go even higher.[7]

Surgical debridement, drainage and antibiotic therapy are the hallmarks of the efficient care of difficult SSTIs.[8] The treatment for SSTIs is still based on antibiotics.[9] However, due to potential overprescribing by healthcare professionals, antibiotic prescribing needs to be closely watched. Antimicrobial resistance (AMR), which is on the rise globally and is currently a significant global public health issue, can result from overprescribing.[10]

Co-morbid disorders are increasingly seriously brought on

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by SSTIs. Skin morbidities are substantially correlated to a lack of consistent hygiene practices.[11] It is commonly known that the main contributing variables include the environment, crowding, subpar housing, and subpar hygiene. Therefore, increasing public knowledge of personal hygiene, health education, healthy lifestyles, and prompt reporting of skin disorders are necessary to improve people's quality of life.

Air pollution has been shown to play an increasing role in most common skin diseases. Acne, hyperpigmentation, atopic dermatitis and psoriasis have been shown to be influenced by air pollution, and in 2019, air pollution was considered by the World Health Organisation (WHO) to be the biggest environmental health risk to humans, responsible for killing more than 7 million people prematurely every year.[12]

The current study was planned to determine the bacterial profile and antibiotic susceptibility in SSTIs among patients in a tertiary care setting.

Materials and Methods

The cross-sectional cohort study was conducted at the Centre for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, Quetta, Pakistan, from June 2021 to May 2022, and comprised bacteria-infected skin samples that were collected from the Bolan Medical Complex Hospital, Quetta, and the Sandeman Provincial Hospital, Quetta. After approval from the institutional ethics review committee, The sample was raised using consecutive sampling technique. The sample size was calculated using the Cochran formula n=z2 p (1- p)/ d2, where n was the required sample size, Z was level of confidence 95% which was 1.96, P was 50% proportion which was 0.5 d was the margin of error which was 0.05.[13] Those included were pus samples, surgical and diabetic wounds, and ulcers on the skin or mucous membranes that had developed an infection due to bacteria, fungus or other pathogens and were referred to as infected ulcers. After consultation with a qualified dermatologist who confirmed the a bacterial infection, the samples were collected from each patient by rubbing the infected area on the skin with a sterile cotton swab. The samples were sent to CASVAB in an ice box for microbiological examination.

Prior to sample acquisition, informed consent was obtained from all the participants, or their parent, and data about their gender, age, education and socioeconomic status (SES) was collected using a predesigned checklist. Those included were both male and female patients aged 1-70 years who had been diagnosed with bacterial skin infection. Patients with history of significant trauma, diabetes mellitus, and those who were on steroids or were immuno-compromised were excluded.

The swab samples were incubated at 37°C for 24 hours after being inoculated on brain heart infusion (BHI) liquid medium. On selective and differentiating medium plates (Mannitol salt agar, Eosin methylene blue agar, *Pseudomonas* Cetrimide agar, and reinforced clostridial medium [RCM] agar plates), the overnight bacterial culture was streaked. Depending on the bacterial condition, the plates were incubated at 37°C for 24 hours. The selected medium plates' pure colonies were employed for additional organism confirmation. To obtain pure growth for gram staining, several biochemical assays (Indole, Simmon citrate, Methyl red, Voges-Proskauer, Urease, Oxidase and Catalase tests), and polymerase chain reaction (PCR) were used, with all isolates undergoing three rounds of cloning. PCR was performed on 15 strains of each isolate.

The Clinical and Laboratory Standards Institute's (CLSI) guidelines were followed when utilising the disc diffusion method to test antimicrobial susceptibility with 10 strains of each isolate.[14]

A suspension of bacterial cells (0.5 McFarland) was produced and spread on a Mueller-Hinton agar media plate (Oxoid, United Kingdom) before being incubated at 37°C for 24 hours.

Deoxyribonucleic acid (DNA) was extracted from culture using a DNA purification kit (Hiper Bacterial Genomic DNA Extraction Teaching Kit, India). DNA templates were extracted and stored for later use at -20°C. Specific forward and reverse primers were employed to amplify the relevant PCR fragments for each of the five strains. For each isolate, the PCR reaction mixture underwent many cycles of initial denaturation, final denaturation, annealing, initial extension and final extension in the thermal cycler. Further, 1.5% agarose gel electrophoresis was utilised to look at the PCR results under ultraviolet (UV) light.

Data was analysed using SPSS 22. Chi-square test was used to assess the significance of association between the variables of interest. $P<0.05$ was considered significant.

Results

Of the 800 samples, 598(74.7%) tested positive for pathogenic bacteria (Figure 1). Among all the infected samples, 380(47.5%) belonged to males, 218(27.25%) to patients aged 5-20 years, 448(56%) to the uneducated subjects, and 462(57.87%) to patients having lower SES. Gender, age and literacy were significant factors ($p<0.05$), while SES, infection type and infection site were not significantly different (Table 1). Through the use of gram staining, biochemical testing and sugar fermentation,

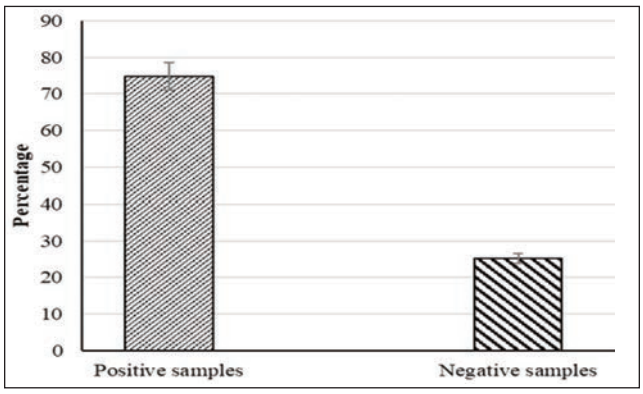

Figure-1: Positive and negative samples.

Table-1: Distribution of positive and negative cases according study variables.

Demographics	Positive (n=598) n (%)	Negative (n=202) n (%)	Total (n=800) n (%)	*p*-value
Gender				
Male	380 (47.5)	131 (16.3)	800 (100)	0.004*
Female	218 (27.25)	71 (8.8)		
Age (years)				
1-4	82 (10.25)	13 (1.62)	800 (100)	0.032*
5-20	218 (27.25)	89 (11.1)		
21-40	138 (17.25)	30 (3.75)		
41-70	160 (20)	70 (8.75)		
Socioeconomic Class				
Lowerclass	463 (57.8)	105 (13)	800 (100)	0.308
Middleclass	113 (14.1)	70 (8.75)		
Higher class	22 (2.75)	27 (3.5)		
Literacy				
Educated	149 (18.7)	53 (6.87)	800 (100)	0.002*
Uneducated	448 (56)	149 (18.37)		
Infection type				
Abscess	187 (23.3)	77 (9.6)	800 (100)	0.210
Furunculosis	177 (22.1)	60 (7.5)		
Infected wound	99 (12.3)	32 (4)		
Cellulitis	84 (10.5)	21 (2.6)		
Impetigo	51 (6.37)	12 (1.5)		
Infection site				
Face	229 (28.7)	81 (10.1)	800 (100)	0.624
Upper extremities	200 (25)	69 (8.6)		
Lower extremities	108 (13.5)	37 (4.6)		
Body	60 (7.5)	15 (1.87)		

several isolated pathogenic bacteria were identified (Table2). *Staphylococcus (S.)* aureus accounted for 316(39.5%) infections, followed by *clostridium (C.) perfringens* 18.96(2.37%), *escherichia (E.) coli* 120(15.12%), *pseudomonas (P.) aeruginosa* 98(12.25%) and *klebsiella (K.) pneumoniae* 44(5.5%).

The antibiotic susceptibility test showed that *P. aeruginosa* had the highest level of resistance against all antibiotics (Table 3).

All PCR findings were noted in detail (Figure 2).

Table-2: Biochemical and sugar characterisation of different pathogenic bacteria isolated from the infected samples.

Tests	Biochemical and Sugar Fermentation Tests				
	Staphylococcus aureus	***Escherichia coli***	***Pseudomonas aeruginosa***	***Klebsiella pneumoniae***	***Clostridium perfringens***
Gram staining	+	–	–	–	+
Indole test	–	+	–	–	–
Simmons citrate test	+	–	+	+	+
Methyl red test	+	+	–	–	+
Voges-Proskauer test	+	–	–	+	–
Oxidase test	–	–	+	–	–
Catalase test	+	+	–	+	–
Urease test	–	–	–	+	+
Motility test	–	+	+	–	–
Glucose test	+	+	+	+	+
Lactose test	+	+	–	+	+
Maltose test	+	+	–	+	+
Mannose test	+	+	–	+	+
Mannitol test	+	+	+	+	–
Raffinose test	–	–	–	+	–
Sucrose	+	–	–	+	+

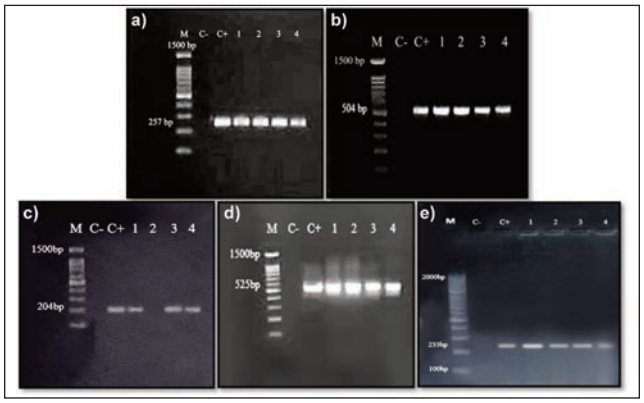

Figure-2: a) Polymerase chain reaction (PCR)-based identification of staphylococcus *(S.) aureus* produced a specific size of the 257-bp fragment of the 16S ribosomal ribonucleic acid (rRNA) gene; **b)** *pseudomonas (P.)* aeruginosa produced a specific size of the 504-bp fragment of the OprL gene; **c)** escherichia *(E.) coli* produced a specific size of the 204-bp fragment of the 16S rRNA gene; **d)** *klebsiella (K.) pneumoniae* produced a specific size of the 525-bp fragment of the 16S rRNA gene; and **e)** *clostridium (C.) perfringens* produced a specific size of 233-bp fragment of the enterotoxin gene.

Discussion

The pattern of skin diseases varies from country to country and even from region to region within a country due to different ecological factors, genetics, hygienic standards and social customs.15 *S. aureus, E. coli, P. aeruginosa, K. pneumoniae* and *C. perfringens* were among the harmful microorganisms that were identified in the current study.

Among positive samples, most were found to be *S. aureus*-positive, followed by *E. coli, P. aeruginosa, K. pneumoniae* and *C. perfringens*. This was in line with an earlier study done in Peshawar, Pakistan.[15]

S. aureus is the most common pathogen involved in skin

Table-3: Antibiotic susceptibility test of the isolated pathogens (n=50).

Antibiotics	Conc (µg)	*S. auerus* (n=10) n (%)		*P. aeruginosa* (n=10) n (%)		*E. coli* (n=10) n (%)		*K. pneumonia* (n=10) n (%)		*C. perfringens* (n=10) n (%)	
		R	S	R	S	R	S	R	S	R	S
Erythromycin	25	3(30)	7(70)	9(90)	1(10)	9(90)	1(10)	2(20)	8(80)	2(20)	8(80)
Meropenem	25	2(20)	8(80)	9(90)	1(10)	3(30)	7(70)	2(20)	8(80)	3(30)	7(70)
Trimethoprim	10	7(70)	3(30)	8(80)	2(20)	2(20)	8(80)	3(30)	7(70)	1(10)	9(90)
Colistin Sulfate	30	8(80)	2(20)	3(30)	7(70)	4(40)	6(60)	3(30)	7(70)	4(40)	6(60)
Vancomycin	5	2(20)	8(80)	8(80)	2(20)	8(80)	2(20)	8(80)	2(20)	1(10)	9(90)
Metronidazole	30	9(90)	1(10)	9(90)	1(10)	8(80)	2(20)	8(80)	2(20)	2(20)	8(80)
Tetracycline	10	3 (30)	7(70)	9(90)	1(10)	7(70)	3(30)	7(70)	3(30)	3(30)	7(70)
Lincomycin	5	1(10)	9(90)	8(80)	2(20)	8(80)	2(20)	9(90%	1(10)	1(10)	9(90)
Spectinomycin	10	2(20)	8(80)	8(80)	2(20)	1(10)	9(90)	3(30)	7(70)	2(20)	8(80)
Pencilline G	30	8(80)	2(20)	9(90)	1(10)	8(80)	2(20)	9(90)	1(10)	9(90)	1(10)

S: Staphylococcus, P: Pseudomonas, E: Escherichia, , K: Klebsiella, C: Clostridium.

infections worldwide, regardless of patient's age, the climate or geographical area. It represents a common global cause of human infection and easily acquires antimicrobial resistance through mutation or horizontal transfer of resistance genes from other bacteria.[16] The current finding of S. aureus being the most prevalent pathogenic isolate were also in line with a 2019 study.[17]

In the current study, male patients had more skin infections than female patients, and this was similar to earlier findings.[18] Age-based categorisation revealed that patients aged 5-20 years had the highest infection rate, while an earlier study indicated that patients aged 11-20 years had the highest infection rates.[19]

The foundations of lifelong responsibility for the maintenance of personal hygiene are laid down in childhood. The role of parent-teacher associations in all schools should be encouraged.[20]

In the current study, uneducated patients were more likely to get bacterial skin infections than educated patients, which was comparable to literature.[21] Abscesses accounted for the highest percentage of skin infections in the current study, followed by furunculosis, infected wounds, cellulitis, and impetigo. According to a study, most patients had surgical wound infections, followed by simple or superficial abscesses, decubitus ulcers, and additional SSTIs.[22]

Antimicrobial resistance (AMR) has emerged as one of the principal public health problems of the 21st century that threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi that are no longer susceptible to the common medicines used to treat them. Faced with this reality, the need for action to avert a developing global crisis in healthcare is imperative.[23]

In the current study, antibiotic susceptibility test showed that *P. aeruginosa* had the highest level of resistance against all antibiotics. A range of findings in this regard have been reported in literature.[24-28]

The organisms identified in the current study were verified in the light of literature(Table 4).[29-33]

The preventive strategies in the hospital setting to control such infections include antimicrobial stewardship programmes administered by multidisciplinary teams of experts, such as infectious diseases physicians, clinical pharmacists and clinical microbiologists. In addition, innovative approaches are needed for the development of new antibiotics and other products to limit AMR.[34]

The finding showed that the importance of local surveillance to guide empirical therapy and the urgent need for antimicrobial stewardship programmes. This study draws attention to establish local surveillance system to monitor the bacterial profile and antibiotic susceptibility patterns that would help them to select appropriate empirical treatment and strengthen the infection control

Table-4: Primer sequences for the diagnosis of bacterial infections.

Strains	Genes	Primers	bps	Tm values	Refer
S. aureus	16SrRNA	F= ACGGTCTTGCTGTCACTTATA R = TACACATATGTTCTTCCCTAATAA	257bp	57	Johnson et al.27, 2016
P. aeruginosa	OprL	F= ATGGAAATGCTGAAATTCGGC R= CTTCTTCAGCTCGACGCGACG	504bp	55	El- Houssien et al.28,2020
E.coli	16SrRNA	F= GGGAGTAAAGTTAATACCTTTGC R= CTCAAGCTTGCCAGTATCAG	204bp	58	Wang et al.29, 2014
K. pneumonia	16SrRNA	F=TCTGAGAGGATGACCAGCCA R= GTTTACGGCGTGGACTACCA	525bp	59	Hamed and Awni30 2019
C. perfringens	Enterotoxin CPE	F= GGAGATGGTTGGATATTAGG R= GGACCAGCAGTTGTAGATA	233bp	53	Tang et al.31, 2012

S: Staphylococcus, P: Pseudomonas, E: Escherichia, , K: Klebsiella, C: Clostridium.

measures to prevent the spread of resistant pathogens and reduce the incidence of skin and soft tissues infections.

Limitation: The Present study was conducted at a single tertiary care hospital which limit the generalizability of the finding to other health care setting.

Conclusion

There was a diverse range of bacterial pathogens causing SSTIs, with *S. aureus* being the most common isolate. A high rate of antibiotic resistance was observed, particularly among *S. aureus* and *P. aeruginosa*, highlighting the need for prudent antibiotic use.

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Author Contribution:

BSA: Literature search, writing and performed the experiments.
MKT: Supervision and design.
IT: Approved the study design and concept.
SA: Data analysis.
SK: Conceived idea.
RR: Drafted the final manuscript.