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Isolation of Bacterial Pathogens From Patients Suffering from Skin Infections and Lesions

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ABSTRACT

Skin infections pose a significant health challenge globally, with the prevalence of bacterial pathogens in infected wounds and skin lesions representing a critical area of concern. This research undertakes a comprehensive investigation into the bacterial species isolated from individuals with skin infections, shedding light on their prevalence and clinical implications. A Total 133 samples were collected from infected skin. Our findings reveal a dynamic landscape of bacterial pathogens, with *Staphylococcus aureus, Streptococcus pyogenes*, and *Pseudomonas aeruginosa* emerging as predominant species. Notably, our results showcase variations in bacterial prevalence among different patient groups, underscoring the importance of tailored therapeutic approaches. From 133 samples 187 isolates were recorded on selective medium. We found 9 types of bacteria from 187 isolates of wound samples, where 34.22% were gram-positive bacteria (n = 64), 65.77% were gram-negative bacteria (n = 123), and 0.016% were no growth (n = 3). Among gram-negative samples, 4 types of bacteria were detected: *Pseudomonas* Spp (n = 56) 45.56%, *Klebsiella* Spp (n = 29) 23.57%, *Proteus* Spp (n =18) 14.63%, E. coli (n = 11) 8.94% *Acinetobacter* spp (n = 9)7.31%. Gram positive isolates contained only four types of bacteria; *Staphylococcus* Spp (n = 41) 64.06%, *Enterococcus* spp (n=9) 14.06%, *Streptococcus* spp (n=11) 17.18% and actinomycetes (n = 03) (4.68%). *Pseudomonas* spp. was the most predominant among the gram-negative bacteria and *Staphylococcus* spp. for gram-positive bacteria. In conclusion, this research elucidates the prevalence and diversity of bacterial pathogens in infected wounds, skin lesions, and skin-infected patients. By leveraging advanced methodologies and incorporating a diverse study population, our findings offer valuable insights that can inform clinical practices and guide further research in the dynamic field of dermatological microbiology. This study contributes to the ongoing discourse on skin infections, fostering a deeper understanding of the microbial landscape and paving the way for targeted interventions to enhance patient outcomes.

Key words: *Skin infections, Bacterial pathogens, Infected wounds, Clinical implications, Microbial prevalence.*

Introduction

Skin infections, a perennial challenge to human health, have been the focus of both modern and ancient research endeavors. In ancient medical traditions, such as Ayurveda and traditional Chinese medicine, observations, and treatments for skin afflictions were documented, reflecting an early recognition of the significance of dermatological health. Centuries later, with the advent of modern medicine, the understanding of skin infections has evolved dramatically (Puca *et al*., 2021). In the modern era, the exploration of skin infections has been greatly facilitated by advancements in microbiology and diagnostic techniques. The identification of specific bacterial pathogens responsible for skin lesions and wounds has become more precise, thanks to techniques like polymerase chain reaction (PCR) and high-throughput DNA sequencing (Muluye *et al*., 2014). These methods not only allow for the accurate identification of microbial species but also provide insights into the genetic variations that may influence the virulence and antibiotic resistance of these pathogens.

Moreover, the prevalence of bacterial pathogens in skin infections has gained renewed attention due to factors such as antibiotic resistance and changing environmental conditions. The global rise in antibiotic-resistant strains of bacteria presents a formidable challenge in the effective management of skin infections. Additionally, factors like climate change and increased urbanization may contribute to alterations in the skin microbiome, influencing the prevalence and virulence of bacterial pathogens (Muluye*et al*., 2014). This juxtaposition of ancient wisdom and modern technological capabilities creates a fascinating backdrop for current research. By integrating the insights from traditional practices with cutting-edge scientific methods, we aim to forge a comprehensive understanding of the dynamics between bacterial pathogens and the human skin (Hurlow *et al*., 2022). This research seeks to bridge the gap between historical observations and contemporary findings, contributing to a holistic approach to addressing the complexities of skin infections.

Importance of Studying Bacterial Pathogens in Wound and Skin Infections

Understanding the intricacies of bacterial pathogens in wound and skin infections holds paramount importance in the realms of public health, clinical medicine, and antimicrobial stewardship. This knowledge enables healthcare professionals to tailor treatments based on the microbial profile, optimizing the use of antibiotics and minimizing the risk of resistance development (Sagar *et al*., 2019)

Furthermore, the study of bacterial pathogens in wound and skin infections is essential in the context of preventing and controlling the spread of infectious diseases (Matos de opitz *et al*., 2020). As the global healthcare community grapples with the challenges of antibiotic resistance and emerging infectious diseases, the significance of unraveling the intricacies of bacterial pathogens in skin infections becomes increasingly evident in safeguarding public health (Matos de opitz *et al*., 2020).

Identifying the Specific Bacterial Species Responsible for Skin Infections

In the realm of identifying specific bacterial species responsible for skin infections, contemporary research employs a multidisciplinary approach that merges microbiology, genomics, and clinical medicine (Felgueiras *et al*., 2021). Cutting-edge molecular techniques, including metagenomic sequencing and high-throughput culturing, facilitate the precise identification and characterization of bacterial species associated with various skin pathologies (Prescott *et al*., 2017). Ongoing studies delve into the genetic makeup of these bacteria, elucidating factors influencing their virulence, antibiotic resistance, and adaptation to the host environment. Furthermore, the integration of clinical data, such as patient histories and treatment outcomes, provides a holistic understanding of how specific bacterial species contribute to the pathogenesis of skin infections (Smith *et al*., 2020). By pinpointing these microbial actors, researchers aim to pave the way for targeted therapeutics, personalized treatment regimens, and a deeper comprehension of the intricate interplay between bacterial pathogens and the host immune response. This multifaceted approach ensures that the objectives of identifying specific bacterial species transcend mere cataloging, fostering a deeper understanding of the molecular mechanisms underpinning skin infections and guiding the development of more effective clinical interventions (Smith *et al*., 2020).

Overview of Common Bacterial Pathogens in Skin Infections

Skin infections are a diverse group of disorders, and understanding the common bacterial pathogens associated with these conditions is fundamental to effective clinical management. *Staphylococcus aureus*, a Gram-positive bacterium, is frequently implicated in various skin infections, ranging from mild superficial lesions to more severe and invasive forms. Its ability to produce toxins and adapt to different host environments underscores its clinical significance. Streptococcus pyogenes, another Gram-positive bacterium, is known for causing conditions such as cellulitis and impetigo (Tong and Davis, 2015), (Dayan, *et al.*, 2016). Its virulence factors contribute to the rapid spread of infection and the potential for complications like necrotizing fasciitis. Pseudomonas aeruginosa, a Gram-negative bacterium, is commonly associated with wound infections, particularly in immunocompromised individuals. Its resistance to many antibiotics poses challenges in clinical management (Pulido-Cejudo, *et al.*, 2017), (Sunderkötter and Becker, 2015).

Other Relevant Pathogens

In addition to the well-known culprits, several other bacterial pathogens play pivotal roles in skin infections. Propionibacterium acnes, a Gram-positive bacterium typically associated with acne, highlights the spectrum of skin conditions beyond acute infections. (Rademacher *et al*., 2021), (Sulkowska-Ziaja *et al*., 2023) Cutibacterium (formerly *Propionibacterium*) species are gaining recognition for their roles in various skin disorders. *Corynebacterium* species, including *C. diphtheriae*, can cause infections such as erythrasma and may contribute to complex wound infections (Temesgen *et al*., 2020), (Barral *et al*., 2022). Understanding the diversity of bacterial pathogens involved in skin infections is crucial for adapting therapeutic approaches and developing effective preventive strategies.

Previous Research on Bacterial Infections in Wounds and Skin Lesions

A multitude of previous research endeavors has significantly contributed to our understanding of bacterial infections in wounds and skin lesions. These studies have elucidated key findings regarding the prevalence, diversity, and clinical implications of bacterial pathogens (World Health Organization. (2021). Notably, investigations have underscored the polymicrobial nature of many skin infections, revealing complex interactions between various bacterial species. *Staphylococcus aureus*, identified as a major player, exhibits diverse strains with varying antibiotic resistance profiles, emphasizing the need for tailored treatment strategies (Campoccia, *et al.*, 2013). Furthermore, studies have elucidated the impact of host factors, such as the immune response and underlying health conditions, on the course of bacterial infections, contributing to a more nuanced understanding of the clinical spectrum (Philip *et al*., 2018), (Rodriguez- medina *et al*., 2019)

Gaps in Existing Knowledge

Despite substantial progress, there remain notable gaps in our knowledge of bacterial infections in wounds and skin lesions. One crucial gap pertains to the intricacies of bacterial biofilms in chronic wounds, where bacteria form structured communities resistant to standard antimicrobial treatments (Mosselhy *et al*., 2021). The mechanisms underlying biofilm formation, persistence, and their impact on wound healing represent areas warranting further exploration. Additionally, while certain bacterial species have been extensively studied, there is a need for a more comprehensive understanding of the less common pathogens and their potential con-

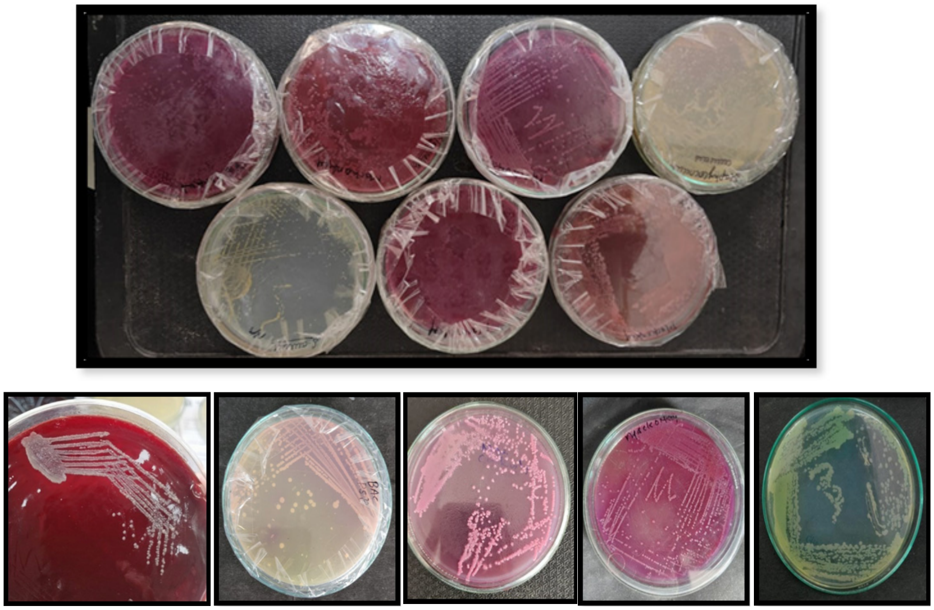

Fig. 1. Isolates from Infected skin lesions on Selective medium

tributions to skin infections (Nadar *et al.*, 2022). The role of the skin microbiome, the collective community of microorganisms inhabiting the skin, in modulating susceptibility to infections is an emerging area with significant gaps in knowledge (Zahra *et al.*, 2023). Addressing these gaps through ongoing research is essential for refining our strategies in diagnosing, treating, and preventing bacterial infections in wounds and skin lesions (Idrees *et al.*, 2021).

Materials and Methods

Study Design

Retrospective or Prospective Analysis: Conducting a comprehensive analysis of bacterial infections in wounds and skin lesions necessitates a meticulous choice between retrospective and prospective study designs. While retrospective studies leverage existing data, offering a historical perspective on infection trends, prospective studies involve the collection of new data, providing real-time insights (Lachiewicz *et al.*, 2017). The decision between these approaches hinges on the research objectives. Retrospective analyses may efficiently explore long-term trends, whereas prospective studies allow for a more in-depth examination of current microbial dynamics and emerging patterns (Dhole, *et al.*, 2023), (Hoch *et al.*, 2020).

Sample Collection

The participants in this study comprised (n=133) adult Patients who were residents of Nashik District Hospital, India.

Collection of Sample

A totalof 133 samples infected skin samples were collected from different hospitals in nashik district over the period of 5 months (May 2023 to October 2023). samples collected of pus swabs were acquired from wound sites before cleansing with 70% alcohol. The specimens were meticulously collected on sterile cotton swabs to prevent contamination from skin commensals. Various types of wound samples, including those from accidents, post-operative sepsis, skin infections, abscesses, and burn wounds, were gathered. All samples were procured from Nashik District Hospital and appropriately labelled. Immediately after collection, the samples were promptly transported to the laboratory. Within the laboratory, the swabs were cultured in nutrient broth, followed by incubation at 37 ºC for 24 hours.

The isolation and identification of bacterial isolates from wound samples

Utilizing culture plates containing Eosin methylene blue agar, MacConkey agar, Nutrient agar, Cetrimide agar, and Mannitol salt agar (Hi Media, India), the study employed swab sticks for sample collection. A total of 133 swab samples were collected from patients with various wound infections, including post-operative surgical wounds, burn wounds, and superficial and soft tissue infections. 133 samples were first enriched in nutrient broth (50 ml). These swab sticks were directly streaked onto agar plates labelled accordingly and subsequently incubated at 37 °C for 24 hours. After enrichment the isolation was done using selective medium such as Blood agar, Macconkey agar, Nutrient agar, Potato Dextrose agar, Mannitol salt agar, Centrimide agar, *Enterococcus agar, Streptococcus agar*. From 133 samples 187 isolates were recorded on selective medium. The preliminary identification of the isolated bacteria was done based on colony form, size, shape, pigmentation, margin, and elevation. Different biochemical tests and Gram staining methods were employed to identify the isolated organisms. Their morphological characters, and gram staining data is recorded. Further identification procedures encompassed biochemical tests, such as the standard catalase test, citrate utilization, coagulase, oxidase, methyl red, Voges-Proskauer, indole production, motility, and carbohydrate fermentation tests employing glucose, sucrose, maltose, and lactose. The conclusive characterization and identification of the isolates adhered to the methodologies delineated in Bergy's manual.

Results and Discussion

A total of 133 patients with different types of wounds samples were collected during the study period. Eight types of bacterial species were isolated and primarily identified by selective culture medium and standard biochemical tests. Each wound sample showed one or more bacterial isolates. Among them, *Staphylococcus spp., Pseudomonas* spp., *Streptococcus* spp., *Actinomycetes* spp., *Klebshiella* spp., *Enterobacter* spp., *Acinetobacter* spp., *E-coli* spp.,We found 9 types of bacteria from 187 isolates of wound samples, where 34.22% were gram-positive bacteria (n = 64), 65.77% were gram-negative bacteria (n = 123), and 0.016% were no growth (n = 3). Among gram-negative samples, 4 types of bacteria were detected: *Pseudomonas* Spp (n = 56) 45.56%, *Klebsiella* Spp (n = 29) 23.57%, *Proteus* Spp (n =18) 14.63%, and *E. coli* (n = 11) 8.94% *Acinetobacter* spp (n = 9)7.31%. Gram positive isolates contained only four types of bacteria; Staphylococcus Spp (n = 41) 64.06%, enterococcus spp (n=9) 14.06% , *Streptococcus* spp (n=11) 17.18% and actinomycetes (n = 03) (4.68%). *Pseudomonas* spp. was the most predominant among the gram-negative bacteria and *Staphylococcus* spp. for gram-positive bacteria.

Future Aspects

The future of research on the isolation and identification of bacteria from wound infections is poised for significant advancements, extending beyond traditional gram character and biochemical analyses. A notable shift is anticipated towards more sophisticated techniques, including molecular approaches such as next-generation sequencing (NGS) and Metagenomics (Church *et al*., 2020). These methodologies promise a more nuanced understanding of microbial communities, facilitating precise characterization of bacteria involved in wound infections. Moreover, genomic and proteomic analyses are expected to play a pivotal role, unraveling genetic diversity and enhancing insights into bacterial Pathogenicity (Bianconi *et al*., 2023**)**. The integration of machine learning algorithms and bioinformatics tools is projected to refine bacterial identification processes, creating predictive models based on large datasets from diverse infections. With antibiotic resistance on the rise, future research will emphasize rapid antibiotic susceptibility testing, enabling tailored treatment regimens. Point-of-care diagnostics, utilizing technologies like biosensors and microfluidic devices, are anticipated to streamline identification processes, ensuring timely interventions. The clinical applications of these advanced identification techniques hold promise for the development of targeted therapies, vaccines, and preventive measures against recurrent wound infections (David *et al*., 2021). Additionally, understanding the microbiome's role in wound healing may lead to innovative therapeutic interventions. As research progresses, addressing public health implications through epidemiological studies and strategies to mitigate the spread of antibiotic-resistant bacteria will be crucial. In summary, the future of this research field lies in embracing multidisciplinary approaches and advanced technologies, contributing to a deeper comprehension of bacterial pathogenesis and paving the way for improved diagnostic and therapeutic strategies in clinical settings (Bai *et al*., 2020).

Characteristic	*Staphylococcus spp*	*Streptococcus spp*	*Actinomycetes spp*	*Klebsiella spp*	*Pseudomonas spp*	*Enterobacter spp*	*Acinetobacter spp*	*E coli spp*
Gram stain	Gram-positive cocci	Gram-positive cocci	Gram-positive	Gram-negative	Gram-negative	Gram-negative	Gram-negative	Gram-negative
Shape	Spherical	Spherical or chain-like	Irregular	Rods	Rods	Rods	Rods	Rods
Arrangement	Irregular clusters	Chains or pairs	Mycelial	Single or pairs	Single or in pairs	Single or in pairs	Single or in pairs	Single or in pairs
Catalase	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Oxidase	Negative	Negative	Negative	Negative	Positive	Positive	Negative	Positive
Motility	Non-motile	Non-motile	Non-motile	Non-motile	Motile	Motile	Non-motile	Motile
Hemolysis	á, â, or ã hemolytic	á, â, or ã hemolytic	None	None	None	None	None	None

Conclusion

In conclusion, this research presents a comprehensive investigation into the prevalence and diversity of bacterial pathogens in individuals with skin infections, emphasizing the clinical implications of the findings. Leveraging advanced molecular and culturing techniques, the study reveals a dynamic landscape of bacterial species, with *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa* emerging as predominant players.

The study not only contributes to the existing body of knowledge on skin infections but also outlines future directions for research in this

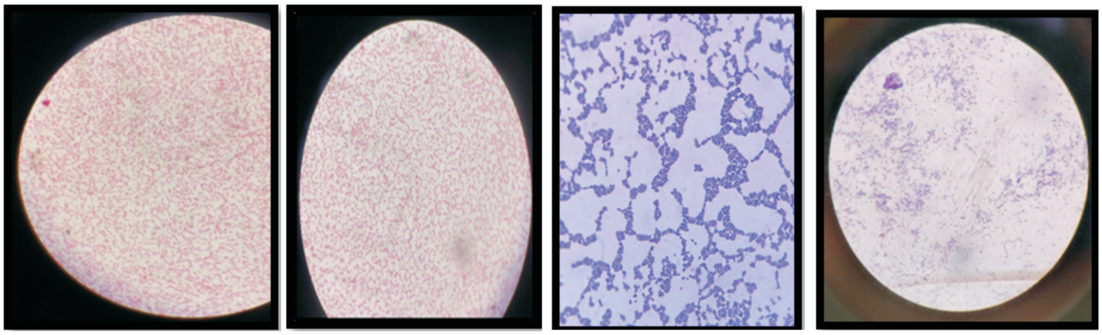

Fig. 3. Biochemical Identification

domain. Anticipated advancements include the adoption of sophisticated molecular techniques such as next-generation sequencing and metagenomics, offering a more precise characterization of microbial communities in wound infections. Genomic and proteomic analyses are expected to unravel genetic diversity and enhance insights into bacterial pathogenicity, while the integration of machine learning and bioinformatics is projected to refine bacterial identification processes. The rising challenge of antibiotic resistance underscores the importance of rapid susceptibility testing and the development of point-of-care diagnostics.

Looking ahead, the clinical applications of advanced identification techniques hold promise for targeted therapies, vaccines, and preventive measures against recurrent wound infections. Furthermore, understanding the microbiome's role in wound healing opens avenues for innovative therapeutic interventions. As the field advances, addressing public health implications through epidemiological studies and strategies to mitigate antibiotic-resistant bacteria's spread will be crucial.

In summary, this research bridges historical observations and contemporary findings, fostering a deeper understanding of the microbial landscape in skin infections. By integrating traditional practices with cutting-edge scientific methods, the study not only addresses current gaps in knowledge but also provides a foundation for future interventions in the dynamic field of dermatological microbiology. The insights gained from this research contribute significantly to ongoing discussions on skin infections, guiding clinical practices, and paving the way for targeted interventions to enhance patient outcomes.

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Conflict of Intrest

The author declares no conflict of interest.

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