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Microbial Profiling and Resistance Patterns in Chronic Non-Healing Wounds Using Next Generation Sequencing

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ABSTRACT

Chronic non-healing wounds, including diabetic foot ulcers, pressure ulcers, and surgical site infections, pose a significant clinical challenge due to their persistent microbial colonization and rising antimicrobial resistance. In this study, 56 samples from such wounds were subjected to next-generation sequencing using the Oxford Nanopore GridION platform. Dominant pathogens included Pseudomonas aeruginosa, Staphylococcus aureus, Acinetobacter baumannii, Enterococcus faecalis, and Klebsiella pneumoniae. Key resistance genes detected were mecA, vanA, blaOXA-23, and blaCTX-M-15. The results revealed polymicrobial communities with notable resistance determinants, underscoring the limitations of culture-dependent methods. This study highlights the clinical utility of metagenomic profiling for pathogen detection and resistance prediction. NGS tools offer enhanced diagnostic accuracy, facilitating targeted antimicrobial therapy and better infection control strategies in chronic wound care.

Keywords: Chronic Wounds, Next-Generation Sequencing, Antimicrobial Resistance, Gridion, Metagenomics

INTRODUCTION

Chronic wounds, particularly those affecting individuals with diabetes [1], peripheral vascular disease, or limited mobility, are a significant global health concern. These wounds, such as diabetic foot ulcers (DFUs), pressure ulcers, and surgical site infections, often fail to progress through the typical stages of wound healing and remain open for prolonged periods. The chronicity of these wounds is frequently compounded by microbial colonization, leading to local infection, delayed healing, and, in severe cases, systemic involvement and amputation. In India, the prevalence of chronic wounds has risen steadily due to the increasing burden of diabetes and other lifestyle-related conditions, placing an enormous strain on healthcare infrastructure. One of the most challenging aspects of managing chronic wounds is the accurate identification and treatment of wound-associated infections. These infections are often polymicrobial in nature, involving both aerobic and anaerobic bacteria, and are frequently embedded in biofilms that shield them from host defenses and antibiotics. Traditional microbiological methods, based on aerobic culturing, often fall short in identifying the full spectrum of pathogens present, particularly those that are fastidious, slow-growing, or viable but non-culturable. As a result, clinicians are often forced to rely on empirical broad-spectrum antibiotic regimens, which can drive the emergence of antimicrobial resistance (AMR) and further complicate treatment outcomes.

In recent years, molecular diagnostic methods have gained prominence for their ability to overcome the limitations of culture-based techniques. Among these, Next Generation Sequencing (NGS) stands out as a transformative tool that allows for culture-independent, high-throughput profiling of microbial communities. Unlike conventional methods that detect only a subset of organisms, NGS captures a comprehensive snapshot of the wound microbiota, including rare or uncultivable species. Moreover, NGS can identify antimicrobial resistance genes directly from clinical samples, providing insight into the resistome—the full complement of resistance determinants within a microbial community.

Oxford Nanopore Technologies' GridION platform has emerged as a powerful NGS tool that offers long-read sequencing, real-time data acquisition, and high scalability. These advantages make it particularly well-suited for clinical microbiology applications, including the profiling of chronic wound infections. By targeting the 16S rRNA gene and concurrently analyzing resistance genes through metagenomic sequencing, GridION enables simultaneous identification of pathogens and prediction of phenotypic resistance patterns.

This study was designed to investigate the microbial diversity and resistance gene profiles in chronic non-healing wounds using the GridION NGS platform in an Indian clinical context. We aimed to characterize the dominant bacterial species colonizing chronic wounds and to detect key resistance determinants such as mecA, blaOXA-23, vanA, and blaCTX-M. Understanding the distribution of these organisms and their resistance mechanisms is critical not only for guiding targeted antimicrobial therapy but also for improving infection control and stewardship strategies in wound management.

By integrating cutting-edge genomic tools into routine diagnostic workflows, this study underscores the potential of NGS to bridge the diagnostic gap in chronic wound care and support evidence-based, precision-guided interventions.

MATERIALS AND METHODS

This study analyzed 56 clinical samples from patients with chronic non-healing wounds (>4 weeks), including wound swabs, pus, surgical wounds, and pressure ulcers. Patients unresponsive to empirical antibiotics were included after obtaining ethical clearance. DNA Sequenced on the Oxford Nanopore GridION platform. Resistance genes were identified using the CARD database, enabling comprehensive profiling of microbial diversity and antimicrobial resistance within chronic wound environments.

RESULTS

A total of 56 clinical samples from chronic non-healing wounds—including diabetic foot ulcers, surgical wounds, pressure ulcers, and pus discharges—were subjected to Next Generation Sequencing (NGS) using the Oxford Nanopore GridION platform. The microbial profile revealed a polymicrobial landscape, predominantly colonized by both Gram-positive and Gram-negative organisms. Pseudomonas aeruginosa was the most frequently detected organism, found in 33% (n=18) of the samples. This was followed by Staphylococcus aureus (30%, n=17), Klebsiella pneumoniae (14%, n=8), Acinetobacter baumannii (15%, n=8), and Enterococcus faecalis (8%, n=4). Each of these organisms harbored critical antimicrobial resistance genes. For instance, S. aureus carried the mecA gene, confirming methicillin resistance (MRSA), while P. aeruginosa demonstrated fluoroquinolone resistance due to mexAB-oprM efflux pump expression and gyrA mutations. A. baumannii was found with blaOXA-23, a marker for carbapenem resistance. E. faecalis carried vanA, indicating vancomycin resistance, and K. pneumoniae was positive for blaCTX-M-15 and blaSHV, identifying it as an extended-spectrum beta-lactamase (ESBL) producer. These findings reflect a high prevalence of multidrug-resistant organisms in chronic wounds, emphasizing the importance of molecular diagnostics in guiding antimicrobial therapy.

DISCUSSION

The most frequently isolated organism was Pseudomonas aeruginosa, identified in 33% [3] (n=18) of the wound samples. Resistance gene analysis revealed the presence of the mexAB-oprM efflux pump system and mutations in the gyrA gene. Staphylococcus aureus was the second most prevalent pathogen [3], present in 30% (n=17) of samples, with the mecA gene detected, confirming methicillin resistance (MRSA).

Acinetobacter baumannii was isolated in 15% (n=8) of samples and was associated with the blaOXA-23 gene, indicative of carbapenem resistance. Enterococcus faecalis, detected in 8% (n=4), harbored the vanA gene indicating vancomycin resistance. Klebsiella pneumoniae, found in 14% (n=8), carried both blaCTX-M-15 and blaSHV resistance genes, confirming its ESBL phenotype.

Compared to conventional culture methods, NGS provides [6] a high-throughput and unbiased view of microbial diversity and resistomes. The GridION platform’s long-read sequencing offers real-time results and is suited for identifying closely related species and resistance genes [7]. The study’s findings underscore the potential of integrating NGS into wound care diagnostics, especially in the Indian context [8] where chronic wounds are prevalent and antibiotic misuse is common [9].

By identifying pathogens and their resistance genes directly from wound samples, clinicians can adopt more targeted antimicrobial therapy, reducing reliance [10] on empirical regimens and improving patient outcomes.

CONCLUSION

This study demonstrates the utility of Oxford Nanopore GridION-based next-generation sequencing for comprehensive identification of microbial pathogens and their resistance determinants in chronic non-healing wounds. The detection of key resistance genes such as mecA, blaOXA-23, vanA, and blaCTX-M-15 highlights the importance of culture-independent diagnostics in wound management. Integrating NGS into clinical workflows may significantly enhance diagnostic accuracy, promote targeted antimicrobial therapy, and support antimicrobial stewardship initiatives in high-burden settings like India.

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Table No 1. Microbial Distribution and Resistance Summary ***(Table showing the organisms, resistance genes, phenotypic predictions, and methods used)***

Sl No	Organism Isolated	%	Sample No.	Resistance Genes Detected	Predicted Resistance	Common Site of Isolation	Method of Isolation
1	Staphylococcus aureus	30%	17	mecA	MRSA (Methicillin-resistant)	Wound swabs, pus wounds, surgical wounds, pressure ulcers	NGS ASSAY, GridION
2	Pseudomonas aeruginosa	33%	18	mexAB-oprM, gyrA mutations	Fluoroquinolone resistance, efflux-mediated	Wound swabs, pus wounds, surgical wounds, pressure ulcers	NGS ASSAY, GridION
3	Acinetobacter baumannii	15%	8	blaOXA-23	Carbapenem-resistant A. baumannii	Wound swabs, pus wounds, surgical wounds, pressure ulcers	NGS ASSAY, GridION
4	Enterococcus faecalis	8%	4	vanA	VRE (Vancomycin-resistant Enterococcus)	Wound swabs, pus wounds, surgical wounds, pressure ulcers	NGS ASSAY, GridION
6	Klebsiella pneumoniae	14%	8	blaCTX-M-15, blaSHV	ESBL-producing Klebsiella pneumoniae	Wound swabs, pus wounds, surgical wounds, pressure ulcers	NGS ASSAY, GridION