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Development and validation of a reference marker for identification of aerobic and anaerobic bacteria associated with diabetes chronic wound ulcers using PCR denaturing gradient gel electrophoresis

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Introduction: Diabetes chronic wounds consist with a diverse microbial community and unculturable species may be highly prevalent.

Objectives: This study aimed to establish a bacterial reference marker consisting of a group of chronic wound related bacteria, using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) for profiling of bacteria in diabetes chronic wound infections.

Methods: DNA was extracted from the known wound bacterial strains. PCR–DGGE was performed using eubacterial specific primers targeting V2-V3 region of 16S rDNA. DGGE was performed using a 30-55% denaturing gradient. Migration position of each organism was detected on DGGE gel and important organisms were selected. Equal volume from PCR products of each selected organism was mixed, diluted with gel loading dye in 1:1.5 ratio and used for all DGGE gels. The ladder was then subjected to species identification of fifteen tissue debridement specimens obtained from diabetes chronic wound ulcers. The identification efficacy was tested by sequencing.

Results: DNA of bacterial pathogens which showed different migration distances on the gel were combined and used as a reference panel. This bacterial ladder consisted of eleven different bacterial species including *Bacteroides* sp., *S. aureus*, *Acineto bacter* sp., *P. aeruginosa*, S*treptococcus* Group A and Group B sp., *E. faecalis*, *Providencia* sp., *Veillonella* sp., *E .coli* and *Enterobacter* sp. According to the reference panel, *Pseudomonas species* were abundant. Further the results were confirmed by sequencing.

Conclusion: Reference marker allows comparative analysis of DGGE patterns and can be used as a tool for presumptive identification of polymicrobial microbiota in chronic wound infections.