OP 8

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*, *Streptococcus* 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.

OP 9

Impact of routine laboratory culture media on *in-vitro* biofilm formation of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus feacalis*

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Objectives: This study was aimed to determine the efficacy of four routine laboratory culture media on biofilm formation of *Pseudomonas aeruginosa, Staphylococcus aureus* and *Enterococcus feacalis*.

Methods: A sterile flat bottom 96 well plate was inoculated using 0.5 McFarland equivalent standard cell suspension of *P. aeruginosa, S. aureus* and *E. feacalis* and the growth rate of planktonic cells was quantified by measuring the optical density (OD492) at two hour intervals. Influence of culture medium on adhesion of bacteria as an initial step of biofilm formation in the presence of four culture media