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Basic science: biofilm pathophysiology

POLYMICROBIAL BIOFILMS BY DIABETIC FOOT CLINICAL ISOLATES

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Objectives:

Diabetes mellitus is a major chronic disease that continues to increase significantly. One of the most important and costly complications of diabetes are foot ulcers that may be colonized by pathogenic and resistant bacteria, which may harbor several virulence factors that can impair its successful treatment. We aimed to evaluate the potential of diabetic foot clinical isolates to form polymicrobial biofilms, since this virulence factor can be responsible for diabetic foot ulcer (DFU) chronicity.

Methods:

Biofilm production by 95 DFU isolates belonging to several bacterial genera, including *Staphylococcus, Corynebacterium*, *Enterococcus*, *Pseudomonas* and *Acinetobacter,* was quantified at three time points (24, 48 and 72 hours) using a microtiter plate assay with resazurin (Pettit et al., 2005). The potential to form polymicrobial biofilms, by bacteria isolated from the same individual, was evaluated using mixed suspensions by this method and a Fluorescent *In Situ* Hybridisation (FISH) assay (Oliveira et al., 2007).

Results:

The study revealed that all isolates produced biofilm at 24 hours and that biofilm forming-ability increased with incubation time. Significant differences were found in biofilm formation between the three time points. *Pseudomonas* was the bacterial genera with a higher biofilm production followed in descending order by *Corynebacterium*, *Acinetobacter*, *Staphylococcus* and *Enterococcus.*

When incubated together, isolates from the same individual produced higher biofilm values. Mixed suspensions including *Pseudomonas*+enterococci, *Acinetobacter*+staphylococci, *Corynebacterium*+staphylococci produced higher biofilm values. However, no significant differences were found between biofilm production for all the mixed suspensions tested.

Conclusions:

To our knowledge this is the first study regarding polymicrobial biofilm formation by bacterial diabetic foot isolates that includes a large variety of bacterial species. Our study is in agreement with previous reports on the occurrence of synergy in biofilm formation between species present in polymicrobial communities. The enhanced biofilm biomass formed by multi-species bacterial communities may be due to quorum-sensing systems and enzyme complementation.

Biological behavior of different bacterial species in these mixed biofilms has important clinical implications for treating effectively biofilm infections, showing that further research is necessary for better understanding these complex communities.

References:

Oliveira M. et al. Time course of biofilm formation by *Staphylococcus aureus* and *Staphylococcus epidermidis* mastitis isolates. Veterinary Microbiology 2007. 124, 187–19.
Pettit R. K. et al. Microplate Alamar blue assay for Staphylococcus epidermidis biofilm susceptibility

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