Polymicrobial biofilms by diabetic foot clinical isolates

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Introduction

Diabetes *mellitus* is a major chronic disease that continues to increase significantly. One of its most important and costly complications are diabetic foot ulcers (DFU) that may be colonized by pathogenic and antimicrobial resistant bacteria, which could impair its treatment. We evaluated the influence of polymicrobial communities in the ability of DFU isolates to produce biofilms, since this virulence factor can be responsible for ulcer chronicity.

Results

All isolates produced biofilm at 24 h and biofilm forming-ability increased with incubation time (Chart 1). Significant differences were found in biofilm formation between the 3 time points (P≤0.05). When incubated together, bacterial isolates from the same individual produced higher biofilm values (Chart 2); however, no statistically significant differences were found between biofilm formation by these polymicrobial communities (P>0.05). Heterogeneous multi-species biofilms were detected with MFISH (Figure).

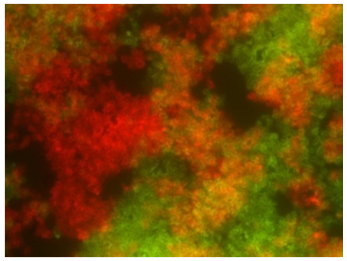

Figure. *Corynebacterium* (green) + *Staphylococcus* (red) biofilm at 24h (x1000)

References

1. Pettit et al 2005 Antimicrob Agents Chemoter, 49:2612-2617.
2. Oliveira et al 2007 Vet Microbiol, 124:187–219.

Materials and Methods

A microtiter plate assay with resazurin (1) and a Multiplex Fluorescent *In Situ* Hybridization protocol (MFISH) (2) were applied to polymicrobial communities at 24, 48 and 72 h, after first evaluating biofilm formation by 95 DFU isolates belonging to the genera *Staphylococcus* (n=57), *Corynebacterium* (n=15), *Enterococcus* (n=12), *Pseudomonas* (n=7) and *Acinetobacter* (n=4). Polymicrobial communities tested (n=34) were formed by clinical isolates obtained from the same DFU sample and included the following combinations: *Corynebacterium* + *Staphylococcus* (n=14), *E. faecalis* + *Staphylococcus* (n=7), *P. aeruginosa* + *Enterococcus* (n=5), *Acinetobacter* + *Staphylococcus* (n=4), *E. faecalis* + *Corynebacterium* (n=4).

Statistical analysis of the plate assay results was performed (SPSS 19.0) to evaluate the significance of the increase in biofilm production with time, by individual isolates and to compare biofilm production by individual isolates and dual-species communities.

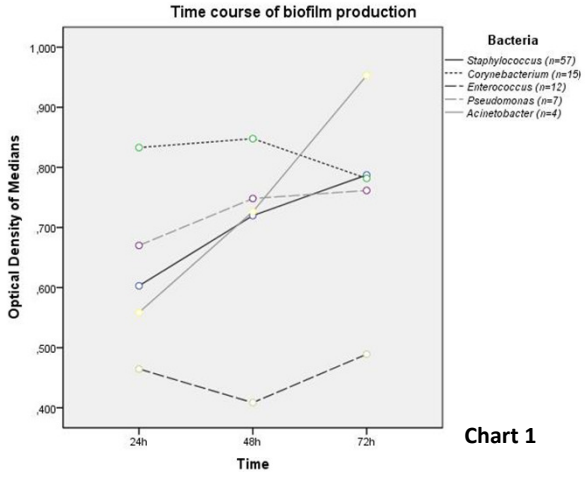

Chart 1

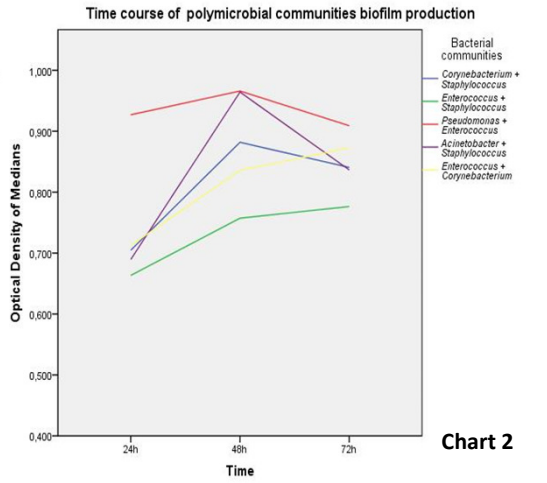

Chart 2

Conclusions

This is the first report on time course biofilm formation by polymicrobial communities from DFU that include several species. Results are in accordance with previous reports on the occurrence of synergy in biofilm formation by multi-species communities. The enhanced biofilm biomass may be due to quorum-sensing and enzyme complementation. Species biological behavior in such biofilms might have important implications for therapeutic success in treating chronic ulcers.

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