



45° CONGRESSO NAZIONALE DELLA SOCIETÀ ITALIANA DI MICROBIOLOGIA

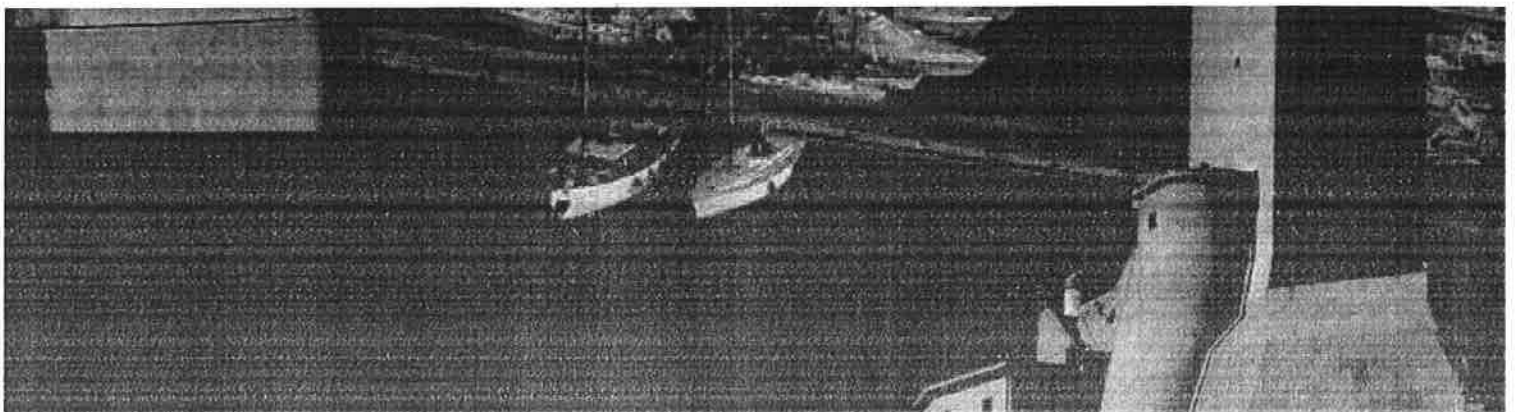
SIM 2017

Genova, Palazzo Ducale

27 - 30 settembre 2017



Abstract Book



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EFFECTS OF DIFFERENT MOUTHWASHES ON BIOFILM FORMATION BY ORAL STREPTOCOCCI

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Introduction: Oral microbiota is an extremely complex and dynamic system including bacteria, archaea and fungi. Different locations of the oral cavity host a large variety of microbiota mostly organized as biofilm, characterized by spatial and temporal differences in their distribution. Several bacteria belonging to the genus *Streptococcus* act as early colonizers, binding to the adhesive pellicle and providing a substrate for the attachment of late colonizers bacteria. The latter, in turn, may be responsible of severe pathologies, such as carious lesions, gingivitis and periodontal lesions. Therefore, we evaluated the effects of commercial mouthwashes (MoWs) on biofilm (BF) formation/persistence of early colonizers Streptococci.

Materials and methods: Fourteen isolates belonging to 5 different species of oral streptococci (*S. salivarius*, *S. mitis/oralis*, *S. sanguinis*, *S. parasanguinis*, *S. vestibularis*) and 3 isolates of *Enterococcus faecalis* were obtained from pharyngeal swabs and employed for the present study. All the bacteria were incubated for 1 minute with 6 commercial MoWs, 4 with (MoWs 1, 2, 3 and 7) and 2 without (MoWs 4 and 5) chlorhexidine digluconate (CHX), which is known to exert antibacterial and antifungal activity. Control groups of each strain were treated for 1 minute with PBS and used as negative controls. After treatment with MoWs or PBS, bacteria were seeded in 96-well plates and allowed to form BF for up to 48 hours. BF formation was assessed by crystal violet assay. The capacity to form BF was expressed as the optical density (OD) percentage of each MoW-treated strain, as compared to the OD of the PBS-treated strain, which was considered as 100%. Statistical analyses were carried out by one-way ANOVA test with Bonferroni post-hoc test.

Results: CHX-containing MoWs were capable to inhibit BF formation in most of the cases. In detail, CHX-containing MoWs significantly reduced BF formation by all the *S. salivarius* and *E. faecalis* isolates, while only several *S. parasanguinis*, *S. mitis/oralis*, *S. sanguinis* and *S. vestibularis* isolates

were affected. One of the 2 CHX-free MoWs was capable to significantly inhibit BF formation by *S. salivarius* and *S. vestibularis*, whereas the remaining CHX-free MoW did not prove to be effective in impairing BF formation by any of the bacterial isolates assessed.

Discussion and conclusions: Similarly to *Candida albicans* (1), BF formation by oral streptococci is affected by MoWs, provided that they include CHX in their formulation. Since the streptococci used in the present study act as early colonizers in the multispecies microbial BF of the dental surface, special attention should be used when choosing MoWs for prevention and/or treatment of oral pathologies of microbial origin.

References

1. Paulone et al. New Microbiol 2017.