Evaluation of the in vitro activity of the gentamicin-collagen sponge against small colony variants of coagulase-negative staphylococci isolated from prosthetic joint infections

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Background: Prosthetic joint infections associated with staphylococcal small colony variant strains (SCVs) present not only a diagnostic challenge but they also constitute a therapeutic problem. SCVs are often auxotrophic for haemin and/or menadione involved in the biosynthesis of electron transport chain components. The resultant defects in electron transport and altered membrane potential may confer on these variants innate resistance to aminoglycosides.

The aim of the study was to evaluate the in vitro effectiveness of a gentamicin-collagen (GM-C) sponge against 15 SCV isolates of coagulase-negative staphylococci (CNS) cultured from 7 patients with prosthetic hip joint infections.

Material/methods: The SCVs were cultured from the sonicate fluid samples and/or from periprosthetic tissue samples collected intra-operatively. All SCVs were auxotrophic for haemin; one isolate was additionally auxotrophic for menadione.

Susceptibility of the isolates to gentamicin (GM) was evaluated using E-tests (bioMérieux, France) according to recommendations of the European Committee on Antibiotic Susceptibility Testing (EUCAST).

The in vitro effectiveness of the GM-C sponge was evaluated using a pilot study whose methodology was based on the diffusion of the drug to the Mueller-Hinton II Agar (M-HA) pre-inoculated with a standardized suspension of each tested SCV isolate (0.5 and 2 in the MacFarland/McF scale). The GM-C sponge (COLLATAMP®EG) of 1 x 1 x 0.5 cm (GM content: 1.3 mg/cm³) was placed in the middle of each M-HA plate. After incubation at 35°C for 48/96 hours under microaerophilic conditions the zone of inhibition (ZOI) of bacterial growth around each sponge was measured. Each SCV isolate was tested in duplicate.

Results: 4 out of the 15 tested SCVs were GM susceptible (MIC values: 0.094-0.38 µg/ml). The 11 GM resistant SCVs demonstrated MIC values between 38- =>256 µg/ml.

The GM-C sponge assay revealed the development of ZOI in 12 (80%) SCVs. The largest ZOI (56-57 mm at 0.5 McF and 50-53 mm at 2 McF) were observed for GM susceptible isolates (MIC: 0.094-0.38 µg/ml). ZOI ranging between 25-37 mm (0.5 McF) and 22-37 mm (2 McF) were observed for GM-resistant isolates (MIC: 24 - 256 µg/ml). ZOI were not observed around GM-C sponges placed on the MH-A inoculated with 3 SCV isolates whose GM MICs were > 256 µg/ml, irrespective of the density of the suspension used.
**Conclusion:** The results of our study indicate potential effectiveness of the GM-C sponge against SCVs even those demonstrating high GM MIC values (24-256 µg/ml). Nevertheless, the obtained results cannot be extrapolated to the *in vivo* conditions. The tendency of SCVs to persist intracellularly and their potential ability to produce biofilms are significant limitations in assessment of the potential clinical usefulness of the GM-C sponge in case of infections associated with SCVs. Hence, further studies are required.