Influence of penicillin on biofilm formation by *Streptococcus agalactiae* serotype Ia/CC23

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BACKGROUND AND AIM

*Streptococcus agalactiae* (Group B Streptococcus, GBS) is a major agent of perinatal infections. Biofilms have been associated with GBS colonization and disease, as well as with infection persistence and recurrence, leading to treatment failure. Although GBS remains susceptible to beta-lactams (including penicillin), it is still unknown how sessile cells present in biofilms respond to these antibiotics. Serotype Ia/CC23 is one of the versatile GBS lineages that lack host tropism or clinical specificity, being recovered from carriages and diseases in humans, as well as in animals. Moreover, serotype Ia/CC23 GBS strains isolated from human oropharynx and urine specimens in Brazil were previously characterized as strong biofilm producers (Alvim et al. 2019). Here we evaluated the effect of different concentrations of penicillin (3-48 mg/L) on in vitro biofilm formation by four GBS strains belonging to the versatile serotype Ia/CC23 lineage that were recovered from the oropharynx or urine of pregnant women.

METHODS

1. **Bacterial strains**
   - 4 GBS serotype Ia/CC23 strains characterized as strong biofilm producers

2. **Penicillin minimal inhibitory concentration (MIC):** antibiotic gradient strips (Liofilchem™ MIC Test Strips; CLSI, 2020).

3. **Assessment of biofilm prevention by penicillin:** Alvim et al., 2019; five different penicillin concentrations (3, 6, 12, 24 and 48 mg/ml) were tested.

4. **Confocal laser scanning microscopy (CLSM) of biofilms:** Biofilms formed by strain Sag 010 in the absence and presence of 48 mg/ml of penicillin were stained with BacLight™ BacLight™ Bacterial Viability Kit (Invitrogen and analyzed by CLSM (Leica TCS-SPI). Data were analyzed using ImageJ and Comstat2.

5. **Statistical analyses:** one-way ANOVA followed by Tukey’s multiple comparisons test or the two-way ANOVA with Bonferroni posttests. (GraphPad Prism v5.0). p<0.05.

RESULTS

**Figure 1.** Mean absorbance values (as measured by the crystal-violet staining method at 570 nm) of biofilms formed in 96-well microtitre plates by four Group B Streptococcus isolates: A, Sag 010; B, Sag 025; C, Sag 033; D, Sag 083, including control (without penicillin) and test wells (with five different penicillin concentrations). **p<0.01 and *** p<0.001.

**Figure 2.** Biomass (A) and thickness (B) with corresponding live and dead cells of biofilms formed by the Group B Streptococcus strain Sag 010 in control (without penicillin) and test wells (with 48 mg/ml of penicillin). Analysis were performed by confocal laser scanning microscopy after staining with LIVE/DEAD™ BacLight™ Bacterial Viability Kit (Invitrogen) using imaging software and COMSTAT plug-in. *p<0.05 and ** p<0.01.

**Figure 3.** Biofilms formed by the Group B Streptococcus strain Sag 010 visualized by confocal laser scanning microscopy after staining with LIVE/DEAD™ BacLight™ Bacterial Viability Kit (Invitrogen) in control (without penicillin) wells (A) and test (with 48 mg/ml of penicillin) wells (B). Live cells are colored in green and dead cells are colored in red. Cocci chains and channels are indicated by purple and blue arrows respectively.

CONCLUSION

Overall, results highlight concerning possible impacts of biofilm formation in penicillin-based treatment and preventive strategies of GBS infections, which can shed light into the involvement of this type of community with treatment failure, recurrent infections and intermittent colonization even when the bacterial strain involved is fully antibiotic-susceptible.