**Antimicrobial Activity of Penicillin G and N-acetylcystein on Planktonic and Sessile Cells of *Streptococcus suis***

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**Abstract**

The aim of this study was to investigate the capacity of *Streptococcus suis* strains to form biofilms and to evaluate the antimicrobial activity of Penicillin G and N-acetylcystein (NAC) on both *S. suis* sessile and planktonic forms. Only non-typeable isolates of *S. suis* were correlated with a greater biofilm formation capacity. The MCI of Penicillin G and NAC required for inhibiting biofilm growth were higher than the required concentration for inhibiting planktonic growth. The combinations of NAC and Penicillin G showed a strong synergistic activity that inhibited biofilm formation and disrupted the pre-formed biofilm of *S. suis*.

**Key words:** *Streptococcus suis*, biofilm, N-acetylcystein, Penicillin G
by (Marois et al., 2007). Serotyping of S. suis was performed by a coagglutination test as previously described (Higgins and Gottschalk, 1990).

The biofilm formation was screened using the conditions previously described (Grenier et al., 2009). Briefly, S. suis strains were cultured in Todd Hewitt Broth (THB) until the late-log phase (1 × 10⁸ CFU · ml⁻¹). Then, aliquots of 0.05 ml were added into wells containing the minimal medium (MM). The adherent cells in the wells were quantified measuring the optical density (OD) at λ = 492 nm using a microtiter plate reader (SUMA, PR-621, Cuba).

The antibacterial activity of Penicillin G and NAC on planktonic cells were determined using the broth microdilution method according to the Clinical and Laboratory Standards Institute, document M100-S18 (CLSI, 2008). Briefly, serial two-fold dilutions of Penicillin G (500–0.5 μg · ml⁻¹) and NAC (16–0.016 mg · ml⁻¹) were respectively prepared in THB. Then, aliquots corresponding to a cell concentration of approximately 5 × 10⁸ CFU · ml⁻¹ were inoculated into wells containing each antimicrobial. After incubation at 37°C for 24 h, the absorbance at 620 nm was determined.

The effect on biofilm formation was evaluated as described previously, but the dilutions of both antimicrobials (Penicillin G and NAC) were prepared in MM. Each well was inoculated with a final concentration of approximately 5 × 10⁸ CFU · ml⁻¹. The antibacterial activity of Penicillin G in combination with NAC (P + NAC) was investigated as previously described (Mackay et al., 2000). Two concentrations of NAC (4 and 8 mg · ml⁻¹) and 10 fold dilutions (500–0.5 μg · ml⁻¹) of Penicillin G were tested. The effect of antimicrobials on preformed biofilms was also evaluated after incubation at 37°C for 4, 8 and 24 h in MM. Preformed biofilms were then exposed to 200 μl of test agent-containing MM broth.

The activity of Penicillin G and NAC was evaluated on planktonic growth of the following strains Ss213, Ss36, SsS9A, while the effect on both planktonic and sessile growth was evaluated in the strains i.e. SsNTF, SsS9Q and SsNTV.

The results of the antimicrobial activity were expressed as percentage of the growth inhibition compared with the untreated wells. All experiments were repeated independently three times. All microtiter plate assays were performed in duplicate wells. One-way ANOVA was used to compare groups followed by Bonferroni’s multiple comparison post-test by using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). The significance level was P < 0.05.

Only one isolate was classified as strongly adherent (SsNTF), one isolate as moderately adherent (SsNTQ), while SsNTV and SsNTY were weakly, the rest were non-adherent. According to our results, only few biofilm-producing isolates could be detected, in agreement with the observations of other researchers who found non-typeable isolates producer of biofilms (Bonifait et al., 2008; 2010). The ability to form biofilms is not required for virulence, but instead contributes towards long-term colonization, transmission and difficulties to eradicate these infections (Huaijie et al., 2013).

Penicillin G is still the drug of choice for the treatment of Streptococcus spp. infections because it remains susceptible to the antibiotic despite its intensive use (Grenier et al., 2009). In this work, the minimum effective concentration of Penicillin G for reducing the 50% of planktonic growth of S. suis was found to be 2 μg · ml⁻¹; however, the needed concentration for reducing their sessile counterparts was 32 μg · ml⁻¹. On the other hand, MIC for reducing the 90% of planktonic cells were over 128 μg · ml⁻¹ and 500 μg · ml⁻¹ for sessile cells (Fig. 1A). Significant limitations to biofilm penetration have been reported for beta-lactams antibiotics, which act at the surface of the bacteria due to the impermeability of their stable architecture. Bacteria within a biofilm multiply very slowly and therefore are much less susceptible to growth-dependent antimicrobial killing (Mackay et al., 2000; Marchese et al., 2003).

Thus, the need for more effective biofilm dissolution treatments becomes imperative. One the ways is to enhance the antimicrobial activity by combining antibiotics with other compounds such as NAC. MIC of NAC for reducing the 90% of planktonic cells were over 2 mg · ml⁻¹ (Fig. 1B). Interestingly, the MCI of Penicillin G in the presence of 8 mg · ml⁻¹ of NAC was reduced from 500 μg · ml⁻¹ to 128 μg · ml⁻¹ for sessile bacteria inhibition and to 1 μg · ml⁻¹ for the inhibition of planktonic cells (Fig. 1C). Therefore, the combinations of Penicillin G and NAC exerted a synergistic inhibition of both planktonic and sessile forms.

Biofilm formation by the S. suis strains was shown to occur earlier than 4 hours. The effects of the tested antibacterial agents on the development of established biofilms is shown in Fig. 2. Among the concentrations of Penicillin G tested, 250 μg · ml⁻¹ and 500 μg · ml⁻¹ showed the highest reduction activity against preformed biofilm, permitting only the following percentages of biofilm formation 47.16 ± 5.54%; P < 0.05 and 48.14 ± 8.64%; P < 0.05, respectively (Fig. 1A). N-acetyl cysteine (16 mg · ml⁻¹) reduced biofilm development approximately to 60.16 ± 2.33% (P < 0.05) compared to the control group during the first 4 hours (Fig. 1B). However, the combination of Penicillin G and NAC showed a higher disruptive effect on the preformed biofilms and reduced the development of biofilm approximately to 80.84 ± 18.73% (P < 0.05) with respect to the control after 4 hours (Fig. 1C). None of the treatments inhibited biofilm development at more than 50% with respect to the untreated group after 8 or 24 hours.
Biofilm formation can be divided into three stages: early, intermediate and mature. During the first stage, the bacteria are still susceptible to antibiotics and perioperative antibiotic prophylaxis can be critical for successful treatment (Merle et al., 2012). Some studies show that, when an antimicrobial agent is used for exopolysaccharide matrix disruption, the penetration of other antimicrobials into the pre-formed biofilm can be facilitated (Hajdu et al., 2009). The results of this study showed the Penicillin G and NAC disrupted pre-formed biofilms by up to 50% and 38% from microtiter plates when were used separately at the concentrations 500 µg/ml and 16 mg/ml respectively. However the application of both mixed compounds permitted only the development of biofilm by up to 20% at 4 hours. NAC is a sulfhydryl group-containing antioxidant and a mucolytic agent that is used in therapy of bronchitis (Marchese et al., 2003; Aslam et al., 2007). Previous studies showed that NAC could decrease biofilm formation by a variety of bacteria and that inhibited bacterial adherence, reduced the production of extracellular polysaccharide matrix, while promoting the disruption of mature biofilms, and reduced sessile cell viability (Aslam et al., 2007).

Antibiotic treatment due S. suis infections in pigs is rarely successful, probably because of poor antibiotic penetration of the porcine tonsillar tissues, which act as a source of infection. Development of new therapeutic...
options against the most prevalent strains might be one possible way to prevent colonization of female pigs and to protect those working with pigs.

Traditionally, microbiologists have evaluated the efficacy of an antibiotic by measuring the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). These measurements are made on freely floating, planktonic, laboratory phenotypes. In conclusion, the results of this study agrees with other works in emphasizing the importance of considering the growth in biofilms when evaluating antimicrobials to control *S. suis*–associated infections, and suggests to use combinations of NAC and Penicillin G to increase their antibiotic activity against *S. suis* because of a strong synergic effect.

**Literature**


