In most parts of the world, *Streptococcus suis* is the predominant agent of streptococcal infections in the swine industry and it is associated with a variety of diseases including meningitis, arthritis and pneumonia (Gottschalk *et al.*, 2007; Lun *et al.*, 2007). Thirty-five serotypes of *S. suis* have been characterized, the most virulent type to swine and humans, being serotype 2 (Gottschalk *et al.*, 2007; Lun *et al.*, 2007; Wang *et al.*, 2011).

Zoonotic disease due to *S. suis* does occur sporadically in Western countries, but is encountered more commonly in countries such as China and Hong Kong. It affects predominantly individuals with occupational exposure to pigs (Huang *et al.*, 2005). The antibiotics are widely used to control *S. suis* infections (in humans and in animals), but the emergence of antibiotic-resistant strains represents a problem for both pig production and public health (Hui *et al.*, 2005).

Bacterial biofilm formation is a mechanism that allows them to become persistent colonizers and enhance their resistance to antibiotics (Guo *et al.*, 2012). Many drugs and compounds have been described as biofilm inhibitors (Aslam *et al.*, 2007), specifically Ampicillin and Penicillin G have been examined on *S. suis* biofilm (Grenier *et al.*, 2009); biocides such as N-acetylcysteine (NAC) decrease biofilm formation for a variety of bacteria (Marchese *et al.*, 2003; Schwandt *et al.*, 2004), but their effect on *S. suis* biofilm remains unknown. Therefore, we investigated the biofilm-forming potential of *S. suis* strains collected from farms in Cuba; the effect of two antimicrobial agents, Penicillin G and N-acetylcysteine, on biofilm formation was also studied.

*S. suis* strains tested for biofilm formation in this study belonged to different serotypes: two serotype 1 (Ss181, ss1S), one serotype 1/2 (Ss1/2M), two serotype 2 (Ss213, Ss211), two serotype 3 (Ss36, Ss364), one serotype 8 (S80), one serotype 9 (Ss9A), one serotype 16 (S16X) and four non-typeable isolates (SsNTF, SsNTV, Ss9Q, SsNTY). The isolates were recovered as predominant bacteria from lungs (pigs with pneumonia) in different farms in Cuba. Appropriate amounts of materials from infected lungs were aseptically transferred to the surface of a Columbia agar plate (Oxoid), supplemented with 5% bovine blood. All the isolates were biochemically typed using the API 20 STREP test kit (Bio Mérieux, France). All the strains are part of the collection of Bacteriology Laboratory at National Centre for Animal and Plant Health (CENSA). Strain confirmation was carried out by PCR assays proposed...
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 Hevit Broth (THB) until the late-log phase (1 × 10⁶ 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 × 10⁶ 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 × 10⁶ 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 (Bonfait 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-acytelycysteine (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 it 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 agree 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**


