INNOVATIVE COMPOUNDS TO BATTLE MULTI-RESISTANCE TO ANTIBIOTICS: USE OF PVA-TANNIC ACID NANOPARTICLES TO INHIBIT STAPHYLOCOCCUS PSEUDOINTERMEDIUS GROWTH

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ABSTRACT

Antibiotic resistance is an increasing public health problem that affects numerous pathogens, including *Staphylococcus pseudintermedius*, which has a high prevalence of methicillin resistance and can be easily transmitted to humans. Nowadays, the scientific community is developing new compounds that not only improve classic therapies in fighting antibiotic resistances but also prevent its appearance, essential to maintain health protection. The main objective of the present study is to synthesize tannic acid and polyvinyl alcohol nanoparticles and to determine their potential as growth inhibitors for *S. pseudintermedius*, to be considered a potential alternative therapy. The study includes diameter and Z-potential measurements for nanoparticles characterization and antimicrobial effect assays with different nanoparticles concentrations. MIC_{90} is determined as 112 µg/mL. Nonetheless, further studies to identify the underlying action mechanisms of these nanoparticles are going on in our group.

INTRODUCTION AND OBJECTIVES OF THE STUDY

S. pseudintermedius is an opportunistic pathogen commonly associated with skin and soft tissue infections (SSTIs) in dogs. The high prevalence of antibiotic resistance, particularly to methicillin, along with its zoonotic potential could lead to treatment implications in human infections (Somayaji *et al.*, 2016). In this sense, searching

for new bactericide molecules that could prevent the appearance of resistance has become a critical issue for WHO priority plan.

Biocompatible polymers, such as polyvinyl alcohol (PVA), have become crucial materials in the development of novel drug delivery systems due to their low toxicity and high biocompatibility (Begines *et al.*, 2015). Tannic acid (TA), existing in plant tissues, has been reported to possess natural antioxidant, antibacterial and antiviral activity (Ivanova *et al.*, 2018). Thus, the presentation of both compounds in the form of nanoparticles, that have shown to be very useful structures in biomedicine, may offer new therapeutic possibilities such as controlled delivery of the TA, increased toxicity to bacteria, etc. (Wu *et al.*, 2017). Furthermore, polyvinyl alcohol- tannic acid nanoparticles (PVA-TA NPs) have been described as non-toxic and biodegradable nanomaterials, which makes them an ideal candidate for developing new alternative therapies (Velázquez *et al.*, 2016).

The purpose of the present study is to describe an easily executable synthesis protocol for PVA-TA NPs and to determine their potential capacity to inhibit *S. pseudintermedius* growth in vitro to be considered a potential alternative drug compared to classic therapies.

MATERIALS AND METHODS

Nanoparticles synthesis and characterization

32 mg of TA were dissolved in 10 mL deionized water and 100 mg of PVA in 10 mL deionized water using different flasks. Then, TA solution was added dropwise to the PVA solution at room temperature. The solution turned white and opaque and it was left stirring for 15 minutes. Nanoparticles' characteristics were measured using Dynamic Light Scattering.

BACTERIAL GROWTH INHIBITION ASSAYS

Different concentrations of PVA-TA NPs in TSB – sterile water liquid culture medium was prepared. TSB was prepared at triple concentration (3x TSB) to be at normal concentration when mixed with the other compounds. "Stock solutions" were prepared with the following proportions:

1. 600 µL of 3xTSB, 1110 µL of sterile H_20 and 90 µL of PVA-TA NPs.
2. 600 µL of 3xTSB, 1020 µL of sterile H_20 and 180 µL of PVA-TA NPs.
3. 600 µL of 3xTSB, 930 µL of sterile H_20 and 270 µL of PVA-TA NPs.

4. 600 µL of 3xTSB, 840 µL of sterile H_20 and 360 µL of PVA-TA NPs.
5. 600 µL of 3xTSB, 750 µL of sterile H_2O and 450 µL of PVA-TA NPs.
6. 600 µL of 3xTSB, 660 µL of sterile H_2O and 540 µL of PVA-TA NPs.

Once prepared, a volume of 300 µL of each was poured in eppendorf tubes. Finally, 5 µL of an overnight grown *S. pseudintermedius* liquid culture were inoculated per tube, except the first of each (negative control). Once the dilutions were prepared, they were incubated for 24 hours at 37 °C. 200 µL of each eppendorf were transferred to a microtiter plate for determining bacterial growth as a measurement of the absorbance at 600 nm (turbidity).

RESULTS AND DISCUSSION

Nanoparticles synthesis and characterization

The synthesis protocol was proven to be effective and nanoparticles always presented identical characteristics. The final solution was a white, non-aggregated and completely opaque colloid.

Three samples of three different batches of nanoparticles were characterized at different times (Table 1). The average NP size was 164 nm, with PDI of 0.16 and a Z-potential of -20 mV. As standard deviations showed, values obtained were almost identical between the three samples.

Table 1. Hydrodynamic diameter (size, nm), Z-potential (Z-pot, mV) and polydispersity index (PDI) obtained from three samples, A, B and C, of PVA-TA NPs.

	Sample A	Sample B	Sample C	Average	St.Dev
Size (nm)	164,3	165,5	163,0	164,3	1,2
PDI	0,155	0,168	0,176	0,16	0,01
Z-pot (mV)	-19,1	-20,7	-20,1	-20,0	0,8

Source: own elaboration.

Low PDI values obtained indicated that nanoparticles size distribution was homogeneous, which is an ideal characteristic for nanoparticles to be considered as therapeutic agents (Clogston & Patri, 2011). Also, the negative values in Z-potential could mean a reduction in possible toxic effects in humans, but further studies are needed to determine this feature.

Bacterial growth inhibition assays

The results of bacterial growth (Table 2) showed a great reduction in the absorbance rate from dilution D (40 µL of nanoparticles), suggesting that the bacterial growth

was inhibited at this concentration. MIC_{90} is determined as 112 µg/mL, a promising result to take them into account as an alternative therapy or combined with regular antibiotic drugs.

Table 2. Absorbance at 600nm of cultures of *S. pseudintermedius* incubated in the presence of increasing concentrations (0-60 µL) of PVA-TA NPs.

Stock solutions	Control	1	2	3	Average
A (10µL NPs)	0.26	0.77	0.748	0.718	0.624
B (20µL NPs)	0.291	0.55	0.73	0.625	0.549
C (30µL NPs)	0.227	0.341	0.392	0.452	0.353
D (40µL NPs)	0.223	0.264	0.347	0.276	0.278
E (50µL NPs)	0.217	0.220	0.247	0.221	0.226
F (60µL NPs)	0.241	0.211	0.294	0.214	0.24

Source: own elaboration.

CONCLUSIONS

These nanoparticles present an outstanding balance among their properties. PDI values obtained indicates that nanoparticles size is very homogeneous, meaning that, along with negative Z-potential values, their potential toxicity for animals and humans is low. Although this is a pilot study, our findings provided insights into the importance of PVA-TA NPs in the treatment of bacterial infections and potential use of this combination that would need further studies that will be conducted in a near future, along with studies focused on describing their underlying mechanisms.

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