INNOVATIVE COMPOUNDS TO BATTLE MULTIRRESISTANCE TO ANTIBIOTICS: USE OF PVA-TANNIC ACID NANO- PARTICLES TO INHIBIT *STAPHYLOCOCCUS PSEUDINTERMEDIUS* GROWTH

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Abstract

Antibiotic resistance is an increasing public health problem that affects to numerous pathogens, including *Staphylococcus pseudintermedius,* which has a high prevalence of methicillin resistance and can be transmitted to humans.

The development of new compounds to prevent the appearance of antibiotic resistances and find alternatives to classic therapies is essential to health protection.

The main objective of the present study is to determine whether the nanoparticles of polyvinyl alcohol and tannic acid "PVA-TA NPs" are effective to inhibit *Staphylococcus pseudintermedius* and can be considered as an alternative therapy.

The study includes increasing concentration essays of the nanoparticles and establishes, using the determination of turbidity by refractometry that could be a useful tool to inhibit bacterial growth, resulting successful.

Nonetheless, further studies to identify the underlying action mechanisms of these nano-particles are going on in our group.

1. Introduction and objectives of the study

Staphylococcus pseudintermedius is a relevant pathogen due to the high prevalence of antibiotic resistance, particularly to methicillin and its zoonotic potential.

In this sense, searching for new molecules or compounds to act as bactericides in order to prevent the apparition of tolerance or resistance to them has become a world-wide top priority.

Therefore, the purpose of the present relevant study is to determine the potential capacity of nanoparticles of polyvinyl alcohol and tannic acid to inhibit *Staphylococcus pseudintermedius* growth in vitro to be considered as an alternative drug to classic antibiotherapy.

2. Materials and Methods

The essay included four different concentrations of PVA-tannic acid nano-particles with TSB liquid culture medium and sterile water. The TSB was prepared at triple concentration, to be at normal concentration when mixed with the other compounds.

The denominated "stock solutions" are prepared with the following proportions:

A. Blanc. 600µl of 3xTSB and 1.2 ml of sterile H_2O.

B. 600µl of 3xTSB, 900 µl of sterile H_2O and 300 µl of NPs.

C. 600 µl of 3xTSB, 600 µl of sterile H_2O and 600 µl of NPs.

D. 600 µl of 3xTSB, 300 µl of sterile H_2O and 900 µl of NPs.

E. 600 µl of 3xTSB and 1.2 ml of NPs.

Once they were prepared, a volume of 300 µl of them was poured in epperndorf tubes. Finally, 5 µl of an overnight grown *Staphylococcus pseudintermedius* liquid culture were inoculated per tube, except the first of each (negative control). The experiment included four repetitions of each solution to obtain statistically significant results. Once the dilutions were prepared, they were incubated for 24 hours at 37 °C using a rotatory wheel to homogenize them. Then, 200µl of each eppendorf were transferred to 96-well microtiter plate for determination of absorbance at 600nm as a measurement of bacterial growth (turbidity) in a microplate absorbance reader.

3. Results

The results of bacterial growth (Table 1) show a great diminution of the absorbance rate from the second dilution with 33.3 µl of nano-particles (B) and the average of the determinations were very similar to the control, suggesting that the bacterial growth was inhibited.

Table 1. Absorbance at 600nm of cultures of *Staphylococcus pseudintermedius* incubated in the presence of increasing concentrations (0-133µl) of PVA-tannic acid nanoparticles (PVA-TA NPs).

Stock solutions	Control	Rep. 1	Rep.	Rep. 3	Rep. 4	Average
A (0µl NPs)	0.210	1.025	1.12	1.057	1.064	0.895
B (33,3µl NPs)	0.182	0.254	0.197	0.209	0.196	0.208
C (66,67µl NPs)	0.191	0.258	0.207	0.234	0.214	0.221
D (100µl NPs)	0.178	0.242	0.186	0.252	0.212	0.214
E (133,3µl NPs)	0.192	0.238	0.195	0.234	0.190	0.210

Source: own elaboration.

In consequence, the experiment was repeated by lowering down the volume of NPs added from 10 to 60 µl, in order to determine the minimum inhibitory concentration (MIC) of PVA-tannic acid NPs. Results are shown in Table 2.

Table 2. Absorbance at 600nm of cultures of *Staphylococcus pseudintermedius* incubated in the presence of increasing concentrations (0-60 µl) of PVA-tannic acid nanoparticles (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 (30 µl NPs)	0.227	0.341	0.392	0.452	0.353
D (40 µl 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 (60 µl NPs)	0.241	0.211	0.294	0.214	0.24

Source: own elaboration.

These new data show a significant reduction of the absorbance rate from of nano – particles, determining the MIC as 50 µl.

4. Discussion and Conclusions

Although *Staphylococcus pseudintermedius* has been commonly associated with skin and soft tissue infections (SSTIs) in dogs, however some human cases have been described. Approximately 90% of healthy dogs are colonized with S.

pseudintermedius, in this sense is an opportunistic pathogen in dogs and the leading cause of pyoderma and otitis externa and is also the most commonly isolated pathogen in urinary tract infections. As an example, methicillin resistance is rapidly emerging in S. pseudintermedius with a higher incidence amongst clinical isolates in dogs, and this may have treatment implications in human infections (Somayaji *et al.*, 2016).

As a result, there is a remarkable increasing problem with this pathogen and consequently, related with the potential risk to human health, thus it is critical to study and work out a possible solution combining biocompatible, chemical structures and polymers.

For instance, PVA (Polyvinyl alcohol) is a water-soluble synthetic polymer. Polymers based on naturally occurring products are promising new materials, with novel technical potential and enhanced properties about biocompatibility and biodegradability (Galbis, *et al.*, 2016). Besides, it is being used, in combination with other products for inhibition of bacterial growth (Yang *et al.*, 2018; Wu *et al.*, 2017). At the same time, tannic acid, existing in plant tissues, has been reported to possess natural antioxidant, antimicrobial and antiviral activity (Ivanova *et al.*, 2018). Finally, Nanoparticles as well, have been demonstrated to be very useful structures, due to their physical and chemical properties and interactions in biomedicine (Dong *et al.*, 2018). Thus, the presentation of both compounds in the form of nanoparticles may offer new therapeutic possibilities such as controlled delivery of the compounds, increased toxicity to bacteria, etc. (Wu *et al.*, 2017).

We consider that a combination of these factors could be a useful tool to inhibit bacterial growth, resulting successful.

Nonetheless, further studies to identify the underlying action mechanisms of these nano-particles are going on in our group.

Although, this is a pilot study, our findings provided insights into the importance of PVA-tannic acid nano-particles 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.

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