

Manuscript:

Title: Activity of biogenic silver nanoparticles in planktonic and biofilm-associated *Corynebacterium pseudotuberculosis*

The subject approached in this manuscript is interesting, but the results is confusing and contradictory.

The biggest question is about the choice of concentrations to evaluate the interference with biofilm formation. It was not possible to understand why concentrations above bactericidal concentrations were used to evaluate the interference with biofilm formation. According to supplementary figure 1 and 2, above 0.3 mg/mL there is 100% inhibition of bacterial growth. Furthermore, the discussion also does not present information that justifies this choice.

However, I send some suggestions for the improvement of the manuscript.

Comment#1: Introduction, lines 57-58. "...since cases of infection in humans were already described in several contries." In this sentence, several countries were mentioned, but there is only one citation. I suggest rephrasing the sentence or finding which countries CL is a public health problem.

Comment#2: Material and Methods, lines 101-102: Inform that four clinical isolates were evaluated.

Comment#3: Material and Methods, line 127: The unit was missing in degrees Celsius.

Comment#4: Material and Methods, line 161: I would like the authors to explain the criteria for choosing AgNP concentrations (0.25, 0.5, 1, 2 and 4 mg/L) to determine interference with biofilm formation. The concentrations used are greater than the MBC for all isolates. Normally for this type of test, subinhibitory concentrations are used. I consulted the article that was cited (Siddique et al., 2020; Santos et al., 2021) and both articles used concentrations below inhibitory.

- ✓ Siddique MH, Aslam B, Imran M, Ashraf A, Nadeem H, Hayat S, Khurshid M, Afzal M, Malik IR, Shahzad M, Qureshi U, Khan ZUH, Muzammil S. 2020. Effect of silver nanoparticles on biofilm formation and EPS production of multidrug-resistant *Klebsiella pneumoniae*. Biomed Res 2020:6398165. doi: 10.1155/2020/6398165
- ✓ Santos LM, Rodrigues DM, Kalil MA, Azevedo V, Meyer R, Umsza-Guez MA, Machado BA, Seyffert N, Portela RW. 2021. Activity of ethanolic and supercritical propolis extracts in *Corynebacterium pseudotuberculosis* and its associated biofilm. Front Vet Sci. 8:700030. doi: 10.3389/fvets.2021.7000

Comment#5: Results, line 210: I suggest combining Supplementary figures 1 and 2 into a single figure and page.

Line 217: I suggest combining figures 2 and 3 into a single figure and page.

Line 224: I suggest combining figures 4 and 5 into a single figure and page.

Comment#6: Results, lines 228 and 234: In this paragraph, figures 6A, 6B and 6F were explained. What about images 6C, D and E? Please insert in the text.

Comment#7: Discussion, lines 269 and 345: The serovar of the *Salmonella* bacteria is not italicized. The correct way to describe it is *Salmonella* Typhimurium and *Salmonella* Enteritidis. Please make the correction.

Comment#8: I thank you for providing raw data, however your supplemental files need translation conference. Some spreadsheets are in "Portuguese".

Comment#9: Do the AgNPs at the concentration used for growth inhibition to *C. pseudotuberculosis* show cytotoxicity? More evidence should be proved in the manuscript.