Excellent antimicrobial activity of novel nanocomposites (pyrophyllite clay based) modified with silver

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Abstract: Novel antimicrobial substances are among the most exploring in scientific circles nowadays. Nanocomposites proved to be one of the promising candidates. Novel research based on nanocomposites goes in the direction of synthesizing hybrid materials based on inorganic matrix of layered structure. Clays possess such specific layered structures and their structure can be modified. In this research pyrophyllite clay was in focus and was modified with silver, well known antimicrobial metal. The main emphasis in this study was on antimicrobial efficiency of nanocomposite (pyrophyllite clay based) against planktonic (individual) isolates and against biofilm (community bacterial forms of living). Antibacterial activity against planktonic isolates of *E. coli* ATCC 8739 and *S. aureus* ATCC 25923 was detected with bujon microdilution method. Microtiter plates were inoculated with 100 µl of bacterial inoculum and treated with 20 and 40 mg of pyrophyllite clay/Ag material during overnight incubation with shaking at 37 °C. Antibiofilm testing with 10 mg of nanocomposite was performed in microtiter plate against biofilm producing clinical isolates of *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. In conclusion, nanocomposites of pyrophyllite clay/Ag have excellent in vitro activity against all living forms of bacteria which could have enormous potential for wide practical usage of these substances.
Key words: nanocomposite (clay based), antimicrobial activity, silver, biofilm

Introduction: Bacterial resistance to common antimicrobial substances is one of the globally widespread problem. In the hospital settings, as well as in community, occurens of multidrug- resistant and pan- resistant bacteria is in arising, leading to increased morbidity and mortality of patients. Clasical antimicrobial testing imply technics such are disc diffusion and bujon dilution method, that examine antibiotic activity against individual or planctonic bacteria, performing their action either on bacterial surface envelopes or on bacterial metabolism and protein synthesis. In last two decades knowledge about bacterial living and formation was improved with development of scanning electron microscopy and discovering of bacterial biofilms. A biofilm is an assemblage of microbial cells that is irreversibly associated (not removed by gentle rinsing) with a surface and enclosed in a matrix of primarily polysaccharide material. In one word, biofilm is bacterial community which protect bacteria from antibiotics, desinfectants and immune system. So, in order to eliminate biofilm, antibiofilm substances must either penetrate through biofilm or mechanically damage it. Evolvement of new antibiotics lag far behind of the resistance spreading that direct the technology and pharmacy to the development of new antimicrobial substances, such are clays, nanoparticles and herbal extracts.

Experimental: Antimicrobial testing

Antimicrobial activity of clay was examined against planktonic (individual) isolates and collective forms of living (biofilm). Antibacterial activity against planktonic isolates of *E. coli* ATCC 8739 and *S. aureus* ATCC 25923 was detected with bujon microdilution method. Microtiter plates were inoculated with 100 µl of bacterial inoculum in Muller Hinton broth (MHB) with final concentration of 106 CFU/ml, and treated with Ag/clay during overnight incubation at 37 °C with shaking at 250 rpm. Serial tenfold dilution of bacterial suspension was plated on agar plate, incubated at 37 °C for 24h and counted next day. As positive control, we used bacteria incubated in MHB, without clay. Antibiofilm testing was performed in microtiter plate against biofilm producing clinical isolates of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* according to Stepanovic et al. Plates were inoculated with 200 µl of bacterial inoculum in brain heart infusin broth, with final concentration of 10^6 CFU/ml, 10 mg of autoclaved clay was added and incubated without shaking at 37 °C. As positive control we used the same bacteria in brain heart infusin broth, but not treated with clay.

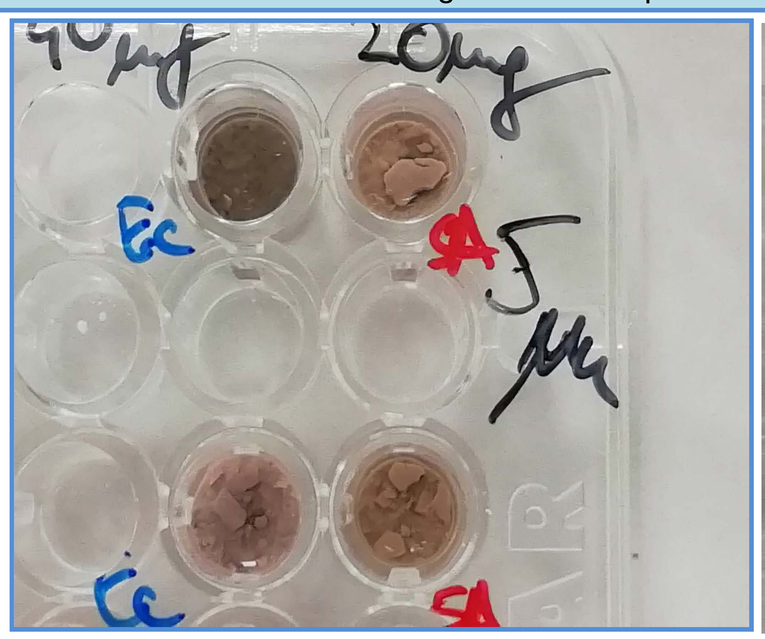


Figure 1 and 2. Antibacterial activity of nanocomposites against planctonic isolates *Staphylococcus aureus* ATCC 25923 and *Echerichia coli* ATCC 8739

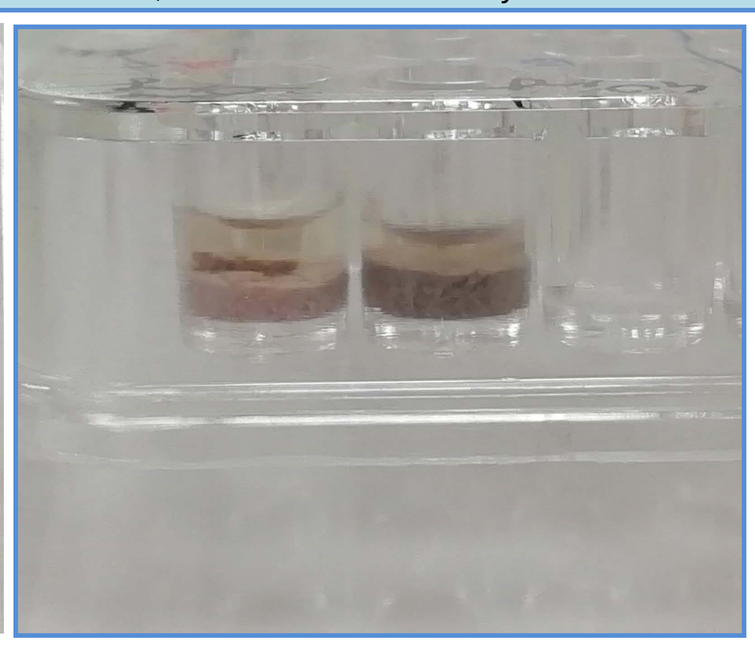
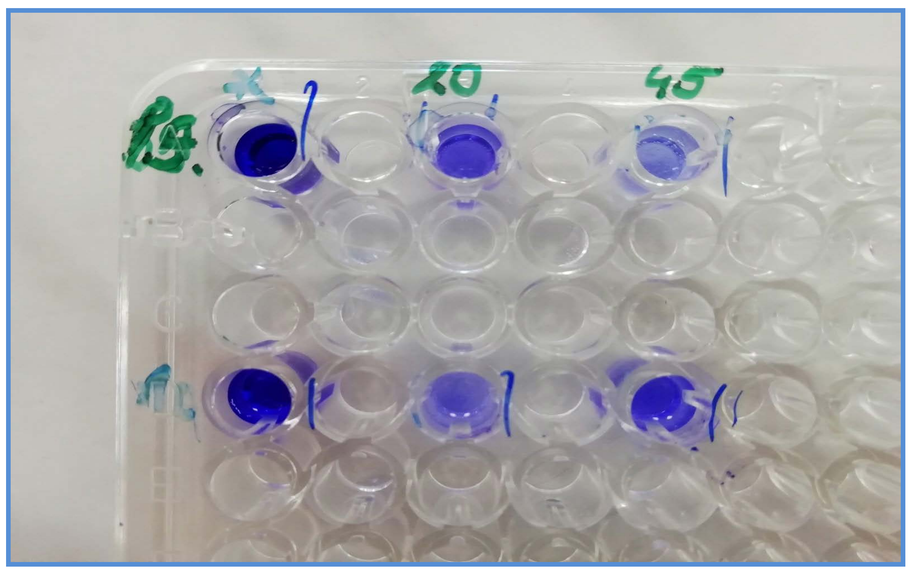


Figure 3. Antibacterial activity of nanocomposites against planctonic isolates *Staphylococcus aureus* ATCC 25923



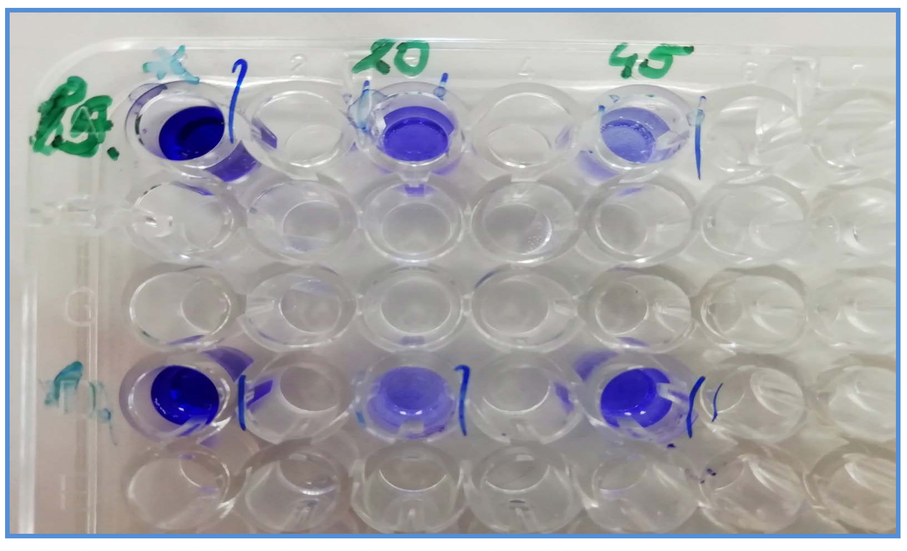


Figure 4. and Figure 5. Antibiofilm activity of nanocomposites against biofilm producing isolates of *Acinetobacter baumannii* and *Pseudomonas aerugionosa*

Conclusions: Incorporation of silver nanoparticles in nanocomposite (pyrophillite based) was successfully performed. According to our experimental results Ag/nanocomposite (pyrophyllite based) showed excellent antimicrobial properties. Results obtained have great potential and will be further developed. We deeply recommend clay nanocomposite modified with Ag nanoparticles as an excellent antibacterial and good antibiofilm substances. We assumed that our result are in relations with mechanical action between bacteria and nanocomposite.

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