Bacteriophages – novel biotechnology tools available in clinical practice in Romania

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Abstract

Staphylococcus species can be the etiological agents of hard-to-treat infections because of their capacity to form biofilm, invade host cells and develop resistance to available antimicrobials. We present the case of a patient who developed periprosthetic dorsolumbarsoft-tissue infection with S. aureus and could not receive effective antibiotic treatment due to a history of multiple drug allergy syndrome. Therapeutic success and eradication of biofilm were obtained by combining topical bacteriophages with oral antibiotic therapy. Bacteriophages can be a solution, and further in vitro and in vivo studies are needed for improvingthe biotechnology behind drug delivery.

Keywords: *Staphylococcus* spp., biofilm, multiple drug allergy syndrome, bacteriophages, nanoparticles.

Staphylococcus species have an important capacity to form biofilm(ZAMBORI& al. [1]), invade human host cells, and develop resistance to available antimicrobials (SULLA & al. [2]). Because they are natural inhabitants of the human skin (PREOȚESCUand STREINU-CERCEL [3]), staphylococcal strains can infect any cutaneous wound and cause significant long term morbidity and disability (IONESCU & al. [4]). We present the case of a patient who developed periprosthetic dorsolumbarsoft tissue infection with *S. aureus* and could not receive effective antibiotic treatment due toa history of multiple drug allergy syndrome (CHIRIAC and PASCAL [5]), with sequential allergic reactions to most available antimicrobial agents. To ensure therapeutic success and eradication of biofilm we resorted to combined therapy of antibiotic plus bacteriophages, and achieved sustained clinical response and favorable evolution. We report the case of a 37 year-old female with morbid deformity of the spine who presented to our clinic in October 2014 for cellulitis of the back with purulent secretion and loss of substance in two adjoining areas (Figure 1), dating back 6 months. The patient had been diagnosed 36 years previously with T10-L1 spinal fibro-sarcoma with important spinal cord compression. She underwent surgery and four series of radiotherapy with complete tumor removal. Afterwards, she developed morbid kyphoscoliosis that required the implantation of two metal rods for deformity correction and trunk balance restoration. Due to the degradation of the rods and important spine malformation, the patient underwent more than 10 surgeries, the last two being performed in November 2012 and August 2013. After the last surgery she experienced skin necrosis due to the metallic friction and developed two areas with loss of substance.The patient's relevant medical history included chronic hepatitis B

since childhood, probably acquired during one of the surgeries, as her parents had negative hepatitis B markers. She currentlyhad low viral load (5268 IU/mL), normal liver enzymes, F0 fibrosis and A0 necro-inflammatory activity on FibroMax (BioPredictive, Paris, France). The patient presented multiple drug allergy syndrome with sensitization developing sequentially, i.e., in the past the patient wasn't allergic to vancomycin, quinolones and clindamycin but allergy developed as soon as they were administered.

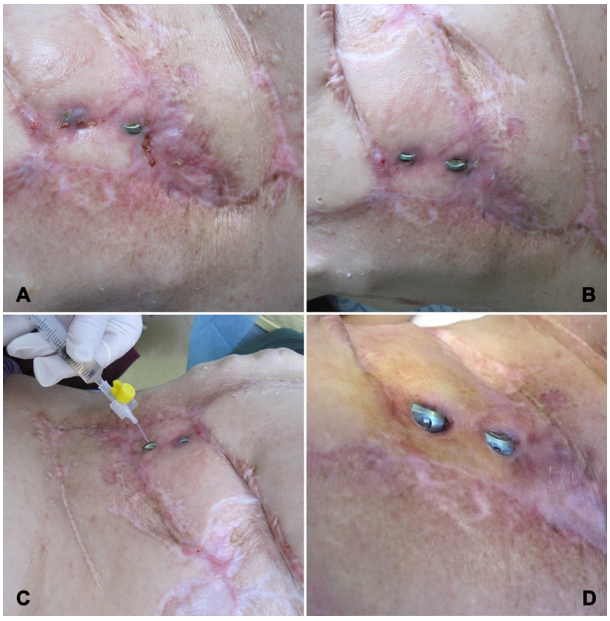

Figure 1. A. Aspect of the lesions at admission; **B.** Aspect during treatment; **C.** Phages instillation into the lesions; **D.** Aspect at the end of phage therapy and before surgery.

Before presenting to our clinic, the patient had received treatment with oral cefuroxime 500 mg every 12 hours for 1 month with no improvement and with persistence of the purulent secretion.

At admission in our ward, the clinical exam revealed: oriented, afebrile patient with morbid deformity of the spine and the body, normal pulmonary and cardiovascular function, presence of erythema, edema and tenderness around two of the screws used for spine stabilization, loss of substance and purulent secretion, pain in the inguinal region and both thighs, paresthesia and tremors of the legs, no neck stiffness. Laboratory tests showed normal completeblood count, mild inflammatory response, normal biochemistry, and cultures positive for methicillin-susceptible *S. aureus* from the ulcers with more than 90% neutrophils on the smear. Being aware of the patient's allergies, we performed cutaneous allergy testing for antibiotics potentially active on her staphylococcal strain: ciprofloxacin, clindamycin, oxacillin, teicoplanin, linezolid and vancomycin. All allergy tests were positive. Cefuroxime was the only antibiotic to which the patient wasn't allergic and the strain displayed *in vitro* susceptibility. Knowing that the patient had taken cefuroxime for 1 month with no result, we decided that combined therapy was needed to obtain a synergistic effect *in vivo*, particularly since no other antibiotics could be administered safely. We evaluated the strain's susceptibility to commercially-available Georgian bacteriophages mixtures; PYO and INTESTI phages

(EliavaBioPreparations, Tbilisi, Georgia) displayed *in vitro* activity against the bacterial strain. We also evaluated the impact of phages on biofilm formation,through the previously described protocol (NEGUȚ& al. [6]). Briefly, a 0.5 McFarland inoculum was plated on Muller Hinton agar and 20 µL phages were added. After 24 hours incubation, plaque formation was evaluated. The existence of confluent plaques is suggestive for the highest phage susceptibility of the bacterial strains (Figure 2). In 96-well plates with liquid media the bacterial strain was cultured alone or with different phage dilutions (1/2 - 1/64) to assess the inhibition of biofilm formation. Bacteriophages were also tested to assess their effect on preformed biofilm: after 24 hours of growth in liquid media (the optimal timespan to ensure biofilm formation), the planktonic bacteria were washed out and 1/2 dilution of phages was added for 24 more hours of incubation. The wells were fixed with cold methanol followed by staining with 1% crystal violet. The colored biofilm was re-suspended with acetic acid and the optical density was read at 492 nm through spectrophotometry. The patient's strain displayed a 54.1% decrease in biofilm formation and a 20.9% decrease in the existing mature biofilm after the addition of 1/2 dilution of PYO phages. Therefore, the advantage of phages would not only reside in the inhibition of biofilm formation, but also in the destruction of mature biofilm, facilitating the action of antibiotics. Having the local bioethics committee approval and the patient's signed informed consent for bacteriophage therapy and having no other clinical option, we initiated local therapy with PYO phages 2mL every 12 hours together with oral cefuroxime, 500 mg every 12 hours. The in vivo response to therapy was evaluated on a daily basis with cultures and smears. The cultures, which had remained positive after one month of cefuroxime alone, became negative after three days of combined treatment. We alsoassessed the in vivo effect of phages by sampling wound secretion 12 hours after the last phage administration and plating it together with a 0.5 McFarland inoculum of the patient's *S. aureus* strain. After 24 hours of incubation, the plate displayed plaques where the secretion was inoculated, suggesting that the trough bacteriophage concentration in the secretion was still high enough to induce lysis of the isolated etiologic agent (Figure 2). After almost 2 weeks of treatment the patient was discharged, after the purulent secretion and the erythema had disappeared. She continued combined therapy for a total of 3 months, with sustained treatment response. In January 2015, while still on combined treatment, the patient received plastic surgery for covering the loss of substance with *latissimus dorsi* flap. Three months, six months and almost one year after surgery the laboratory results and local aspect showed no recurrence of infection.

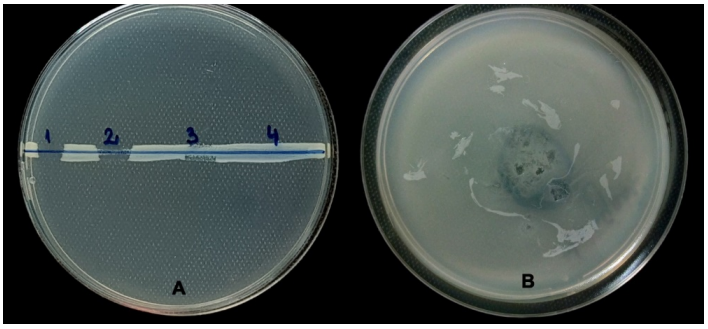

Figure 2.A. Phage susceptibility testing of the patient's *S. aureus* strain – confluent plaques for PYO (1), semiconfluent for INTESTI (2) and non-susceptibility to PHAGYO (3) and PHAGESTI (4); **B.** *In vitro* effect of the trough concentration of phages from the wound secretion. Figure 2 legend: PYO and INTESTI were acquired from EliavaBioPreparations, Tbilisi, Georgia. PHAGYO and PHAGESTI from JSC "Biochimpharm", Tbilisi, Georgia. All tested mixtures contain, among others, phages active on *Staphylococcus* spp.

Allergic patients may posesignificant treatment issues, even for infections with germs susceptible to most antibiotics. In cases where the pathogenic agent also displays multidrug resistance, the treatment options truly become limited. Multiple drug allergy syndrome is characterized by an irregularresponse of T lymphocytes to cross-reactive stimuli, and as time passes the patient may acquire more and more allergies, becoming a hard to treat patient in any disease. When it comes to infections, the patient might end up having no antibiotic options in the future.In the case of our multi-allergic patient, we administered bacteriophagestopically. We chose this administration route over the oral or intravenous ones because of the patient's high risk of sensitization following systemic exposure, with the potential subsequent development of phage allergy. We thus tried to avoid compromising phages as future option.

Given the patient's poor previous response to simple cefuroxime therapy, bacteriophages were most likely responsible for providing an added benefit to ensure treatment success, recovery of skin lesions, and allowing a curative surgery (SANSOM [7]). The patient's medical background was also complicated by the existence of multiple foreign bodies (metallic rods and screws), which can provide an inert substrate for germs capable to form biofilm, and which were successfully decontaminated during treatment. To our knowledge, this is the first clinical case successfully treated in Romania with combined therapy with antibiotics and bacteriophages since the '70s. In hard to treat infections due to multidrug-resistant bacteria with intracellular invasion, biofilm formation on metal prostheses inserted in hard to sterilize sites, antibiotics might need an adjuvant for destroying bacteria. Bacteriophages can be a solution, and further *in vitro* and *in vivo* studies are needed, particularly to improve drug delivery techniques. In the case we have presented, phages were administered locally, but for systemic administration bacteriophage-loaded nanoparticles could aid in targeting the exact site of infection. In conclusion, biology provides the necessary tools (bacteriophages that eliminate bacteria), but technology is needed to improve the use of these tools in actual clinical practice.

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References

1. C. ZAMBORI, A.A. MORVAY, C. GURBAN, M. LIKER, G. TANASIE, O. COLIBAR, C. CUMPANASOIU, C.G. SONEA, A.C. SONEA, E. TIRZIU, Biofilm formation of *Staphylococcus*, *Streptococcus*, *Pasteurella* and *Neisseria* strains. *Rom. Biotechnol. Lett.,* 20, 10718 (2015).
2. F. SULLA, D.T. BUSSIUS, F. ACQUESTA, A. NAVARINI, S.M. SASAGAWA, M.J. MIMICA, Vancomycin minimum inhibitory concentrations and lethality in *Staphylococcus aureus* bacteremia. *Germs*, 5, 39 (2015).
3. L.L. PREOȚESCU, O. STREINU-CERCEL, Prevalence of nasal carriage of *S. aureus* in children. *Germs*, 3, 49 (2013).
4. B. IONESCU, D. IONESCU, I. GHEORGHE, C. CURUTIU, O. BANU, C. BLEOTU, G. MIHAESCU, V. LAZAR, R. GRIGORE, E. BEZIRTZOGLOU, S. BERTESTEANU, Virulence patterns of *Staphylococcus aureus* hospital strains isolated in Bucharest, Romania. *Rom. Biotechnol. Lett.*, 20, 10536 (2015).
5. A. CHIRIAC, D. PASCAL, Multiple Drug Hypersensitivity Syndrome. *Curr. Opin. Allergy Clin. Immunol.*, 13, 323 (2013).
6. A.C. NEGUȚ, O. SĂNDULESCU, M. POPA, A. STREINU-CERCEL, Z. ALAVIDZE, I. BERCIU, C. BLEOTU, M.I. POPA, M.C. CHIFIRIUC, A. STREINU-CERCEL, Experimental approach for bacteriophage susceptibility testing of planktonic and sessile bacterial populations – Study protocol. *Germs*, 4, 92 (2014).
7. C. SANSOM, Phage therapy for severe infections tested in the first multicentre trial. *Lancet Infect. Dis.*, 15, 1384 (2015).