Short communication

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The mutual alteration of bacterial susceptibility to antimicrobials in dual-species biofilm

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ABSTRACT. The biofilm formation by pathogenic bacteria significantly reduces the efficacy of antimicrobial therapy, leads to chronic diseases and slows down the wounds healing. Many infectious processes are caused rather by consortium of bacteria than by one microorganism. As a result, mixed bacterial biofilms are formed, in which the resistance to antimicrobials increases due to the survival of some microorganisms in the biofilm of resistant strains and the transition to an uncultivated state. Here we show that the bacterial susceptibility to antimicrobials in dual-species biofilms significantly differs from monoculture biofilms depending on both conditions and chosen antimicrobial agents. While *Staphylococcus aureus* could completely avoid some antimicrobials by embedding into the biofilm of various gram-negative bacteria, the very same consortium was characterized by 10–fold increase in susceptibility to broad-spectrum antimicrobials like ciprofloxacin and aminoglycosides compared to monocultures. These data clearly indicate that efficient treatment of biofilm-associated mixed infections requires antimicrobials active against both pathogens, since the interbacterial antagonism would enhance the efficacy of treatment.

Keywords: Microbial biofilms, susceptibility to antimicrobials, interbacterial interactions, bacteria.

1. Introduction

While in the biofilm, bacteria are extremely resistant to bactericidal drugs, antibiotics and the immune system of the host, and survive at antibiotics concentrations of 500-1000 times greater than their minimum inhibitory concentration in vitro (Sanchez-Vizuete et al., 2015). Despite of active investigation of monomicrobial biofilms, studies of mixed communities have been made only for several bacteria, for example, *S. aureus – P. aeruginosa* biofilms for which the mutual changes in antibiotic resistance driven by various metabolies synthesis has been shown. On the other hand, saprophytic staphylococci are able to displace pathogenic microflora in atopic dermatitis (Nakatsuji et al., 2017). However, there are only few investigations for many combinations of bacteria, such as *E. coli, Klebsiella*, *Bacilli* etc. As well, less data on inter-bacterial interactions in such consortia are known. Understanding the mechanisms of interspecies interactions in mixed consortia of pathogenic bacteria will allow adjusting the recommendations for the use of different antibiotics depending on the microbial composition of the infection and improve the efficacy of the therapy.

2. Materials and methods

Staphylococcus aureus (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (MG1655), *Klebsiella pneumoniae* (clinical isolate) were used in this study. For the biofilm assay bacteria were grown under static conditions at 35°C for 48 hours in BM broth (Kayumov et al., 2015a; 2015b; Baidamshina et al., 2017). The mannitol-salt agar, Endo agar and Cetrimide agar were used to distinguish bacteria in mixed cultures. Biofilm formation was assessed in 24-well polystirol plates (Eppendorf) by staining with crystal violet (Sharafutdinov et al., 2017). The minimum inhibitory concentration (MIC) of antimicrobials was determined by the broth microdilution method according to the recommendation of EUCAST. The viability of cells was assessed by CFUs count (Sharafutdinov et al., 2017). Experiments were carried out in three biological repeats with three technical repeats in each of them.

3. Results and discussion

We have shown that *S. aureus* and *P. aeruginosa* form a stable mixed biofilm *in vitro* with prevalent location of *S. aureus* in upper layers of the biofilm.

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When mixed biofilms were treated with *S. aureus*-specific antimicrobials like vancomycin, tetracycline, ampicillin, and ceftriaxone, *P. aeruginos*a cells in the biofilm remained viable regardless of the presence of antibacterial substances. At the same time, *S. aureus* cells also survived, and were located in bottom layers of the biofilm suggesting that *S. aureus* is able to integrate into the matrix of *P. aerugunos*a biofilm and form separate microcolonies within it, and thus avoid of antimirobial drugs. On the other hand, antibiotics such as ciprofloxacin and aminoglycosides, which are effective against both strains, led to no re-arrangements of bacterial distribution in mixed biofilm and 10-fold stronger effect in compare with biofilms of monocultures. Similar results were obtained in experiments on mixed cultures of *S. aureus* and *K. pneumonia*e, *S. aureus* and *E. coli*. These data confirm that interactions between bacteria in the mixed biofilm significantly change the sensitivity to antibiotics of the microbial consortium. Moreover, these changes are bi-directional and the correct choice of antimicrobials determines whether the interbacterial interaction pattern will diminish or enhance the efficacy of antimicrobial.

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