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TIME-KILL ASSESSMENT OF INTERACTION BETWEEN CHEMICAL AND BIOLOGICAL COMPOUNDS AGAINST METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

Dmitri IUNAC, Greta BALAN

The discipline of microbiology and immunology, Department of Preventive Medicine,
PI *NicolaeTestemitanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova

Autor corespondent: Dmitri Iunac, e-mail: dmitri.iunac@usmf.md

Introduction

Staphylococcus aureus (*S. aureus*) is a Gram-positive bacterium that may cause life-threatening diseases and some minor infections in living organisms. However, it shows notorious effects when it becomes resistant to antibiotics. Methicillin is a semisynthetic antibiotic drug that was used to inhibit staphylococci pathogens. The *S. aureus* resistant to methicillin is known as methicillin-resistant *Staphylococcus aureus* (MRSA), which became a superbug due to its defiant activity against the antibiotics and medications most commonly used to treat major and minor infections. MRSA was first found in 1961. It is resistant to many antibiotics such as methicillin, penicillin, oxacillin, cloxacillin, cefazolin, cefoxitin, and other common antibiotics. MRSA can spread via close contact with infected people. It can transmit from an object which contains MRSA to a human, or from a human carrier to another human. MRSA becoming the predominant cause and representing a significant burden to the healthcare system. The emergence of resistance to antibiotics is a serious public health problem worldwide and can be a cause of mortality. For this reason, antibiotic treatment is compromised, and we have few therapeutic options to treat infections.

The use of combinations of diverse compounds with antimicrobial effects to achieve synergistic activities against MRSA is a potential strategy for overcoming bacterial resistance.

The **main goal** of our study is to search for new treatment options for infections caused by difficult-to-treat resistant germs.

Materials and methods

Time-kill studies were performed to analyze the activity of the selected chemical and biological compounds in combination. Stock solutions of chemical ($C_{14}H_{20}N_4S$) and biological (carotenoid pigment myxoxanthophyll from *Spirulina platensis* biomass) compounds were prepared according to the CLSI (Clinical Laboratory Standardization Institute) method or manufacturer's recommendations. The tests were performed on the methicillin-resistant strain *Staphylococcus aureus* ATCC 700699. All experiments were performed in triplicate. The kill measurement and the rate of bacterial death were determined by plotting the viable colony counts as a log10 (CFU/ml) against the time. The interaction was classified as bacteriostatic or bactericidal. Bacteriostatic action was defined as a decrease of < 3 logs CFU/ml and bactericidal effect was defined as a decrease of ≥ 3 log CFU/ml after 24 h of incubation compared with the size of the initial inoculum.

Results

All treated cultures were affected in a concentration-dependent manner which means that the reduction in CFU count of MRSA was increased by increasing the concentrations of biological in each combination in comparison with the initial inoculum. Positive control reflects MRSA's ideal growth behavior during 24 h of incubation. The combination of 64.5 + 340.5 (μg/μg)/ml of chemical and biological compounds respectively did not allow the CFU count of MRSA to increase from the onset of the experiment to its end; it significantly

reduced the CFU count of the initial inoculum during all time intervals of the experiment, especially after 24 h of incubation where the reduction of CFU count was (– 3.7). Also, the combination at 62.5 + 170.25 (µg/µg)/ml of chemical and biological compounds respectively reduced the CFU count after 24 h by (– 1.8). On the other hand, the combination at 62.5 + 85.125 suppressed the growth of MRSA for 18 hours only. After that, it was regrown until reaching (6.1) which means an increase of (0.1) compared to the CFU count of the initial inoculum.

Conclusions

Combining diverse compounds such as chemical and biological can improve safe and cost-effective patient care delivery in an era where research into discovering new agents is limited and expensive.

Keywords: methicillin-resistant *Staphylococcus aureus*, time-kill, interaction between chemical and biological compounds