ABSTRACT

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Title of the diploma thesis: **Searching for a rational strategy in combat with multi-drug resistant staphylococci – options of combination therapy**

Background

With the rise of multi-drug resistance in microbes, treating many infectious diseases has become challenging. Among clinically important bacteria with higher priority, pathogens from the ESKAPE group are included. The S in the acronym ESKAPE stands for *Staphylococcus aureus* (*S. aureus*). This bacterial agent is a common human pathogen and can cause various infectious diseases. *S. aureus* strains are often resistant to antibiotics such as β-lactams, chloramphenicol, lincomycin, aminoglycosides, tetracyclines, macrolides, sulfonamides, and rifampicin. The need for new antibiotics or alternative strategies to combat infections caused by multi-drug-resistant pathogens has become more apparent in recent years. A combination therapy could cover the requirement for an alternative treatment strategy. Although it is already used in clinical practice, it is mainly due to empirical knowledge, and proven evidence about treatment benefits or potential pitfalls is lacking.

Aim

This diploma thesis is focused on evaluating the mutual interaction and impact on the activity of selected commercially available antibiotics in combinations. The *Staphylococcus aureus,* MRSA, American Type Culture Collection (ATCC) 43300, CCM 4750, purchased from the Czech Collection of Microorganisms (CCM) was used to determine the effect of selected pair-wise combinations. Selected antibiotics for this thesis were ciprofloxacin (CIP), cotrimoxazole (COT), daptomycin (DAP), linezolid (LIN), rifampicin (RIF), tigecycline (TIG), and vancomycin (VAN). Combinations that show promising results will be recommended for further testing.

Methods

A universal bipolar solvent dimethylsulfoxide was used to prepare a stock solution of selected antibiotics. Cation-adjusted Müller-Hinton broth was used as a medium for a final antibiotic solution and bacterial suspension.

The checkerboard microdilution method was applied to assess the interaction of antibiotics in combinations. Spectrophotometric measurement was used to determine the degree of inhibition of bacterial growth. The potency of the antibiotic pair-wise combinations was expressed by creating a heat map of each combination using a percentage of inhibition values, and the categorization of mutual antibiotic drug interactions was determined by calculating the FIC (fractional inhibitory concentration) index.

Results

Seventeen pair-wise combinations, each comprising of thirty-six sub-combinations, were evaluated. The result of the evaluation of most pair-wise antibiotic combinations was indifference. One combination expressed an outright antagonistic effect, and two others expressed indifference bordering on antagonism. Two combinations, which showed mostly indifference, had a small number of sub-combinations where the additive effect was registered. Two combinations expressed additive effect.

Conclusion

In summary, out of seventeen evaluated pair-wise antibiotic drug combinations, two combinations, namely CIP+RIF and COT+RIF, expressed promising mutual interaction (additive effect) in at least three drug concentration ratios. These drug combinations will undergo further advanced assessments— they will be incorporated into antimicrobial cocktails (e.g., with antimicrobial peptides, efflux pump inhibitors, or biosurfactants), and the antibiofilm activity will also be studied.

Keywords: MRSA, combination therapy, *in vitro* susceptibility testing, checkerboard microdilution method, drug combinations interactions