

Abstract

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Title of diploma thesis: The study of the impact of the mutual interaction of selected antibacterial drugs *in vitro* on activity against enterococci

Background: The point of the experimental part of this thesis was to evaluate the effect of the mutual interaction of two selected, clinically approved antibiotics in combination on two selected bacterial strains of genus *Enterococcus*. The effect of the combination was evaluated using the FIC index. Based on the calculation of the FIC index, the mutual interaction was further classified as synergistic, additive, indifferent or antagonistic.

Methods: To evaluate the interaction of antibacterial drugs in combination, four antibiotics were selected and employed to a pair-wise combinations. The main antibiotic was daptomycin, which was combined with tigecycline, linezolid and vancomycin. The effect of pair-wise combinations was monitored against the clinical isolate *Enterococcus faecium* and against the collection bacterial strain *Enterococcus faecalis* (ATCC 29212). Determination of MIC values was performed using the microdilution broth technique according to the EUCAST methodology. To determine the MIC values, the growth of bacteria was monitored with the naked eye and by using spectrophotometric detection. The evaluation was carried out after a 24-hour or 48-hour incubation. For the clinical isolate strain, also the method of metabolic activity evaluation using the Alamar Blue indicator was chosen to confirm the antibacterial effect of antibiotics in combination. The mutual drug interactions of the antibiotics in combination were classified as synergistic, additive, indifferent or antagonistic, based on the calculation of the FIC index parameter.

Results: Pair-wise combinations of antimicrobial agents were tested against two bacterial strains (*Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* clinical isolate). The most promising results based on the evaluation of the FIC index with the determination of the additive effect were revealed for the combination of daptomycin and vancomycin against *Enterococcus faecalis* (ATCC 29212) in concentration ratios of 0.25 : 4 (mg/L); 0.5 : 4 (mg/L); 1 : 4 (mg/L); 2 : 2 (mg/L) and 4 : 2 (mg/L). Additionally, an additive effect was recorded for the combination of daptomycin with linezolid, in a concentration ratio 1 : 4 (mg/L). For the combinations of daptomycin with tigecycline, there were additive effects in ratios of 0.25 : 4 (mg/L) and 0.25 : 0.25 (mg/L). The additive effect of the combination of daptomycin with linezolid in a concentration ratio 2 : 64 (mg/L) was evaluated against the *Enterococcus faecium* strain. In the other tested concentration ratios of pair-wise combinations acting against both strains, the indifferent effect was revealed. No synergistic effect was demonstrated in any concentration ratio for any of the tested combinations.

Conclusion: Enterococci are classified as "superbugs", or strains of bacteria with a high level of resistance to several classes of antibiotics. This resulted in difficulties with the treatment of the infections caused by enterococci. Inappropriate use of antibiotics can possibly lead to failure of pharmacotherapy due to antimicrobial resistance. Thereby it is desirable to look for new therapeutic options in the treatment of enterococcal infections. One of the possible approaches for successful treatment is represented by rational combination therapy. Within the rational combination therapy implementation, it is necessary to choose combinations of antibiotics, which show a synergistic effect. As a part of the evaluation of the combined antienterococcal effect of pair-wise combinations of four selected antimicrobial substances, additive effect was demonstrated for the drug combination of daptomycin with vancomycin. Furthermore, the additive effect was demonstrated in combination of daptomycin with linezolid and daptomycin with tigecycline against the collection strain *Enterococcus faecalis* ATCC 29212, and in the combination of daptomycin with linezolid against the clinical isolate *Enterococcus faecium*. No synergistic effect was noted for any of the selected combinations in any concentration ratio of the selected antimicrobial compounds.

Keywords: enterococci, checkerboard test, ESKAPE, microdilution broth method