Title: ANTIBACTERIAL ACTIVITY BY TIME-KILL ASSAY OF LINEZOLID IN COMBINATION OF ANTIMICROBIAL DRUGS AGAINST VANCOMICIN-RESISTANT ENTEROCOCCUS FAECIUM (VREfm) ISOLATE.

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Abstract:

Enterococci are ubiquitous bacteria of environment and normal colonizing of human host. However, E. faecium is an important pathogen often recovered from infections as endocarditis and sepsis. Acquired antimicrobial resistance to clinically useful antibiotics as glycopeptides and beta-lactams has been associated by high genome plasticity, and this specie has emerged as an important nosocomial pathogen. The aim of this study was to evaluate by time-kill assay the potential for synergy of a combination of antimicrobial agents against a multiresistant vancomycin-resistant E. faecium (VREfm) recovered from a bloodstream infection. The MIC for antimicrobial agents used in the study was performed according CLSI guidelines. Time-kill assay was performed in triplicate at free drug concentration achieved in therapeutic regimen: ampicillin (AMP-70 µg/mL), vancomycin (VAN-15 µg/mL), gentamicin (GEN-5 µg/mL), rifampin (RIF-8 µg/mL), and linezolid (LIN-4.4 µg/mL). Mean of colony counts (log10 CFU/mL) versus time at 0, 4, 8 and 24h was analysed and classified by CLSI criteria regarding antimicrobial activity as bactericidal or bacteriostatic (reduction ≥3log10 and reduction <3log10 of the original inoculum, respectively), synergy (reduction of ≥2log10 at 24h, compared with the most active single drug or combination result), antagonism (a bacterial growth ≥1log10 compared with of least active single agent or combination) or indifferent. The VREfm isolate (VAN ≥256 µg/mL) was resistant to AMP (≥256 µg/mL) and RIF (≥32 µg/mL), intermediate to LIN (4 µg/mL) and non-high level aminoglycoside resistant (GEN 4 µg/mL). No antibiotic combination was classified as bactericidal. Only linezolid as a single drug and combination was bacteriostatic at 24h, with a reduction -1.46 log10 to LIN+VAN+GEN and -1.81 log10 to LIN+AMP+GEN. AMP+GEN and VAN+GEN drug combination were considered bacteriostatic at 4h and 8h, followed by a regrowth at 24h. Only AMP+RIF showed synergy (-2.11 log10, respectively) although AMP+RIF showed regrowth at 24h. In conclusion, the combination of linezolid, ampicillin and gentamicin improved antibacterial activity enhancing the bacterial killing (reduction of -1.10 log10 at 24h) compared to single and the most active drug combination, even if the isolate was ampicillin resistant. This preliminary data support the continued evaluation of in vitro drug combination as alternative to antimicrobial therapy of multiresistant bacteria, especially VREfm isolates.

Keywords: antimicrobial agents, synergy, time-kill assay, Enterococcus faecium

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