

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

**Authors:** Paim, T. G. S.<sup>1</sup>, Sambrano, G. E.<sup>1,2</sup>, Soares, R. O.<sup>1</sup>, Moura, T. M.<sup>1</sup>, d'Azevedo, P. A.<sup>1</sup>

**Affiliations:** <sup>1</sup> Universidade Federal de Ciências da Saúde de Porto Alegre (Rua Sarmento Leite, 245 – Rio Grande do Sul, Porto Alegre, Brasil), <sup>2</sup> National University of Ireland Galway (University Road, Galway, Ireland)

**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 potencial 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 (log<sub>10</sub> 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  $\geq 3\log_{10}$  and reduction  $< 3\log_{10}$  of the original inoculum, respectively), synergy (reduction of  $\geq 2\log_{10}$  at 24h, compared with the most active single drug or combination result), antagonism (a bacterial growth  $\geq 1\log_{10}$  compared with of least active single agent or combination) or indifferent. The VREfm isolate (VAN  $\geq 256$  µg/mL) was resistant to AMP ( $\geq 256$  µg/mL) and RIF ( $\geq 32$  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 log<sub>10</sub> to LIN+VAN+GEN and -1.81 log<sub>10</sub> 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 log<sub>10</sub>, 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 log<sub>10</sub> 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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