Communication/Comunicação

Evaluation of bacterial growth inhibition by mercaptopropionic acid in metallo-β-lactamase detection on multidrug-resistant *Acinetobacter baumannii*

Avaliação da inibição do crescimento bacteriano pelo ácido mercaptopropiônico na detecção de metalo-β-lactamases em *Acinetobacter baumannii* multirresistente

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ABSTRACT

Introduction: Metallo-β-lactamase (MBL) has been reported all over the world. **Methods**: The inhibitory effect of mercaptopropionic acid (MPA) on bacterial growth was evaluated by comparison between disk diffusion and broth dilution methodology with determination of the minimum inhibitory concentration (MIC) for multidrug-resistant *Acinetobacter baumanni* strains. **Results:** MPA significantly inhibited growth of the strains. **Conclusions**: The use of MPA can affect the results in phenotypic methods of MBL detection.

Keywords*:* Drug resistance. *Acinetobacter baumannii*. Microbial sensitivity tests.

RESUMO

Introdução: Metalo-β-lactamases (MBL) têm sido reportadas em todo o mundo. **Métodos**: Avaliamos o efeito inibitório do ácido mercaptopropiônico (AMP) sobre o crescimento bacteriano mediante a comparação entre a difusão em disco e a metodologia de diluição em caldo com a determinação da concentração inibitória mínima (CIM) entre cepas multirresistentes de *Acinetobacter baumanni*. **Resultados**: O AMP inibiu o crescimento das cepas do presente estudo de maneira significativa. **Conclusões**: O uso do AMP pode afetar os resultados dos métodos de detecção fenotípica de MBL.

Palavras-chaves: Resistência a medicamentos. *Acinetobacter baumannii*. Testes de sensibilidade microbiana.

Metallo-β-lactamase (MBL) has been reported all over the world and its fast spread has not been accompanied by standardization of a suitable method for phenotypic detection in the laboratory[1,2]. The proliferation of MBLs-producing strains must be regarded as a potential public health problem because of the limited therapeutic options, increased morbimortality rates and serious concerns in relation to infection control[3-5]. In this context, *Acinetobacter baumannii* is an opportunistic Gram-negative pathogen with increasing relevance among nosocomial infections[1,3,6]. The phenotypic methods to detect metalloenzyme producers are based on the fact that these enzymes are affected by the removal of zinc from the active site, which can be performed by chelating agents[1,2], or by alteration of the active site of the enzyme caused by thiol-based compounds[7], including mercaptopropionic acid (MPA).

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Thus, the fact that the metallo-β-lactamase inhibitor (IMBL) may possess its own inhibitory activity against bacterial growth in phenotypic tests should be taken into account. Depending on the test concentration of the IMBL, interpretation of the test results may be compromised[4].

In this study, the inhibitory effect of MPA on bacterial growth was evaluated by comparison between disk diffusion and broth dilution methodology with determination of the minimum inhibitory concentration (MIC) for multidrug-resistant (MDR) *A. baumanni* strains.

A total of 30 MDR *A. baumannii* isolates were tested. The strains form part of the bacteria collection of the Bacteriology Laboratory, Department of Clinical and Toxicological Analysis, Center for Health Sciences, Federal University of Santa Maria, Santa Maria, Brazil. *Pseudomonas aeruginosa* ATCC 27853 (MBL-negative control) and *P. aeruginosa* SPM-1 (MBL-positive control) were included as controls.

The broth microdilution method was developed according to the criteria of the CLSI[8] but instead of an antimicrobial agent solution, 20μL of MPA was used, which was dispensed into the first well, obtaining a concentration of 0.74mg/L. Dilutions were condcuted in subsequent wells to obtain the following concentrations: 0.37mg/L, 0.185mg/L, 0.0925mg/L, 0.0463mg/L, 0.0231mg/L, 0.0116mg/L, 0.0058mg/L, 0.0029mg/L and 0.0014mg/L.

In order to determine the minimum bactericidal concentration (MBC), strains inhibited in the visual reading of MIC were subcultured in Mueller-Hinton agar and incubated for at least 24h in a bacteriological incubator at 35°C ± 2.

Evaluation of MPA activity by the disk diffusion method was performed by applying a filter paper disk on a plate of Mueller-Hinton agar, previously inoculated with a bacterial suspension equivalent to the 0.5 McFarland standard. Three microliters (3μL) of MPA (undiluted) were added to the filter paper disk. The diameter of the inhibition zone produced by the MPA in the strains tested was measured after incubation at 35 ± 2°C for 24h.

The inhibitor effect of MPA on bacterial growth in the strains studied was evaluated according to the size of the inhibition zone. Inhibition ranged from 14mm to 28mm; a halo of 21mm was the most frequent (n = 7) (23.3%). Picão et al[4] reported an increase in the size of the inhibitor zones produced by MPA (from 0.3 to 12.4mm).

A minimum inhibitory concentration of 0.0116mg/L (n = 16) (53.3%) was the most frequent. The MBC of most strains was

0.0231mg/L (n = 20) (66.7%). In the majority of the strains tested, the bactericidal effect was observed at a dilution greater than the MIC (MIC = 0.0116mg/L and MBC = 0.0231mg/L).

In conclusion, no assays have been published in the national or international literature using the MIC technique to evaluate the activity of MPA against *A. baumannii* growth. MPA significantly inhibited bacterial growth in the strains analyzed in this study, which could affect the results when using phenotypic methods of MBL detection. Analysis of the results demonstrated that the use of MPA as a IMBL should be reconsidered. In the case of preliminary results, a larger number of strains will be analyzed.

CONFLICT OF INTEREST

The authors declare that there are no conflict of interest.

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