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Original article

Comparison of phenotypic tests for the detection of metallo-beta-lactamases in clinical isolates of *Pseudomonas aeruginosa*

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ABSTRACT

Metallo-β-lactamase (MBL)-producing gram-negative bacteria are an increasing public health concern worldwide. Screening tests for the rapid and specific identification of these pathogens are essential, and should be included among routine diagnostics in laboratories. This study aimed to determine the MBL frequency among carbapenem-resistant *Pseudomonas aeruginosa* isolates, and to evaluate the accuracy of different tests in screening for MBL production. From January 2001 to December 2008, a total of 142 imipenem-non-susceptible *P. aeruginosa* strains were isolated from distinct clinical samples from hospitalized patients. These isolates were examined by PCR, MBL E-test, double-disk synergy test (DDST), and combined disk (CD) test. The minimal inhibitory concentration (MIC; μg/mL) was determined by agar dilution, and pulsed field gel electrophoresis (PFGE) was performed on all samples. Sequencing was performed to confirm and define the MBL variant and subtype. Using PCR and DNA sequence analysis, 93 strains were confirmed positive for MBLs, 91 strains for the blaSPM-1 gene, 1 strain for the blaIMP-1 gene, and 1 strain for the blaIMP-16 gene. PFGE displayed a clonal pattern. The sensitivities, specificities, positive and negative predictive values were evaluated for all tests. The DDST assay (CAZ-MPA) was the optimal method for screening MBL production in *P. aeruginosa* strains. However, the results of the CD assay (IMP/EDTA) showed close agreement with those of the DDST. In addition, the CD assay allowed a more objective interpretation and did not require the use of a toxic substance.

Comparación de las pruebas fenotípicas para la detección de metalo-beta-lactamasas en aislados clínicos de *Pseudomonas aeruginosa*

Palabras clave:
Pseudomonas aeruginosa
Metalo-beta-lactamasa
Pruebas de detección

RESUMEN

Las metalo-β-lactamasas (MBL) que producen las bacterias gram-negativas son un creciente problema de salud pública en todo el mundo. Las pruebas de detección para la identificación rápida y específica de estos patógenos son esenciales y deben ser incluídas entre los diagnósticos de rutina de los laboratorios. Este estudio tiene como objetivo determinar la frecuencia de MBL en aislamientos de *Pseudomona aeruginosa* resistentes a carbapenem y evaluar la precisión de diferentes pruebas en la detección de la producción de MBL. Entre enero de 2001 y diciembre de 2008 un total de 142 cepas de *P. aeruginosa* no susceptibles a imipenem fueron aisladas de muestras clínicas provenientes de pacientes hospitalizados. Estas cepas fueron examinadas por PCR, prueba de MBL-E, prueba de sinergia de doble disco (DDS), y prueba de disco

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combinado (DC). La concentración inhibitoria mínima (CIM; g/ml) se determinó mediante dilución en agar. Se realizó electroforesis en gel de campo pulsado (PFGE) a todas las muestras. La secuenciación se realizó para confirmar y definir la variante de MBL y subtipo. Por PCR y análisis de secuencia de ADN, 93 cepas fueron confirmadas como positivas para MBL. A su vez, 91 cepas fueron confirmadas para el gen blaSPM-1, 1 cepa para el gen bla IMP-1, y 1 cepa para el gen bla IMP-16. La prueba de PFGE muestra un patrón clonal. Se evaluó la sensibilidad, especificidad, valores predictivos positivos y negativos para todas las pruebas. El ensayo DDS (CAZ-MPA) fue el método óptimo para la detección de la producción de MBL en las cepas de *P. aeruginosa*. Sin embargo, los resultados del ensayo de DC (IMP/EDTA) mostraron una estrecha concordancia con los de la DDS. Adicionalmente, el ensayo de DC permitió una interpretación más objetiva de los resultados, no requiriendo el uso de una sustancia tóxica.

Introduction

Metallo-β-lactamases (MBLs) are resistance determinants of increasing clinical relevance in gram-negative bacteria, especially in *P. aeruginosa*, *Acinetobacter* spp., and members of the *Enterobacteriaceae* family.[1,2] The worldwide dissemination of acquired metallo-β-lactamases genes and the emergence of new variants are becoming an emerging threat to public health because they usually are carried by mobile genetic elements that disseminate rapidly.[3–5] Increased mortality rates have been documented for patients infected with MBL-producing *P. aeruginosa*, rates that have been exacerbated by inadequate empirical therapy.[6] Therefore, early detection and identification of MBL-producing organisms is of crucial importance for the prevention of nosocomial dissemination through appropriate treatment, as well as the implementation of infection control measures.[2,7]

Several phenotypic methods used to detect microorganisms carrying MBL have been reported.[8–14] Currently, the most frequently used tests are the double-disk synergy test (DDST), the combined disk (CD) assay, and the MBL E-test. However, these tests have shown discordant results depending on the employed methodology, β-lactam substrates used, presence of MBL inhibitors (IMBL), bacterial genus tested and local prevalence of MBL types. Although there are numerous studies evaluating screening tests with IMP and VIM producing *P. aeruginosa*, there is no inclusion of SPM producing isolates, the most prevalent in our country.[15] Therefore, hospital microbiology laboratories should evaluate a variety of assays and identify the most appropriate one for local routine application. The aim of this study was to determine the MBL frequency among carbapenem-resistant (CR) *P. aeruginosa* isolates and evaluate the accuracy of different tests in screening for MBL production.

Materials and methods

This study was performed at the Hospital de Clínicas da Universidade Federal do Paraná (HC-UFPR), a 640-bed tertiary care academic hospital in Curitiba, Brazil. The study was approved by The HC-UFPR Institutional Review Board (IRB#0248.0.208.000-09).

Bacterial isolates

From January 2001 to December 2008, a total of 142 non-duplicate imipenem (IP)-nonsusceptible *P. aeruginosa* (MIC $\geq$ 8 μg/mL) isolates were collected from different units of the hospital. All of these samples were isolated from different patients and were identified by conventional biochemical tests in accordance with published recommendations.[15] *P. aeruginosa* ATCC 27853 was used as an MBL-negative control. *P. aeruginosa* strain P1088 producing SPM, *A. baumanii* strain 17–4 producing IMP-1 and *P. aeruginosa* producing VIM were used as MBL-positive controls.

Susceptibility testing

The agar dilution method was used to determine the minimal inhibitory concentrations (MICs) of the following drugs: imipenem (IP), meropenem (MEM), piperacillin/tazobactam (PTZ), ceftazidime (CAZ), ciprofloxacin (CIP), gentamicin (GEN), amikacin (AMI), aztreonam (ATM), cefepime (CPM), and B polymyxin (POL). The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI; 2009). *P. aeruginosa* ATCC 27853 was used as a control strain for susceptibility testing.

Phenotypic detection of MBL

DDST: The DDST phenotypic tests were performed by following the CLSI recommendations for the disk diffusion method (CLSI 2011, M100-S21). Briefly, a 0.5 McFarland bacterial suspension was inoculated on a Mueller–Hinton (MH) agar plate. One ceftazidime (CAZ) disk was placed into the agar, aligned 20 mm apart a blank filter disk (edge-to-edge) containing 5 μL of 1.4 mM (1:8) mercaptopropionic acid (MPA; Sigma; St. Louis, MO, USA) solution.[14] Each agar plate was incubated at 35 °C ± 1 °C overnight. Enhancement of the zone of inhibition in the area between the MPA and CAZ disk was interpreted as a positive test result.

CD: Two IP (10 μg) disks (Becton Dickinson, Franklin Lakes, NJ, USA) were placed on an agar MH plate containing the bacterial suspension (0.5 McFarland), and 5 μL of a 0.5 M EDTA solution (pH 8.0)[11] was added to one of the IP disks. After incubation overnight at 35 °C ± 1 °C, the inhibition zones of the IP disks in the presence and absence of EDTA were compared.

E-test MBL: The MBL E-test (AB Biodisk, Solna, Sweden) was performed according to the manufacturer's recommendations.

MBL gene PCR amplification and sequencing

PCR assays were performed to amplify the sequences of the bla_{IMP}, bla_{VIM}, bla_{GIM}, bla_{SPM-1}, bla_{SIM} and bla_{KPC} genes, as previously described.[16–20] The PCR products were purified and sequenced (MegaBACE; ABI PerkinElmer, Waltham, MA, USA) to confirm and define MBL variant and subtype. These tests were used as gold standard in the evaluation of screening testes.

Genetic similarity

Genetic relatedness among the IP-nonsusceptible *P. aeruginosa* isolates was evaluated by pulsed-field gel electrophoresis (PFGE) using the restriction enzyme *Spe*I (Invitrogen, Carlsbad, CA, USA) at 37 °C. Electrophoresis was performed on a CHEF-DRIII (Bio-Rad