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Leila Azimi

Iran University of Medical Sciences

Abdolaziz Rastegar Lari

Iran University of Medical Sciences

Malihe Talebi

Iran University of Medical Sciences

Parviz Owlia

Molecular Microbiology Research Center

Reza Alaghebandan

Washington University School of Medicine in St. Louis

See next page for additional authors

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Authors

Leila Azimi, Abdolaziz Rastegar Lari, Malihe Talebi, Parviz Owlia, Reza Alaghebandan, Babak Asghari, and Elnaz Rastegar Lari

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Inhibitory-based method for detection of *Klebsiella pneumoniae* carbapenemase *Acinetobacter baumannii* isolated from burn patients

Leila Azimi¹, Abdolaziz Rastegar Lari², Malihe Talebi³, Parviz Owlia⁴, Reza Alaghebandan⁵, Babak Asghari⁶, Elnaz Rastegar Lari⁷,

¹ Antimicrobial Resistance Research Center, Iran University of Medical Sciences, Tehran, Iran

² Antimicrobial Resistance Research Center, Iran University of Medical Sciences, Tehran; Department of Microbiology, Iran University of Medical Sciences, Tehran, Iran

³ Department of Microbiology, Iran University of Medical Sciences, Tehran, Iran

⁴ Molecular Microbiology Research Center, Tehran, Iran

⁵ Department of Pathology and Immunology, Washington University School of Medicine, Barnes Jewish Hospital, St. Louis, MO, USA

⁶ Department of Microbiology, Tehran University of Medical Sciences, Tabriz, Iran

⁷ Luxembourg University, Faculty of Science Technology and Communication and Liege University, Faculty of Science and Environment, Belgium

Correspondence Address:

Abdolaziz Rastegar Lari

Department of Microbiology, Iran University of Medical Sciences, Tehran
Iran

Abstract

Background: *Klebsiella pneumoniae* carbapenemase (KPC) is one of the carbapenemases that can cause multi-antibiotics resistance in *Acinetobacter baumannii*. A simple phenotypic rapid and accurate test for the detection of *A. baumannii* - KPC-producer can be useful in treating related infections. The aim of this study was to determine the synergism effect of boronic acid (BA), as an inhibitor, and meropenem to confirm modified Hodge test (MHT) positive strains for KPC-production. **Materials and Methods:** Totally, 126 *A. baumannii* isolates were used as clinical strains. Imipenem resistant isolates were identified by disk diffusion method according to the Clinical Laboratory Standards Institute recommendations. Presence of KPC in imipenem resistant isolates was determined using the MHT. In addition, we used BA as a KPC inhibitor for final confirmation of the species of interest. Additionally, we employed the use of synergism effect of meropenem and cloxacillin to detect false positive cases. **Results:** Of 126 strains, 108 were resistant to imipenem, for which 93 strains were MHT positive. Totally, 68 out of 93 MHT positive isolates had at least 5 mm enlargement of the diameter of the zone of growth inhibition between the meropenem alone and meropenem combined with BA. Of these 68 isolates, 8 had at least 5 mm enlargement of the diameter of the zone of growth inhibition with BA alone and in 60 strains it was observed by cloxacillin. **Conclusion:** Our study suggests that MHT alone cannot confirm KPC-producer microorganisms and that it requires other complementary tests such as the usage of inhibitors.

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Full Text

Introduction

Gram-negative bacteria are common causes of nosocomial infections and wound infections among burn patients. [1],[2],[3] *Acinetobacter baumannii* is one of the most important and common Gram-negative bacteria in this setting. [1],[2],[3] These patients are at increased risk for these types of infections considering their immune-compromised status and the destruction of their skin barrier as a defensive mechanism. [2],[3] On the other hand, multi drug resistance strains among these organisms can cause therapeutic challenges. [2],[4] During the last decade, resistant to beta-lactam antibiotics such as carbapenems, as a broad spectrum antimicrobial agent, has increasingly become prevalent. [5] Resistance associated with *Klebsiella pneumoniae* carbapenemase (KPC) is an alarming problem in the health care systems. [5] This mechanism of resistance is important given it can cause resistant to all beta-lactam antibiotics. [5],[6],[7] Prevalence of KPC-producing microorganisms has emerged worldwide (USA, Greece, Iran, etc.). [5],[8],[9] Accurate detection and identification of KPC-producing bacteria in the clinical isolates is crucial for prevention and control of spread of this mechanism of resistant since the gene for KPC is located in transferable elements such as plasmid or transposon. [5],[10],[11]

According to the Clinical Laboratory Standards Institute (CLSI) recommendations, modified Hodge test (MHT) can be a confirmatory test for the detection of KPC-producer bacteria. [12] Some authors considered MHT positive isolates to be KPC-producer bacteria. [5],[13],[14],[15] It has been suggested that the use of boronic acid (BA) as a KPC inhibitor can be more useful than MHT, with less false positive results compared to MHT. [7],[9],[16],[17],[18],[19] The aim of this study was to determine the synergistic effect of BA as an inhibitor and meropenem to confirm MHT positive strains for KPC-production.

Materials and Methods

Bacterial strains

In this study, 126 *A. baumannii*, all confirmed by API system test and polymerase chain reaction of OXA51 gene, were surveyed. Primers are shown in [Table 1].{Table 1}

Antimicrobial susceptibility test

Imipenem resistance isolates were detected by disk diffusion method according to the CLSI recommendations and MAST company antibiotic discs. Strains with <13 mm diameter inhibition zone around imipenem disk were considered as an imipenem resistant isolate with comparison reference to the CLSI table. Subsequently, the presence of KPC in imipenem resistant was examined with MHT.

Modified hodge test

The modified cloverleaf test was performed according to the CLSI guidelines using *Escherichia coli* ATCC 25922 and Ertapenem disk.

Use of inhibitor for phenotypic detection

The phenotypic detection of KPC-producing *A. baumannii* was evaluated using BA combined-disk tests as an inhibitor of KPC production 400 µg of BA. The stock solution of BA (benzene BA; Sigma-Aldrich, Germany) in dimethyl sulfoxide and distilled water were mixed at a concentration of 20 mg/ml. From this solution, 20 µl (containing 400 µg/disk) was added on to commercially meropenem disks.

The test was considered positive when the inhibition zone diameter around the disk containing meropenem and BA was ≥5 mm compared with meropenem alone.

Considering the use of both BA and cloxacillin inhibitor of AmpC [20],[21] has been recommended for increasing the specificity of the test in detecting KPC, we used synergism effect of meropenem and cloxacillin for the elimination of false positive cases. Strains that had at least 5 mm difference between meropenem alone and meropenem combined with 800 µg cloxacillin were considered false positive.

Results

Of 126 strains, 108 were resistant to imipenem, for which 93 strains were MHT positive. Totally, 68 out of 93 MHT positive isolates had at least 5 mm enlargement of the diameter of the zone of growth inhibition between the meropenem alone and meropenem combined with BA. Of these 68 isolates, 8 had at least 5 mm enlargement of the diameter of the zone of growth inhibition with BA alone and in 60 strains it was observed by cloxacillin. Of the remaining of 108 isolates, 7 had at least 5 mm enlargement of the diameter of the zone of growth inhibition only between meropenem with cloxacillin, and in 18 strains no enlargement of the diameter of the zone of growth inhibition was seen.

Discussion

Although MHT is a useful test in detecting carbapenemases, it is not able to further subtype them. [14],[15],[17] On the other hand, other authors believe that MHT can be positive in the presence of overexpression of cephalosporinase (AmpC) and some of the extended-spectrum beta-lactamases such as CTXM. [21],[22],[23] Other studies suggest that BA can be useful as an inhibitor for detection of KPC. [7],[9],[16],[17],[18],[19] In the present study, 65% (60/93) of MHT positive strains showed synergistic effect of meropenem with BA and cloxacillin. Joint synergism with meropenem - BA and cloxacillin are considered in an AmpC-producer microorganism. Further, our study showed a synergism effect between meropenem and BA in 9% of tested isolates [see interpretation of results in [Figure 1],[Figure 1]

In a study from Argentina, all 36 KPC-producer *Pseudomonas aeruginosa* showed synergism with meropenem and BA. [24] Researchers from Greece reported 57 KPC-producer *K. pneumoniae* (were confirmed genotypically) showed an increase in inhibition zone diameter surrounding beta-lactam with BA compared with beta-lactam alone. [19] In a study from Australia, all 10 *K. pneumoniae* and *E. coli* KPC positive isolates showed synergism between meropenem or imipenem with BA. [7]

We believe that considering only 9% of MHT positive isolates were KPC after utilizing BA as an inhibitor, this test cannot establish KPC alone in *Acinetobacter* despite its high sensitivity.

Low specificity of MHT for *Acinetobacter* in this study is comparable to findings reported by a study from Taiwan (7%) [23] as well as Argentina (57%). [24] Thus, it seems that MHT has different specificity in different geographical areas with different species of bacteria.

In summary, our findings along with those of others in the literatures suggest that MHT alone cannot confirm KPC-producer microorganisms and that it requires complementary tests such as the use inhibitors. Therefore, applying inhibitors and performing other tests such as Carba-NP Test [25],[26] may be reasonable alternatives to molecular testing for detection of KPC due to the high cost and the need for expert personable. On the other hand, BA are being used in clinical applications and well suited as candidates for the inhibitor of bacterial antibiotic resistance enzymes in clinic.

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