Inhibitor-based methods for the detection of KPC carbapenemase-producing Enterobacteriaceae in clinical practice by using boronic acid compounds

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Enterobacteriaceae clinical strains that produce the class A carbapenem-hydrolysing enzyme KPC (Klebsiella pneumoniae carbapenemase) are increasingly reported worldwide, and are already endemic in North and South America, China, Israel and Greece. The accurate detection of KPC enzymes is of utmost importance for containing the global spread of KPC producers. Currently, the detection of putative carbapenemase production is based on an initial phenotypic screen for carbapenem resistance followed by the modified Hodge test (MHT) as a confirmatory test. However, the MHT is often difficult to interpret, is not specific for carbapenemase activity due to KPC and there are reports of false-positive results with CTX-M-positive or AmpC-hyper-producing Enterobacteriaceae. Boronic acid compounds are serine-type β-lactamase inhibitors that were employed originally for the detection of class C plasmidic AmpCs in Enterobacteriaceae. Recently, they have also been evaluated for the differentiation of KPC-producing Enterobacteriaceae. In that respect, combined-disc tests using carbapenem discs with and without phenylboronic acid (PBA) have been proposed as the most accurate phenotypic tests for detecting KPC production. When these disc tests are extended to include carbapenem discs with EDTA or both PBA and EDTA on the same plate, the production of metallo-β-lactamase (MBL) or both KPC and MBL, respectively, can also be accurately detected. Phenotypic tests based on the inhibitory activity of boronic acid compounds are very easy to perform and interpret, and may be applied from the first day of isolation of the suspected resistant Enterobacteriaceae. We think that they could effectively replace MHT for the convenient and early detection of KPC carbapenemases in regions where these enzymes are common.

Keywords: screening test, phenotypic test, combined-disc test, modified Hodge test, phenylboronic acid

Introduction

Klebsiella pneumoniae carbapenemase (KPC) enzymes have become increasingly prevalent among K. pneumoniae and other species of Enterobacteriaceae on the East Coast of the USA since the beginning of the 2000s.¹ KPC-producing bacteria have also caused outbreaks in Israel and recently have become emerging public health concerns in several regions worldwide, such as China, Latin America and Greece.² Given the limited therapeutic options available, the accurate detection of KPCpossessing Enterobacteriaceae is crucial in controlling their spread. The current guidelines for the phenotypic detection of KPC-producing organisms in US hospitals are based on reduced susceptibility to carbapenems, which has to be confirmed by the modified Hodge test (MHT).³ While the MHT has been found to be useful for the phenotypic detection of KPC β-lactamases in hospitals where they are common,³ it is recognized that the MHT is often difficult to interpret, not specific for KPC production and may give positive results with any enzyme with carbapenemase activity, thus requiring PCR for the differentiation of the carbapenemase present. Furthermore, there is increasing evidence that MHT frequently gives false-positive results with CTX-M extended-spectrum β-lactamase (ESBL)-positive or AmpC-hyperproducing Enterobacteriaceae.⁴,⁵ Thus, practical, highly sensitive and highly specific methods for the detection of KPC-producing isolates are needed for regions where carbapenem resistance determinants are prevalent. In this regard, there is increasing interest in the use of boronic acid compounds, which are β-lactamase inhibitors that seem to be promising candidates for the phenotypic detection of potent β-lactamases, including KPC.

Boronic acid compounds as β-lactamase inhibitors

Boronic acid compounds are virtually the only active-site-directed serine-type β-lactamase inhibitors that are not based on...
a β-lactam structure. The inhibitory activity of these modules has been extensively studied against a variety of potent β-lactamases. Kinetic studies have shown that different boronic acid compounds reversibly and rapidly inhibit class C AmpC enzymes, several class A β-lactamases, such as the chromosomal penicillinase of Bacillus cereus, and some of the CTX-M-type ESBLs. From an inhibitor design standpoint, the observation that a glycolyboronic acid compound showed not only inhibition of AmpCs (K_i of 20 nM) but also an unexpected potent inhibition of the CTX-M-16 ESBL (K_i of 4 nM) was intriguing, as compounds with good activity across β-lactamase classes are very rare and could have significant implications in designing global inhibitors for treating infections due to bacteria producing significant β-lactamases. However, kinetic studies on the inhibitory activity of boronic acid compounds against class A carbapenemases, such as the KPC enzymes, have not been performed.

**Boronic acid disc tests for the detection of KPC enzymes in the clinical laboratory**

Further to their investigation for potential use as β-lactamase inhibitors in clinical practice, boronic acid compounds have also been used for the phenotypic detection of plasmid-mediated AmpC enzymes. Tests using boronic acid compounds, mainly 3′-aminophenylboronic acid (APBA), with discs of cefotetan or cefoxitin were found to be successful for detecting AmpC enzymes in organisms that classically did not produce these enzymes. These tests, using expanded-spectrum cephalosporins combined with clavulanic acid, have also been useful for the detection of isolates that harbour both ESBLs and AmpCs.

A provisional observation of the inhibitory effect of boronic acid on KPC was made during an evaluation of a chromogenic test that detects AmpCs, ESBLs and metallo-β-lactamases (MBLs) using the hydrolysis of a chromogenic oximinocephalosporin in the presence of benzothiophene-2-boronic acid. In this study, it was observed that AmpC-negative KPC-possessing isolates may be wrongly inferred to have AmpC enzymes. Also, two isolates producing KPC-2 unexpectedly showed synergy between APBA and cefotaxime, ceftazidime or carbapenem.

Following on from the initial observations of the inhibitory activity of boronic acid on KPC, specific phenotypic tests were developed for the detection of KPCs. This investigation initially revealed that in isolates that did not produce AmpC-type enzymes, production of KPC was associated with positive combined boronic acid disc tests using cephemycins and cefotaxime, and, more specifically, ceftipime and carbapenem as substrates. The synergy of phenylboronic acid (PBA) with these antibiotics was applied to the phenotypic identification of the first KPC-producing isolate in Greece. A further evaluation with a large collection of KPC-producing isolates showed a clear synergistic effect between PBA and carbapenems, inferring an apparently enhanced interaction of the PBA moiety with the active-site serine residue of the class A KPC β-lactamase. The combined-disc tests were considered positive when addition of PBA to a β-lactam disc resulted in a ≥5 mm enlargement of the diameter of the zone of growth inhibition, compared with the zone of inhibition around the β-lactam disc alone. In these studies, combined-disc tests using 400 μg of PBA with and without cefepime, imipenem or meropenem exhibited the highest sensitivities and specificities for the detection of KPC enzymes. PBA caused an 8–11 mm increase of the meropenem inhibition halo for the majority of KPC-positive isolates, thus making this combined test very easy to interpret, even by inexperienced personnel. Additionally, a modified CLSI ESBL confirmatory test using PBA has been shown to accurately detect ESBL production among KPC producers.

Synergy between APBA and carbapenems has also been evaluated for the detection of KPC production in limited numbers of isolates. However, the rate of detection of KPC production was low when 300 μg of APBA was combined with imipenem, or the recommended augmentation of the inhibition zone for the detection of class A carbapenemase production was low (≥4 mm), rendering the interpretation of the results difficult. These observations possibly suggest that the inhibitory activity of APBA is lower against KPC enzymes when compared with PBA.

It should also be mentioned that the combinations of either PBA or APBA with ertapenem tend to exhibit some false-positive results in AmpC-producing strains, which are not observed with imipenem or meropenem. Isolates that produced a plasmid-mediated AmpC also showed a weak synergy when a double-disc synergy test using imipenem and APBA was applied, though the combined-disc test with imipenem and APBA was consistently negative.

In recent years, Enterobacteriaceae that produce carbapenemases of either KPC or MBL types have become endemic in several regions worldwide. To address the issue of detecting these potent carbapenemases, clinical laboratories may consider the implementation of a simple screening method using four meropenem discs with and without 400 μg of PBA, 10 μL of 0.1 M EDTA, or both 400 μg of PBA and 10 μL of 0.1 M EDTA, on the same agar plate. In this method, augmentation of the inhibition zone by ≥5 mm around the meropenem disc with both PBA and EDTA is indicative for the co-production of KPC and MBL enzymes by the test isolate. This very convenient procedure, which can be incorporated into the daily routine, can be applied to large numbers of Enterobacteriaceae from the first day of isolation of suspected pathogens.

The inhibitory activity of several boronic acid compounds for AmpC enzymes has been tested by investigating and modelling their complementarity into the AmpC active site. It should be noted that APBA is a more active inhibitor of AmpC enzymes (K_i = 7.3 ± 0.9 μM) compared with other derivatives, such as PBA (K_i = 10.5 ± 2.6 μM). It is possible that APBA might interfere more with AmpCs and be somewhat less specific than PBA for class A enzymes such as KPC, which may partly explain the differences in combined-disc test results from the use of the two different compounds. Efforts have to be applied to the identification of boronic acid derivatives that more specifically inhibit KPC enzymes. In that respect, the wide variety of chemical functionality present in the available boronic acid compounds will allow the mapping of the KPC binding site and suggest modifications to enhance the affinity of boronic acid-based inhibitors for class A KPC enzymes. Further structural and kinetic analyses along these lines seem warranted.

It is crucial for clinical laboratories to promptly report any isolate with carbapenemase activity to the hospital infection control team, to allow the implementation of appropriate contact isolation precautions. In that respect, we suggest that a combined-disc test using meropenem with and without PBA
may be effectively used for the detection of KPC carbapenemases in routine practice, not only in hospitals where such enzymes have emerged or are already endemic, but also when there is clinical suspicion of carbapenem resistance.

Transparency declarations
None to declare.

References