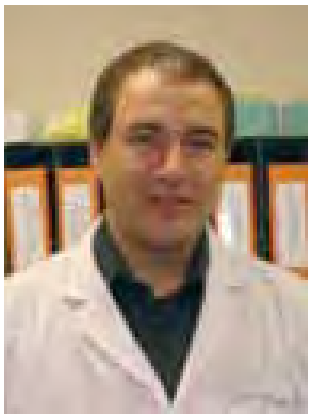
Detecting the dual presence of AmpC and ESBL enzymes



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Introduction

Inducible-chromosomal AmpC cephalosporinase enzymes have been recognised for several years in the ESCAPPM (Enterobacter spp., Serratia spp., *Citrobacter freundii*, Acinetobacter spp., *Proteus vulgaris*, Providencia spp. and *Morganella morganii)* group of gram-negative organisms, which result in the potential resistance to third-generation cephalosporin drugs. More recently several non-ESCAPPM Enterobacteriaceae (particularly *E coli*, *Klebsiella* and *Proteus mirabilis*) have been found to harbour a non-inducible-plasmid form of AmpC. This is particularly important when found in bacteremic patients where third-generation cephalosporins are often the first line drugs of choice.

It was thought that the induction test (inducing resistance to a third-generation cephalosporin, using a stable β lactam, such as cefoxitin, to produce a 'D' or flattened zone) could be used to distinguish between chromosomal (inducible) and plasmid mediated AmpC (non-inducible). However, it has been shown that 'D' zone induction can be positive for both chromosomal and plasmid-mediated AmpC carrying organisms.

False–negative test results can occur when screening for ESBL (Extended spectrum β-lactamase) in organisms harbouring both AmpC and ESBL enzymes. This is due to the presence of AmpC, which overrides the susceptibility to clavulanic acid in ESBL-producing organisms.

Principle of the test

Boronic acid is a known inhibitor of AmpC that can therefore be used to identify this enzyme. A primary screen for AmpC is the resistance to cefoxitin 30μg (≤14mm) but sensitivity to cefoxitin with boronic acid (≥18mm). The dual presence of AmpC and ESBL can also be detected by incorporating boronic acid with a third-generation cephalosporin, with and without clavulanic acid.

Method

Boronic acid solution was made up by dissolving 0.24g phenylboronic acid (P20009 – Sigma Aldrich) in 1ml of alcohol, then adding 5ml of water. We added 10μL of this boronic acid solution to cefoxitin discs and to the third-generation cephalosporin discs, with and without 10μg clavulanic acid. The discs were then incubated at 37°C for 3 hours to dry and then stored at –70°C in an airtight container. The discs were found to be stable following 2 years' storage at –70°C.

Previous trials with boronic acid have used dimethyl sulphoxide as the solvent. This released an extremely unpleasant odour during the drying phase and has therefore been substituted with 100% alcohol in this study.

Mueller Hinton agar was inoculated with the test organisms as per Clinical and Laboratory Standards Institute (CLSI) standards. The screening plate for AmpC consisted of cefoxitin with and without boronic acid. Screening for the dual presence of AmpC and ESBL requires the following four discs to be placed onto the inoculated Mueller Hinton plate: (zone size increases of >5mm are regarded as positive)

1. Third-generation cephalosporin.
2. Third-generation cephalosporin + clavulanic acid.
3. Third-generation cephalosporin + boronic acid.
4. Third-generation cephalosporin + clavulanic acid +boronic acid.

It should be noted that substrate specificity is important when testing for ESBL. Cefotaxime and ceftriaxone are specific for CTXM; however, some SHV ESBLs may need to be tested with other substrates such as ceftazidime, cefpodoxime, cefepime or aztreonam.

Results

Plate 1. AmpC positive

This organism shows resistance to cefoxitin and sensitivity to cefoxitin with boronic acid (a known inhibitor of AmpC).

Plate 2. ESBL positive

AmpC negative

Typical profile for ESBL with the 'activated' sensitivity to the third-generation cephalosporin with clavulanic acid. Note the resistance to cefotaxime with boronic acid and the sensitivity to clavulanic acid indicating the absence of AmpC.

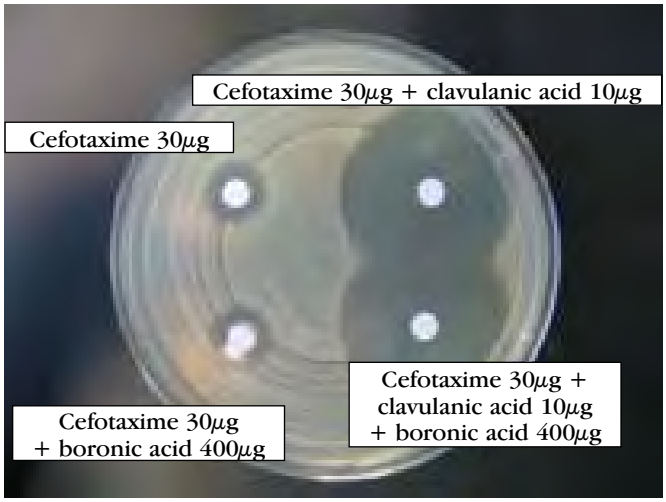


Plate 3. **ESBL negative**
AmpC positive

This case is negative for ESBL production but positive for AmpC. Note activation of the cephalosporin with boronic acid.

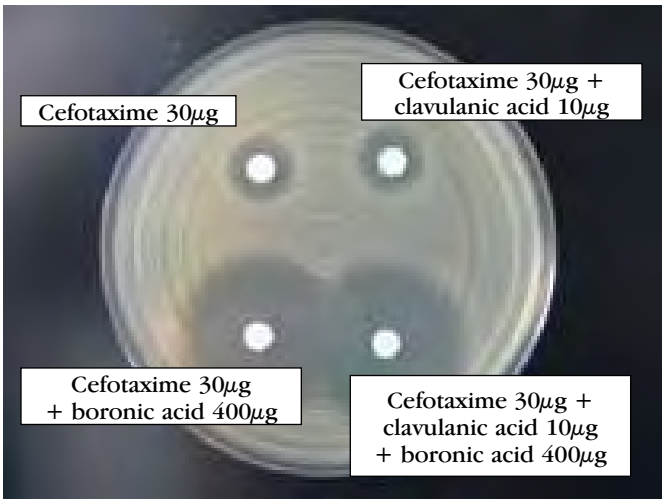
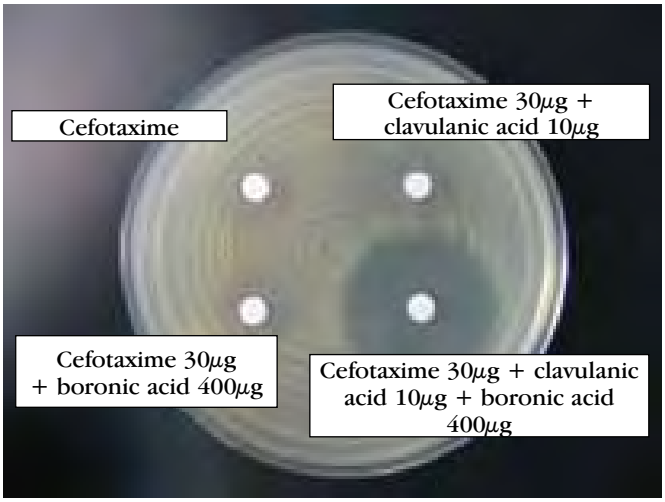


Plate 4. ESBL positive

AmpC positive

Note the resistance to clavulanic acid, due to the presence of AmpC (a routine screen for ESBL would show false–negative results since the clavulanic acid is overridden by the presence of AmpC). In addition, the cephalosporin with boronic acid is 'falsely' resistant or negative for AmpC, due to the presence of ESBL. Only the combination of both boronic and clavulanic acid with the cephalosporin detects the dual presence of both AmpC and ESBL enzymes.



Conclusion

Boronic acid is a known AmpC cephalosporinase inhibitor, which can be used in conjunction with cefoxitin to screen for AmpC. This is particularly important for non-ESCAPPM Enterobacteriaceae in bacteremic patients where third-generation cephalosporins are often the first line of drugs of choice.

It is clearly important to test for AmpC when screening for ESBL to avoid false–negative results. The underlying presence of AmpC can mask sensitivity to clavulanic acid, which is commonly used to detect ESBL production. Many papers have shown the boronic acid test to be useful in detecting AmpC, but at best this test is 95% accurate, while polymerase chain reaction is the gold standard and is often used to check indeterminate results.

It is worth mentioning that there is also a need to test for metallo-β lactamase (MBL). For example, Imp4 is prevalent in Australian isolates of *E coli* and Klebsiella. The presence of MBL can also make the detection of coexisting AmpC or ESBL difficult.

As many serious infections are treated with a carbapenem, for example meropenem or imipenem, a positive MBL finding in such cases would require alternative treatment.

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From the Editors

This is the last *Microbiology Australia* issue for 2009. The Editorial Board works hard to ensure that there is an array of topics of interest for ASM members and this year's issues provide examples of the diversity of our discipline. The first issue (March) focused on the Microbiology of water reuse and alternative supplies. This topic not only remains a high priority for Australia, but its importance continues to increase, as our population grows and our water supplies decrease in many areas.

In May, Microbes and Global Climate Change was covered. ASM's contribution to this issue was very authoritative, describing changing to microbial populations. There were also suggestions of microbes mitigating global climate change.

In July we celebrated ASM's Golden Jubilee with a special extra issue on the History of ASM. This was a very authoritative issue researched by historians commissioned by ASM. It reflected on the origins of ASM and is a great issue to keep for posterity.

Then in September the topic was Emerging Infectious Diseases. Among the many emerging diseases which have serious consequences for Australians, this issue coincided with the outbreak of Hendra virus and H1N1 flu which received numerous media attention.

This issue is on Indigenous Health and we found it very sobering to think that despite the sophistication of the Australian health care system, our Indigenous people have incredibly high rates of infectious diseases. Hopefully this issue may contribute towards the alleviation of their dilemma.

We want to say thanks again to the Editorial Board of *Microbiology Australia* for their support throughout this year. It has been great working under the leadership of Ailsa Hocking over our past four year's with the journal. Now Chris Burke is providing that leadership as the new chair of the Editorial Board. Chris is also a Guest Editor for a special issue on Education that will appear in March 2010.

We also thank our Guest Editors who work with the Editorial Board to plan our special issues. Efforts are made to get cover all facets of a theme and to recruit authoritative contributors to provide authoritative reports to educate ASM members. In addition, however, *Microbiology Australia* is released to the media, and science writers, promoting follow up reporting of microbiology issues to the public.

We also thank readers for their contributions – please continue to send us comments and requests. Next year we plan to have further improvements to *Microbiology Australia* that will add to its utility as an educational resource. We wish you all the best for Christmas and for 2010.

Ian and Jo Macreadie