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Poster Session IV
Molecular detection of bacterial resistance

DETECTION OF CARBAPENEMASES BY REAL-TIME PCR AND MELT-CURVE ANALYSIS ON THE BD MAX SYSTEM

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Objectives

Carbapenem-resistant gram-negative bacteria are increasing in frequency and require rapid detection for initiation of adequate antimicrobial therapy, epidemiological investigations and infection control measures. We set out to develop a multiplex SYBR-Green real-time PCR assay for the new automated BD MAX instrument.

Methods

We developed a method for detection of frequent carbapenemases ranging from Ambler class A (KPC and GES), class B (IMP-1, IMP-2, VIM-1, VIM-2 and NDM) and class D (OXA-48 and OXA-23) in Enterobacteriaceae and nonfermenters such as *Pseudomonas aeruginosa* or *Acinetobacter baumannii* on the BD MAX™ System, a fully automated PCR platform that facilitates molecular diagnostic. We developed a multiplex SYBR-Green real-time PCR assay for the detection of different carbapenemases in two reaction panels. Identity of the amplicons was determined by melt-curve analysis in the 'PCR only' mode on the BD MAX instrument. To evaluate our assay we analysed 2 collections of isolates. (i) First, 128 strains with reduced carbapenem susceptibility and defined presence or absence of specific carbapenemases were tested. (ii) Second, 152 consecutive isolates sent to the German National Reference Laboratory (NRL) in a half-month period (Jan, 21st to Feb, 6th 2013) were analyzed in a blinded study. The blinding was lifted only after completion of the investigation, compared to the NRL data and discrepant results were analyzed on new isolates once.

Results

By melt curve analysis all tested carbapenemases could be differentiated unambiguously. A definite result from culture isolates was obtained in more than 95% of the analyzed samples. Even combined presence of two amplicons within one strain could be differentiated.

In the collection (i) 80/88 carbapenemase-positive strains were detected correctly, 8 were missed, the latter comprising IMP variants not covered by the primer panels. All 40 negative strains were determined correctly. In the collection (ii) 65/65 of the carbapenemase-positive and 87/87 of the negative strains were identified correctly showing 100% concordance with the results from the NRL. Based upon the carbapenemases covered by the applied primer panels this assay would have covered 99%, 89.6% and 87.6% of all carbapenemases detected in isolates of Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, respectively, sent to the NRL in the whole year 2012.

Conclusion

In this study we show that multiplex SYBR-Green real-time PCR combined with melt-curve analysis run on the BD MAX instrument is a valuable tool to discriminate simultaneously carbapenemases from different Ambler classes among Enterobacteriaceae and nonfermenters. Combining this assay with fully automated nucleic acid extraction within the BD MAX allows for simple and rapid identification of molecular mechanisms among carbapenem non-susceptible isolates.