The accuracy of combined disk assay to screen for metallo-β-lactamas in carbapenem resistant *Acinetobacter baumannii*

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### Background

The global spread of metallo-β-lactamas (MBLs) producing Gram-negative bacteria is a major challenge, and significant effort is taken to detect MBL positive isolates. The metal chelator EDTA is known to inhibit MBLs. Based on this fact, different phenotypic methods have been described to identify MBL producers. One of the common and quick methods described is the combined disk assay. However, some studies reported the assay’s inaccuracy to be used as a reliable phenotypic screening test for MBL producing *Acinetobacter*. In this study we are reporting our experience with combined disk assay with *A. baumannii* (1, 2).

Additionally, meropenem and imipenem disks containing 5μl (930μg) of 0.5M EDTA disodium salt dihydrate were also placed on the same plate. As a negative control, three different *A. baumannii* strains were each tested with six disks containing 1-6μl of 0.5M EDTA disodium salt dihydrate. Isolates were considered ‘MBL positive’ when the inhibition zone was increased more than 5mm around the disks containing meropenem or imipenem with EDTA compared with inhibition zones of disks containing meropenem or imipenem alone. Positive isolates were PCR screened for the common MBL genes (*bla*\textsubscript{IMP}, *bla*\textsubscript{NDM}, and *bla*\textsubscript{VIM}) and for the class D *bla*\textsubscript{OXA-23}, *bla*\textsubscript{OXA-24}, *bla*\textsubscript{OXA-58}.

### Methods

One hundred and eleven carbapenem non-susceptible *A. baumannii* were recovered from six different Middle Eastern countries as part of a large regional surveillance study. Meropenem and imipenem susceptibility were tested using disks placed on Muller Hinton plates with a 0.5 McFarland bacterial suspension.

### Results

Ninety eight per cent (n=109) of the *A. baumanii* strains were positive for the combined disk screening assay. However, the disks containing EDTA alone also showed growth inhibition, and none of the isolates were found to be encoding NDM, IMP, or VIM. All of the isolates were OXA-51 positive. Sixty seven per cent (n=76) and five per cent (n=5) had positive results for OXA-23 and OXA-40 PCR respectively.

### Conclusions

It is apparent that the combined disk screening is not an accurate method to identify MBL-positive *A. baumannii*.

### References


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*Image: Inhibition zones around disks containing imipenem (IMI), meropenem (MEM), as well as EDTA and EDTA combined with imipenem and meropenem respectively. Increased inhibition zones can be observed around disks containing EDTA.*