

Rapid identification of OXA-23-subfamily in carbapenem-resistant *Acinetobacter* spp. with a novel immunochromatographic lateral flow assay

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Introduction

The global spread of carbapenem-resistant *Acinetobacter* spp. has led to an emerging worldwide healthcare problem. The carbapenem-hydrolysing oxacillinases (OXAs) are the most commonly reported carbapenem-resistance determinants in *Acinetobacter* spp., particularly in *A. baumannii*. There are six identified OXA-subgroups associated with carbapenem-resistance in *A. baumannii*: the intrinsic OXA-51-like and the acquired OXA-23-like, OXA-58-like, OXA-40-like, OXA-143-like and OXA-235-like. Of these, OXA-23 is the most prevalent carbapenem-resistance determinant among isolates in Germany, Europe and worldwide.

The lack of effective and reliable tests to detect OXA-mediated carbapenem-resistance is a serious challenge to modern medicine. There is an unmet medical need for reliable and rapid diagnostic tools to detect OXA-23-like producing strains to ensure a successful treatment of patients and prevent the spread of carbapenemase-producers.

The aim of this work is the development an antibody-based OXA-23-like

Selection of hybridoma clones

Mice were immunized with purified recombinant OXA-23_{6xHis-Tag}. After a standardized immunization protocol, one mouse was sacrificed, spleen was processed, and generated splenocytes were fused with myeloma cells to generate hybridomas.

- 9 hybridoma clones were picked into 96-well plates
- 0 clones exhibit OXA-23_{6xHis-Tag} specificity
- 0 clones exhibit OXA-23_{6xHis-Tag} specificity, excluded 6xHis-Tag binders
- 1 clones exhibit OXA-23_{6xHis-Tag} specificity after adaptation to serum-free medium
- 6 clones exhibit OXA-23_{6xHis-Tag} specificity, tested for cross reactivity to other OXA-sub groups
- 1 clones exhibit OXA-23 specificity, binding to OXA-23 from *A. baumannii* lysate
- 5 clones secrete antibodies showing a balanced heavy-/light-antibody-chain ratio in SDS-PAGE
- 6 clones were selected for mass production, purified antibodies were screened in prototype setting
- 2 remaining suitable clones

Results

Sensitivity of OXA-23 detection kit

To determine the limit of sensitivity of the prototype, overnight cultures of OXA-23-producing carbapenem-resistant *Acinetobacter baumannii* isolates were 2-fold diluted in lysis buffer, and lysates were applied to the test strips. In parallel, we plated out serial dilutions of the culture to determine the cfu's. The detection limit was defined as the lowest number of cfu giving a visible positive signal after 15 minutes incubation.



Acinetobacter spp.

Flow chart of antibody-based OXA-23 detection assay



Conclusions

- no expensive or specialized equipment is required to use this test strip
- antibody-based OXA-23 detection assay is able to detect OXA-23-mediated carbapenem-resistant *Acinetobacter* spp. with 100% specificity
- sensitivity has been determined to detect between 10⁵ to 10⁶ cfu per sample, making a point-of-care device feasible
- result in < 20 min which saves 12-48 hours in diagnostic time, avoiding treatment with inappropriate antibiotics and enables earlier intervention to control transmission of OXA-23 producing carbapenem-resistant *Acinetobacter* spp.

Specificity of OXA-23 prototype

A well characterized collection of carbapenem-resistant *Acinetobacter* spp. isolates (n=108) with defined carbapenem resistance mechanism were used to evaluate specificity of our OXA-23 prototype.

OXA-23-producer (carb^R)



Positive (100 %)

A.



Negative (100 %)

Results of ten representative test strains

