Rapid identification of OXA-40 and OXA-58-subfamily in carbapenem-resistant *Acinetobacter* baumannii with a novel immunochromatographic lateral flow assay

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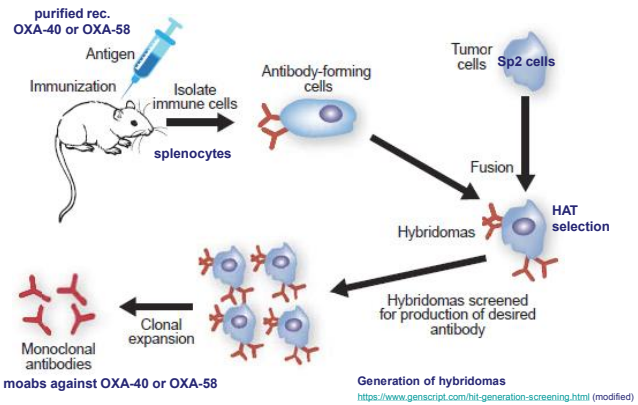
Introduction

The global spread of carbapenem-resistant *Acinetobacter baumannii* 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, OXA-40** and **OXA-58** are the most prevalent carbapenem-resistance determinants among isolates 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-23/40/58-like producing strains to ensure a successful treatment of patients and prevent the spread of carbapenemase-producers.

=> The aim of this work is to expand OXA-detection abilities in the OXA-23 *K*-SeT to OXA-40/OXA-58-like carbapenemases in *A. baumannii* clinical isolates.

Methods



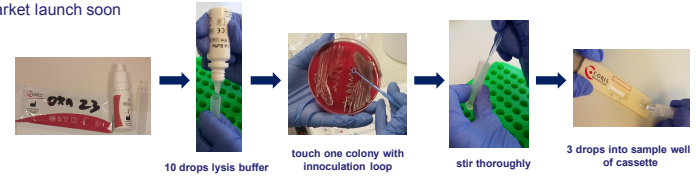
Generation of hybridomas
https://www.genscript.com/hit-generation-screening.html (modified)

1. Generation of antibody producing hybridoma cell clones.
2. Screening for specific antibodies against recombinant OXA-40 and OXA-58 by ELISA.
3. Screening for specific antibodies against native OXA-40 and OXA-58 (*A. baumannii* lysate).
4. Determination of antibody cross-reactivity to other OXA-subclasses.
5. Cultivation and securing of selected hybridoma clones.
6. Antibody purification.
7. Identification of suitable antibody pairs able to detect recombinant OXA-40 and OXA-58 (ELISA and ICT).
8. Evaluation of ICT prototype with clinical *Acinetobacter* spp. isolates.

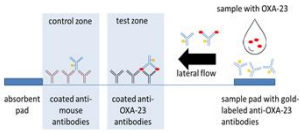
OXA-23 *K*-SetT – antibody-based detection assay (ICT)

Development of OXA-23 *K*-SeT:

- „Proof-of-Concept"
- 100 % specificity
- high sensitivity
- market launch soon



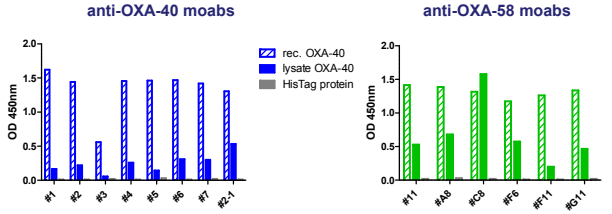
Principal of lateral flow assay



Results

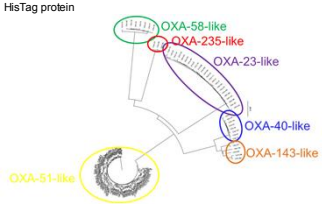
Antibody screening

Hybridoma cell culture supernatants were screened for moabs binding to immobilized recombinant OXA-40/OXA-58 carrying a His-Tag, *A. baumannii* lysate and irrelevant His-Tag protein by ELISA.

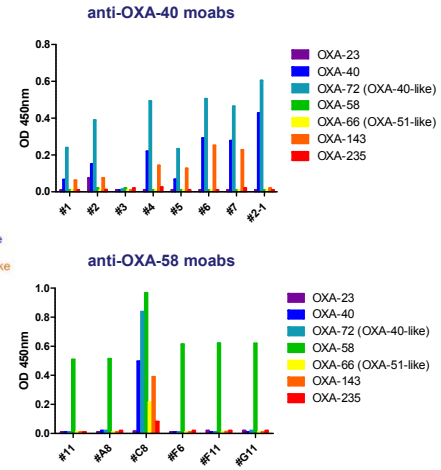


Antibody cross-reactivity

Selected hybridoma cell culture supernatans were screened for antibody cross-reactivity to other OXA-subclasses by ELISA.

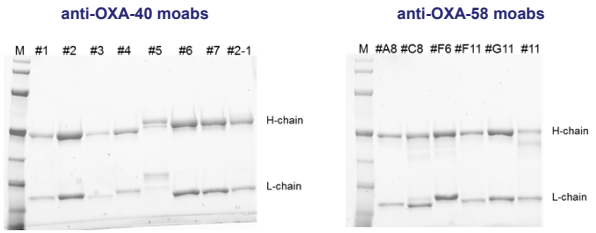


Phylogenetic tree of OXA-subgroups.



Antibody-purification

Hybridoma cell culture supernatants were collected and antibodies were purified by Protein G affinity chromatography. Purified antibodies were analysed by SDS-PAGE.



SDS-PAGE with 10 µg (or less) loaded purified antibody. H= heavy, L= light

Conclusion

- specific anti-OXA-40 and anti-OXA-58 antibodies were obtained
- anti-OXA-40 antibodies show cross-reactivity with OXA-143
- purified antibodies will be delivered to cooperation partner Coris BioConcept to generate various ICT prototypes for validation

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