Detecting Aminoglycoside Resistance by Mass Spectrometry

Irene Burckhardt, Viktoria Pauker, Konrad Bode and Stefan Zimmermann
Dept. of Infectious Diseases, Medical Microbiology and Hygiene, University Hospital Heidelberg

Introduction

The spread of multi-resistant bacteria is a big threat to hospital patients. In the last century many antibiotic treatment regimens against bacterial infections included aminoglycoside antimicrobial agents. But the widespread use of the effective antibiotics also increased resistance in bacteria towards aminoglycosides. Severe infections are still difficult to treat and the mortality for example in septicemia is still close to 30%. Therefore a fast detection method for the resistance of bacterial strains is necessary. The aim of this study is to determine a fast detection method for aminoglycoside resistance in bacteria and matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) was the method of choice.

Aim

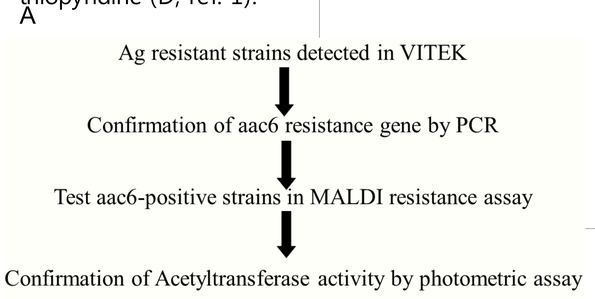
We aimed to develop a rapid, accurate and reliable detection system for aminoglycoside resistance transferred by acetyltransferase enzymes using mass spectrometry. The goal was to establish an assay which delivers same day results on the basis of recently published MS techniques for ß-lactamase resistance.

Methods

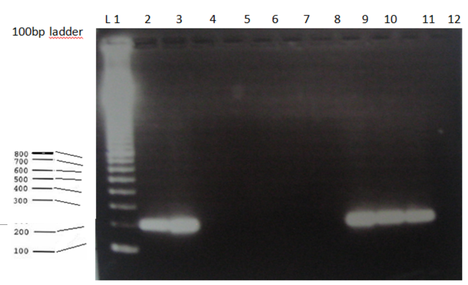
Formatvorlage des Untertitelmasters durch Klicken bearbeiten

42 gram-negative rods from blood cultures and other specimens phenotypically showing aminoglycoside resistance in automated AST (detected in VITEK-2) were tested for aminoglycoside resistance in a mass spectrometry assay. The presence of the resistance gene aac-6 was confirmed by specific PCR (B). The bacterial suspension was incubated with the aminoglycosides (kanamycin, tobramycin) for up to 3 hours. The supernatant was tested by mass spectrometry for specific modifications (C). In addition the enzymatic activity was confirmed by an absorbance test (λ 324 nm) in which aldrithiol-4 was converted to 4-thiopyridine (D; ref. 1).

A



B

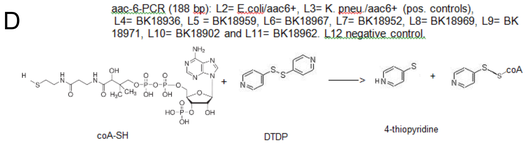


aac-6-PCR (188 bp): L2= E.coli/aac6+, L3= K. pneu /aac6+ (pos. controls), L4= BK18936, L5 = BK18959, L6= BK18967, L7= BK18952, L8= BK18969, L9= BK 18971, L10= BK18902 and L11= BK18962. L12 negative control.

C

Acetylation of 2-deoxystreptamine aminoglycosides with acetyltransferase-6' and the substrate n-propionyl-coA (n-prop-coA)

D



Formation of the 4-thiopyridine through the reaction of coenzyme A-SH and aldrithiol-4 (DTDP)

Results

28 of the 42 gram-negative rods were positive for the resistance gene aac-6. These strains induced detectable shifts in the molecular weight of the different aminoglycosides. Bacterial N-acetyltransferases trans- ferred an ester group to them and thereby destroyed their antibacterial activity (ref. 1). This effect was measurable, if the samples were analysed in a LC-ESI-TOF (A). The enzymatic test system confirmed the data seen in mass spectrometry showing a significant increase of produced 4-thiopyridine within 3 hours (B). The mass shifts on the aminoglycoside molecules could not yet been detected in MALDI-TOF MS as a reproducible and accurate result (C).

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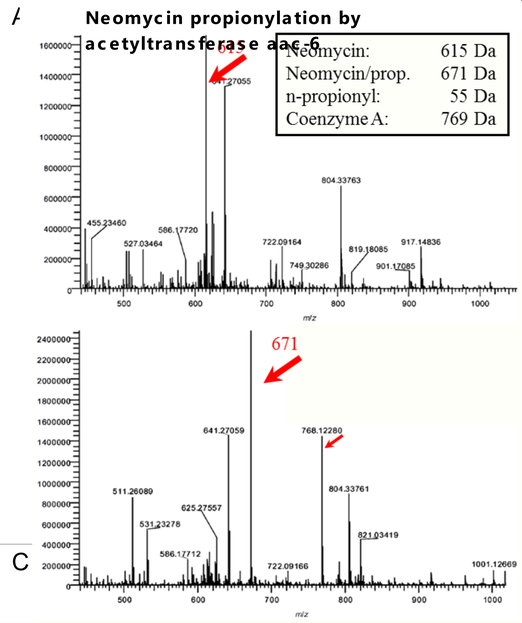

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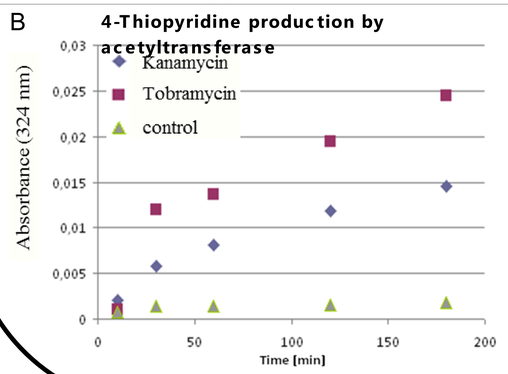

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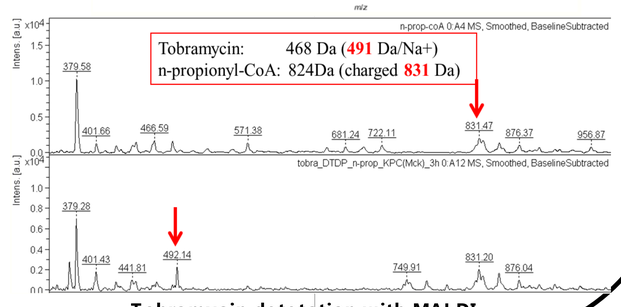

Tobramycin detetction with MALDI-MS

Conclusions

We tried to developed an assay to detect aminoglycoside resistance in gram-negative rods mediated by N-acetyltransferases within less than three hours. As we and others have already described MS assays for the detection of ß-lactam antibiotics recently, we were interested to expand this short time assay giving a same-day-result to other classes of antibiotics, like aminoglycosides. An empiric antibiotic treatment regimen in septicaemia or severe pneumonia often contains a combination of these antibiotic groups. Therefore this assay is a promising approach for a faster determination of susceptibility or resistance enabling de-escalation or escalation of the antibiotic treatment. Still reproducibility and accuracy of the MALDI-MS approach is not good enough to use it as a routine test yet.

[Ref. 1: Green, K. D.; Chembiochem. 2010]

contact: stefan.zimmermann@med.uni-heidelberg.de

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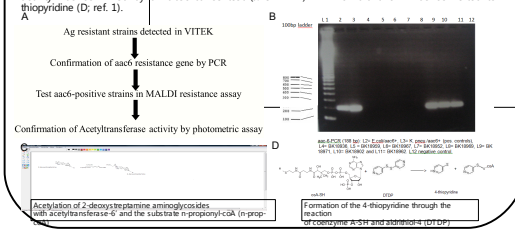

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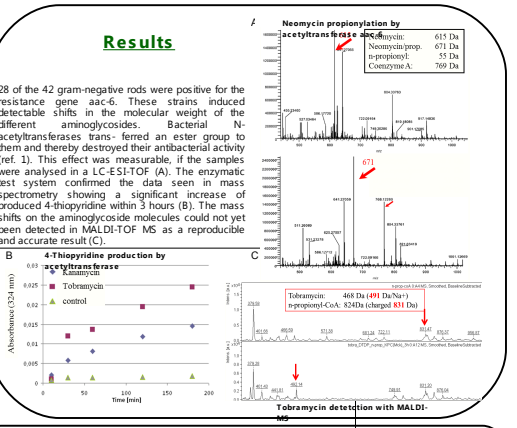

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