

MALDI-TOF AST

NordicAST 2019

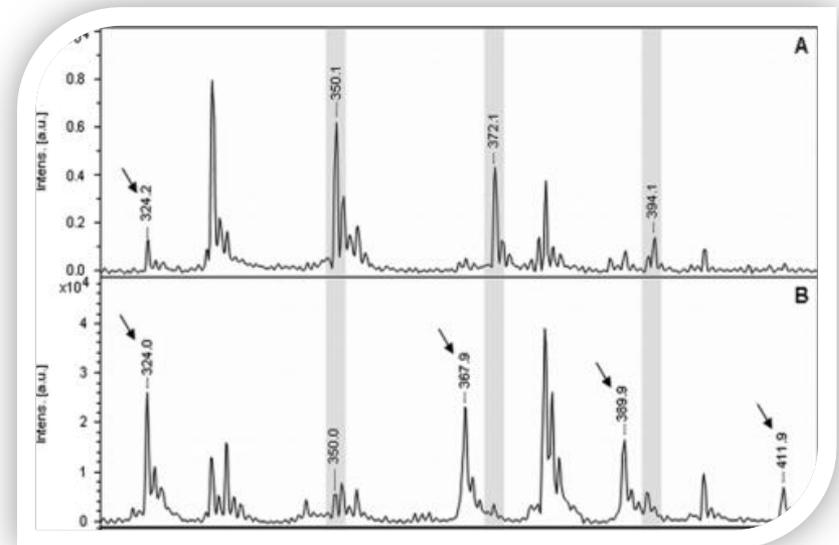
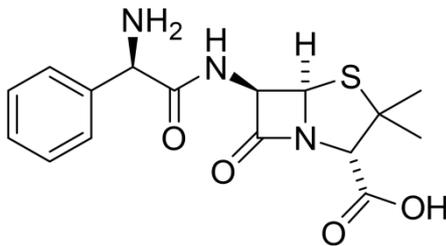
Åsa Johansson

Biomedical scientist
Deputy Head of Dep of Clinical Microbiology
Kronoberg/Blekinge
PhD-student Gothenburg University

1. Analysis of the antibiotic compound (enzymatically modified)

- Beta-lactams
- Quinolones
- (*Aminoglycosides*)

- Incubate a loop of bacteria in suitable concentration of the compound in 35-37C
- Centrifuge
- Analyze supernatant (low mass range)



Ampicillin	Sensitive				Resistant			
	Mol wt	$[M+H]^+$	$[M+Na]^+$	$[M+2Na]^+$	$[M_{hydr}+H]^+$	$[M_{hydr}+Na]^+$	$[M_{hydr}+2Na]^+$	$[M_{hydr}+decarb+Na]^+$
	349.4	350.4	372.4	394.4	368.4	390.4	412.4	324.4

Beta-lactams

- Sparbier *et al*: Meropenem, Imipenem, Ertapenem, Penicillin, Ampicillin, Piperacillin,
- Cefotaxime, Ceftazidim. 3h. Blood cultures.
- Inhibitors: APBA (3-aminophenylboronic acid), DPA (dipicolinic acid), clavulanic acid, tazobactam
- KPC (15 min), MBL (2h), OXA-48 (1-24h)
- **MBT-STAR**



Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry-Based Functional Assay for Rapid Detection of Resistance against β -Lactam Antibiotics

Katrin Sparbier,^a Sören Schubert,^b Ulrich Weller,^c Christiane Boogen,^c and Markus Kostrzewa^a

Johansson *et al* BMC Microbiology 2014, 14:89
<http://www.biomedcentral.com/1471-2180/14/89>

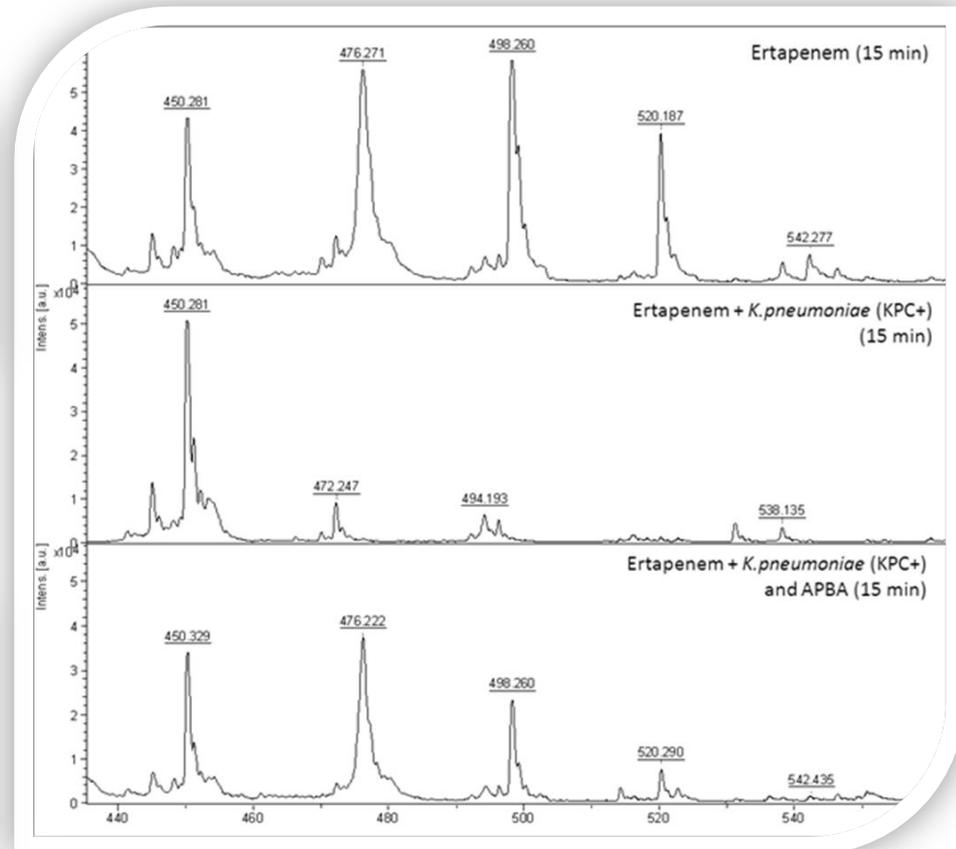


RESEARCH ARTICLE

Open Access

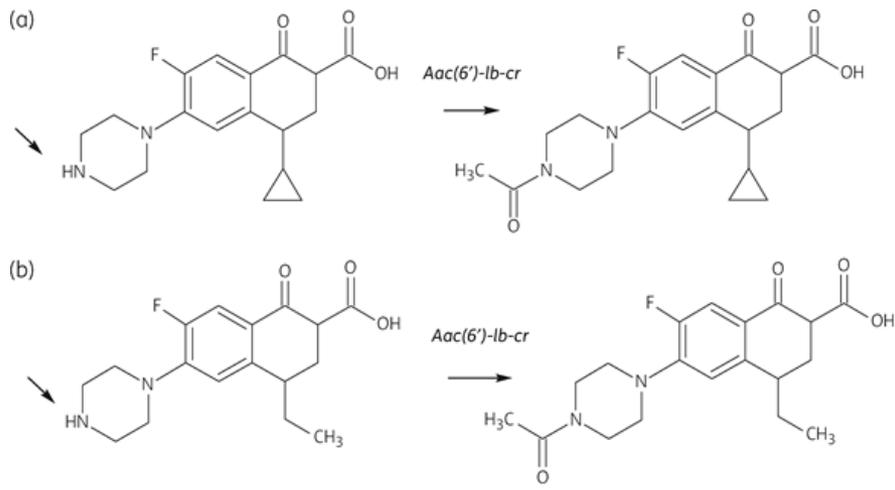
The detection and verification of carbapenemases using ertapenem and Matrix Assisted Laser Desorption Ionization-Time of Flight

Åsa Johansson¹, Josefine Ekelöf², Christian G Giske^{3*} and Martin Sundqvist^{1,4}



Quinolones

- Most common: chromosomal mutations targeting quinolone targets (DNA gyrase and topoisomerase IV)
- Plasmid-mediated:
 - Qnr proteins: protects the quinolone target
 - QepA and OqxAB: efflux pumps
 - AAC(6')-Ib-cr enzyme: acetylation of quinolone



Cip, Nor

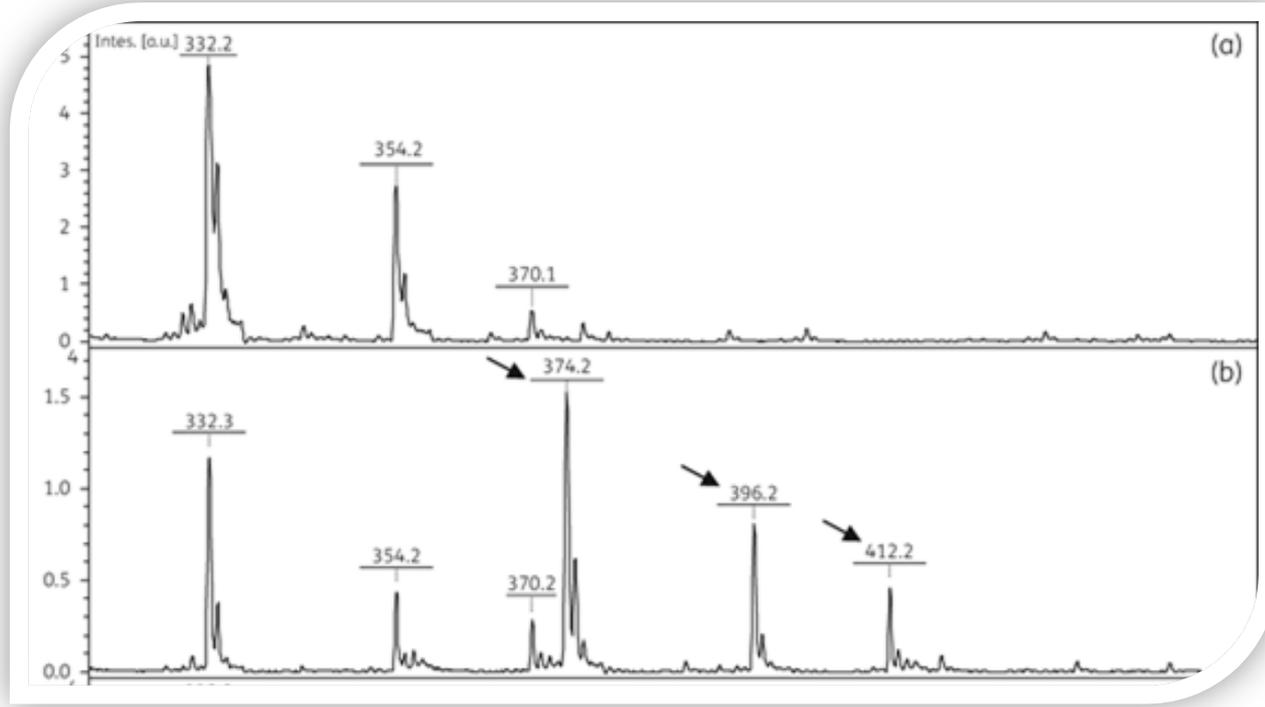
J Antimicrob Chemother 2017; **72**: 1074–1080
doi:10.1093/jac/dkw552 Advance Access publication 8 January 2017

**Journal of
Antimicrobial
Chemotherapy**

Rapid detection of the plasmid-mediated quinolone resistance determinant AAC(6′)-Ib-cr in Enterobacteriaceae by MALDI-TOF MS analysis

Marina Oviño¹, Jose Manuel Rodríguez-Martínez², Álvaro Pascual^{2,3} and Germán Bou^{1*}

E coli ctr
E coli AAC(6′)-Ib-cr producer



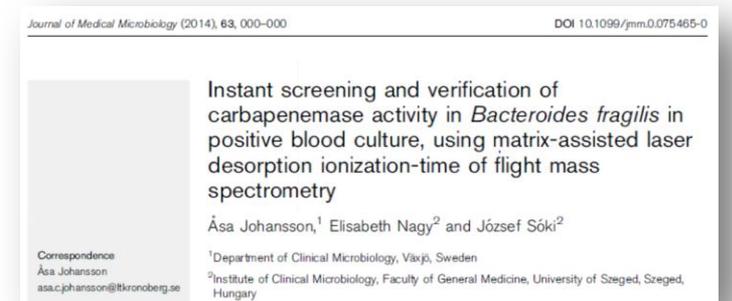
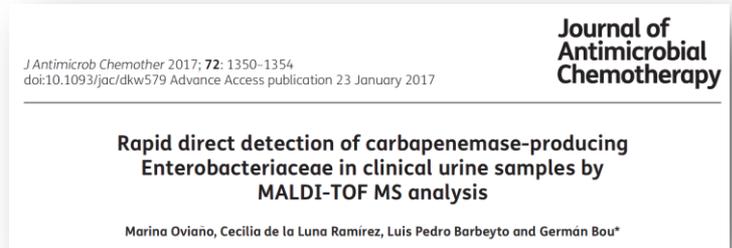
Analysis of antibiotic compounds

PRO

- Simple visual read-out
- Possible to automate interpretation
- Use of inhibitors to confirm type of enzyme
- Rapid
- Simple
- In-house
- Direct from blood and urine
- Aerobes and anaerobes

CON

- No detection of other possible mechanism (efflux pumps, porin loss etc)
- No detection of other Ab-groups



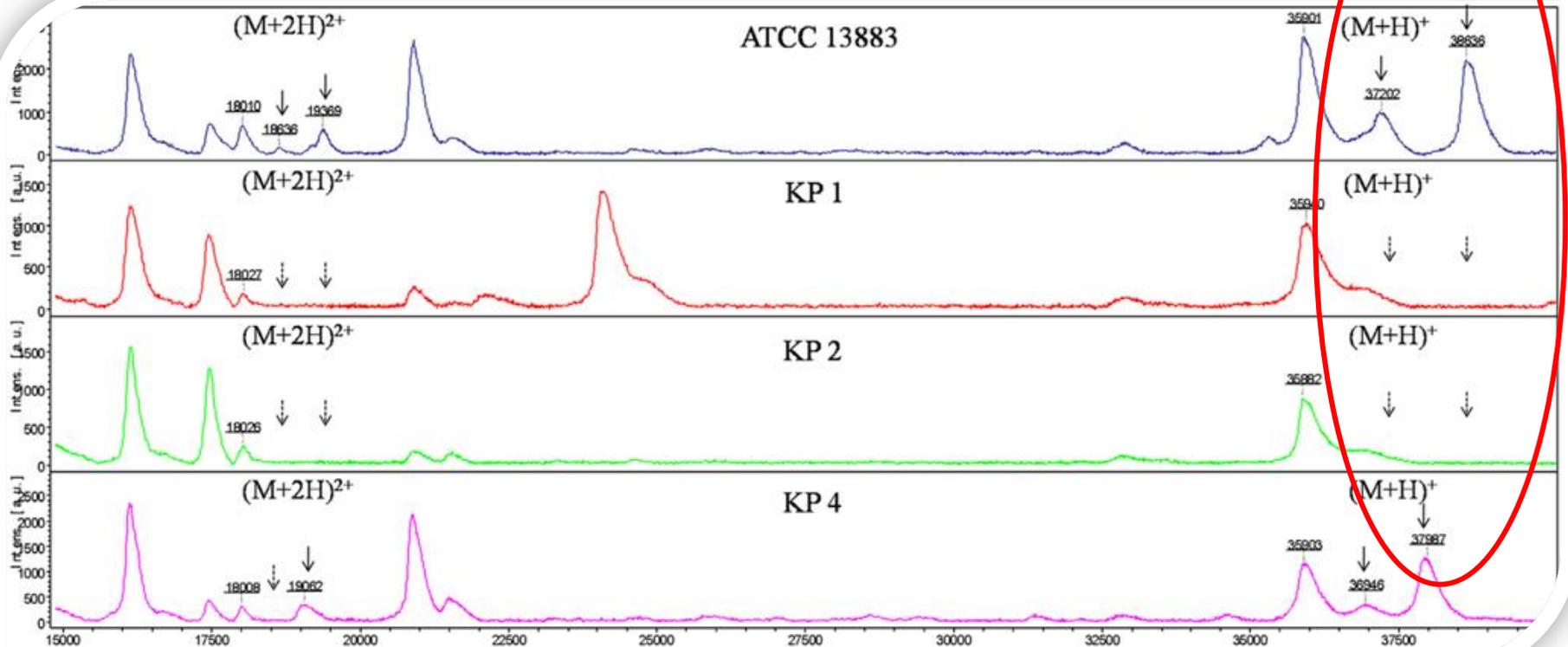
2. Detection of altered protein expression ("biomarkers")

- Detection of the enzyme
(Ampicillin resistance in *F nucleatum*)
- Detection of (loss of) porins (*K pneumoniae*)
- Different protein pattern in suscept vs resist strain
 - Constant expression
(MRSA/ VRE, *CfiA B fragilis*)
 - Induced expression
(Fluconazole resistance in *C tropicalis*)

Detection of (loss of) porins

Rapid detection of porins by matrix-assisted laser desorption/ionization-time of flight mass spectrometry

Yan-Yan Hu, Jia-Chang Cai, Hong-Wei Zhou, Rong Zhang and Gong-Xiang Chen*

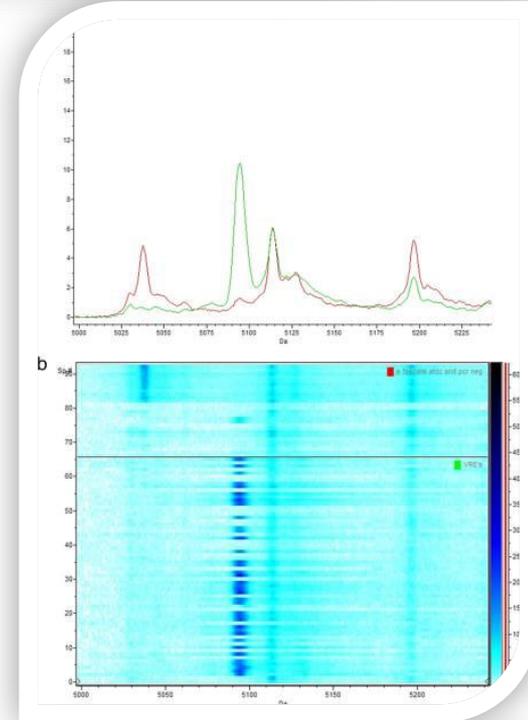


Detection of altered protein expression ("biomarkers")

- Detection of the enzyme
(Ampicillin resistance in *F nucleatum*)
- Detection of (loss of) porins
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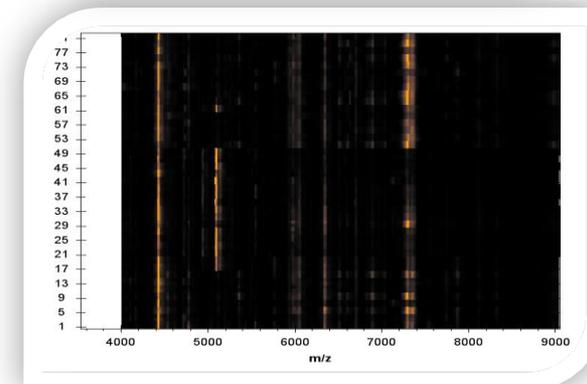
MRSA

- No MRSA peak found
- (Spa-type, Clonal complex?)
- PSM-mec detection (toxin m/z 2413, class A mec)
 - Bruker application



VRE

- No VRE peak found
- Griffin *et al* JCM 2012
- *E faecium* VanB –local biomarker?

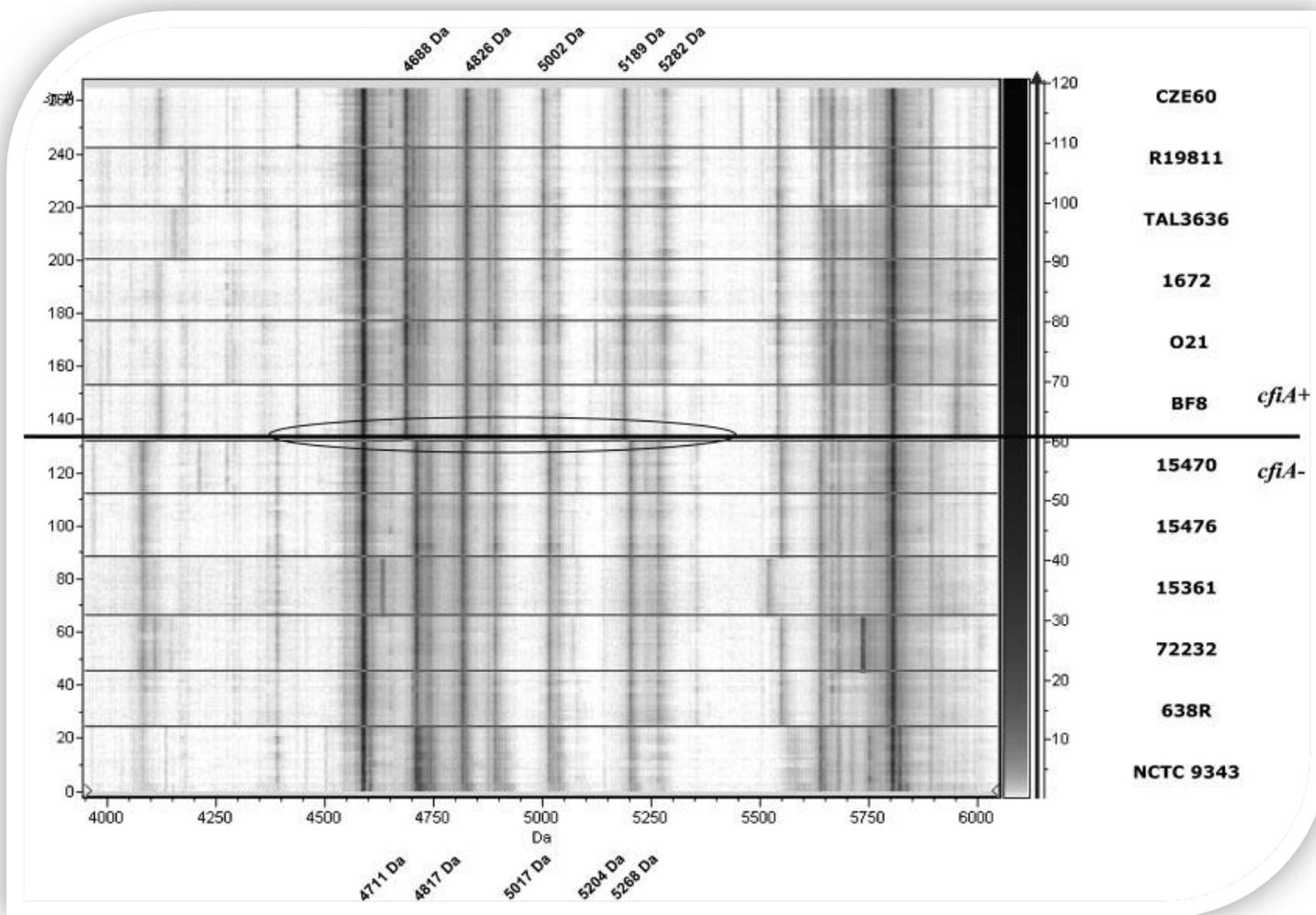


Differentiation of division I (*cfiA*-negative) and division II (*cfiA*-positive) *Bacteroides fragilis* strains by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

Elisabeth Nagy,¹ Simone Becker,² József Sóki,¹ Edit Urbán¹ and Markus Kostrzewa²

cfiA B fragilis

- Bruker application
- Works from dID blood cultures



Detection of altered protein expression ("biomarkers")

- Detection of the enzyme
(Ampicillin resistance in *F nucleatum*)
- Detection of (loss of) porins
(*K pneumoniae*)
- **Different protein pattern in susceptible vs resistant strains**
 - **Constant expression**
(*MRSA/ VRE, CfiA B fragilis*)
 - **Induced expression**
(Fluconazole resistance in *C tropicalis*)

Very few studies ms-AFST
MPCC –minimal profile change concentration

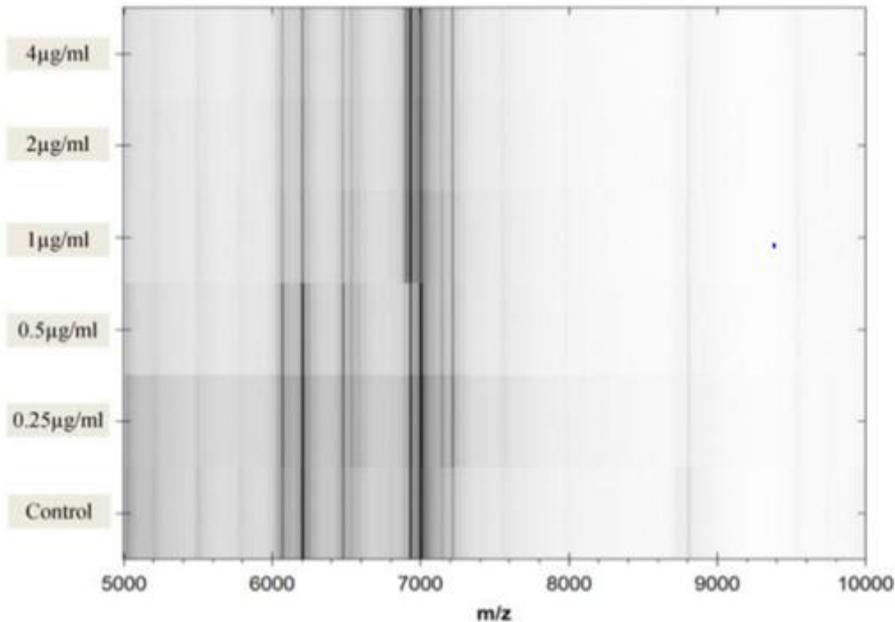


Original Article

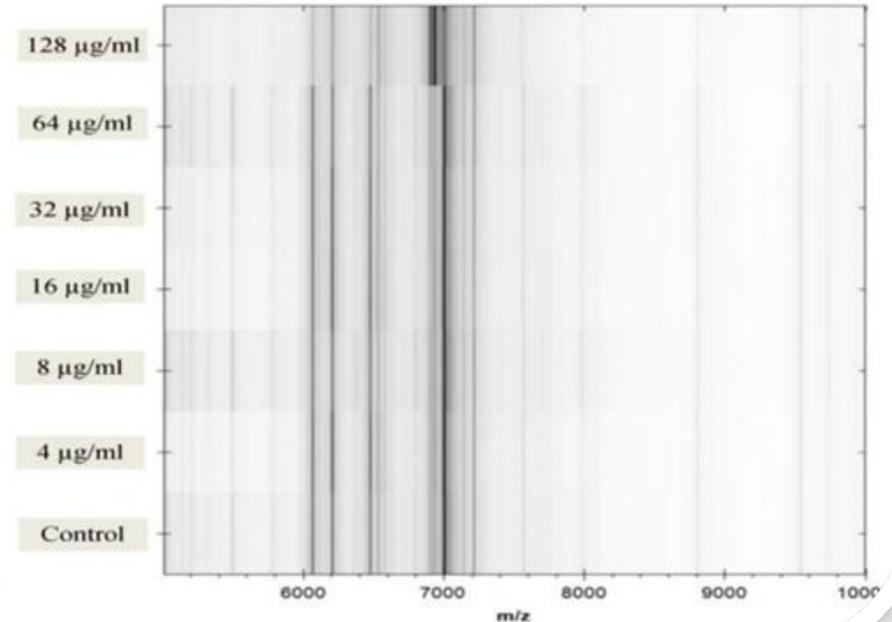
Rapid detection of fluconazole resistance in *Candida tropicalis* by MALDI-TOF MS

Saikat Paul, Pankaj Singh, Shamanth A S,
Shivaprakash M. Rudramurthy, Arunaloke Chakrabarti
and Anup K Ghosh*

Virtual gel of NCCPF 420203 (*C.tropicalis* sensitive strain)



Virtual gel of NCCPF 420193 (*C.tropicalis* resistant strain)



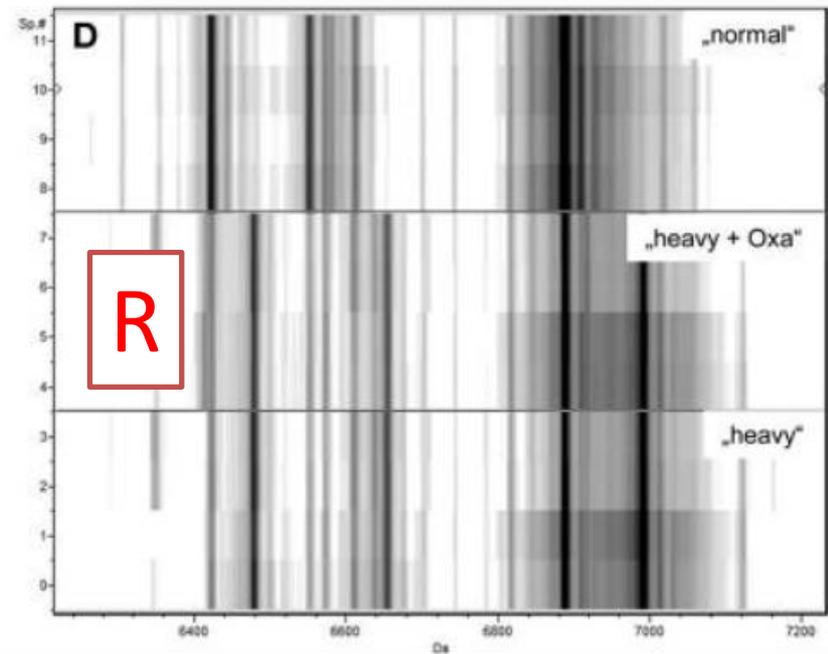
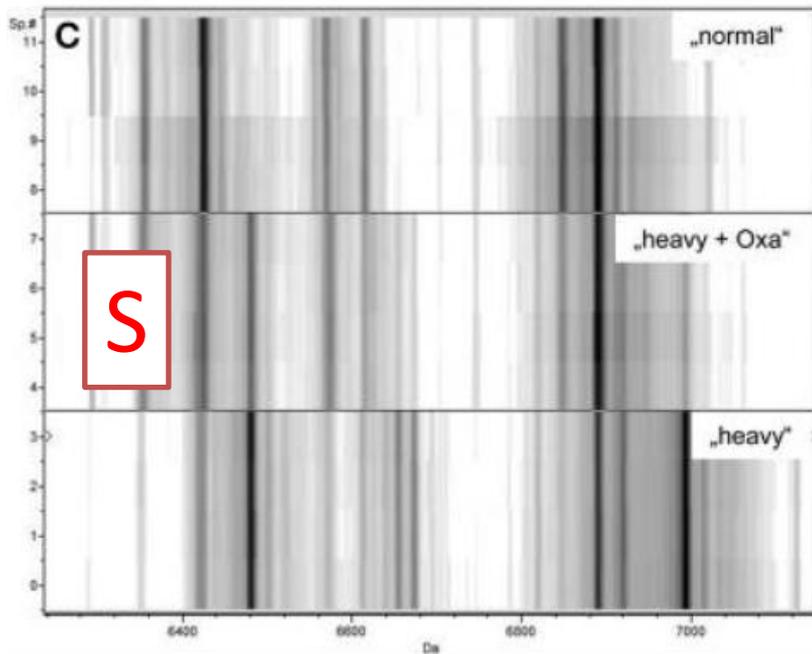
3. Analysis growth/no growth *peak shift as read-out*

- Isotope labelling (incorporation of ^{13}C)
- Normal and heavy Lysine \rightarrow +/- oxacillin



MALDI Biotyper-Based Rapid Resistance Detection by Stable-Isotope Labeling

Katrin Sparbier,^a Christoph Lange,^a Jette Jung,^b Andreas Wieser,^b Sören Schubert,^b Markus Kostrzewa^a
BrukerDaltonik GmbH, Bremen, Germany^a; Max von Pettenkofer-Institut, Ludwig-Maximilians-Universität, Munich, Germany^b



Analysis of growth/no growth

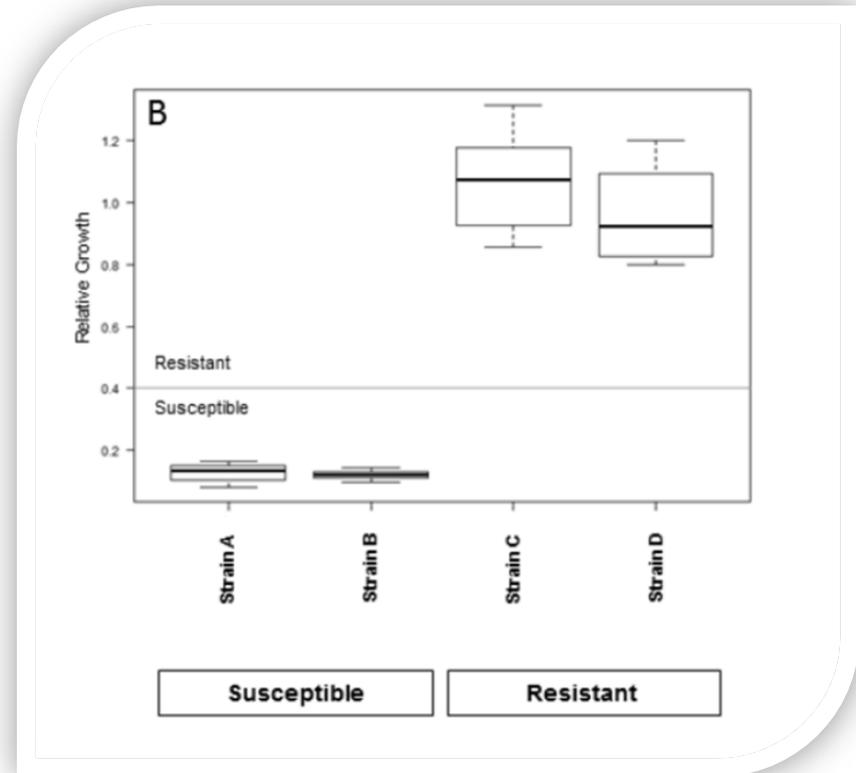
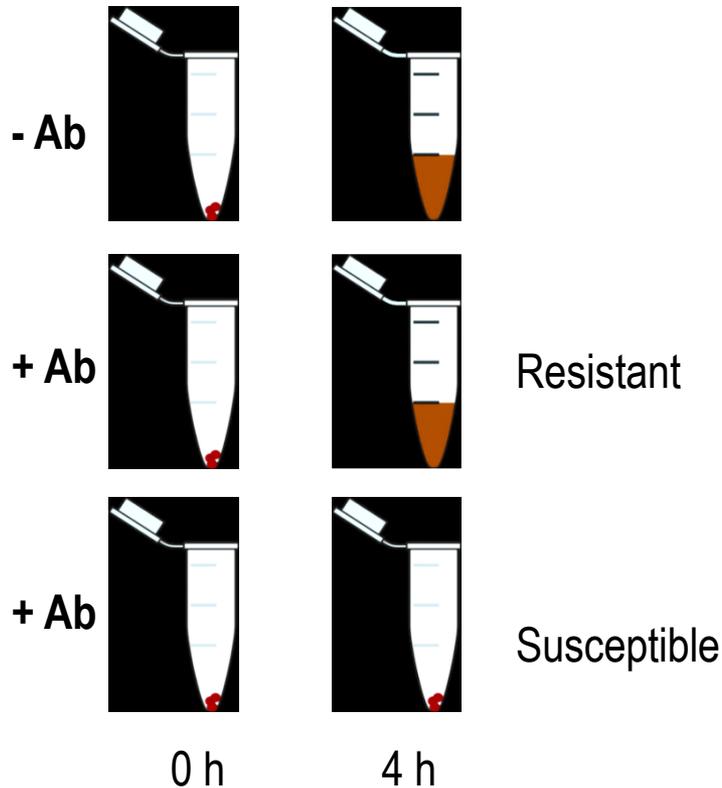
AUC as read out

- MBT-ASTRA



Quantitative Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Rapid Resistance Detection

Christoph Lange,^a Sören Schubert,^b Jette Jung,^b Markus Kostrzewa,^a Katrin Sparbier^a
Bruker Daltonik GmbH, Bremen, Germany^a; Max von Pettenkofer-Institut, Munich, Germany^b



$$\text{Relative growth} = \text{AUC}_{\text{Ab}} / \text{AUC}_{\text{Broth only}}$$

- **Anaerobes (*B fragilis*)**

Contents lists available at ScienceDirect

Anaerobe

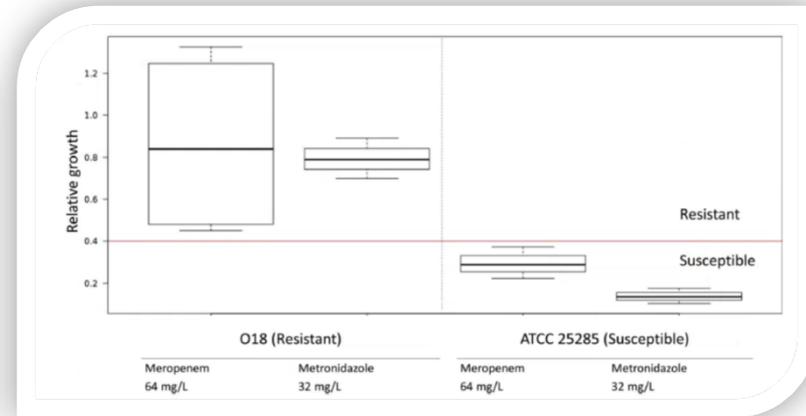
ELSEVIER journal homepage: www.elsevier.com/locate/anaerobe

Antimicrobial susceptibility of anaerobic bacteria

Antimicrobial susceptibility testing of *Bacteroides fragilis* using the MALDI Biotyper antibiotic susceptibility test rapid assay (MBT-ASTRA)

Ulrik Stenz Justesen ^{a,*}, Ziyap Acar ^a, Thomas Vognbjerg Sydenham ^a, Åsa Johansson ^b, ESGAI (ESCMID Study Group on Anaerobic Infections)

^a Department of Clinical Microbiology, Odense University Hospital, Odense, Denmark
^b Department of Clinical Microbiology, Central Hospital, Växjö, Sweden

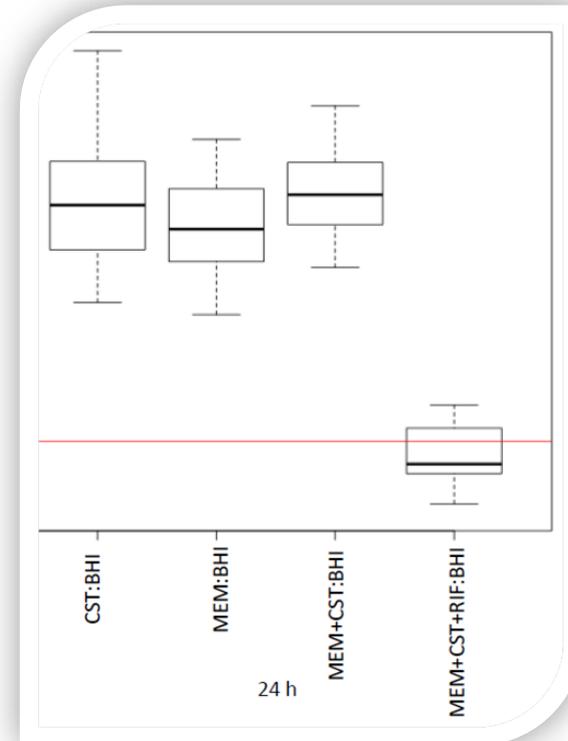


Cli, Mer, Mzl

- **Synergistic effects**

M Sundqvist et al unpublished

- Mero, Col, Rif
- 1-24h



Analysis of growth/no growth

- DOT-MGA: direct on target microdroplet growth assay
- Microtiter assay

Rapid detection of antibiotic resistance by MALDI-TOF mass spectrometry using a novel direct-on-target microdroplet growth assay

E.A. Idelevich¹, K. Sparbier², M. Kostrzewa², K. Becker^{1,*}  
Editor: F. Allerberger

CMI (2017)

Use of target transport box as „humidity chamber“

Separation of broth from microbial cells by “touching” of microdroplets with an absorptive strip

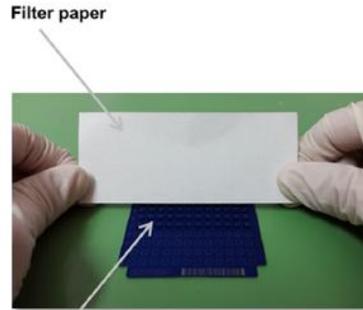
Evaluation of acquired spectra

MSP 96 target box



Inoculated MBT Biotarget 96 Water

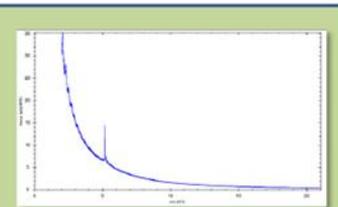
Incubation at 35±1°C



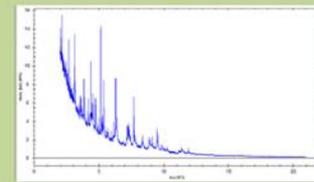
Filter paper

Microdroplets

Adding 1 µl matrix, followed by MALDI-TOF MS measurement



Sample with antibiotic
MALDI Biotyper score <1.7
No identification = susceptibility



Sample with antibiotic
MALDI Biotyper score ≥1.7
Identification = non-susceptibility

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BACTERIOLOGY

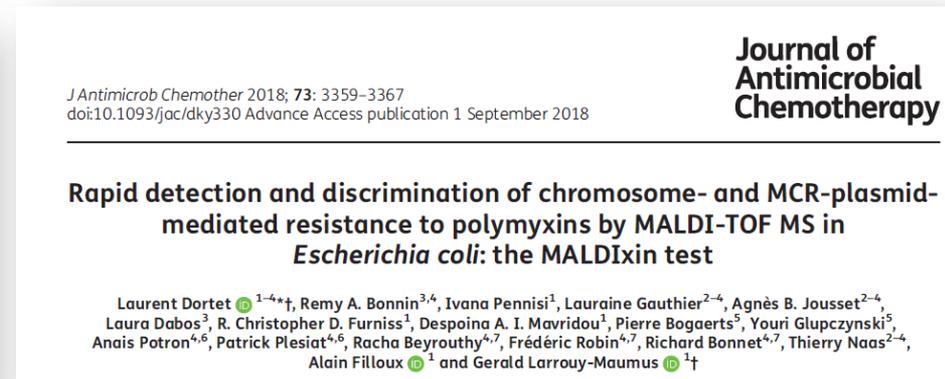
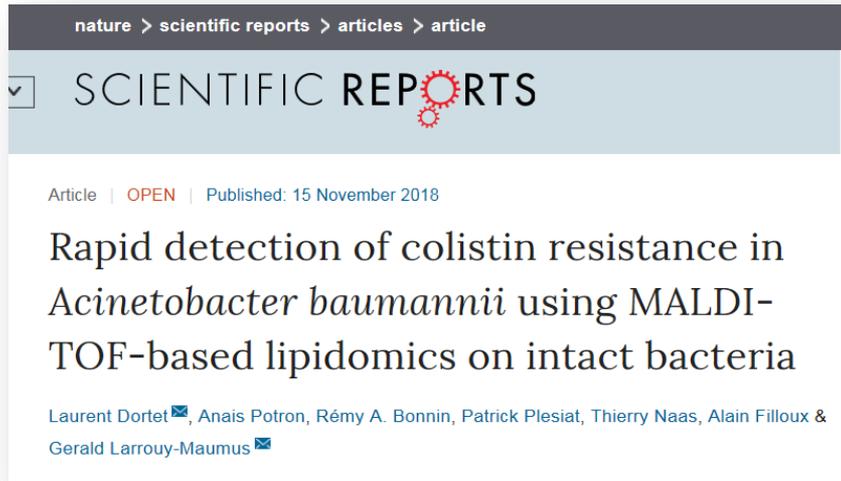


Rapid Direct Susceptibility Testing from Positive Blood Cultures by the Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry-Based Direct-on-Target Microdroplet Growth Assay

Evgeny A. Idelevich,^a Luise M. Storck,^a Katrin Sparbier,^a Oliver Drews,^b Markus Kostrzewa,^b  Karsten Becker^a

Improvements:
Humidity chamber
Incubation time (4h-20h)
Whatman paper
Lysis protocol

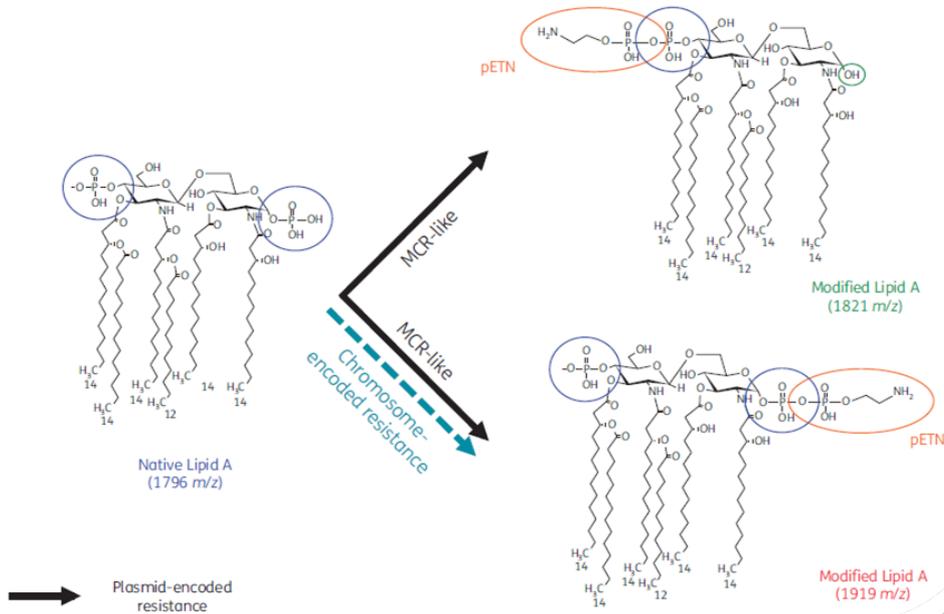
3. Analysis of modification of lipid structures



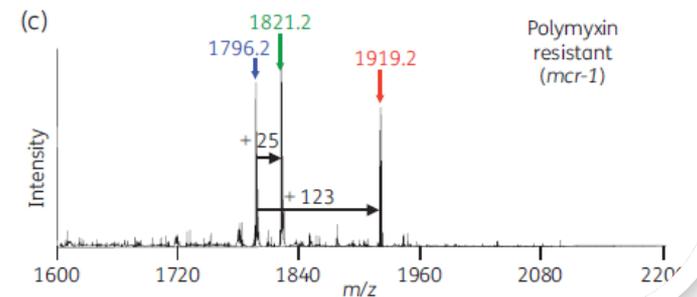
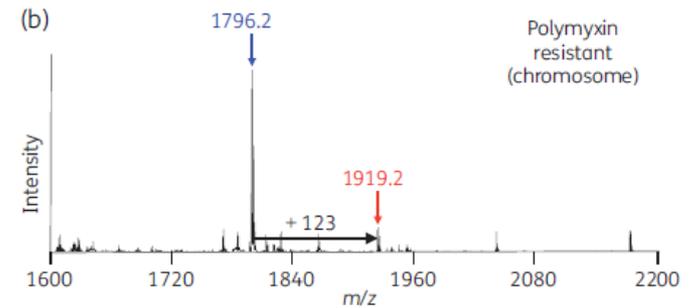
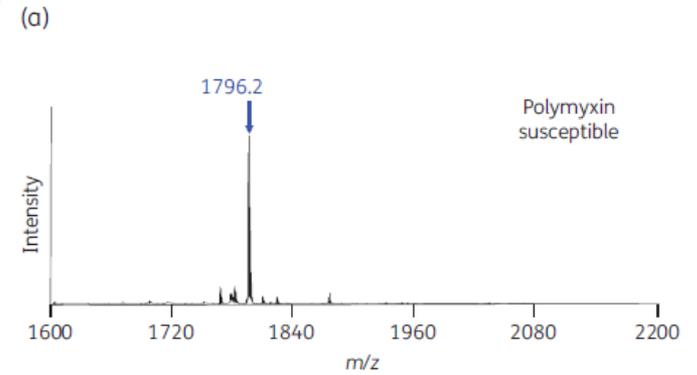
- Colistin resistance
- Modification of LipidA, phosphoethanolamine (pETN)
- Negative ion mode
- 5' protocol

SUSCEPTIBLE

RESISTANT



(Lipid profiles might differentiate between species/complexes that are difficult to discriminate on a protein level. e.g. *E coli* and *Shigella*, *mycobacteria*)



MALDI-TOF AST

- **Species identification!**

Other useful applications:

- Confirmation (e g carbapenemases)
- HMW-typing *C difficile*
- Targeted studies (e g porins)
- "Local biomarkers" in an outbreak
- DOT-MGA?

Thank you!