

MALDI-TOF zur Erregerdifferenzierung

VITEK MS™



MALDI Biotyper

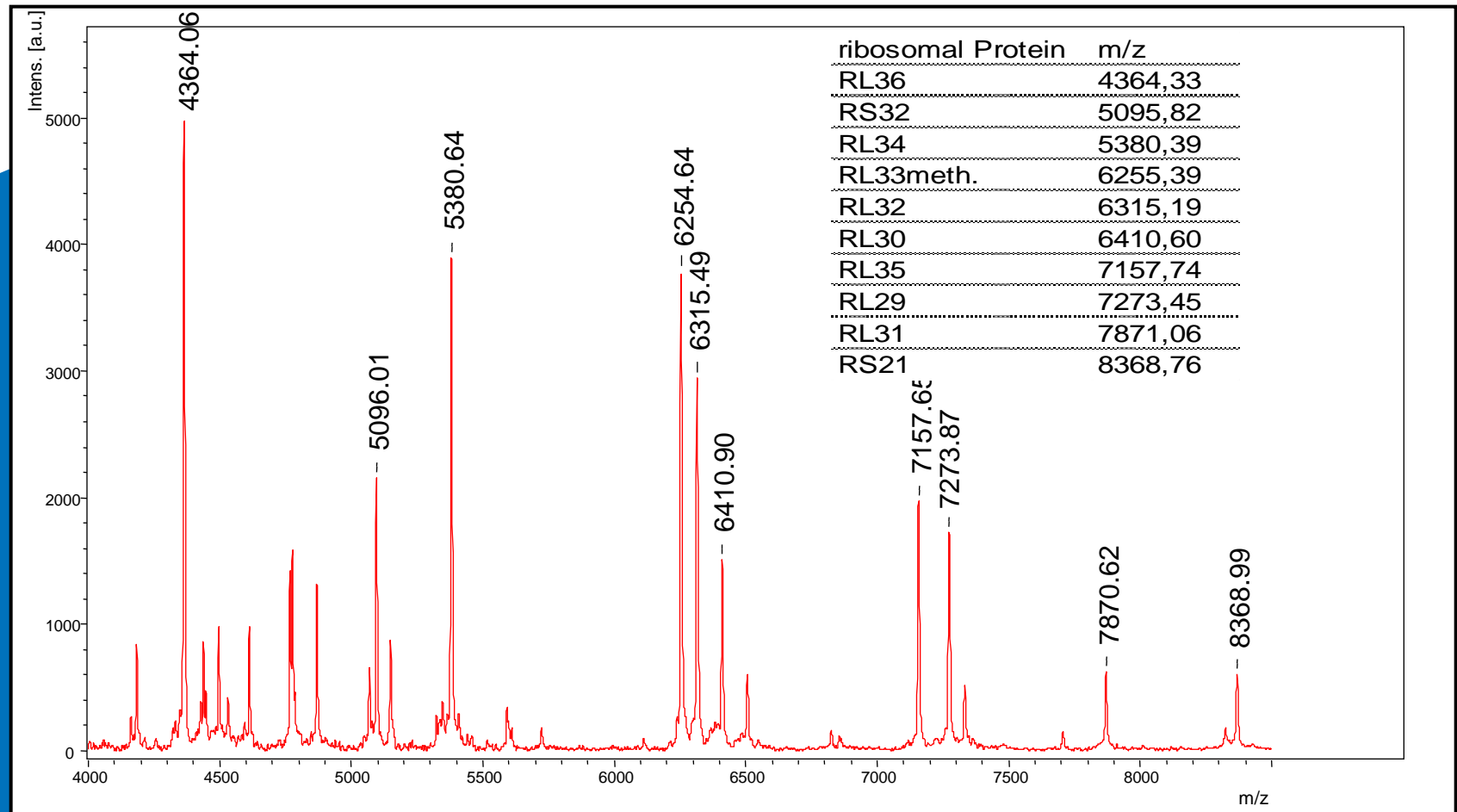


BIOMÉRIEUX

SHIMADZU

Die MALDI TOF Differenzierung ist robust,

➔ beruht auf Messung hochprävalenter (ribosomaler) Proteine



E. coli



Positive blood culture bottle



Harvest 1 ml blood culture liquid in an Eppendorf tube
1 min

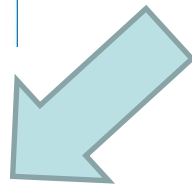
Add **Solution 1+** and mix
30 sec

Centrifuge (1 min., 13.000 rpm)
discard supernatant
1 min

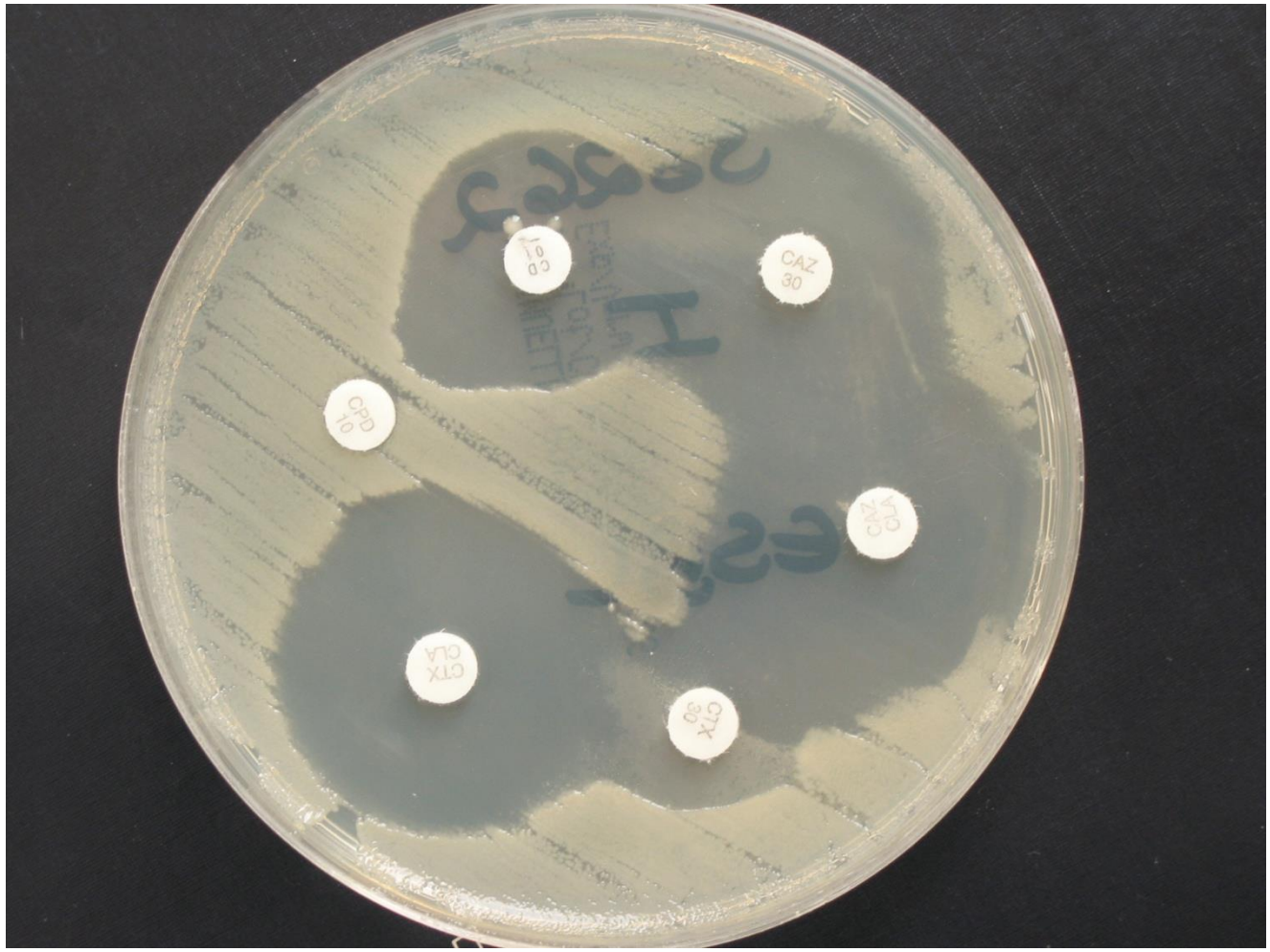
Add **Solution 2** and mix
1.5 min

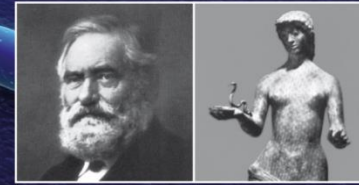
Centrifuge (1 min., 13.000 rpm)
discard supernatant
1 min

Suspend pellet in 300 µl water

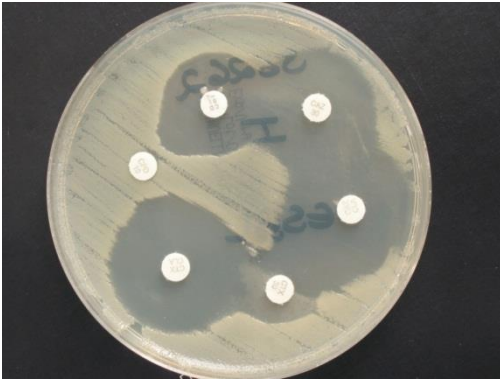


Identifikation





Konventionelle Testung: Wachstum in Gegenwart von Antibiotika

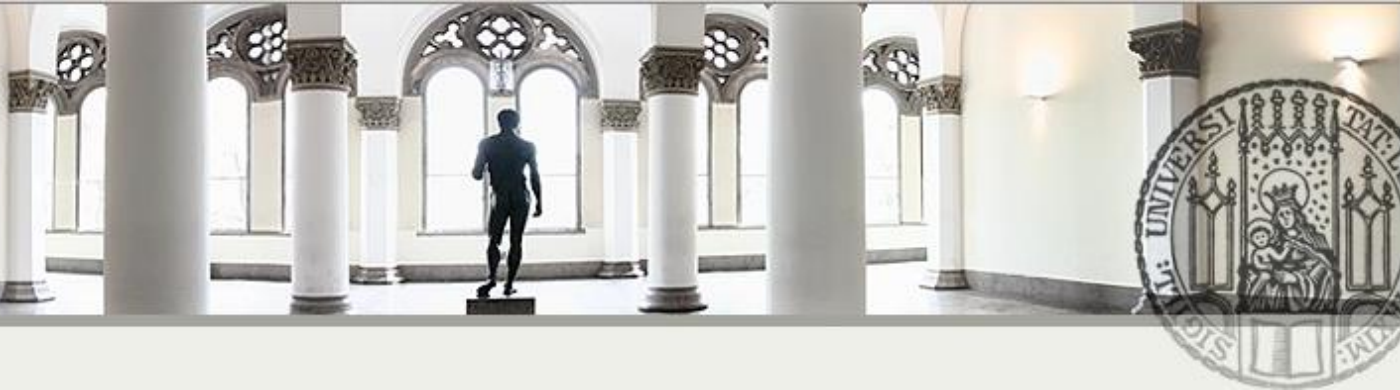


Vorteile:

- Breites AB Spektrum K
- Korrelation von *in vitro* Testung und klinischem Ansprechen
- z.T. sehr gut automatisiert

Nachteil:

- Zeitverzögerung etwa 1 Arbeitstag



MALDI-TOF MS zum Nachweis von Resistenzen gegen Antiinfektiva: Möglichkeiten und Grenzen

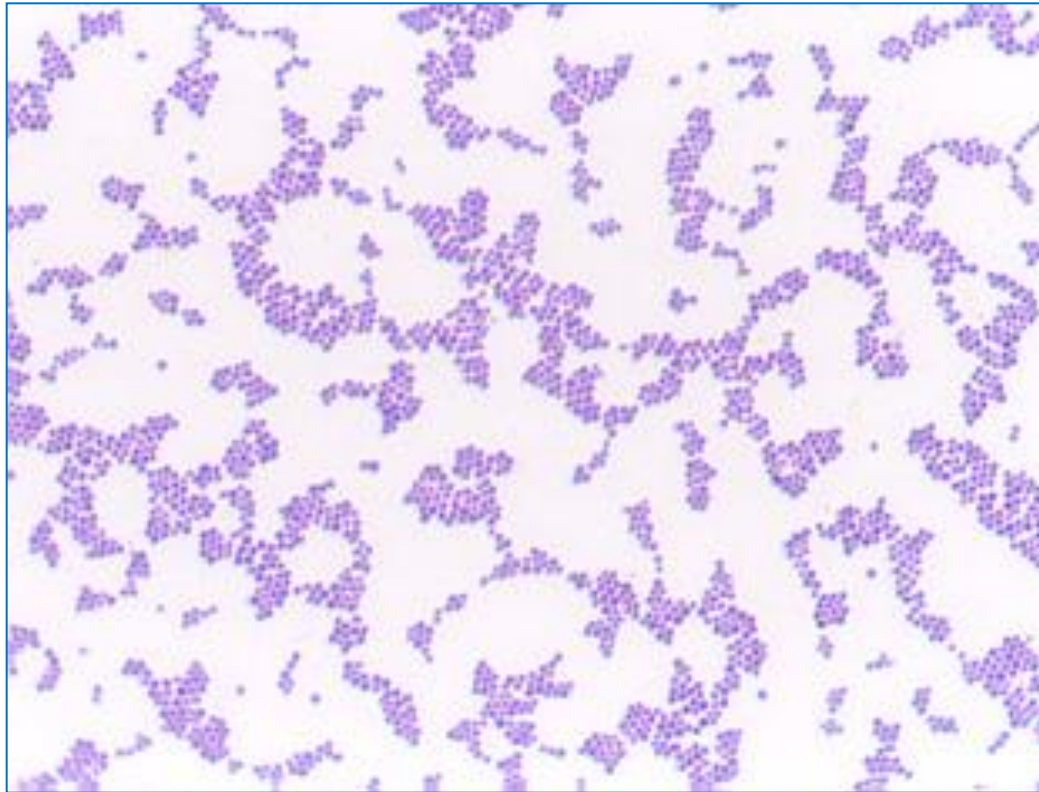
Prof. Dr. med. Sören Schubert

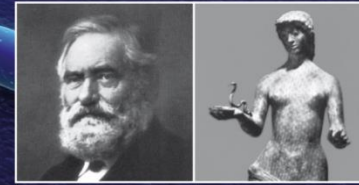
Max von Pettenkofer-Institut
LMU München

MALDI-TOF MS

1. Direktnachweis von Resistenzfaktoren

MALDI-TOF **MRSA** Nachweis?





MRSA



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BIOFILM RESEARCH ASSOCIATION

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Identical MALDI TOF MS-derived peak profiles
in a pair of isogenic SCCmec-harboring and
SCCmec-lacking strains of *Staphylococcus aureus*

Florian Szabados*, Martin Kaase, Agnes Anders, Sören G. Gatermann

J. Med. Microbiol. — Vol. 49 (2000), 295–300
© 2000 The Pathological Society of Great Britain and Ireland
ISSN 0022-2615

TECHNICAL NOTE

Rapid discrimination between methicillin-sensitive
and methicillin-resistant *Staphylococcus aureus* by
intact cell mass spectrometry

VALERIE EDWARDS-JONES*, M. A. CLAYDON*, D. J. EVASON*, J. WALKER†, A. J. FOX**† and
D. B. GORDON*

Proteomics 2002, 2, 747–753

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Cologne, Germany

747
Identification and discrimination of *Staphylococcus aureus* strains using matrix-assisted laser desorption/ionization-time of flight mass spectrometry

Staphylococcus aureus is an important human pathogen frequently resistant to a wide range of antibiotics. Methicillin-resistant *S. aureus* (MRSA) strains are common nosocomial pathogens that pose a world-wide problem. Rapid and accurate discrimination between methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* is essential for appropriate therapeutic management and timely intervention for infection control. We report here the application of matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) for monitoring the bacterial fingerprints expressed by two well characterized *S. aureus* strains ATCC 29213 (MSSA) and ATCC 4330 (MRSA). Consistent strain-specific data were obtained from subcultures analyzed over a period of three months as well as after changing the growth media from Mueller-Hinton to blood agar indicating the reliability of the method. The bacterial fingerprints obtained proved to be specific for persistent clinical isolates of *S. aureus*. A uniform signature profile for MRSA could not be identified. However, the bacterial fingerprints obtained proved to be specific for nosocomial outbreaks of MRSA and for epidemiologic studies of infections diseases in general.

Anal. Chem. 2002, 74, 5467–5491
Identification of *Staphylococcus aureus* and Determination of Its Methicillin Resistance by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry
Zongmin Du, Ruli Yang*, Zhaobiao Guo, Yajun Song, and Jin Wang
Institute of Microbiology and Epidemiology, 20 Donggongjie, Fengtai District, Beijing 100071, P. R. China



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Journal of Microbiological Methods 62 (2005) 273–284

Journal
of Microbiological
Methods

www.elsevier.com/locate/jmimeth

Optimisation of intact cell MALDI method for fingerprinting of
methicillin-resistant *Staphylococcus aureus*

Kathryn A. Jackson^{a,*}, V. Edwards-Jones^b, C.W. Sutton^a, A.J. Fox^c

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^bDepartment of Biological Sciences, Manchester Metropolitan University, Queen Street, Manchester, M13 9PL, UK

^cHealth Protection Agency, Manchester Medical Microbiology Partnership, Clinical Science Building, Manchester, M13 9WE, UK

Received 19 March 2005; accepted 6 April 2005
Available online 5 July 2005

The discriminatory power of MALDI-TOF mass spectrometry
to differentiate between isogenic teicoplanin-susceptible
and teicoplanin-resistant strains of methicillin-resistant
Staphylococcus aureus

Paul A. Majcherczyk¹, Therese McKenna², Philippe Moreillon¹ & Pierre Vaudaux³

¹Department of Fundamental Microbiology, University of Lausanne, Lausanne, Switzerland; ²Waters Corporation, Atlas Park, Manchester, UK; and

³Service of Infectious Diseases, University Hospital of Geneva, Geneva, Switzerland



MALDI TOF „Nachweis“ von MRSA

International Journal of Medical Microbiology 301 (2011) 64–68



Contents lists available at ScienceDirect

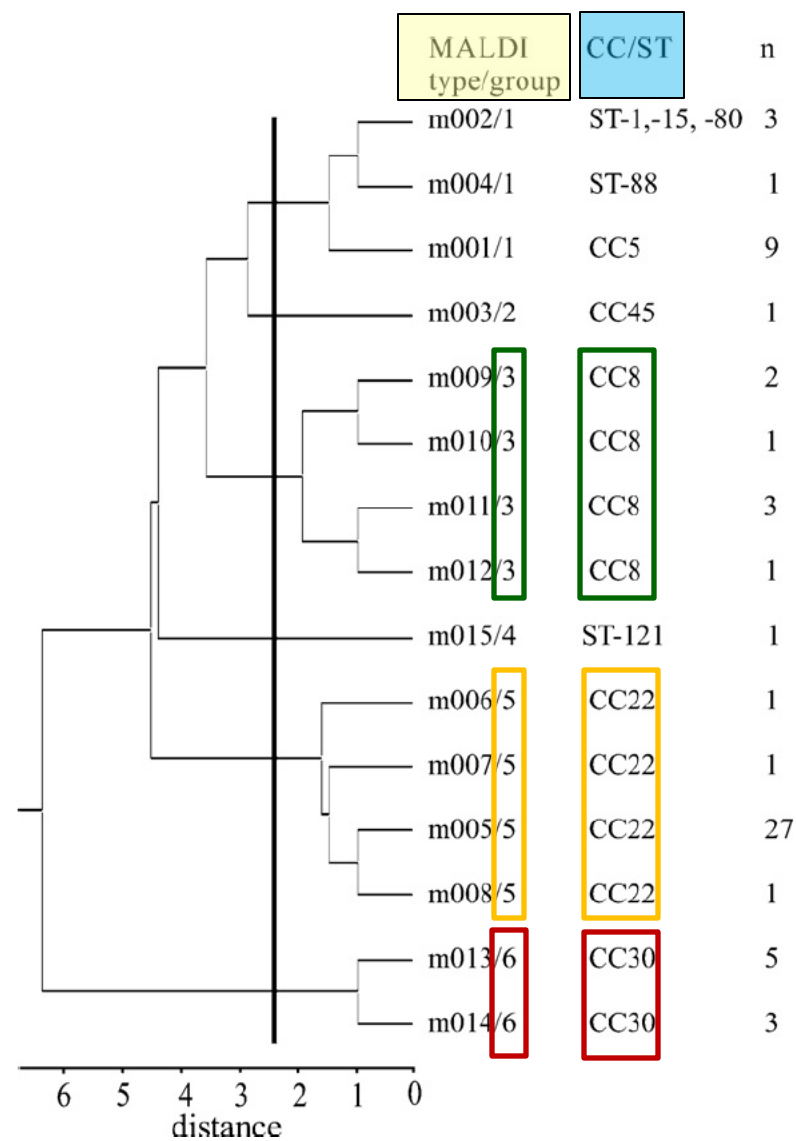
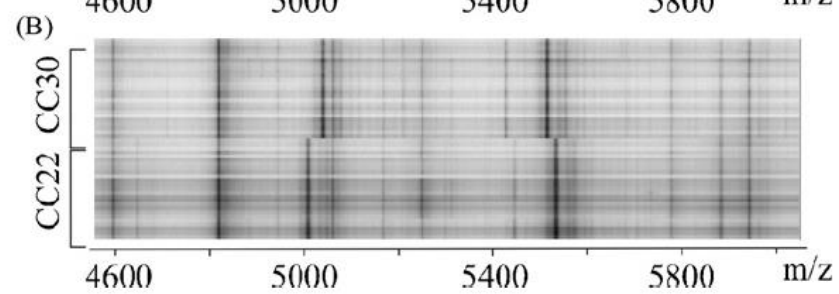
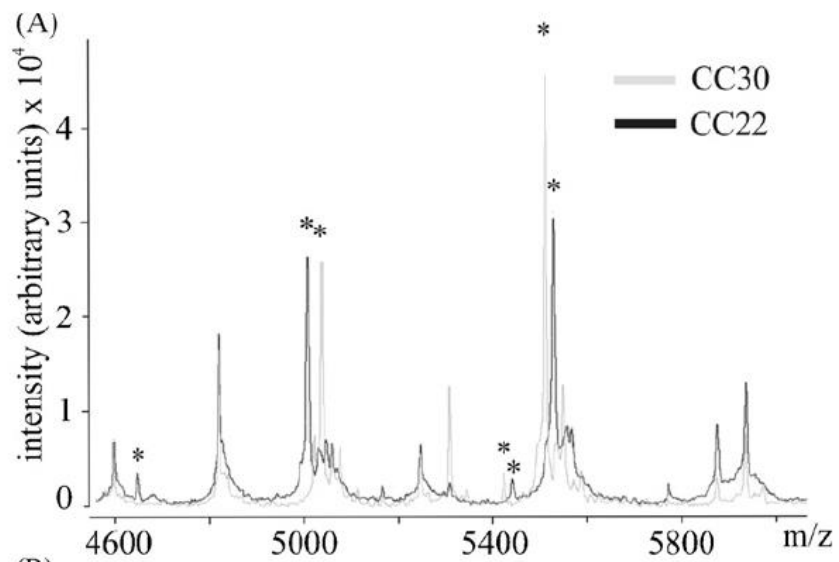
International Journal of Medical Microbiology

journal homepage: www.elsevier.de/ijmm



MALDI-TOF MS fingerprinting allows for discrimination of major methicillin-resistant *Staphylococcus aureus* lineages

Manuel Wolters^a, Holger Rohde^{a,*}, Thomas Maier^b, Cristina Belmar-Campos^a, Gefion Franke^a, Stefanie Scherpe^a, Martin Aepfelbacher^a, Martin Christner^a



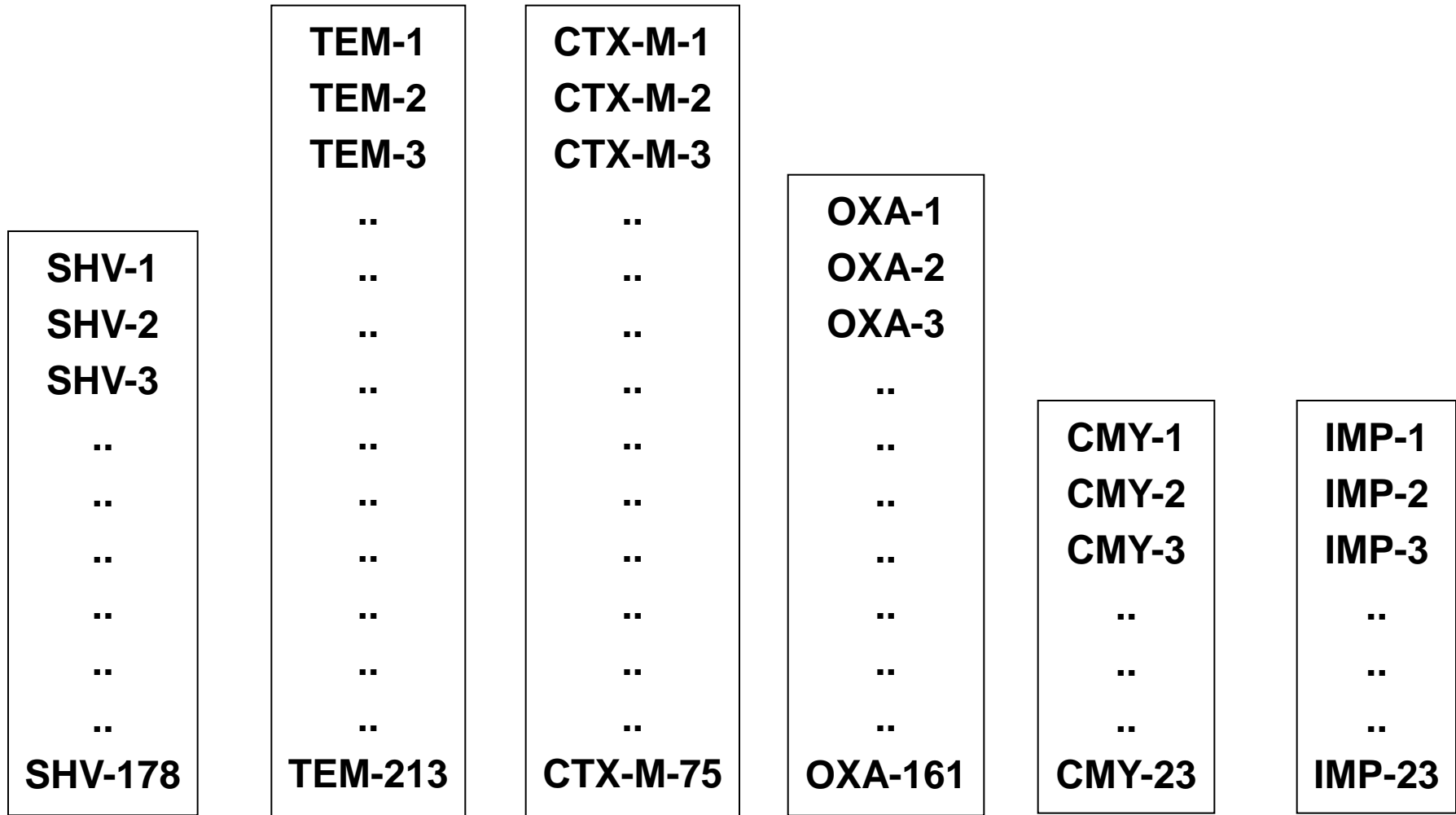
MALDI-TOF Detektion von Antibiotika-Resistenzen

→ β -Laktamasen (ESBL)

→ Carbapenemasen (NDM-1, KPC, ...)

Problem





> 700 β -Laktamase - Subtypen

Fazit:

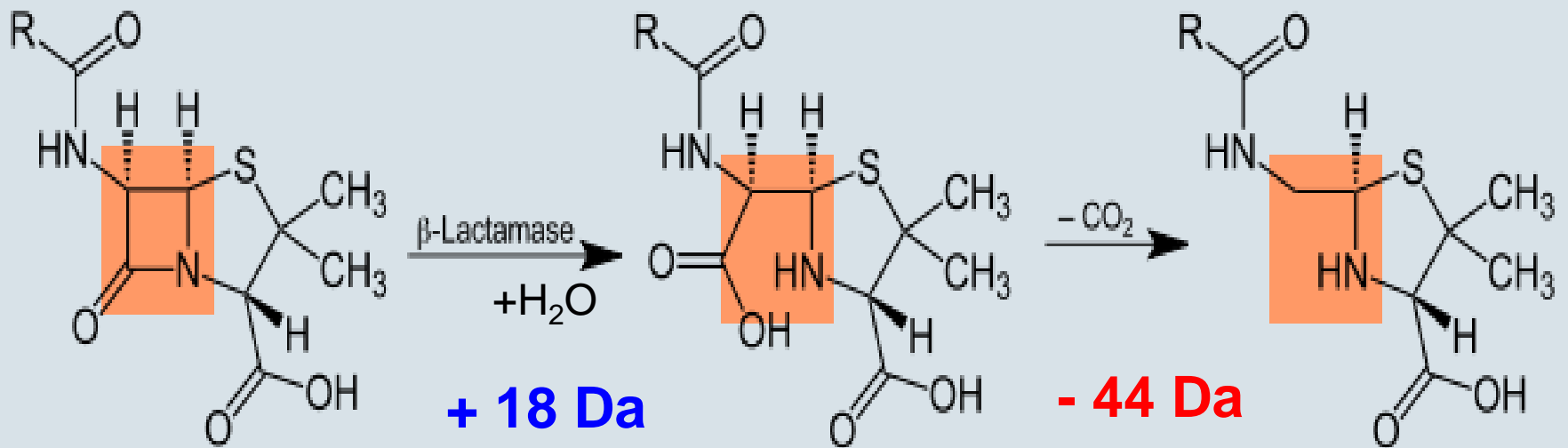
Direktnachweis von Resistenzfaktoren

- Nachweis klonaler Gruppen !
- Nachweis MRSA-assoziiierter Peptide !
- Nicht verlässliche Resistenz-Identifikation
→ β -Laktamasen, PBP2a, Van A / Van B

MALDI-TOF MS

1. Direktnachweis von Resistenzfaktoren (z.B. PBP2a)
2. β -Laktamase Aktivitätstest (MS-BL)

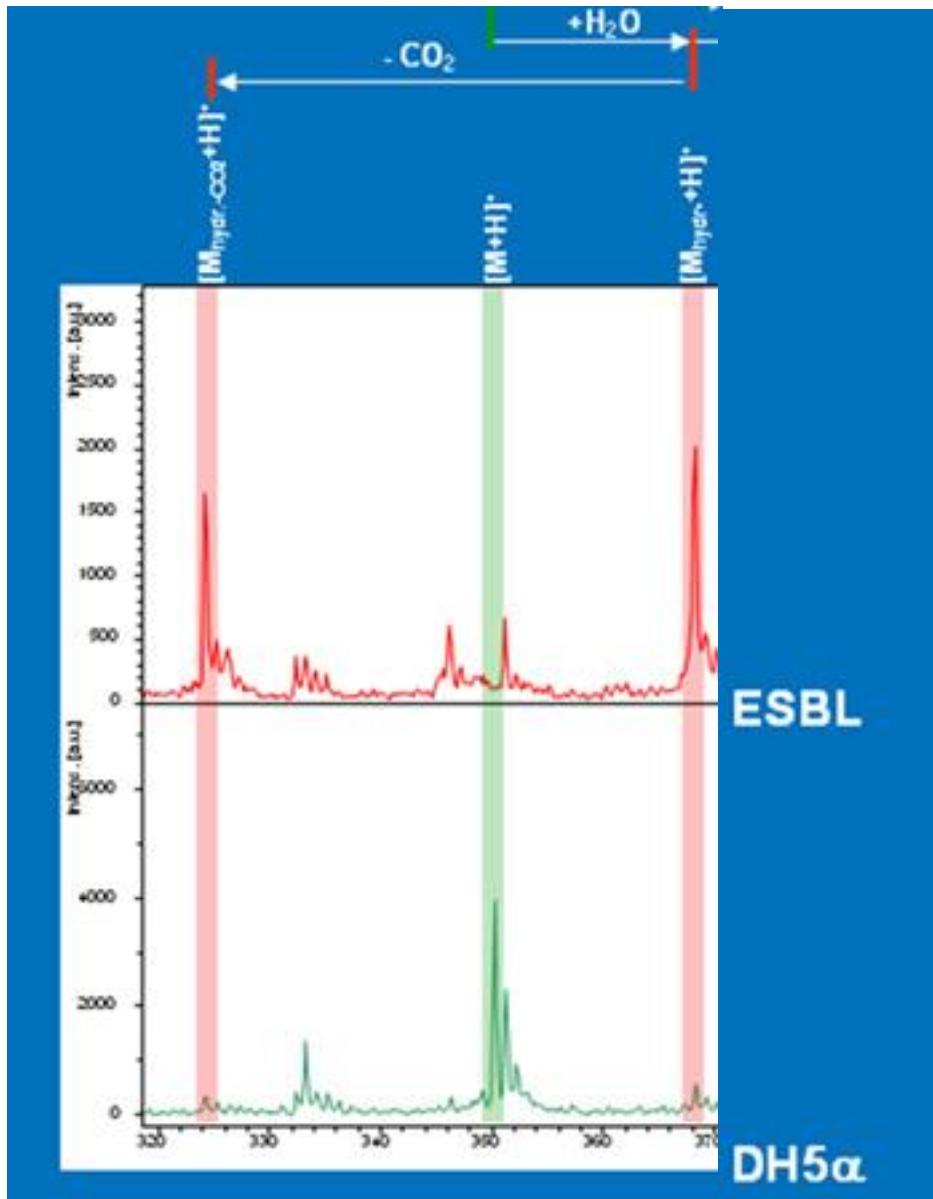
β -Laktamase Aktivität



A

B

C



C

A

B

Ampicillin

+

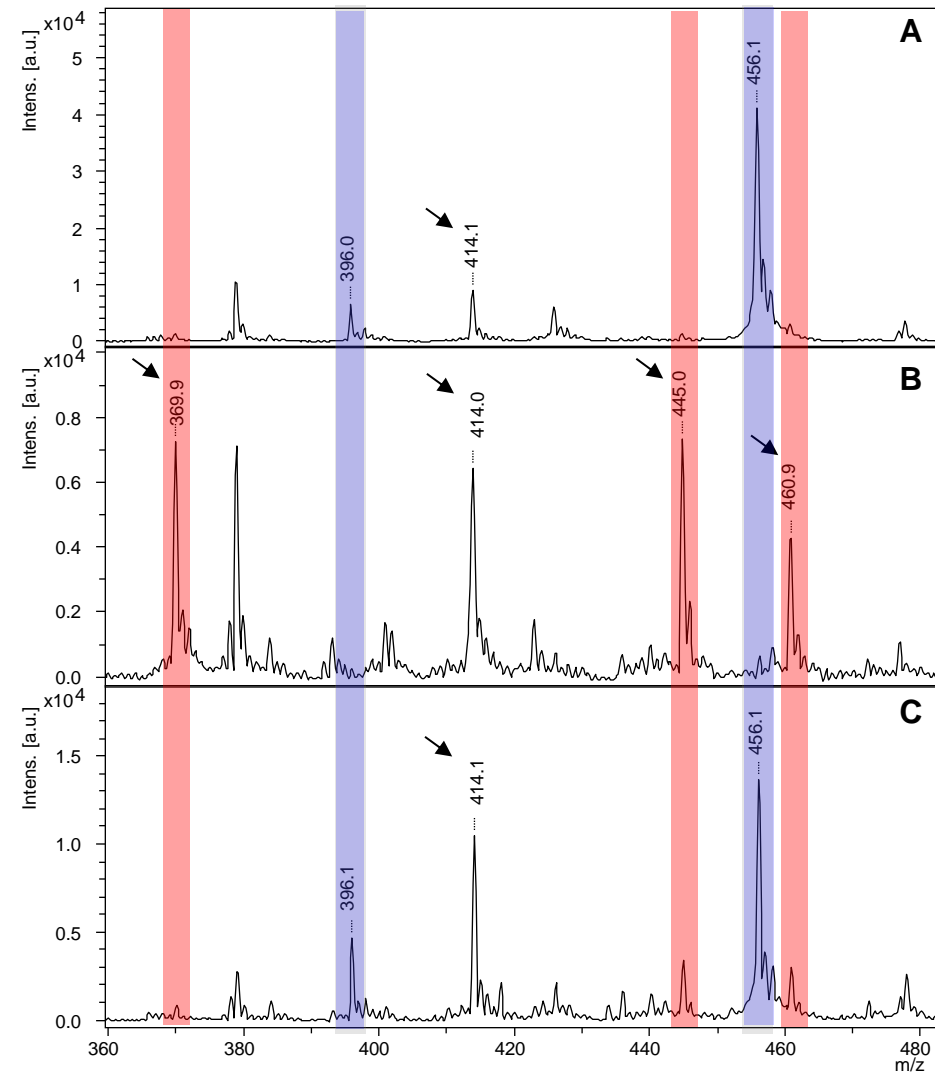
ESBL-E. coli

Ampicillin

+

β -Lact.-neg.
E. coli

Cefotaxim (CTX)

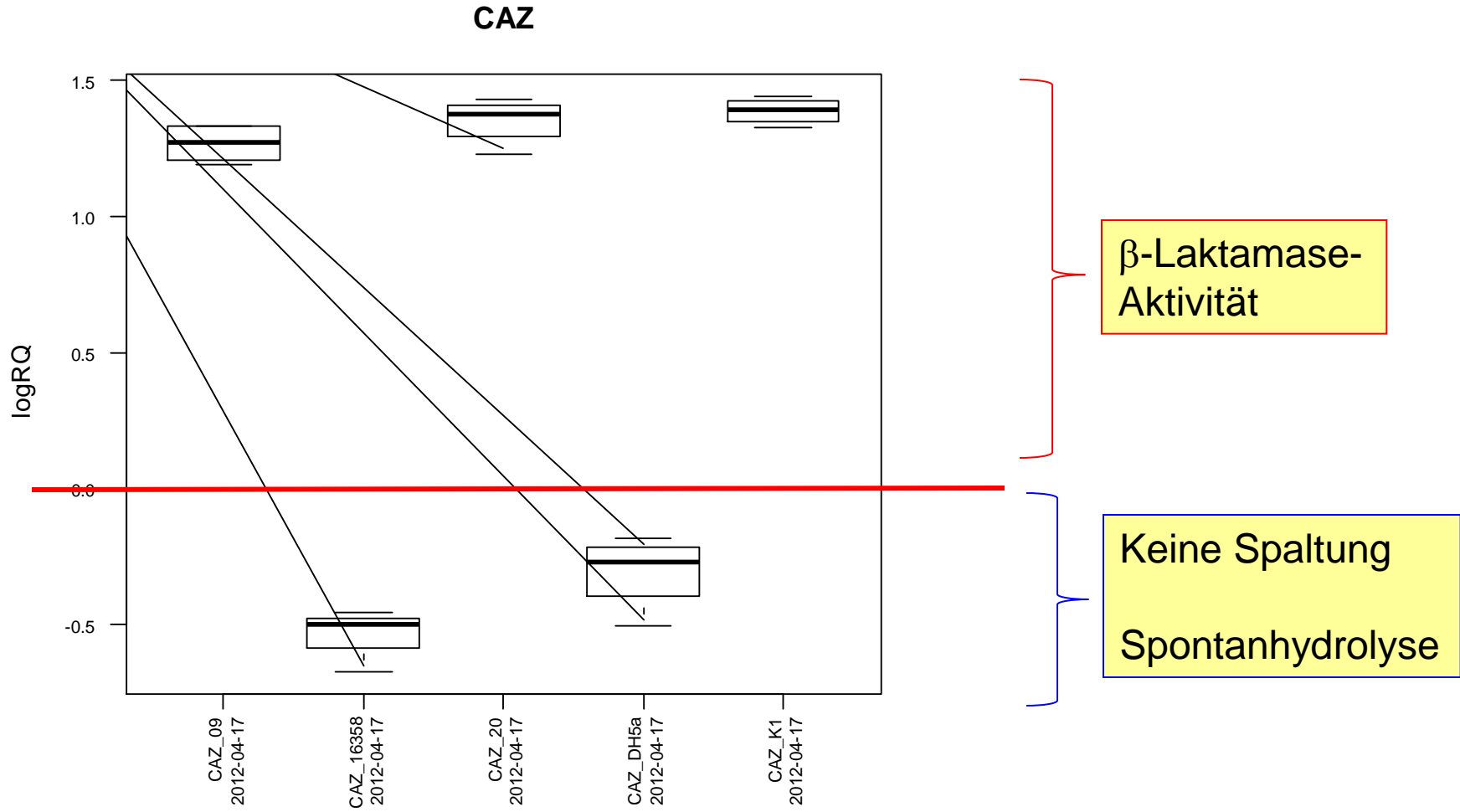


CTX
+
ESBL-*neg.* *E. coli*

CTX
+
ESBL-*pos.* *E. coli*

CTX / CLAV
+
ESBL-*pos.* *E. coli*

0.5 mg/ml CAZ in 10 mM NH₄-hydrogen carbonate
2 h incubation
15 µl sup plus 5 µl 0.5 ng/µl reserpine
1.5 µl spotted





Positive blood culture bottle



Harvest 1 ml blood culture liquid in an Eppendorf tube
1 min

Solution 1+

Add **Solution 1+** and mix
30 sec



Centrifuge (1 min., 13.000 rpm)
discard supernatant
1 min

Solution 2

Add **Solution 2** and mix
1.5 min



Centrifuge (1 min., 13.000 rpm)
discard supernatant
1 min

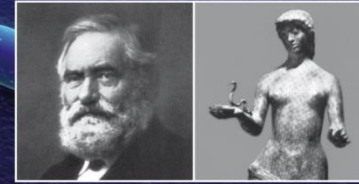
Sample Preparation

Suspend pellet in 300 µl water

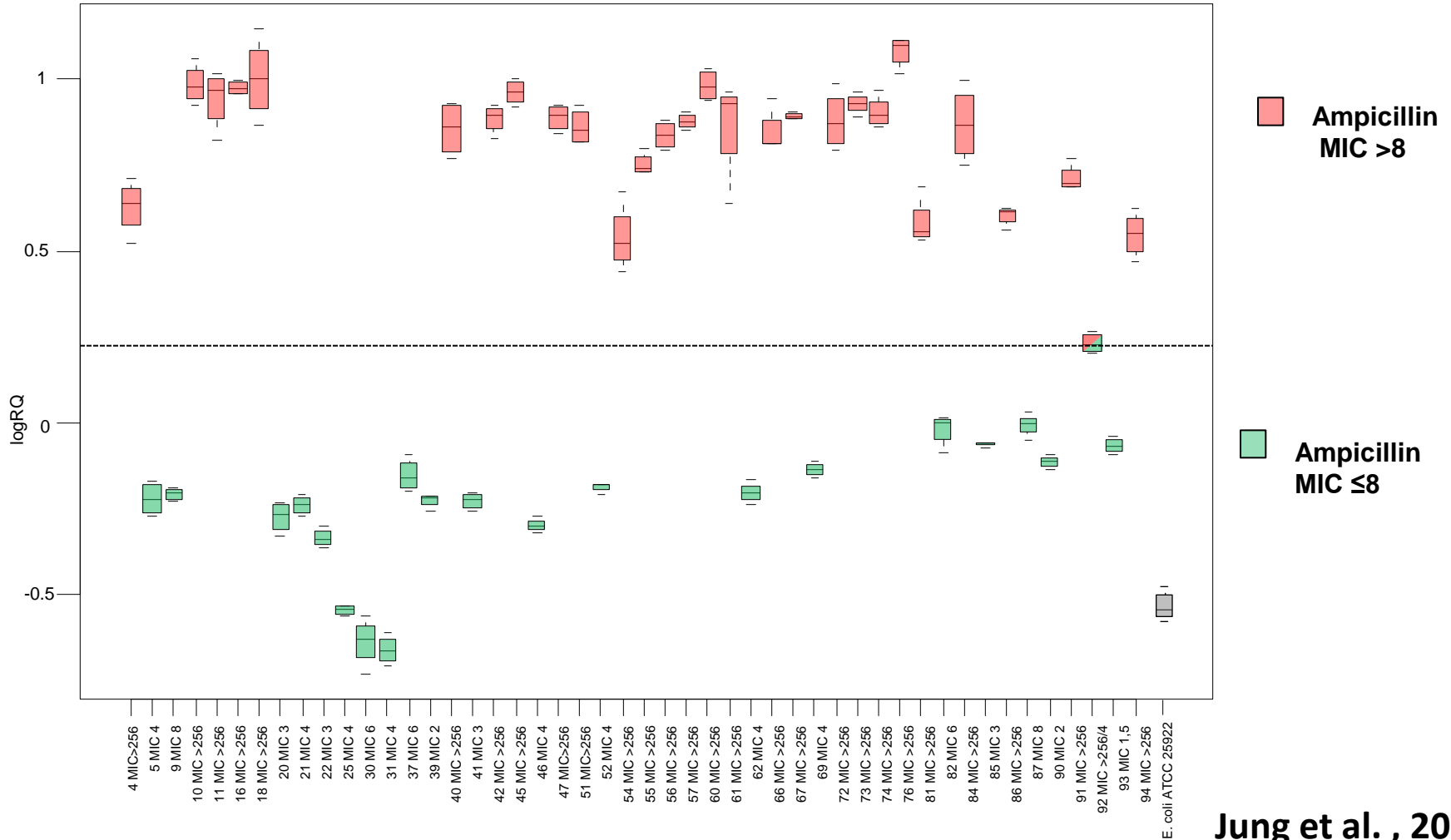


Identifikation

β -Laktamase
Aktivität



E. coli direkt aus positiven Blutkulturen → Ampicillin



Fazit 2:

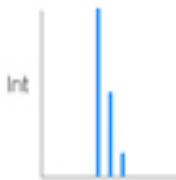
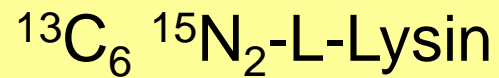
MALDI-TOF β -Laktamase Aktivitätstest

- Schneller Test 1,5 – 3 h
- Automatische Analyse
- Direkt aus positiven Blutkulturen
- Beschränkt auf bestimmte Antibiotika-Resistenzen
 - β -Laktamasen (z.B. ESBL, Carbapenemasen)

MALDI-TOF MS

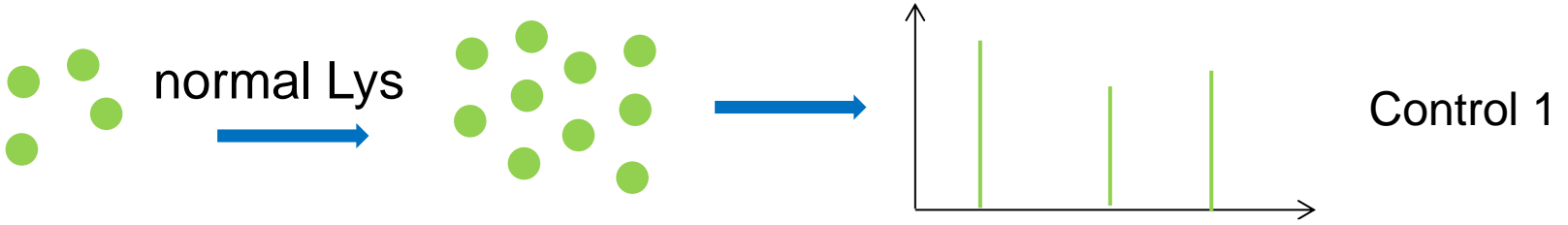
1. Direktnachweis von Resistenzfaktoren (z.B. PBP2a)
2. MALDI-TOF β -Laktamase Aktivitätstest (MS-BL)
3. Antibiotika Resistenzbestimmung - phänotypisch

Phänotypischer Resistenztest (MS-RESIST)



→ Für alle kultivierbaren Bakterien einsetzbar

Antibiotika-Resistenztest mit stabilen Isotopen



S. aureus

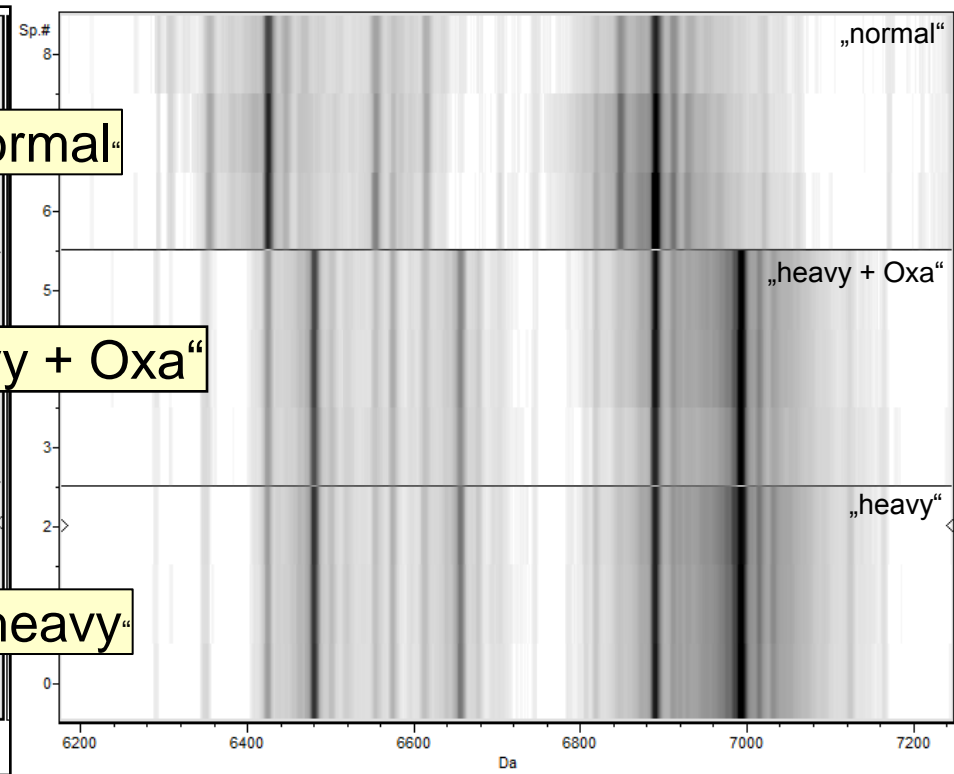
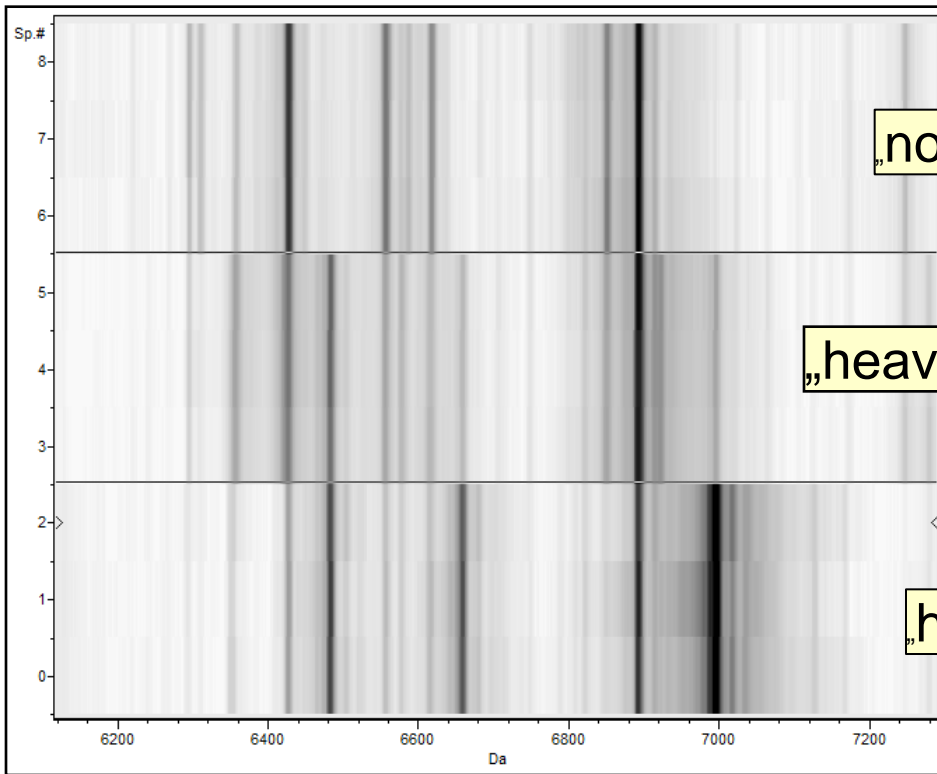
Massenspektrum – Gel-Ansicht

MSSA

MRSA

OXA – sensibler Stamm

OXA - resistenter Stamm



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

Eur J Clin Microbiol Infect Dis
DOI 10.1007/s10096-013-2031-5

14 December 2013

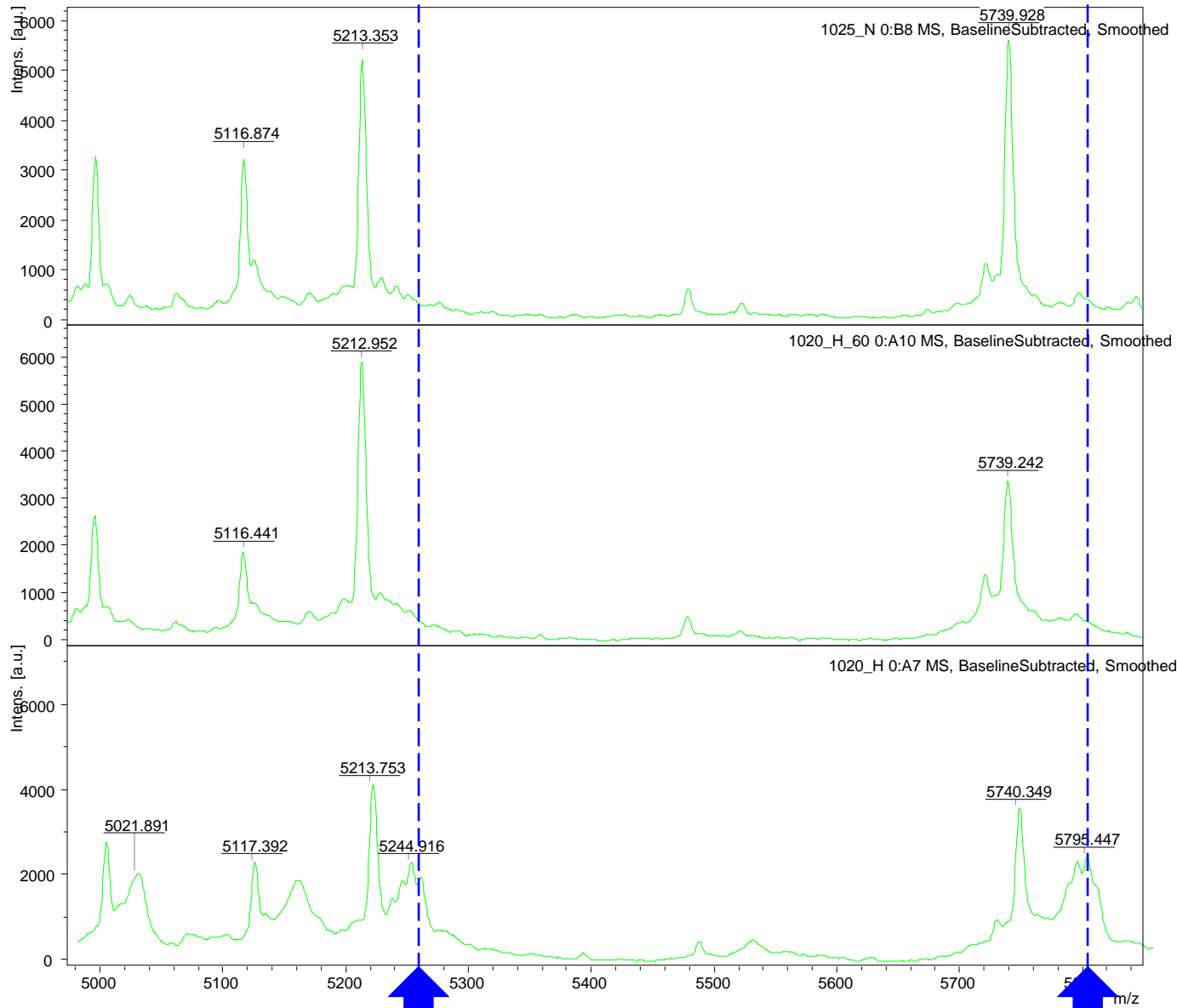
ARTICLE

Rapid detection of antibiotic resistance based on mass spectrometry and stable isotopes

J. S. Jung • T. Eberl • K. Sparbier • C. Lange •
M. Kostrzewa • S. Schubert • A. Wieser

P. aeruginosa

MBT MS-RESIST / *P. aeruginosa* (Tobramycin- susceptible)

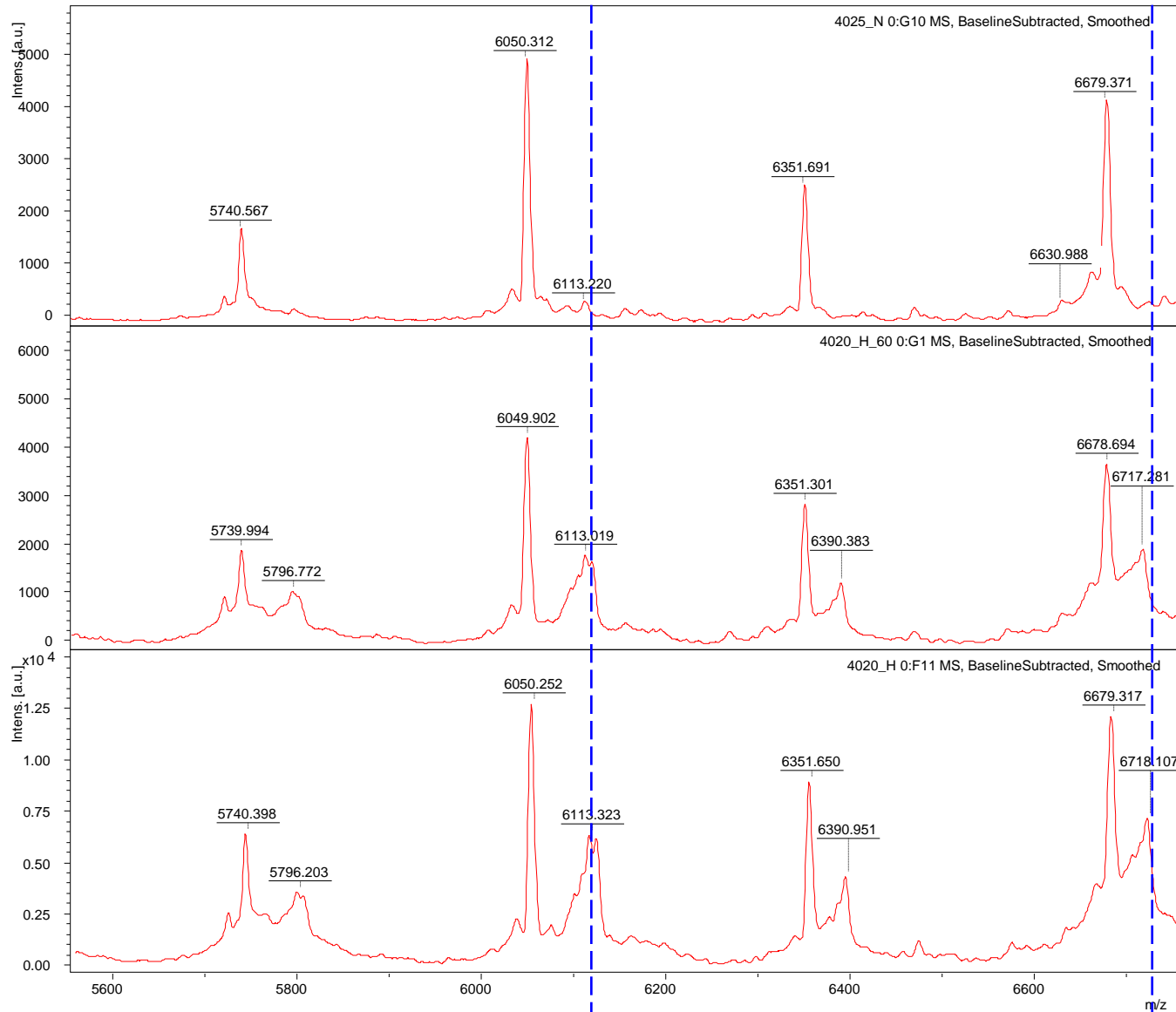


normal

**heavy
+
TOB**

heavy

MS-RESIST/ *P. aeruginosa* (Tobramycin-resistant)



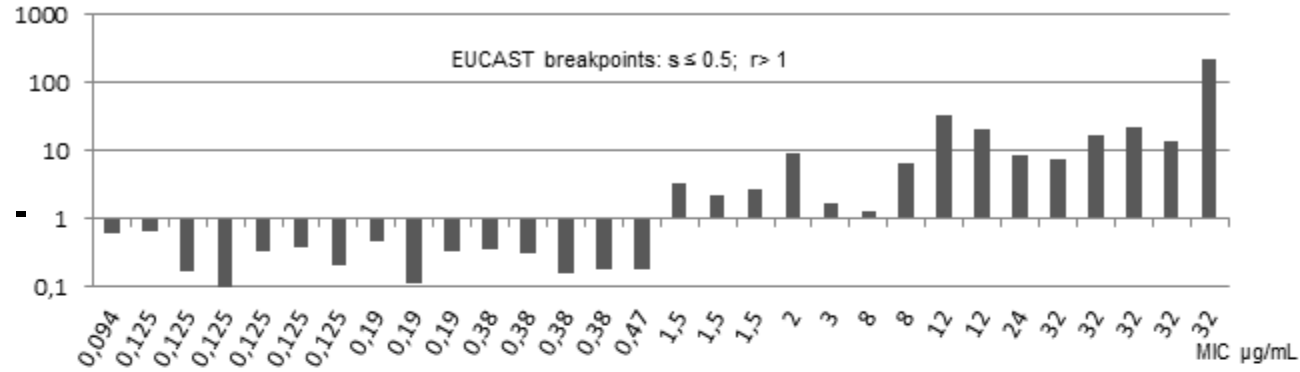
normal

heavy
+
TOB

heavy

Ciprofloxacin

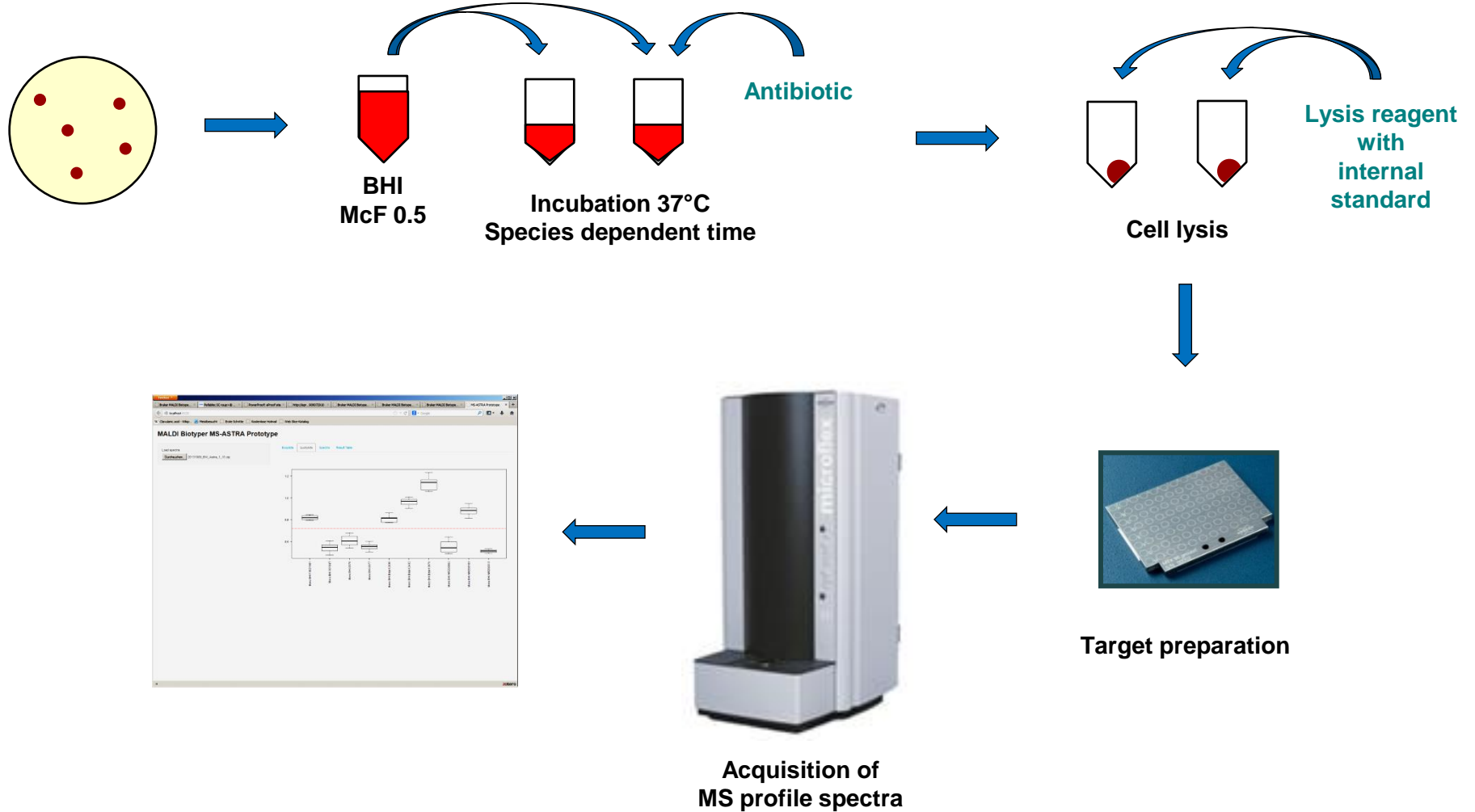
Pseudomonas aeruginosa



3. Antibiotika-Resistenbestimmung mit phänotypischen MALDI-TOF-Tests

Phenotypischer Test ohne Isotope !

MALDI-TOF MS zur Wachstumsmessung (quantitativ)



spectra view

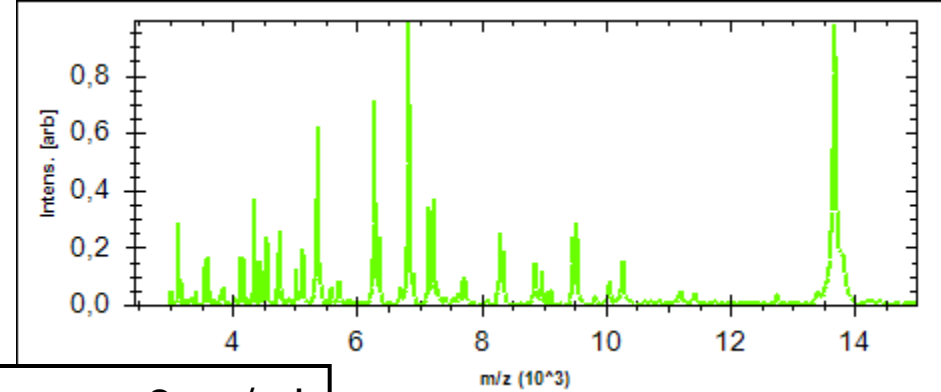
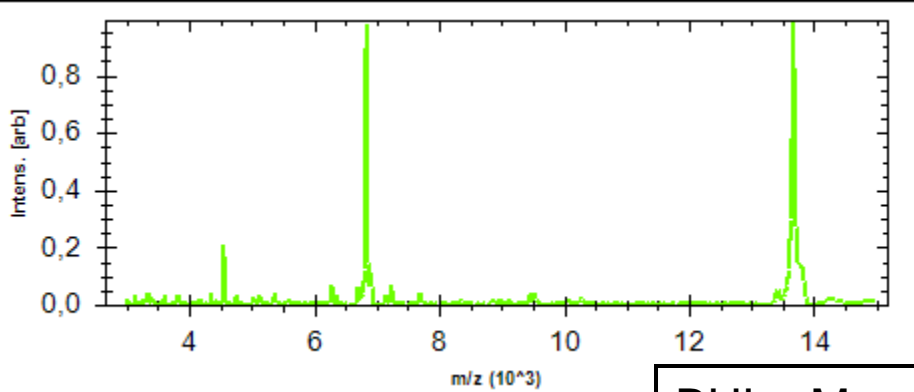
Klebsiella pneumoniae

Standard $[M+H]^{2+}$

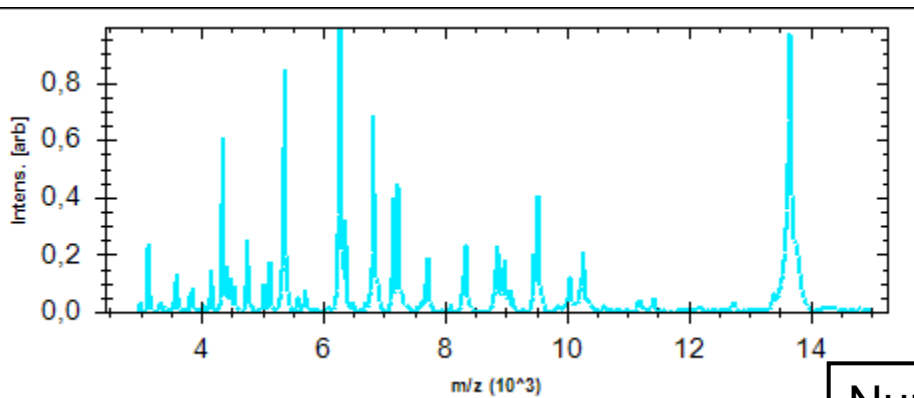
Standard $[M+H]^+$

Standard $[M+H]^{2+}$

Standard $[M+H]^+$

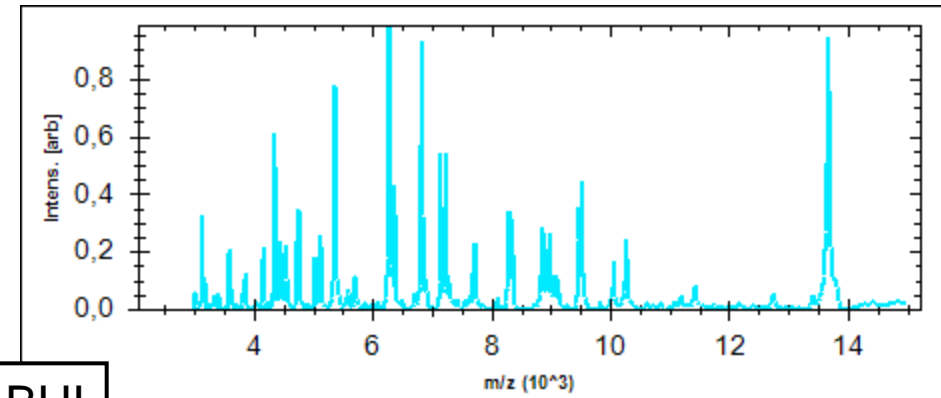


BHI + Meropenem 8 µg/ml



Nur BHI

sensibel



resistent

Zusammenfassung (1)

Direktnachweis von Resistenzfaktoren

- Nachweis klonaler Gruppen
 - Kann den Nachweis klonal verbreiteter Resistenzfaktoren ermöglichen
- Zweifelhaft !
- (bisher) kein Direktnachweis von β -Laktamasen, PBP2a, Van A/B, ...

Zusammenfassung (2)

β -Laktamase Aktivitätstest (MS-BL)

- Schneller Test 1.5 – 3 h
- Automatische Analyse
- Direkt von positiven Blutkulturen anwendbar
- Beschränkt auf bestimmte Resistenzen
 - β -Laktamasen
- Alle β -Laktamasen nachweisbar ?
- Einsatz ?

Zusammenfassung (3)

Phänotypischer Resistenztest (**MS RESIST** / **MS ASTRA**)

- Schneller Test 2 – 3 h
- Universell
- Automatische Analyse
- **MS RESIST**: Stabile Isotope: Komplexer Workflow
→ Kits und Karten wären notwendig
- **MS ASTRA**: Einfache Handhabung
- Alle bug-drug Kombinationen analysierbar?
- Einsatz: Wann ?

Bruker Daltonik

- Katrin Sparbier
- Markus Kostrzewa
- Christoph Lange

Max von Pettenkofer-Institut

- **Jette Jung**
- Theresa Eberl
- Christina Popp
- Julia Walker
- Christina Hamacher
- Lukas Schmidt



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