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MALDI-TOF mass spectrometry for the detection of antimicrobial resistance mechanisms

Jaroslav Hrabák

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22nd ECCMID London, United Kingdom 31 March – 3 April 2012

Presentation Content

- Mechanisms of antibiotic resistance; possible applications of MALDI-TOF MS
- Detection of β-lactamases
- Detection of ribosomal RNA methyltransferase activity
- Resistance determination by the detection of specific peaks
- Proteomic approach
- Minisequencing
- Future perspectives

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Mechanisms Of Antibiotic Resistance

Possible Applications For MALDI-TOF MS

- Production of **enzymes** that inactivate antibiotic molecules (e.g., β-lactamases and aminoglycoside-modifying enzymes);
- Hyperproduction or production of novel **efflux pumps** and other changes in the **cell wall** (e.g., porin alteration);
- **Mutations** in target genes (e.g., in ribosomal protein genes or in genes coding for penicillin-binding proteins – PBPs);
- **Bypass** of a metabolic pathway (e.g., the expression of acquired PBPs with a low affinity for antibiotic molecules);
- Production of proteins that **preserve** the target site (e.g., quinolone resistance mediated by Qnr) or of **target site-modifying enzymes**

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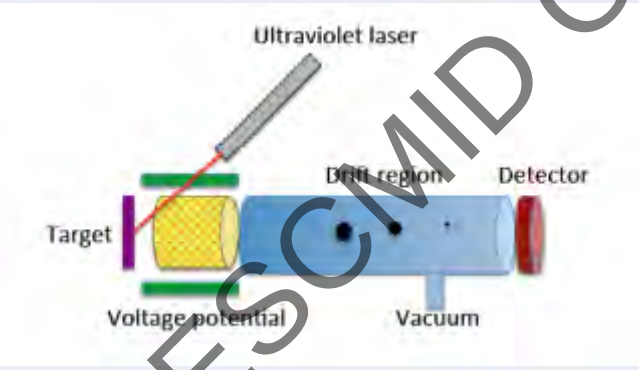
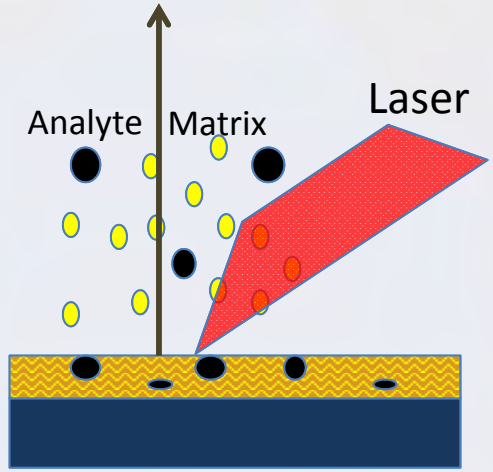
Already Published Applications For MALDI-TOF MS

- Analysis of **antibiotic molecules** and their **modified products**
- Analysis of **bacterial cell components**
- Analysis of **ribosomal RNA methylation**
- Detection of **mutations with mini-sequencing**





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Detection Of β-Lactamases

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Detection Of β-Lactamases

- MALDI-TOF MS is able to detect small molecules (e.g., antibiotics, hormones)
- **Could we detect β-lactams and their degradation products ?**

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2011, p. 3225–3227 Vol. 49, No. 9
0095-1137/11/$12.00 doi:10.1128/JCM.00984-11

Carbapenemase Activity Detection by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry[▽]

Jaroslav Hrabák,* Radka Walková, Vendula Študentová, Eva Chudáčková, and Tamara Bergerová
Department of Microbiology, Faculty of Medicine and University Hospital in Plzen, Charles University in Prague, Plzen, Czech Republic

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2011, p. 3325–3334 Vol. 49, No. 9
0095-1137/11/$12.00 doi:10.1128/JCM.00257-11

NOTES

Using Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry To Detect Carbapenem Resistance within 1 to 2.5 Hours[▽]

Irene Burckhardt* and Stefan Zimmermann
Department for Infectious Diseases, Microbiology and Hygiene, University of Heidelberg, Im Neuenheimer Feld 324, D-69115 Heidelberg, Germany

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,[a] Christiane Boogen,[c] and Markus Kostrzewa[a]
Bruker Daltonik GmbH, Bremen, Germany[a]; Max von Pettenkofer-Institute, Munich, Germany[b]; and Praxis für Laboratoriumsmedizin, Ärztliche Gemeinschaft für Diagnostik Köln-Bonn, Cologne, Germany[c]

0095-1137/12/$12.00 Journal of Clinical Microbiology p. 927–937 jcm.asm.org 927

Carbapenemase Detection By MALDI-TOF MS

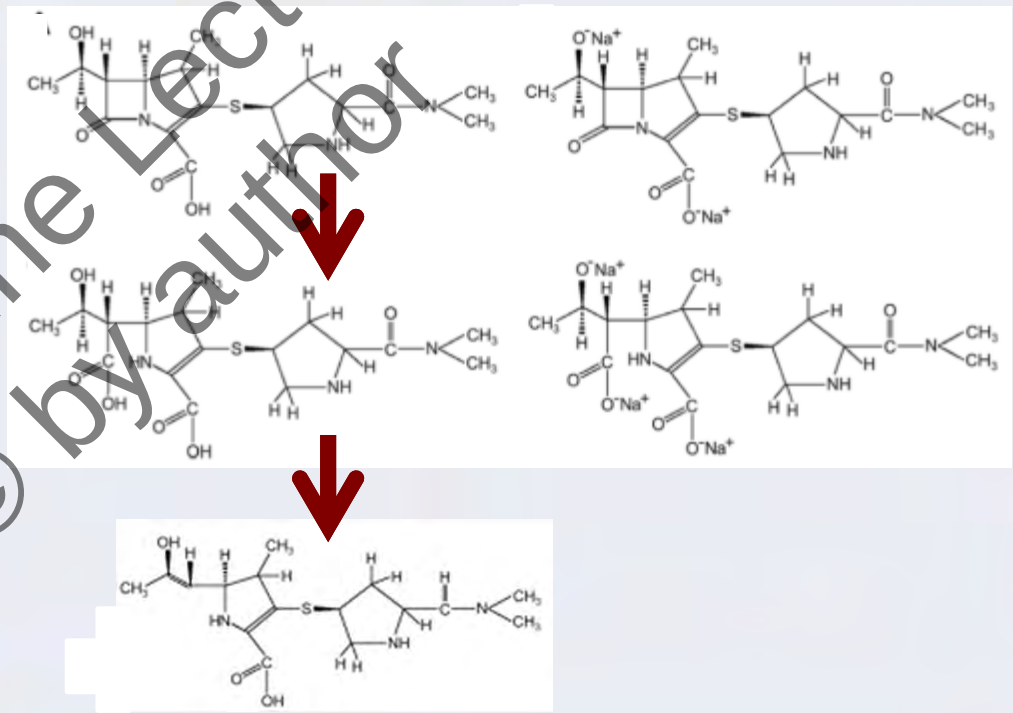
Principle Of the Meropenem Degradation

1. **Hydrolysis of amide bound**

2. **Decarboxylation**

Molecule	*m/z*
Meropenem MW	383,5
[Meropenem+H]$^+$	384,5
[Meropenem+Na]$^+$	406,5
[Meropenem+2Na]$^+$	428,5
[Meropenem$_{hydr}$+H]$^+$	402,5
[Meropenem$_{hydr}$+Na]$^+$	424,5
[Meropenem$_{hydr}$+2Na]$^+$	446,5
[Meropenem$_{hydr}$+3Na]$^+$	468,5
[Meropenem$_{decarbox}$+H]$^+$	358,5
[Meropenem$_{decarbox}$+Na]$^+$	380,5
[Meropenem$_{decarbox}$+2Na]$^+$	402,5

Meropenem Sodium salts



Walková *et al.* Carbapenemase identification by matrix assisted laser desorption/ionisation time-of-flight mass spectrometry P1714, 22nd ECCMID, London
Hrabák *et al.*, MS in review

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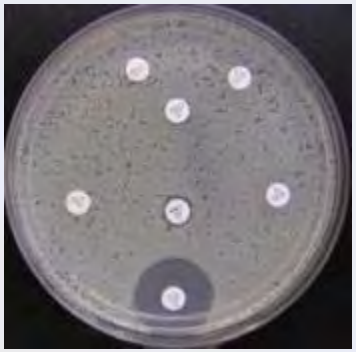
Carbapenemase Detection By MALDI-TOF MS

In *Enterobacteriaceae*, *Pseudomonas* spp., *Acinetobacter spp.*



3 McFarland

20 mM Tris-HCl
20 mM NaCl
pH 7.0

Centrifugation of 1 ml of the suspension

Resuspension of pellet (in 50µl)

20 mM Tris-HCl
0.01% SDS
0.1 mM meropenem
pH 7.0

Incubation at 35°C for 2 hours

Centrifugation of the mixture

MALDI-TOF MS (matrix: DHB in 50% ethanol)

Walková *et al.* Carbapenemase identification by matrix assisted laser desorption/ionisation time-of-flight mass spectrometry P1714 , 22nd ECCMID, London
Hrabák *et al.*, MS in review

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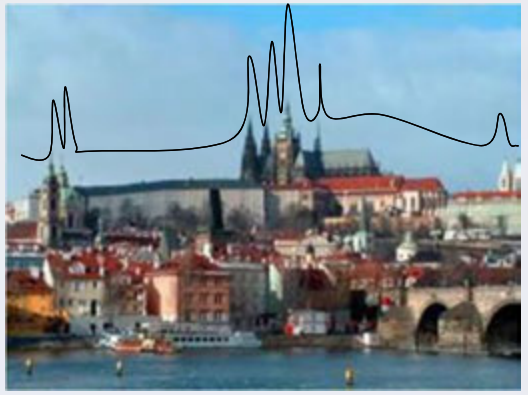
Carbapenemase Detection By MALDI-TOF MS

Spectra Analysis



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Carbapenemase Detection By MALDI-TOF MS

OPEN ACCESS Freely available online PLoS one

Rapid Detection of Carbapenem Resistance in *Acinetobacter baumannii* Using Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry

Marie Kempf[1*], Sofiane Bakour[1,2*], Christophe Flaudrops[1], Meryem Berrazeg[1], Jean-Michel Brunel[1], Mourad Drissi[3], Esma Mesli[3], Abdelaziz Touati[2], Jean-Marc Rolain[1*]

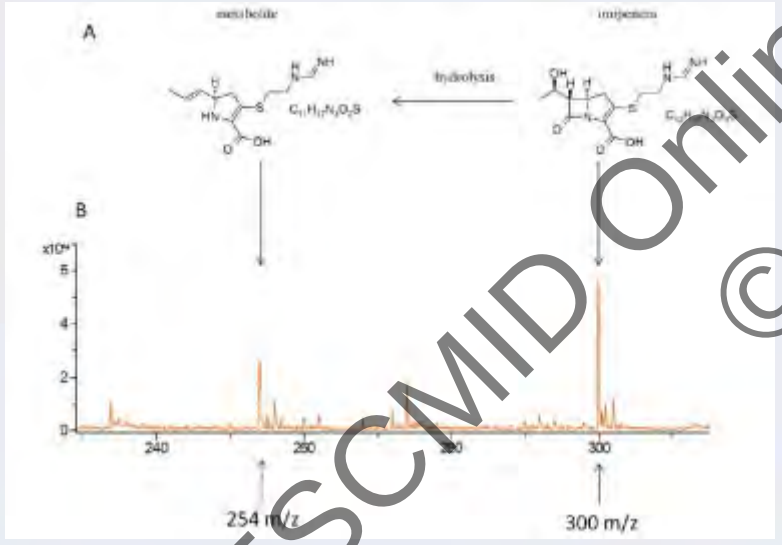

Table 1. Isolates used in the study.

Carbapenemase-producing isolates:	
Carbapenemase	**Species (number of isolates)**
NDM-1	*Escherichia coli* (30)
	Enterobacter cloacae (22)
	Citrobacter spp. (6)
	Klebsiella pneumoniae (5)
	Providencia rettgeri (2)
	Acinetobacter baumannii (2)
KPC-2, KPC-3	*Klebsiella pneumoniae* (10)
VIM-1	*Klebsiella pneumoniae* (16)
	Enterobacter cloacae (4)
	Serratia marcescens (3)
OXA-48	*Escherichia coli* (3)
	Enterobacter cloacae (1)
	Klebsiella pneumoniae (2)
OXA-162	*Escherichia coli* (2)
	Raoultella ornithinolytica (1)
	Citrobacter freundii (1)
Total number of carbapenemase-producing isolates:	**110**
Non-carbapenemase-producing isolates resistant to carbapenems:	
	Klebsiella pneumoniae (28)
	Enterobacter cloacae (4)
	Citrobacter spp. (3)
Total number of non-carbapenemase-producing isolates:	**35**

Walková *et al.* Carbapenemase identification by matrix assisted laser desorption/ionisation time-of-flight mass spectrometry P1714, 22nd ECCMID, London
Hrabák *et al.*, MS in review

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β-Lactamase Detection By MALDI-TOF MS

- Cheap and quick method (turnaround time ca. 3 h!)
- **Detection of a real carbapenemase activity**
- Comparable with spectrophotometric assay
- Possible application to detect carbapenemases in *Acinetobacter baumannii*
- Detection of degradation products depends on a sample preparation (e.g., buffer system)

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Detection Of Ribosomal RNA Methyltransferase Activity

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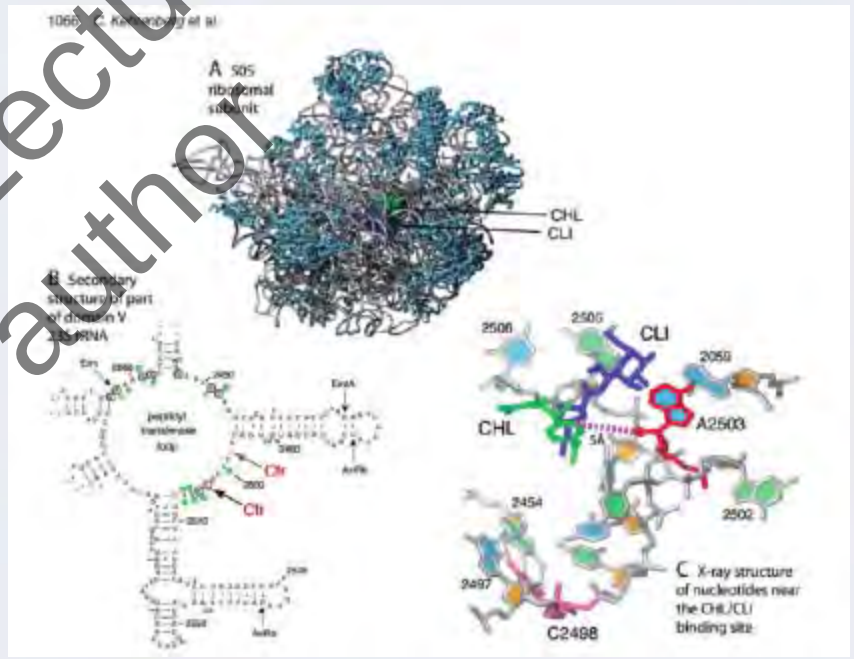
Detection Of Methyltransferase Activity

- **Methylation of the RNA ribosomal subunit**
- Resistance to:
  - Aminoglycosides
  - Chloramphenicol
  - Clindamycin



5420–5431 Nucleic Acids Research, 2009, Vol. 37, No. 16 Published online 9 July 2009
doi:10.1093/nar/gkp575

Determination of the target nucleosides for members of two families of 16S rRNA methyltransferases that confer resistance to partially overlapping groups of aminoglycoside antibiotics

Miloje Savic[1], Josip Lovrić[1,*], Tatjana Ilic Tomic[2], Branka Vasiljevic[2] and Graeme L. Conn[3,*]

Molecular Microbiology (2003) 47(4), 1094–1103

A new mechanism for chloramphenicol, florfenicol and clindamycin resistance: methylation of 23S ribosomal RNA at A2503

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Detection Of Methyltransferase Activity

- In the assay, purified ribosomes and enzymes are used
- rRNA is then digested by a specific Rnase
- Small products are then analyzed with MALDI-TOF MS
- Methylation causes increasing of the mass by 14 Da
- **Still for research use only**

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Antibiotic Resistance Determination By a Detection Of Specific Peaks In Mass Spectra

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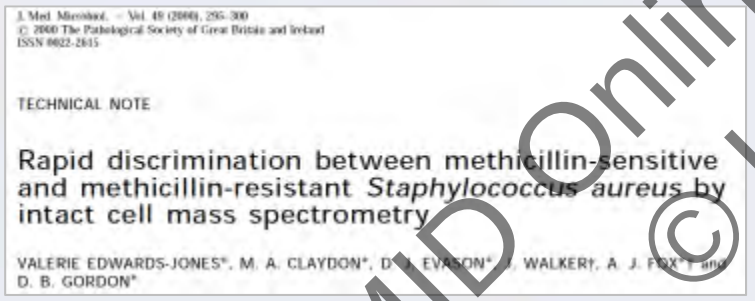
MRSA Detection

- Difference in mass spectra comparing MRSA and MSSA
- Direct detection of PBP2a has not been yet published

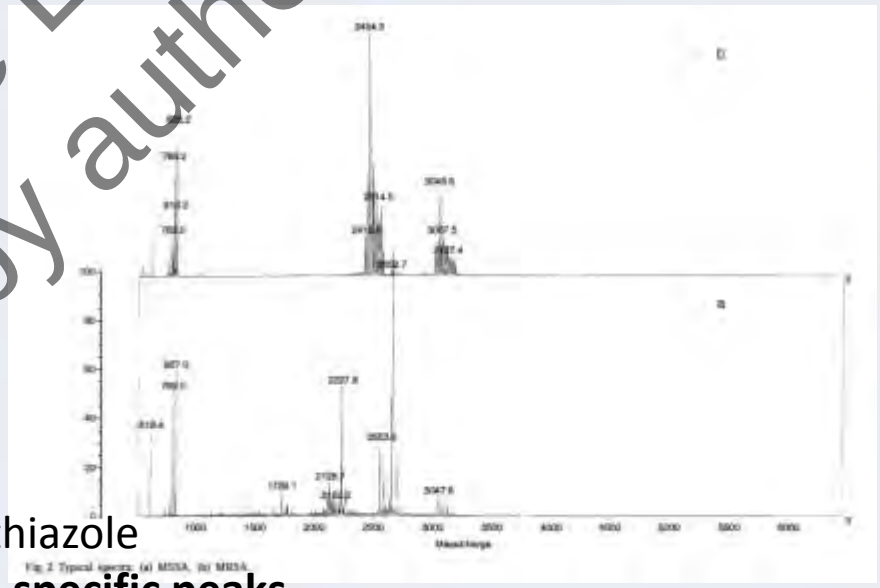


J. Med. Microbiol. – Vol. 49 (2000), 295–300
© 2000 The Pathological Society of Great Britain and Ireland
ISSN 0022-2615

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*



- Intact cells
- **Matrix:** 5-chloro-2-mercapto-benzothiazole
- **14 MRSA-specific peaks and 2 MSSA-specific peaks**

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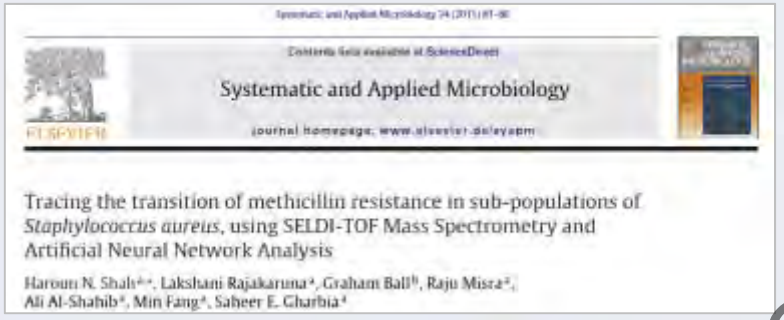
MRSA Detection


Systematic and Applied Microbiology

journal homepage: www.elsevier.de/syapm

Tracing the transition of methicillin resistance in sub-populations of *Staphylococcus aureus*, using SELDI-TOF Mass Spectrometry and Artificial Neural Network Analysis

Haroun N. Shah, Lakshani Rajakaruna, Graham Ball, Raju Misra, Ali Al-Shahib, Min Fang, Saheer E. Gharbia

- **SELDI-TOF MS:** surface-enhanced laser desorption/ionization time-of-flight mass spectrometry
- Protein Chip Array is used

- **Specific preparation of sample via lysis solution**
- **Matrix:** sinapinic acid

MSSA specific peaks	MRSA specific peaks
3 081	5 709
5 893	7 694
9 580	15 308
	18 896

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Antibiotic Resistance Determination By a Detection Of Specific Peaks In Mass Spectra

- **Specific resistance markers should be established for common resistant bacteria** (e.g., MRSA, VRE, Gram-negatives?, multidrug-resistant *Mycobacterium tuberculosis*?)
- Modification of sample preparation process
- **This methodology can be a useful tool for a quick resistance mechanism detection** (during bacterial identification?)

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Proteomic Approach In the Determination Of Antibiotic Resistance

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Proteomic Approach

- **Proteome:**

  the entire **PROTE**in complement expressed by a gen**OME**,
  or by a cell or tissue type

- Methodology:
  - Cell lysis – specific or non-specific
  - Electrophoretic separation (usually 2D – isoelectric focusing/SDS-PAGE)
  - Comparison of protein profiles
  - Identification of proteins by MALDI-TOF MS
    - peptide mass fingerprinting
    - amino acid sequencing

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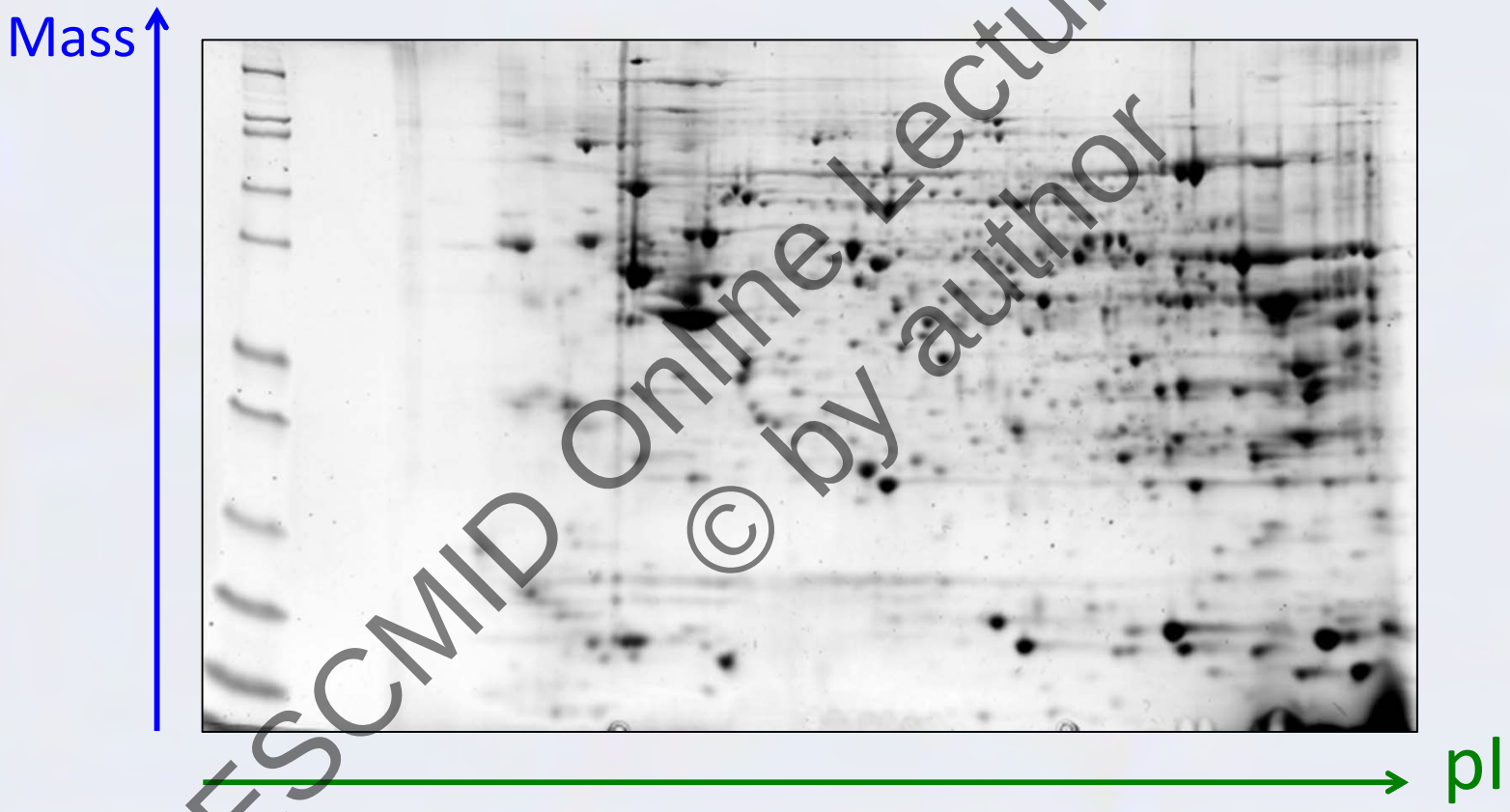
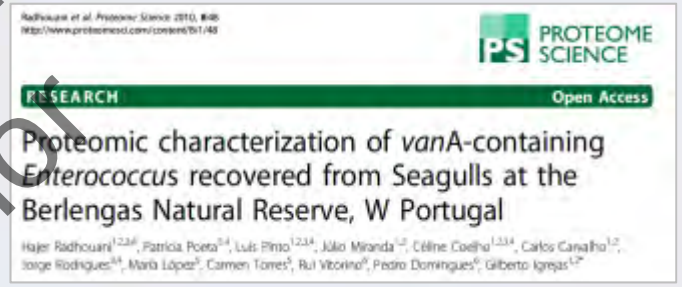
Proteomic Approach

Example of 2D gel electrophoresis:



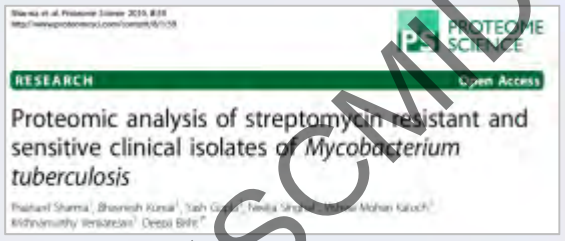
Proteomic Approach In Antibiotic Resistance

- vancomycin-resistant *Enterococcus* spp. isolates recovered from seagulls
- ampicillin-resistant *Escherichia coli*
- ampicillin-resistant *Fusobacterium nucleatum*
- protein identification in resistant *E. coli, Salmonella enterica, Mycobacterium tuberculosis* strains.


PROTEOME SCIENCE

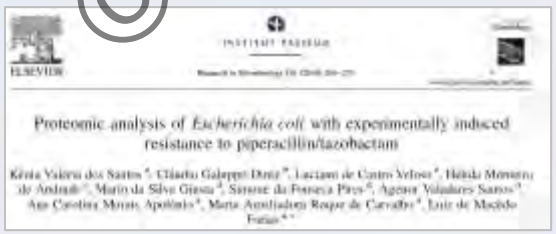
RESEARCH Open Access

Proteomic characterization of *van*A-containing *Enterococcus* recovered from Seagulls at the Berlengas Natural Reserve, W Portugal


PROTEOME SCIENCE

RESEARCH Open Access

Proteomic analysis of streptomycin resistant and sensitive clinical isolates of *Mycobacterium tuberculosis*


Proteomic analysis of *Escherichia coli* with experimentally induced resistance to piperacillin/tazobactam


ScienceDirect

PROTEOMICS

Genomic and proteomic evaluation of antibiotic resistance in *Salmonella* strains

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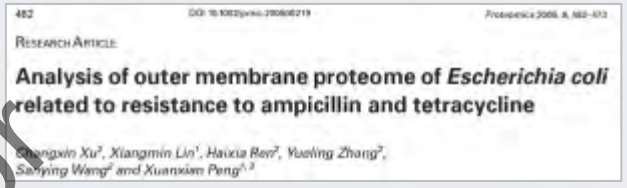
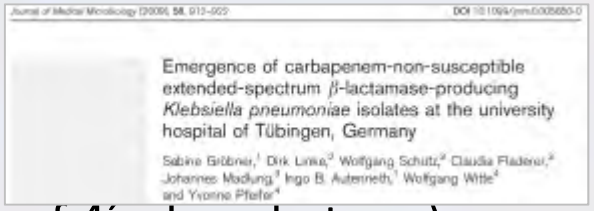
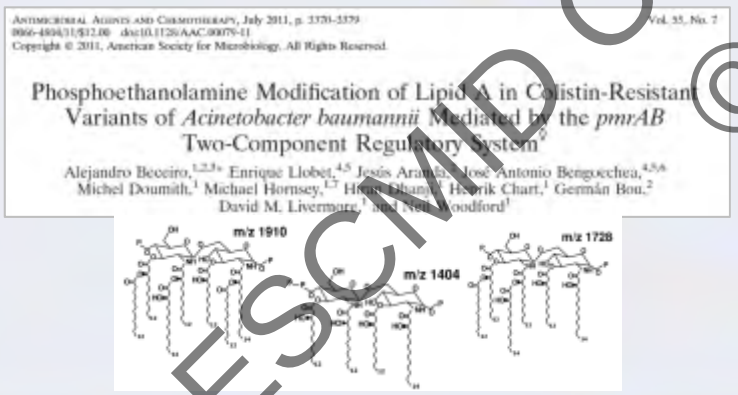
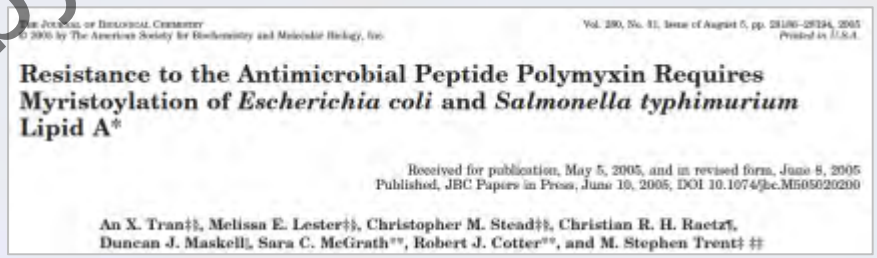
Proteomic Approach – Cell-Wall Components

- **Porin identification –** resistance to carbapenems
  - Proteomic identification of porins
- **Efflux identification**
  - Proteomic identification of efflux pumps
- **Lipopolysaccharide** – resistance to polymyxins
  - Detection of Lipid A modification (activity of 4´-phosphatase)









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Proteomic Approach In Antibiotic Resistance

- **Needs for a standardization of cultivation** (expression of resistance determinants)
- **Development of specific extraction methods** (e.g., for cell-wall components, lipopolysaccharide)
- **Labor intensive method**
- **More complex picture of resistant determinants**
- Should be available in reference centres (???)

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MALDI-TOF MS-based DNA Sequencing

(Minisequencing)

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Minisequencing By MALDI-TOF MS

- **SNP genotyping**

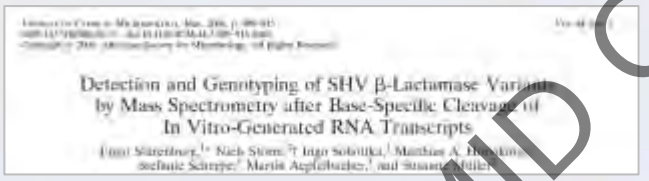
- Labor intensive
- Lenght limitation

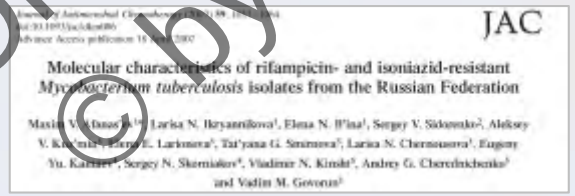

MALDI-TOF mass spectrometry-based SNP genotyping

537

Table 1. Mass differences resulting from single nucleotide exchanges.

Wild type	Mutant	Mass difference	Wild type	Mutant	Mass difference
A	C	+ 24 Da	C	A	+ 24 Da
A	G	- 16 Da	G	A	- 16 Da
A	T	+ 9 Da	T	A	+ 9 Da
C	G	+ 40 Da	G	C	- 40 Da
C	T	+ 15 Da	T	C	- 15 Da
G	T	- 25 Da	T	G	+ 25 Da


Detection and Genotyping of SHV β-Lactamase Variants by Mass Spectrometry after Base-Specific Cleavage of In Vitro-Generated RNA Transcripts


JAC

Molecular characteristics of rifampicin- and isoniazid-resistant *Mycobacterium tuberculosis* isolates from the Russian Federation


Journal of Microbiological Methods

A MALDI TOF MS-based minisequencing method for rapid detection of TEM-type extended-spectrum beta-lactamases in clinical strains of Enterobacteriaceae


ScienceDirect

Journal of Microbiological Methods

Mass-spectrometry based minisequencing method for the rapid detection of drug resistance in *Mycobacterium tuberculosis*

DNA preparation

DNA amplification

Purification

Allele-specific reaction

Purification

Data acquisition

Automated allele calling

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Future Perspectives

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Future Perspectives

- Quantification of the molecules
- Selective extraction


Analytica Chimica Acta

Nanoparticle-assisted MALDI-TOF MS combined with seed-layer surface preparation for quantification of small molecules

Yi-Chi Ho[a], Mei-Chun Tseng[b], Ying-Wei Lu[c], Chun-Cheng Lin[c], Yu-Ju Chen[b,*], Ming-Ren Fuh[a,**]

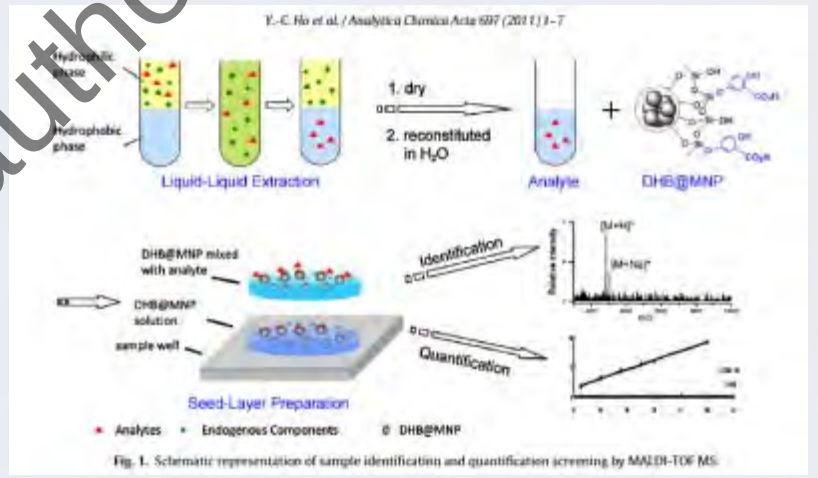
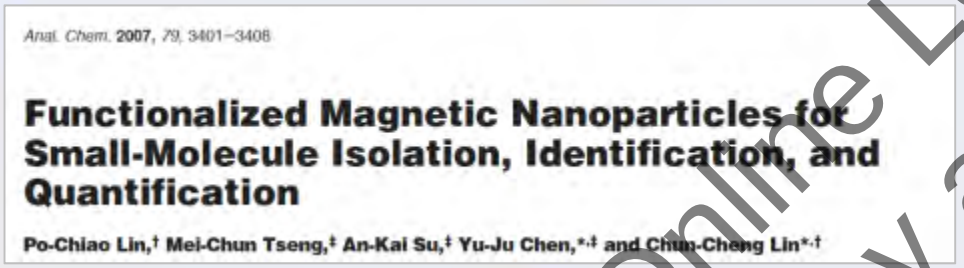
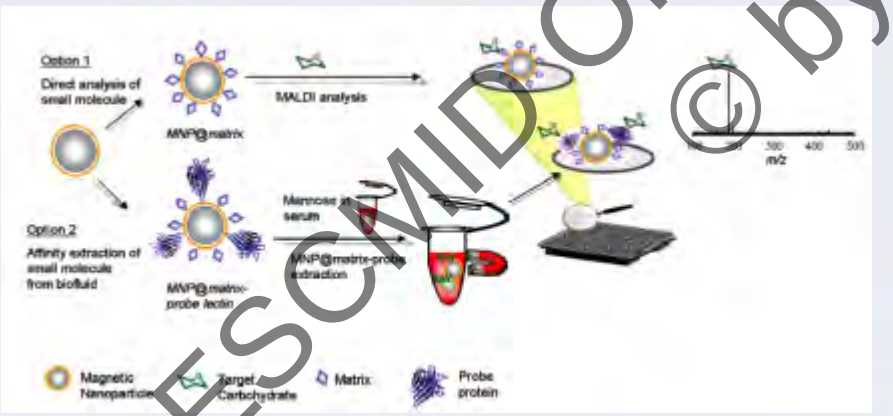


Fig. 1. Schematic representation of sample identification and quantification screening by MALDI-TOF MS.


Anal. Chem. **2007**, *79*, 3401–3408

Functionalized Magnetic Nanoparticles for Small-Molecule Isolation, Identification, and Quantification

Po-Chiao Lin,† Mei-Chun Tseng,‡ An-Kai Su,‡ Yu-Ju Chen,*,‡ and Chun-Cheng Lin*,†




Analytica Chimica Acta

Quantification in MALDI-TOF mass spectrometry of modified polymers

Zuzana Walterová, Jiří Horský*

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Future Perspectives

- **Detection of enzymes that degrade antibiotic molecules** through the direct detection of antibiotic modifications;
- **Detection of the resistance mechanism determinants** (e.g., Qnr proteins and modified PBPs) and **proteomic studies** on multi-resistant bacteria that allow the construction of a complete database of protein fingerprints of resistant isolates for detection of the main proteins responsible for resistance;
- **Analysis of the modification of target sites** (e.g., ribosomal methylation);
- **Quantification of antibiotics**. Developing a method for the determination of antibiotic concentrations could aid in the analysis of influx and efflux of an antibiotic.

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Conclusion

- **Very fast and cheap detection of antimicrobial resistance**
- **New applications are expected**
- **More complex view on determining the resistance mechanisms** (should be available in the national reference centres?)

Applying this technique to determine resistance mechanisms cannot replace standard susceptibility testing.

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Acknowledgements

MALDI-TOF MS team:

Tamara Bergerová, M.D.

Eva Chudáčková, M.D.

Radka Walková, M.D.

Vendula Študentová, BSc.

Thank you for your attention

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