MALDI-TOF MS: a new tool to rapidly assess antibiotic susceptibility

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Learning Objectives

After this presentation, you should...

• know the principles of MALDI-TOF MS and its application in identification of microorganisms
• be able to identify and to trace the development of MALDI-TOF MS for the $\beta$-lactamase activity testing of bacteria
• record and compare facts about different approaches using MALDI-TOF MS for comprehensive antibiotic resistance testing
• be able to identify the pros and cons of using MALDI-TOF MS for resistance testing in diagnostic laboratory and will retrace possible future perspectives
MALDI-TOF MS

MALDI Biotyper
Bruker Daltonics

Vitek MS
bioMérieux
MALDI-TOF: principle

Matrix Assisted Laser Desorption/Ionization,

Time Of Flight
MALDI TOF – Identification of bacteria and fungi

**E. coli**

<table>
<thead>
<tr>
<th>ribosomal Protein</th>
<th>m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>RL36</td>
<td>4364.33</td>
</tr>
<tr>
<td>RS32</td>
<td>5095.82</td>
</tr>
<tr>
<td>RL34</td>
<td>5380.39</td>
</tr>
<tr>
<td>RL33meth.</td>
<td>6255.39</td>
</tr>
<tr>
<td>RL32</td>
<td>6315.19</td>
</tr>
<tr>
<td>RL30</td>
<td>6410.60</td>
</tr>
<tr>
<td>RL35</td>
<td>7157.74</td>
</tr>
<tr>
<td>RL29</td>
<td>7273.45</td>
</tr>
<tr>
<td>RL31</td>
<td>7871.06</td>
</tr>
<tr>
<td>RS21</td>
<td>8368.76</td>
</tr>
</tbody>
</table>
MALDI-TOF MS bacteria identification

Workflow
Positive blood culture bottle

- Harvest 1 ml blood culture liquid in an Eppendorf tube
- Add Solution 1\textsuperscript{st} and mix
  - 30 sec
- Centrifuge (1 min., 13,000 rpm)
  - 1 min
discard supernatant
- Add Solution 2 and mix
  - 1.5 min
- Centrifuge (1 min., 13,000 rpm)
  - 1 min
discard supernatant
- Suspend pellet in 300 µl water

Identification
## Antibiotic resistance testing (AST)

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Time to result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disc Diff.</td>
<td>12 – 24 h</td>
</tr>
<tr>
<td>Walkaway</td>
<td>12 – 48 h</td>
</tr>
<tr>
<td>Vitek 2</td>
<td>7 – 18 h</td>
</tr>
<tr>
<td>Phoenix</td>
<td>8 – 18 h</td>
</tr>
</tbody>
</table>
MALDI-TOF MS

1. Direct detection of resistance factors
MRSA detection by MALDI-TOF MS?
The discriminatory power of MALDI Tof mass spectrometry and methicillin-resistant Staphylococcus aureus and methicillin-sensitive Staphylococcus aureus

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

identical MALDI Tof MS-derived peak profiles in a pair of biogenic SCCmeC-harboring and SCCmeC-lacking strains of Staphylococcus aureus

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

Manuel Wolters\textsuperscript{a}, Holger Rohde\textsuperscript{a,}\textsuperscript,*\textsuperscript{,} Thomas Maier\textsuperscript{b}, Cristina Belmar-Campos\textsuperscript{a}, Gefion Franke\textsuperscript{a}, Stefanie Scherpe\textsuperscript{a}, Martin Aepfelbacher\textsuperscript{a}, Martin Christner\textsuperscript{a}
MALDI-TOF for detection of antibiotic resistance

→ $\beta$-lactamases (ESBL)

→ carbapenememases
Problem
<table>
<thead>
<tr>
<th>β-lactamase - subtypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHV-1</td>
</tr>
<tr>
<td>SHV-2</td>
</tr>
<tr>
<td>SHV-3</td>
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<tr>
<td>SHV-178</td>
</tr>
<tr>
<td>TEM-1</td>
</tr>
<tr>
<td>TEM-2</td>
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<tr>
<td>TEM-3</td>
</tr>
<tr>
<td>CTX-M-1</td>
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<td>CTX-M-2</td>
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<tr>
<td>CTX-M-3</td>
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<tr>
<td>OXA-1</td>
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<tr>
<td>OXA-2</td>
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<td>OXA-3</td>
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<tr>
<td>TEM-213</td>
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<tr>
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<tr>
<td>OXA-161</td>
</tr>
<tr>
<td>IMP-1</td>
</tr>
<tr>
<td>IMP-2</td>
</tr>
<tr>
<td>IMP-3</td>
</tr>
</tbody>
</table>

> 700 β-lactamase - subtypes
Conclusion 1:

Direct detection of resistance factors

• Detection of clonal groups
• No reliable resistance identification
  → β-lactamases, PBP2a, Van A – Van B
MALDI-TOF MS

1. Direct detection of resistance factors (e.g. PBP2a)

2. β-lactamase activity test
β-lactamase activity
Ampicillin + ESBL- 

Ampicillin + β-Lact.-neg. 

Sparbier et al., 2012
Procedure

• 1 colony of fresh o/n culture
• 10 µl antibiotic solution (e.g. 0.5 µg/ml CTX)
• 1 - 2 h incubation, 37°C
• Spin down bacteria
• Supernatant on MALDI-target plate
• Mass range 100 – 1000 Da
• Calibration with suitable molecules
Cefotaxime (CTX)

CTX
+ ESBL-neg. E. coli

CTX
+ ESBL-pos. E. coli

CTX / CLAV
+ ESBL-pos. E. coli
\[ \log \left( \frac{\sum \text{peak-intensity}_{\text{hydrolysed}}}{\sum \text{peak-intensity}_{\text{non-hydrolysed}}} \right) \]
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

β-lactamase-activity
No cleavage
spontaneous hydrolysis
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

Identification

β-lactamase activity
E. coli directly from positive blood cultures $\rightarrow$ ampicillin

Jung et al., 2014
Conclusion 2:

MALDI-TOF $\beta$-lactamase activity test

- Rapid test 1.5 – 3 h
- Automated analysis
- Directly from positive blood cultures

- Restricted to certain antibiotic resistances
  - $\beta$-lactamases, e.g. ESBL, carbapenemases
MALDI-TOF MS

1. Direct detection of resistance factors (e.g. PBP2a)

2. β-lactamase activity test

3. Antibiotic susceptibility test - phenotypic assays
   - MBT-RESIST
Phenotypic Susceptibility Testing (MBT-RESIST)

$^{13}\text{C}_6\ ^{15}\text{N}_2$-L-Lysin

$\Rightarrow$ For all growing bacteria applicable
Susceptibility testing using stable isotopes

- normal Lys
- heavy Lys + antibiotic
- heavy Lys

Control 1
Susceptible
Resistant
Control 2
S. aureus  

mass spectra – gel view

MSSA
OXA - susceptible strain

MRSA
OXA - resistant strain
MALDI Biotyper-Based Rapid Resistance Detection by Stable-Isotope Labeling

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

Eur J Clin Microbiol Infect Dis

14 December 2013

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


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

Jung et al., EJCMID 2013
MS-RESIST/ *P. aeruginosa* (Tobramycin-resistant)

Jung et al., EJCMID 2013
Pseudomonas aeruginosa

**Ciprofloxacin**

EUCAST breakpoints: $s \leq 0.5$; $r > 1$

**Meropenem**

EUCAST breakpoints: $s \leq 2$; $r > 8$

**Tobramycin**

EUCAST breakpoints: $s \leq 4$; $r > 4$

Jung et al., 2014
MALDI-TOF MS

1. Direct detection of resistance factors (e.g. PBP2a)

2. β-lactamase activity test

3. Antibiotic susceptibility test - phenotypic assays
   - MBT-RESIST
   - MBT-ASTRA
3. Antibiotic susceptibility testing by phenotypic assays

Phenotypic assay without isotopes?
MALDI-TOF MS as a quantitative growth monitor MBT-ASTRA

- BHI McF 0.5
- Incubation 37°C Species dependent time
- Antibiotic
- Cell lysis
- Lysis reagent with internal standard
- Target preparation
- Acquisition of MS profile spectra

MALDI-TOF MS as a quantitative growth monitor MBT-ASTRA
spectra view

**Klebsiella pneumoniae**

- Standard $[\text{M+ H}]^{2+}$
- Standard $[\text{M+ H}]^{+}$

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**BHI + Meropenem 8 µg/ml**

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**susceptible**

**BHI only**

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**resistant**

Meropenem

Lange et al., J Clin Microbiol. 2014
Conclusion (1)

Direct detection of resistance factors

– Detection of clonal groups
  • May help to identify distinct clonally distributed resistance factors

– False positive and negative results possible!

– (yet) no direct detection of β-lactamases, PBP2a, Van A/B, …
Conclusion (2)

**β-lactamase activity test**

- Rapid test 1.5 – 3 h
- Automated analysis
- Directly from positive blood cultures
- Restricted to certain resistances
  - β-lactamases

- All β-lactamases detectable?
Conclusion (3)

Phenotypic resistance test (**MBT-RESIST** / **MBT-ASTRA**)

- Rapid tests 2 – 3 h
- Automated analysis available

- Stable Isotopes: A rather complex workflow
  → kits and cards are needed
- All bug-drug combinations analyzable?
MALDI-TOF MS for antibiotic resistance testing

**Pro**
- Reduction of time to result: 12 h → 2.5 h
- Phenotypic assay – irrespective of underlying molecular mechanism
- High-throughput feasible
- Low costs of consumables

**Cons**
- Initial culture necessary → plus 12 – 24 h
- High costs of MALDI-hardware
- (Yet) no determination of MIC values
- (Yet) no test kits available → hands on time is high
Max von Pettenkofer-Institut
- Jette Jung
- Theresa Eberl
- Christina Popp
- Julia Walker
- Christina Hamacher
- Lukas Schmidt
- Birgit Gross
- Andreas Wieser