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 β-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
MALDI-TOF MS bacteria identification
Workflow
Identification

Antibiotic resistance testing (AST)

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

1. Direct detection of resistance factors
MRSA detection by MALDI-TOF MS?

MALDI TOF „detection“ of MRSA

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

MALDI-TOF for detection of antibiotic resistance

\rightarrow \beta\text{-lactamases (ESBL)}

\rightarrow \text{carbapenemases}
> 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 + \( \beta \)-Lact.-neg. \( E. \) coli

Ampicillin + ESBL-\( E. \) coli

Sparber et al., 2012

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**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)

\[ \log \left( \frac{\sum \text{peak-intensity}_{\text{hydrolysed}}}{\sum \text{peak-intensity}_{\text{non-hydrolysed}}} \right) \]

0.5 mg/ml CAZ in 10 mM NH₄hydrogen carbonate
2 h incubation
15 µl sup plus 5 µl 0.5 ng/µl reserpine
1.5 µl spotted

\[ \beta \]-lactamase-activity
No cleavage spontaneous hydrolysis
E. coli directly from positive blood cultures → ampicillin

**Conclusion 2:**

MALDI-TOF β-lactamase activity test

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

For all growing bacteria applicable

Susceptibility testing using stable isotopes

normal Lys → Control 1
heavy Lys + antibiotic → Susceptible
heavy Lys → Resistant
                  → Control 2
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

spectra view

Klebsiella pneumoniae

sustiable

BHI + Meropenem 8 µg/ml

BHI only

resistant

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

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**Max von Pettenkofer-Institut**
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**ForBLMed**

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**FöFoLe**