Genotypic methods for detection of resistance mechanisms

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Necessity of molecular detection

Phenotypic detection is not always sufficient:

• phenotypic detection is slow
• phenotypes do not always correlate to one single resistance mechanism
• no hints on mobility of resistance mechanisms
• no epidemiological data
Necessity of molecular detection

- TEM?
- SHV?
- CTX–M?
- PER? VEB? GES–1?...
Epidemiology

The Trade Routes of the CTX-M Enzymes

- CTX-M-1
- CTX-M-2
- CTX-M-3
- CTX-M-9
- CTX-M-14
- CTX-M-15
- Others

★ = Faecal isolates

a = Lebanon
b = Israel
c = Kuwait

Hawkey & Jones, JAC 2009
Therapy guidance

positive BC

MALDI
*S. aureus*

Yes

Genotypic detection

MRSA

Cover MRSA

Not MRSA

Do not use MRSA agent

Unneeded coverage of MRSA

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.1%</td>
<td>29.2%</td>
</tr>
</tbody>
</table>

Clerc *et al.*, Clin Microbiol Infect. 2014
Relevance for isolation

Molecular detection of MRSA
- sensitivity 85%
- specificity 97–99%
- prevalence 10%

1000 patients
- 900 MRSA negative
  - 18 positive
  - 882 negative
- 100 MRSA positive
  - 85 positive
  - 15 negative

PPV 82.5%
NPV 98.3%
Relevance for isolation

Molecular detection of MRSA

- sensitivity 85%
- specificity 97–99%
- prevalence 2%

1000 patients

980 MRSA negative

20 MRSA positive

+ 960 17
- 3

PPV 45.9% NPV 99.7%
Utility of molecular detection

- Epidemiological data
- Identification of carriers (high PPV needed)
- Identification of resistance mechanisms
- Species confirmation/identification
- Therapy guidance
  - MRSA
  - ESBLs
  - Carbapenemases
Molecular detection can be difficult…

- you only find what you know
- MRSA: *mecA, mecC*
Molecular detection can be difficult...

**β-lactamases**

<table>
<thead>
<tr>
<th>Class</th>
<th>Enzymes</th>
<th>Most common bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>KPC, CTX-M, TEM, SHV,...</td>
<td>Enterobacteriaceae</td>
</tr>
<tr>
<td>B</td>
<td>VIM, NDM, IMP,...</td>
<td>P. aeruginosa, Enterobacteriaceae, (Acinetobacter spp.)</td>
</tr>
<tr>
<td>C</td>
<td>CMY-2, DHA-1,...</td>
<td>Enterobacteriaceae</td>
</tr>
</tbody>
</table>
Molecular detection can be difficult...

### Other classes of antibiotics

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resistance genes</th>
<th>Most common bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoroquinolones</td>
<td><code>qnrA, B, C, S</code>&lt;br&gt;<code>qepA</code>&lt;br&gt;<code>aac(6')-Ib-cr</code></td>
<td><code>Enterobacteriaceae</code></td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td><code>armA</code>&lt;br&gt;<code>rmtH-A</code>&lt;br&gt;<code>aac, ant, aph</code></td>
<td><code>Enterobacteriaceae</code>&lt;br&gt;<code>Acinetobacter spp.</code></td>
</tr>
<tr>
<td>Polymyxins</td>
<td><code>mcr-1, mcr-2</code></td>
<td><code>Enterobacteriaceae</code></td>
</tr>
</tbody>
</table>
Molecular detection can be difficult...
Molecular detection can be difficult...  

- you only find what you know  
  → unknown mechanisms will not be detected  
- necessity for local adaption  
- variants of resistance genes may not be detected
Molecular detection can be difficult...
Molecular principles

- PCR
- qPCR
- Hybridization
- LAMP/isothermal amplification
- Whole genome sequencing (WGS)
PCR

Pro:
• cheap
• high sensitivity / high specificity
• flexible
• detects almost all variants
• sequencing

Con:
• slow (endpoint analysis)
• hands-on-time, TAT 3–6 h
• establishment & evaluation
• Possibility of cross-contaminations
qPCR

Pro:
• fast (real-time detection)
• TAT: 2–3 h
• high sensitivity / high specificity
• (flexible)
• hands-on-time (depending on system/assay)
• directly from clinical samples (depending on system/assay)

Con:
• variants of resistance genes might be undetected
• more expensive than classical PCR
• (flexible)
qPCR

Amplification Plot

QXA-48
KPC
IC
VIM/NDM

Check-Points Check-Direct CPE
qPCR

• Cepheid GeneXpert®
• BD MAX™ MRSA XT / CRE
• BioMérieux EasyQ KPC
• Check–Points Check–Direct CPE/ESBL
• Roche cobas®/lightcycler® LightMix®
• and many more...
Hybridization
Hybridization

Pro:
• lots of target genes
• clinical samples

Con:
• hands-on-time (~2 h)
• TAT 4–8 h
• Some need culturing
• Problems with variants
• Amplex hyplex®
• Check-Points Check-MDR
• Alere Arraymate®
### PCR-based methods

<table>
<thead>
<tr>
<th>Study</th>
<th>Nr. of isolates (CPE)</th>
<th>Targets</th>
<th>Method</th>
<th>Sens.</th>
<th>Spec.</th>
<th>Time to result (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swayne (2011 &amp; 2013)</td>
<td>59 (41) &amp; 965 (343)</td>
<td>KPC, GES, IMI, SME, OXA-48, IMP, VIM, NDM, SPM, SIM, GIM</td>
<td>RT TaqMan PCR</td>
<td>100</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>Van der Zee (2014)</td>
<td>226</td>
<td>OXA-48, VIM, IMP, NDM, KPC</td>
<td>RT TaqMan PCR</td>
<td>100</td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>Kaase (2012)</td>
<td>132 (94)</td>
<td>KPC, VIM, NDM, IMP, OXA-48</td>
<td>PCR reverse hybridization ELISA</td>
<td>97</td>
<td>99</td>
<td>3–4</td>
</tr>
<tr>
<td>Cuzon (2012)</td>
<td>187 (89)</td>
<td>ESBLs, AmpCs, KPC, VIM, NDM, IMP, OXA-48</td>
<td>Multiplex Ligation PCR microarray</td>
<td>98</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>Monteiro (2013)</td>
<td>58 (30)</td>
<td>KPC, GES, VIM, NDM, IMP, OXA-48</td>
<td>RT PCR melting curves</td>
<td>100</td>
<td>100</td>
<td>3</td>
</tr>
</tbody>
</table>

Slide taken & modified from Pierre Bogaerts (UCL Namur, Belgium)
LAMP

Loop-mediated isothermal amplification
LAMP

Amplex eazyplex® SuperBug CRE
Amplex eazyplex®

- can be used with clinical samples
- assays for CRE/MRSA/VRE/C. diff/mcr-1
- Kaase *et al.*, JCM 2012:
  - 100% sensitivity for $\text{bla}_{KPC}$, $\text{bla}_{VIM}$, $\text{bla}_{NDM}$, $\text{bla}_{OXA-48}$
  - 99% specificity for $\text{bla}_{OXA-48}$, 100% for others
- IMP variants were not detected
Whole genome sequencing

Pro:
• lots of data (80–100 % of genome)
• complete resistome
• additional information (cgMLST, epidemiology)
Whole genome sequencing

Databases for analysis:
- The comprehensive antibiotic resistance database (CARD) http://arpcard.mcmaster.ca
- Antibiotic resistance gene database (ARDB) http://ardb.cbcb.umd.edu
Whole genome sequencing

Ridom SeqSphere+ Software
Whole genome sequencing

Pro:
• lots of data
• complete resistome
• additional information (cgMLST, epidemiology)

Con:
• lots of data
• slow (days to weeks until results)
• (expensive)
• again: you only find what you know
  → mcr-1
Conclusions

- molecular detection can guide therapy and identify carriers
- high PPV needed
- required for detailed epidemiology
- detects only known genes
- many techniques & systems available
- adaption for purpose required
- almost all are (partly) PCR-based
- WGS is the most comprehensive but slowest/most expensive method

→ Phenotypic AND genotypic detection is necessary