Clinical Relevance of Molecular Detection of Carbapenemases

Focus on carbapenemase-producing Enterobacteriaceae (CPE)

Pierre Bogaerts Ir PhD
Belgian NRC for AR in Enterobacteriaceae and non-fermenters
Clinical Relevance of Molecular Detection of Carbapenemases

- Why is it time for action?
- Phenotypic detection of CPE
  - Focus on OXA-48
- Speeding up the detection
- Added value of a rapid detection
- Conclusions
Evolving resistance in *Enterobacteriaceae*

- **Wild-type**
- **Penicillinases (TEM-1, SHV-1)**
- **ESBLs (CTX-M >> SHV, TEM)**
- **Carbapenemases**

Year:
- 1940
- 1970
- 1990
- 2000

Carbapenemases detection April 2014
4. DEVELOPING NEW ANTIBIOTICS AND DIAGNOSTIC TESTS

Because antibiotic resistance occurs as part of a natural evolution process, it can be significantly slowed but not stopped. Therefore, new antibiotics will always be needed to keep up with resistant bacteria as well as new diagnostic tests to track the development of resistance.

Tomorrow’s Antibiotics: The Drug Pipeline

The number of new antibiotics developed and approved has steadily decreased in the past three decades, leaving fewer options to treat resistant bacteria.

FOUR CORE ACTIONS
PREVENTING INFECTIONS, PREVENTING SPREAD.
TRACKING RESISTANCE PATTERNS.
IMPROVING USE OF ANTIBIOTICS.
DEVELOPING NEW ANTIBIOTICS AND DIAGNOSTIC TESTS.
CPE in the top three agents considered as Urgent Public Health Threats

CDC, April 23rd, 2013

MICROORGANISMS WITH A THREAT LEVEL OF URGENT

- *Clostridium difficile*
- Carbapenem-resistant *Enterobacteriaceae*
- Drug-resistant *Neisseria gonorrhoeae*

Nightmare bacteria

Carbapenemases detection April 2014
Carbapenemases: the triple difficulty (DDD)

- Difficulty of detection in the clinic and in the laboratory
- Difficulty of treatment (owing to their multidrug-resistant character, with few drugs remaining active)
- Difficulty to limit transmission and spread and to control outbreak (local, regional, national, pandemic)
# CARBAPENEM RESISTANCE: MECHANISMS

<table>
<thead>
<tr>
<th>Genus</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacteriaceae</td>
<td>Cephalosporinase/ESBL + porin loss</td>
</tr>
<tr>
<td></td>
<td><strong>Carbapenemase</strong></td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>Porin loss</td>
</tr>
<tr>
<td></td>
<td>Up-regulated efflux</td>
</tr>
<tr>
<td></td>
<td><strong>Carbapenemase</strong></td>
</tr>
<tr>
<td>Acinetobacter spp.</td>
<td>Cephalosporinase + porin loss</td>
</tr>
<tr>
<td></td>
<td><strong>Carbapenemase</strong></td>
</tr>
</tbody>
</table>
# Carbapenemases

<table>
<thead>
<tr>
<th>Classification</th>
<th>Enzyme (variant)</th>
<th>Most Common Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Class A</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Active Serine70)</td>
<td>SME (1-5)</td>
<td><em>Enterobacteriaceae</em></td>
</tr>
<tr>
<td></td>
<td>IMI (1-5)</td>
<td>(reports in <em>non fermenters</em>)</td>
</tr>
<tr>
<td></td>
<td>NMC-A</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>KPC</strong> (2-18)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GES (2-24)</td>
<td></td>
</tr>
<tr>
<td><strong>Class B</strong></td>
<td>Chromosomal</td>
<td></td>
</tr>
<tr>
<td>(metallo-β-lactamase)</td>
<td><strong>IMP</strong> (1-48),</td>
<td><em>Bacteroides, Aeromonas, Steno, ...</em></td>
</tr>
<tr>
<td></td>
<td><strong>VIM</strong> (1-41),</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GIM, SPM, SIM, ...</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>NDM</strong> (1-11)</td>
<td></td>
</tr>
<tr>
<td><strong>Class D</strong></td>
<td><strong>OXA</strong> (-23,40,58)</td>
<td></td>
</tr>
<tr>
<td>(Active Serine70)</td>
<td><strong>OXA-48</strong> (162, (163), 181, 204, 232, 244, 245, 370)</td>
<td><em>Acinetobacter spp.</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Enterobacteriaceae</em></td>
</tr>
</tbody>
</table>
Figure 3 Occurrence of carbapenemase-producing Enterobacteriaceae in 38 European countries based on self-assessment by the national experts, March 2013

Epidemiological stages
- No cases reported
- Sporadic occurrence
- Single hospital outbreak
- Sporadic hospital outbreaks
- Regional spread
- Inter-regional spread
- Endemic situation
- Data not available
- Not participating
- Uncertain

Cyprus
Luxembourg
Malta

Grundmann et al. July 2013
Detection of OXA-51 Carbapenemase Gene in *Klebsiella pneumoniae*: A Case Report and a New Dimension on Carbapenemase Resistance

Budak S¹, Aktaş Z², Oncul O¹, Acar A¹, Ozyurt M³, Turhan V¹ and Gorenek L¹

¹Gebze Technical University, Fatsa, Sariyer, Türkiye, 21480
²Istanbul University, Faculty of Veterinary Medicine, Department of Microbiology, Istanbul, Türkiye, 34320
³Acıbadem Haydarpasa Training Hospital, Uskudar-Istanbul 34668, Turkey

OXA-48 91.5 %, 4.3 % NDM-1; 1%

Both

2014

10
Evolution of the distribution of resistance mechanisms of carbapenemase-producing Enterobacteriaceae (CPE) isolates, National Reference Centre, Belgium, January 2007 – December 2013 (n=657)

Number of CPE isolates

Year

- OXA-48
- IMI-2
- GES-5
- KPC-2
- NDM-1
- VIM-2
- VIM-1

Carbapenemases detection April 2014
Species and carbapenemase encoding enzyme distribution in Enterobacteriaceae isolates referred to the NRC in 2013 (n=664)

**K. pneumoniae** isolates (n=312)
- **Negative**: 38,5%
- **OXA-48**: 0,6%
- **KPC**: 14,7%
- **VIM**: 44,9%
- **NDM**: 19,7%
- **IMI**: 1,3%
- **GES**: 1,3%

**E. coli** isolates (n=122)
- **Negative**: 61,6%
- **OXA-48**: 1,8%
- **KPC**: 32,1%
- **VIM**: 4,5%
- **NDM**: 9,5%
- **IMI**: 1,1%
- **GES**: 6,7%

**E. cloacae** isolates (n=89)
- **Negative**: 67,4%
- **OXA-48**: 14,6%
- **KPC**: 10,1%
- **VIM**: 1,1%
- **NDM**: 6,7%
- **IMI**: 6,7%
- **GES**: 1,1%

**K. oxytoca** isolates (n=42)
- **Negative**: 28,6%
- **OXA-48**: 2,4%
- **KPC**: 54,8%
- **VIM**: 4,8%
- **NDM**: 9,5%
- **IMI**: 4,8%
- **GES**: 2,4%

**C. freundii** isolates (n=19)
- **Negative**: 42,1%
- **OXA-48**: 15,8%
- **KPC**: 21,1%
- **VIM**: 15,8%
- **NDM**: 5,3%
- **IMI**: 5,3%
- **GES**: 5,3%

**Enterobacter spp. (other than E. cloacae)** isolates (n=60)
- **Negative**: 93%
- **OXA-48**: 5%
- **KPC**: 2%
- **VIM**: 93%
- **NDM**: 93%
- **IMI**: 93%
- **GES**: 93%

Carbapenemases detection April 2014
How to detect CPE?

From antibiogram and confirmatory testing

Focus on OXA-48
OXA-48 carbapenemase (Class D)

*Klebsiella pneumoniae*

- ~70 Kb plasmid-mediated resistance
- Resistance to penicillins
  - (TEMOCILLIN INCL. *)
- No effect of Beta-lactamases inhibitors
- Variable level of resistance to carbapenems (meropenem MICs ≤0.5- >128)
- Susceptible to 3-4 gen ceph when not associated with other mechanisms
- Difficult to detect (low-level R, algorithms of expert systems not adapted)

*Glupczynski et al. IJAA, 2012*
### When should screening of CPE be carried out?

EUCAST Clinical breakpoints (v 3.1) (2013-02-11)

<table>
<thead>
<tr>
<th>Carbapenem</th>
<th>MIC (mg/L)</th>
<th>Disk diffusion zone diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S/I</td>
<td>Screening cut-off</td>
</tr>
<tr>
<td>Meropenem (10 µg)¹</td>
<td>≤2</td>
<td>&gt;0.125</td>
</tr>
<tr>
<td>Imipenem (10 µg)³</td>
<td>≤2</td>
<td>&gt;1</td>
</tr>
<tr>
<td>Ertapenem (10 µg)⁴</td>
<td>≤0.5</td>
<td>&gt;0.125</td>
</tr>
</tbody>
</table>

¹ EUCAST Clinical breakpoints (v 3.1) (2013-02-11)
Meropenem 10 µg paper disc zone diameter distribution of Enterobacteriaceae isolates referred to National Reference Centre, Belgium, January 2013 – December 2013 (n=664)
Temocillin and piperacillin/tazobactam disk for the detection of carbapenemase-producing Enterobacteriaceae

(n=1354)

TD Huang et al., JAC 2014

Scattergram of piperacillin/tazobactam 100/10-µg and of temocillin 30-µg disk inhibition zones (DID) according to carbapenemase enzyme for Enterobacteriaceae isolates referred to the NRLs in 2012 (n=1354)

- Pip/tazo (100/10 µg) and (Temo 30 µg) are better markers for the detection of OXA-48
- PPV of 95% in K. pneumoniae

Temo <12 mm AND Pip/tazo <16 mm
## Phenotypic confirmation disc method

EUCAST guidelines for detection of resistance mechanisms (v 1.0; 2013-12-11)

<table>
<thead>
<tr>
<th>B-lactamase</th>
<th>Synergy observed as increase in meropenem zone diameter (mm) with 10µg disk</th>
<th>Temocillin MIC &gt; 32 mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DPA/EDTA</td>
<td>APBA/PBA</td>
</tr>
<tr>
<td>MBL</td>
<td>≥5</td>
<td>-</td>
</tr>
<tr>
<td>KPC</td>
<td>-</td>
<td>≥4</td>
</tr>
<tr>
<td>MBL+KPC&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Variable</td>
<td>Variable</td>
</tr>
<tr>
<td>OXA-48-like&lt;sup&gt;3&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AmpC + porin loss</td>
<td>-</td>
<td>≥4</td>
</tr>
<tr>
<td>ESBL + porin loss</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>1</sup> Temocillin is recommended only in cases where no synergy is detected, in order to differentiate between ESBL + porin loss and OXA-48-like enzymes.

<sup>2</sup> Commercial tablets containing double inhibitors (DPA or EDTA plus APBA or PBA). This phenotype is rare outside of Greece and confers high-level resistance to carbapenems.
Comparison of laboratory methods for detection of carbapenemases in Enterobacteriaceae

Doyle et al., JCM 2012; 50: 3877-3880

142 genotypically characterized CPE (Worldwide source of isolates, SMART surveillance program)
28 non CPE isolates (AmpC/ESBL + impermeability)
All included isolates: I or R to carbapenems (MICs mero, Imi > 1 µg/ml; MIC Ert > 0.5 µg/ml)

<table>
<thead>
<tr>
<th>Enterobacteriaceae</th>
<th>Mastdiscs inhibitor Combination (%)</th>
<th>Rosco Diagnostica Sensitabs (%)</th>
<th>MBL Etest (%)</th>
<th>Multiplex PCR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (n=114)</td>
<td>78</td>
<td>80</td>
<td>58</td>
<td>55</td>
</tr>
<tr>
<td>KPC (49)</td>
<td>98</td>
<td>100</td>
<td>0</td>
<td>98</td>
</tr>
<tr>
<td>NDM-1 (27)</td>
<td>100</td>
<td>100</td>
<td>74</td>
<td>11</td>
</tr>
<tr>
<td>VIM (19)</td>
<td>53</td>
<td>63</td>
<td>53</td>
<td>11</td>
</tr>
<tr>
<td>IMP (5)</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>OXA-48 (14)</td>
<td>14</td>
<td>14</td>
<td>0</td>
<td>93</td>
</tr>
<tr>
<td>Specificity (n=28)</td>
<td>93</td>
<td>93</td>
<td>100</td>
<td>93</td>
</tr>
</tbody>
</table>

Confirmatory Disc Test and MBL Etest did not intend to detect OXA-48

Mast & Rosco combination disc tests: lack of sensitivity for detection of VIM/IMP

Carbapenemases detection April 2014
Speeding up the detection at the laboratory
How to speed up the detection process

On clinical samples

On colonies/antibiogram

Molecular or rapid testing

Modified from Didelot X, Nat Rev Genet, 2012
Rapid testing on colonies/antibiogram
CARBA NP test

- **Principle of the test**
  - *In vitro* hydrolysis of imipenem
  - Change of pH with red phenol indicator (red to yellow: visual reading)

- **Advantages:**
  - Fast (TAT: 15 to 120 min +30 min lysis)
  - Accurate (sensitive, specific)
  - Easy to perform and to interpret
  - Inexpensive
  - No special equipment/material
  - No special expertise

- **Drawbacks:**
  - Need for multicenter studies (in different areas)
  - By eye
  - Inter-laboratories technical variations (reproducibility?) (extract concentration, lysis, some faint reactions, robustness, preparation of solutions)

---

*Nordmann et al., EID 2012; 18(9): 1503
Dortet et al., AAC 2012: 56: 6437
Dortet et al., JCM 2012; 50: 3773*
Evaluation of Carba NP test for rapid detection of carbapenemases

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Organisms</th>
<th>N° CP &amp; non-CP</th>
<th>Carba types</th>
<th>Sens (%)</th>
<th>Spec (%)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nordmann (2012)</td>
<td>ETB</td>
<td>162 / 46</td>
<td>All</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Dortet (2012)</td>
<td>ETB/Pyo</td>
<td>108 / 90</td>
<td>All</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Vasoo (2013)</td>
<td>ETB/Pyo</td>
<td>131 / 140</td>
<td>Mostly KPC</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Tijet (2013)</td>
<td>ETB/Pyo</td>
<td>145 / 99</td>
<td>All</td>
<td>72.5-80</td>
<td>100</td>
<td>OXA-48, GES-5 missed</td>
</tr>
<tr>
<td>Dortet (2013)</td>
<td>ETB</td>
<td>193</td>
<td>All</td>
<td>97.9</td>
<td>100</td>
<td>Spiked blood culture (10% OXA-48 missed)</td>
</tr>
</tbody>
</table>

False negative: - incomplete lysis (*mucoid colonies*)
- weak carbapenemase expression
  (more concentrated extract needed)
Detection of carbapenemases by MALDI-TOF

- 110 CPE isolates characterized by PCR-sequencing
- 35 CRE not producing carbapenemases

**Sensitivity: 100%; Specificity: 100% (...)**

- Lysis buffer mixed with overnight culture extract
- 2h incubation at 35°C with 0.1 mM meropenem
- Mass spectrometer (Bruker Daltonics)
- Software FlexAnalysis 3.3
- TAT: 3-4 h (20 min?)
- Possible on positive hemoculture

Detection of meropenem (salts) degradation products

**Hands-on time for preparation, lecture and interpretation of results**

More studies needed
Rapid CARB Screen Kit® (Rosco)

TD Huang et al. RICAI 2013
TD Huang et al. ECCMID 2014

Procedure:

✓ Similar to Carba NP (imipenem hydrolysis)
  ▪ Bacterial lysis with B-PER (commercial)
  ▪ 2 tubes (one test disk and one neg control disk)
  ▪ Reading up to 2 hours at 37°C
  ▪ Interpretation (score 0=neg; score 1,2,3=pos)

✓ Advantage: rapid, easy to perform

Performance on EB and PA:

✓ Excellent sensitivity
✓ Poor specificity
✓ Uninterpretable results ++
Molecular testing on colonies
End-Point multiplex PCR Mont-Godinne


6-Plex, Qiagen multiplex mix, QIAxcel electrophoresis
## PCR based method as confirmatory test from isolated bacterial colonies

**Gold standard: PCR-sequencing (detects all variants, costly, labor-intensive)**

<table>
<thead>
<tr>
<th>Author (yr)</th>
<th>N° of isolates (CPE)</th>
<th>Targeted bla genes</th>
<th>Method</th>
<th>Sens.</th>
<th>Spec.</th>
<th>Time to result (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swayne (2011)</td>
<td>59 (41)</td>
<td>KPC, GES, IMI, SME, OXA-48</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>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>
<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>
</tbody>
</table>

**ETC ....**
Check-Points: Ligation-PCR-Array

Multicenter Evaluation of a New DNA Microarray for Rapid Detection of Clinically Relevant bla Genes from β-Lactam-Resistant Gram-Negative Bacteria

Pierre Bogaerts,1 Andrea M. Hujer,2,3 Thierry Naas,4 Roberta Rezende de Castro,5 Andrea Endimiani,2,3 Patrice Nordmann,3 Youri Glupczynski,1 and Robert A. Bonomo2,3,5,6

Evaluation of a DNA Microarray (Check-MDR CT102) for Rapid Detection of TEM, SHV, and CTX-M Extended-Spectrum β-Lactamases and of KPC, OXA-48, VIM, IMP, and NDM-1 Carbapenemases

Thierry Naas,1* Gaelle Cazou,1 Pierre Bogaerts,2 Youri Glupczynski,3 and Patrice Nordmann1

About 7 hours TAT Working by batch

NDM-1, KPC, AmpC and ESBL

Check-MDR CT101*

NDM-1, KPC, OXA-48, VIM, IMP and ESBL

Check-MDR CT102*

ESBL, AmpC, Carbapenemases

Check-MDR CT103*

3 strains/array

1 strain/array

Carbapenemases detection April 2014
## Check-Points CE-IVD assays

<table>
<thead>
<tr>
<th>Check-ESBL</th>
<th>Check-MDR CT101</th>
<th>Check-MDR CT103</th>
<th>Check-MDR CT103 XL*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microarray</strong></td>
<td></td>
<td></td>
<td>Unique panel on the market</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>On colonies By batch 7 hours process</td>
</tr>
<tr>
<td><strong>Check-ESBL</strong></td>
<td><strong>Check-MDR CT101</strong></td>
<td><strong>Check-MDR CT103</strong></td>
<td><strong>Check-MDR CT103 XL</strong>*</td>
</tr>
<tr>
<td></td>
<td>• KPC</td>
<td>• KPC</td>
<td>• KPC, GES</td>
</tr>
<tr>
<td></td>
<td>• NDM</td>
<td>• NDM, VIM, IMP</td>
<td>• NDM, VIM, IMP, GIM, SPM</td>
</tr>
<tr>
<td></td>
<td>• SHV (ESBL vs. wt)</td>
<td>• SHV (ESBL vs. wt)</td>
<td>• SHV (ESBL vs. wt)</td>
</tr>
<tr>
<td></td>
<td>• TEM (ESBL vs. wt)</td>
<td>• TEM (ESBL vs. wt)</td>
<td>• TEM (ESBL vs. wt)</td>
</tr>
<tr>
<td></td>
<td>• CTX-M</td>
<td>• CTX-M</td>
<td>• CTX-M</td>
</tr>
<tr>
<td></td>
<td>• CMY, DHA, FOX, MOX, ACC, MIR, ACT</td>
<td>• CMY, DHA, FOX, MOX, ACC, MIR, ACT</td>
<td>• CMY, DHA, FOX, MOX, ACC, MIR, ACT</td>
</tr>
</tbody>
</table>

| **Check-MDR ESBL** | **Check-MDR Carba** | **Check-Direct CPE** (& for BD MAX™) | **Check-Direct ESBL** (& for BD MAX™)* |
| **Real-time PCR** | | | |
| | • SHV | • KPC | • CTX-M-1 group |
| | • TEM | • NDM, VIM, IMP | • CTX-M-2, M-9 group |
| | • CTX-M | • OXA-48 | • SHV-ESBL |

On colonies: by batch, 4 hours process

* CE-IVD validation in progress.. Launch Q2 ’14.
Proposed algorithms for laboratory detection of carbapenemases

Adapted from Nordmann et al., JAC 2013

Day 0
- Infecting strains

Day 1
- Screening of carriers
- Fecal swab
- Stool samples
- Selective agar for Carba-R isolates
- In areas with high prevalence of OXA-48

Day 2
- Carba hydrolysis test positive
- Hydrolysis test negative

Day 3
- MHT and/or inhibitor combination disks (PBA/DPA) + temocillin

For epidemiology

Hydrolysis test positive
- Strain isolation and AST (decreased suscept. to Carbapenems)
- Day > 3

Hydrolysis test negative
- Day 2
- Selective or non selective Media
- Day 1
- Day 0

Day 0
- Lysis
- 30 min lysis + 15-120 min
- Day 2

Day 1
- Carba hydrolysis test
- Screening of carriers

Carbapenemases detection April 2014
Molecular testing on clinical samples
# Molecular methods for rapid screening of CPE from rectal swab/stools

<table>
<thead>
<tr>
<th>Author (yr)</th>
<th>Targets</th>
<th>Method</th>
<th>Sens. %</th>
<th>Spec. %</th>
<th>Detection limit (CFU/PCR)</th>
<th>Process Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAAS (2013)</td>
<td>OXA-48 and variant</td>
<td>RT PCR (LNA)</td>
<td>100</td>
<td>100</td>
<td>20-50 CFU/mL of stool (100mg)</td>
<td>4h</td>
</tr>
<tr>
<td>Giani (2012)</td>
<td>KPC</td>
<td>In house end-point PCR</td>
<td>100</td>
<td>86</td>
<td>1</td>
<td>3-4h</td>
</tr>
<tr>
<td>Pournaras (2012)</td>
<td>KPC, VIM,</td>
<td>In house end-point PCR</td>
<td>94.4</td>
<td>86</td>
<td>NR</td>
<td>4h</td>
</tr>
<tr>
<td>Singh (2012)</td>
<td>KPC</td>
<td>RT PCR (TaqMan) FAM labeled reporter probes</td>
<td>97</td>
<td>96.6</td>
<td>1-10</td>
<td>24 h* (culture: 64-72h)</td>
</tr>
<tr>
<td>Richter (2012)</td>
<td>KPC-2/-12</td>
<td>Fast RT PCR (TaqMan) MGB probe</td>
<td>100</td>
<td>98</td>
<td>1</td>
<td>&lt; 2h</td>
</tr>
<tr>
<td>Vasoo (2013)</td>
<td>KPC, NDM</td>
<td>RT PCR (Light Cycler) Simple lysis extraction (soiled spec.)</td>
<td>89.1</td>
<td>-</td>
<td>2-10</td>
<td>1.5-4 h</td>
</tr>
</tbody>
</table>

*PCR performed from overnight enrichment broth culture
Cepheid GeneXpert® System

On swabs from Cepheid

Xpert® Carba-R⁺: Projected Release (CE-IVD) Q1 2014

- FDA cleared, all in one inclusive extraction
- Random access
- Hands-on time < 5 min
- So easy RT-PCR
- Expensive + Redo because of inhibition
- Available soon

5 targets for carbepenemase-producing Enterobacteriaceae (CPE)

- For detection of bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48}, and bla_{IMP-1}

Do not detect OXA-181 variant

To be evaluated
The swab is taken using E-swab
Transfer 25 µl from the E-Swab into 500 µl RALF – buffer
The LAMP assays are run on the Genie®II at 66°C for 30 minutes
Carbapenemases/ESBL genes
- KPC
- NDM
- VIM
- OXA-48
- CTX-M-1 (Group 1 ?)
- CTX-M-9 (Group 9 ?)
Commercial Real-Time kits

Detect in a single assay
KPC - OXA-48 - VIM - NDM

- OXA-48/VIM
- OXA-48/VIM/KPC
- CTX-M G1-2-9
- pAmpC
- Pyo/ Acineto/mecA

Not highly multiplexed
Not evaluated

Not easy to use in routine (ELISA)

Do detect OXA-181
Commercially available
Evaluated

Real Time PCR kit for detecting the 4 most common carbapenemases

Key features:
Dynex Laboratories, office@dynex.cz,
Surveillance – Targeted screening with laboratory extractor and RT thermocycler

1. Perianal swab
2. DNA Extraction
3. OXA-48, KPC, NDM/VIM

40 min

2 h

*KPC-1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15; OXA-48, 162, 163, 181, 204, 232, 244, 245; VIM-1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37; NDM-1, 2, 3, 4, 5, 6, 7, 8.

Carbapenemases detection April 2014
Check-Direct CPE for BD MAX™

1. Or

50 min
Extraction

1.5 h
PCR

OXA-48
KPC
NDM
VIM

Carbapenemases detection April 2014
Evaluation of Check-Direct CPE

- 100% Sensitivity and Specificity on 62 characterized clinical isolates
- LoD = 200-2X10⁴ CFU/mL for spiked stool

- 81 rectal swabs during an OXA-48 outbreak
- 4 hours enrichment in BHI + 0.25 µg/mL ertapenem
- 23 samples Check-Direct CPE positive
- All confirmed by culture
Check-direct CPE detection of OXA-48 carbapenemase-producers directly in screening rectal swabs

Amplification Curves

Culture positive sample (OXA-48)

Control +

Culture negative samples (OXA-48)

Pierre Bogaerts, in preparation
Added value of rapid detection
Clinical relevance

Rapid Detection of Antibiotic-Resistant Organism Carriage for Infection Prevention

Daniel J. Diekema¹ and Michael A. Pfaller¹²
¹University of Iowa Carver College of Medicine, Iowa City; and ²JMI Laboratories, North Liberty, Iowa

CID, 2013. 56:1614-1620

Preanalytic
• Collection
• Transport

Analytic
• Processing
• Testing

Postanalytic
• Reporting
• Intervention

Carbapenemases detection April 2014
Rapid detection = Reducing transmission/infection?

- Yes! seems the natural answer (intuitively) but the answer could be probably often No.

- Routine MultiDrug Resistant Organism rapid detection
  - Published data are weak and conflicting
  - On MRSA, rapid detection did not prove to reduce transmission
  - Turn Around Time of rapid PCR tests: 19-22 hours! Post analytical and intervention!
    - Little benefit at substantially increased cost
    - Currently no evidence for reducing transmission or infection
  - But we need more evidences
Rapid detection = Reducing transmission/infection?

- **Upon admission** (all or selected patients?):
  - Could reduce the number of contact precaution if preemptive isolation

- **During outbreaks**
  - Prompt detection and isolation of carriers can be important in containing spread
Conclusions

- What is the question, what will I do with the answer and when?
  - Routine
  - Screening
  - Outbreak
  - Epidemiology/surveillance

- Laboratory testing is just one step
  - Reception of the samples
  - Analysis (random access or by batch)
  - Transmission of the results to the clinician
  - Implementation of the measures on the field (after 5 PM?)

- Complexity of the interpretation: what means detected?
- Rapid detection: Kits exist and alternatives to PCR too
- Costs
- Patient benefit

Carbapenemases detection April 2014
Working partners & Acknowledgements

**UCL Mont Godinne**
- Youri Glupczynski
- Daniel Te-Din Huang
- Pierre Bogaerts
- Caroline Bauraing
- Catherine Berhin
- Warda Boucharhouf
- Marion Massart

**Hôpital ULB-Erasme**
- Olivier Denis
- Sandrine Roisin
- Ariane Deplano
- Claire Nonhoff
- Ricardo De Mendonca

**Hôpital de Bicêtre, Paris**
- Thierry Naas
- Laurent Poirel
- Patrice Nordmann

National reference center for Resistant Enterobacteriaceae and non-fermenters

**Université de Fribourg, Suisse**
- Laurent Poirel
- Patrice Nordmann

**Institute of Public Health**
- Béatrice Jans
- Boudewijn Catry
- Mathias Goossens