Carbapenem resistance detection: update and advances

Matthew Oughton, MD, FRCPC
bioMérieux Users’ Group
Toronto
2014 June 3
<table>
<thead>
<tr>
<th><strong>In the past 2 years I have been an employee of:</strong></th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In the past 2 years I have been a paid consultant:</strong></td>
<td>YES (Optimer - fidaxomicin)</td>
</tr>
<tr>
<td><strong>In the past 2 years I have held investments in the following pharmaceutical organizations, medical devices companies or communications firms:</strong></td>
<td>N/A</td>
</tr>
<tr>
<td><strong>In the past 2 years I have been a member of a Scientific advisory board:</strong></td>
<td>YES (Cubist - daptomycin)</td>
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<td><strong>In the past 2 years I have been a paid speaker:</strong></td>
<td>YES (Cubist - daptomycin)</td>
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<td><strong>In the past 2 years I have received research support (grants) from:</strong></td>
<td>N/A</td>
</tr>
<tr>
<td><strong>In the past 2 years I have participated in a clinical research trial:</strong></td>
<td>YES (Merck – monoclonal Abs for C. difficile; Sanofi Pasteur – C. difficile toxoid vaccine)</td>
</tr>
<tr>
<td><strong>I agree to disclose approved and non-approved indications for medications in this presentation:</strong></td>
<td>YES</td>
</tr>
<tr>
<td><strong>I agree to use generic names of medications in this presentation:</strong></td>
<td>YES</td>
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</table>
Objectives

• To describe current laboratory methods for detecting carbapenemase production
• To discuss new methods for detection and confirmation of carbapenemase production
• To review the evidence supporting current screening and confirmatory methods
Outline

- Background
- Screening methods
- Established methods
- Novel methods
- Challenges
- Summary
Background
## The carbapenemases in *Enterobacteriaceae*

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Penicillins</th>
<th>Cephalosporins 1st et 2nd generation*</th>
<th>Cephalosporins 3rd/4th generation cephalosporins</th>
<th>β-lactams/Inhibitors of β-lactamases</th>
<th>Carbapenems</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Penicillinases: KPC, IMI, GES..</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Metallo-enzymes: VIM, IMP, NDM-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Oxacillinases = OXA-48, OXA-181</td>
<td></td>
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</tr>
</tbody>
</table>

* Cephamycins excluded for most class As
KPCs; Klebsiella Pneumoniae Carbapenemase

Novel Carbapenem-Hydrolyzing β-Lactamase, KPC-1, from a Carbapenem-Resistant Strain of Klebsiella pneumoniae

Rosa Yigit, Anne Marie Queenan, Gregory J. Anderson, Antonio Domenech-Sanchez, James W. Biddle, Christine D. Stewart, Sebastian Alberti, Karen Bush, and Fred C. Tenover

Hospital Infections Program, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333; The R. W. Johnson Pharmaceutical Research Institute, Raritan, New Jersey 08867; and Unidad de Investigación, Hospital Son Dureta, Andorra Doris, Palma de Mallorca, 07014, and Área de Microbiología, Universidad de las Islas Baleares, Crna, Villedemosa, Palma de Mallorca, 07071, Spain

Received 19 September 2000/Returned for modification 21 November 2000/Accepted 23 January 2001
Isolated cases

Outbreaks

Endemicity

USA

Province

s:

Jiangsu
Shanghai
Zhejiang

Puerto Rico

South Korea

Europe

Canada

Taiwan

Puerto Rico

Columbia

Brazil

Argentina

China

Provinces:

Jiangsu
Shanghai
Zhejiang
Hong Kong

Isolated cases
Characterization of a New Metallo-β-Lactamase Gene, \( \text{bla}_{\text{NDM-1}} \), and a Novel Erythromycin Esterase Gene Carried on a Unique Genetic Structure in \emph{Klebsiella pneumoniae} Sequence Type 14 from India

Dongeun Yong, Mark A. Toleman, Christian G. Giske, Hyun S. Cho, Kristina Sundman, Kyungwon Lee, and Timothy R. Walsh

Yonsei University College of Medicine, Research Institute of Antimicrobial Resistance, Seoul, Republic of Korea; Department of Medical Microbiology, Cardiff University, Cardiff, United Kingdom; Clinical Microbiology, MTC—Karolinska Institute, Karolinska University Hospital, Stockholm, Sweden; Yonsei University College of Life Science and Biotechnology, Seoul, Republic of Korea; and Department of Clinical Microbiology, Örebro University Hospital, Örebro, Sweden
Wordwide distribution of carbapenemases in Enterobacteriaceae
NDM-2013
The global spread of OXA-48-like producers

Ellis et al – DMID 2013
Lascols et al – AAC 2013
Mathers et al – JCM 2013
Oteo et al – JAC 2013
Oteo et al - AAC 2013

Poirel et al - JAC 2012 & Refs therein
Brink et al – JCM 2013
Gomez et al – CMI 2013
Potron et al – Eurosurv 2013
Abdelaziz et al – JCM 2013
Huang et al – JAC 2013

Nagano et al - JJID 2013
Balm et al – BMC ID 2013
Espedido et al – Plos ONE 2013
Stolle et al – JAC 2013
Dortet et al – CMI 2012
Media supplemented with cephalosporins

The challenge of detecting OXA-48+/ESBL- isolates

*K. pneumoniae* OXA-48 ESBL +

*K. pneumoniae* OXA-48 ESBL -
• 26 *E. cloacaee* isolates (16 pts at one centre)
  ♦ Sept 2010-Dec 2011
• 4 main pulsovars all carrying *bla*$_{KPC}$ inserted into *Tn4401* on plasmids
  ♦ 28-103 kb
• outbreak of a gene rather than a strain or even plasmid
Screening for carbapenem resistance
Selective media for screening purposes

Drigalski lactose + ertapenem disk

media containing a 3rd gen ceph

Media supplemented with a carbapenem

CHROMAGAR
KPC Brillance CRE
ChromID Carba
SUPERCARBA
Selective media containing carbapenem

- **CHROMagar KPC** (CHROMagar) : meropenem + chromogens

  - uses high concentration of carbapenem ⇒ lack of detection for weak hydrolysis of carbapenems
    - e.g. OXA-48, some metallo-beta-lactamases
  - dehydrated medium requires on-site plate preparation (unless prepared via third party at added cost)
  - relatively poor performance characteristics when compared to other commercial media for CRE (Girlich *et al*, Diag Microb Infect Dis 2012):
    - Sensitivity 43%
    - Specificity 68%
Selective media containing carbapenem

- **Brillance CRE agar** (Oxoid) : carbapenem (?) + chromogens

  - good test performance characteristics in one study using a panel of 255 *Enterobacteriaceae* (Cohen Stuart *et al*, Eur J Clin Microbiol Infect Dis 2013)
    - overall sensitivity 94%, specificity 71%
    - sensitivity varied according to carbapenemase:
      - KPC, NDM, GIM: 100%
      - VIM: 90%
      - OXA-48: 84% (only 1 of 5 ESBL- OXA-48+ isolates)
    - lower specificity due to AmpC and ESBL overexpressors
  - moderate test performance when compared directly to other commercial CRE detection media (Girlich *et al*, Diag Microb Infect Dis 2012):
    - Sensitivity 76%
    - Specificity 57%
**ChromID Carba** (bioMérieux): carbapenem (?) + chromogen

- one study found good performance of ChromID Carba relative to selective enrichment with ertapenem, MacConkey+meropenem, and chromID-ESBL media
  - sensitivity 92%, specificity 97%
- However, no commercial carbapenem-resistance detection media were included as comparators
- manufacturer’s data claims high sensitivity (97.4% [93.4-99.3]) and specificity (99.7% [98.9-100.0])
- test performance characteristics require more published direct comparative data
- may have difficulties detecting organisms that express OXA-48 (L. Poirel, personal data)
Selective media containing carbapenem

- **SUPERCARBA** (bioMérieux): Drogalski with ertapenem + cloxacillin + zinc
  - Uses low carbapenem concentration to detect OXA-48 producers
  - Uses cloxacillin to inhibit growth of AmpC-overexpressers
  - Uses zinc to detect metallo-β-lactamase producers (e.g. NDM)
  - Overall high sensitivity (96.5%) and moderate specificity (61%) when compared to other commercial CRE detection media (Girlich et al, Diag Microb Infect Dis 2012)

<table>
<thead>
<tr>
<th></th>
<th>CHROMagar KPC</th>
<th>Brillance CRE</th>
<th>SUPERCARBA</th>
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<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>43</td>
<td>76.3</td>
<td>96.5</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>67.8</td>
<td>57.1</td>
<td>60.7</td>
</tr>
<tr>
<td>Ambler class A</td>
<td>70</td>
<td>85</td>
<td>100</td>
</tr>
<tr>
<td>Ambler class B</td>
<td>58.8</td>
<td>78.4</td>
<td>92</td>
</tr>
<tr>
<td>Ambler class D</td>
<td>11.6</td>
<td>69.8</td>
<td>100</td>
</tr>
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Confirmatory methods for detection of carbapenemase production
Detection of carbapenemase producers

- **Susceptibility testing**: updated CLSI, EUCAST guidelines

- **Phenotypic detection**
  - modified Hodge test
  - carbapenemase inhibition:
    - clavulanic acid, boronic acid; EDTA, dipicolinic acid

- **Carbapenem hydrolysis** (UV spectrophotometry, MALDI-TOF)

- **Nucleic acid amplification testing**
  - conventional vs. real-time PCR
  - usually in multiplex PCR assays or DNA microarrays
  - permits gene sequencing
Susceptibility to carbapenems?

Current debate on breakpoints

<table>
<thead>
<tr>
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<th>EUCAST</th>
<th>CLSI</th>
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<tr>
<td><strong>Imipenem</strong></td>
<td>$R &gt; S \leq$</td>
<td>$R &gt; S \leq$</td>
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<tr>
<td></td>
<td>Imipenem</td>
<td>Imipenem</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><strong>Meropenem</strong></td>
<td>$R &gt; S \leq$</td>
<td>$R &gt; S \leq$</td>
</tr>
<tr>
<td></td>
<td>Meropenem</td>
<td>Meropenem</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><strong>Ertapenem</strong></td>
<td>$R &gt; S \leq$</td>
<td>$R &gt; S \leq$</td>
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<tr>
<td></td>
<td>Ertapenem</td>
<td>Ertapenem</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Doripenem</strong></td>
<td>$R &gt; S \leq$</td>
<td>$R &gt; S \leq$</td>
</tr>
<tr>
<td></td>
<td>Doripenem</td>
<td>Doripenem</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>ND</td>
</tr>
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</table>

ND: Not determined.
Value of the modified Hodge test for detection of emerging carbapenemases in *Enterobacteriaceae*

from Girlich D, Poirel L, Nordmann P. J Clin Micro 2011
Carbapenemase inhibition tests

**Principal**
- KPC inhibited by boronic acid or clavulanic acid
- MBLs inhibited by EDTA or dipicolinic acid

**Tests available**
- Combined Test (ROSCO) : meropenem+/− cloxacillin / dipicolinic acid / boronic acid
- E-test MBL (bioMerieux)
- Inhibition by EDTA (« home-made » technique)
Mass spectrometry: MALDI-TOF

Protocol:
1) Broth culture with the strain to be tested + carbapenem: 3-6h
2) Mass spectrometry
3) if carbapenemase +:
   hydrolysis of the carbapenem molecule leading to degradation product(s)

Advantages:
Specific / sensitive
Fast +
Cheap (cost per test)

Disadvantages:
Equipment price
Expertise

Hrabák et al. JCM. 2011
Burckhardt et al. JCM. 2011
Hrabák et al. JCM. 2012
Molecular biology : PCR-based Techniques

• Real-time PCR :

- Check-MDR Real-Time PCR
- detects the presence of known carbapenemase genes
- TAT: 4-5 h
- Expertise ++, Cost +++

• Specific PCR +/- sequencing :

- OXA-48-like / KPC / VIM / IMP / NDM
- TAT: 3-5 h or longer (sequencing)
- Expertise ++++, Cost + (on top of PCR)
- requires known gene(s)
A novel method for confirmation of carbapenemase production
The Carba NP test

Carbapenemase

Carbapenems
- Imipenem
- Meropenem
- Ertapenem
- Doripenem

Acid production

Colorimetric detection

pH
The Carba NP test; the kit

- Lysis buffer
- Diluted red phenol pH=7.8 + ZnSO₄ 0.1 mM
- 96 wells plate
- 3 mg of imipenem powder
Results

$K. \text{pneumoniae CTX-M-15} + \text{impermeability}$

$K. \text{pneumoniae OXA-48}$

$K. \text{pneumoniae KPC-2}$

$E. \text{coli VIM-1}$

$E. \text{coli IMP-1}$

$E. \text{coli NDM-1}$

Yellow = carbapenem hydrolysis
The Carba NP test

<table>
<thead>
<tr>
<th>Ambler class</th>
<th>Carbapenemase type</th>
<th>Mean time for positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>KPC</td>
<td>15 min- 1h</td>
</tr>
<tr>
<td>A</td>
<td>GES-2, -5</td>
<td>1h-1h30</td>
</tr>
<tr>
<td>B</td>
<td>NDM</td>
<td>20-50 min</td>
</tr>
<tr>
<td>B</td>
<td>VIM</td>
<td>20-50 min</td>
</tr>
<tr>
<td>B</td>
<td>IMP</td>
<td>5-30 min</td>
</tr>
<tr>
<td>D</td>
<td>OXA-48</td>
<td>30-40 min</td>
</tr>
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</table>
Comparison of a Novel, Rapid Chromogenic Biochemical Assay, the Carba NP Test, with the Modified Hodge Test for Detection of Carbapenemase-Producing Gram-Negative Bacilli

Shawn Vasoo, a Scott A. Cunningham, a Peggy C. Kohner, a Patricia J. Simner, a Jayawant N. Mandrekar, b Karen Lolans, c Mary K. Hayden, c,d Robin Patel a,e

TABLE 2 Performance characteristics of the Carba NP and modified Hodge tests

<table>
<thead>
<tr>
<th>Performance characteristic (compared to presence of carbapenemase gene)</th>
<th>Value for isolate group and test (n%) a</th>
<th>All isolates (Enterobacteriaceae and nonfermenting Gram-negative bacilli)</th>
<th>Enterobacteriaceae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carba NP (269)b</td>
<td>Modified Hodge (251)b</td>
<td>Carba NP (200)c</td>
</tr>
<tr>
<td>Sensitivity, % (95% CI)</td>
<td>100 (96.4–100)</td>
<td>97.7 (92.9–99.4)</td>
<td>100 (96.3–100)</td>
</tr>
<tr>
<td>Specificity, % (95% CI)</td>
<td>100 (96.7–100)</td>
<td>80.0 (71.5–86.5)</td>
<td>100 (94.1–100)</td>
</tr>
<tr>
<td>Positive predictive value, % (95% CI)</td>
<td>100 (96.4–100)</td>
<td>84.2 (77.2–89.4)</td>
<td>100 (96.3–100)</td>
</tr>
<tr>
<td>Negative predictive value, % (95% CI)</td>
<td>100 (96.7–100)</td>
<td>97.0 (90.8–99.2)</td>
<td>100 (94.1–100)</td>
</tr>
</tbody>
</table>
Comparative evaluation of two chromogenic tests for the rapid detection of carbapenemase in Enterobacteriaceae and in Pseudomonas aeruginosa isolates.

- 100 Enterobacteriaceae, 35 Pseudomonas aeruginosa well-characterized carbapenem-resistant isolates
  - 66 carbapenemase, 69 other mechanisms
- Rosco CARB Screen (RCS) vs Carba NP (CNP), using PCR-based carbapenemase gene detection as gold standard

Comparative evaluation of two chromogenic tests for the rapid detection of carbapenemase in Enterobacteriaceae and in Pseudomonas aeruginosa isolates.

- Results (sens, spec, PPV, NPV)
  - CNP:
    - Enterobacteriaceae: 97%, 100%, 100% and 95%
    - Pseudomonas: 91%, 100%, 100%, 96%
  - RCS:
    - Enterobacteriaceae: 98%, 83%, 81%, 95%
    - Pseudomonas: 96%, 54%, 39%, 97%
- out of 73 Enterobacteriaceae with OXA-48:
  - CNP: 62 strong-positive, 8 weak-positive, 3 negative, 0 uninterpretable
  - RCS: 49 strong-positive, 14 weak-positive, 2 negative, 8 uninterpretable

D0

Infection

Urine

Others

Blood cultures

?? Direct ??
Carba NP test
< 2h

D1

Carba NP test
< 2h

D2

Carba NP test
< 2h
Challenges

- rising incidence of carbapenem-producing organisms in nosocomial infections

- limitations of conventional methods and screening for dealing with epidemics of genes rather than organisms

- slow emergence of new antimicrobial options from the development pipeline

- persistent creativity of pathogens
Increased prevalence of carbapenemase producers worldwide.

Diagnostic techniques are now available for accurate screening for, and confirmation of, carbapenemase producers.

Detection alone is insufficient:
- Improved carbapenem stewardship
- Outbreak prevention and control
Acknowledgements

• Professor Laurent Poirel, Medical and Molecular Microbiology Unit, Dept of Medicine, University of Fribourg, Switzerland
• Yves Longtin, MD, FRCPC, Head of Infection Control, Jewish General Hospital