Laboratory Challenges in the Detection of Carbapenemase Resistant Enterobacteriaceae (CRE)

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Objectives

- Introduction:
  - Overview of \( \beta \)-Lactamases
  - Types of Carbapenemases
- Significance of CRE
- Laboratory Detection:
  - Importance
  - Difficulties
  - Screening Media
  - FH screen Method
- Rapid Phenotypic Confirmation of Carbapenemase production
  - Available methods
  - Method used by Fraser Health (including evaluation data)
- Confirmatory Molecular Methods
- Conclusion
Introduction

- Overview of β-lactamases
- Types of Carbapenemases
Overview of β-Lactamases

- Definition: enzymes that hydrolyze β-lactam antibiotics
- Penicillinases: target penicillins
- Cephalosporinases: target cephalosporins
- CLASSICAL Broad spectrum β-lactamases: target penicillins and narrow spectrum cephalosporins (ceph/cefaz/cefaclor)
- ESBL (Extended Spectrum β-lactamases): target same as broad spectrum plus newer cephalosporins (cefurox/CTX/CTZ)
- Carbapenemases: target all types of β-lactam antibiotics including carbapenems (ertapenem, meropenem, imipenem)
Types of Carbapenemases

- **Ambler Class A:**
  - Hydrolyze all β-lactams
  - Eg: KPC

- **Ambler Class B (Metallo-β-Lactamases):**
  - Zinc dependent
  - Hydrolyze all β-lactams except aztreonam
  - Eg: NDM, VIM, IMP

- **Ambler Class D:**
  - Hydrolyze carbapenems and +weak/- broad-spectrum cephalosporins
  - Eg: OXA-48
Significance of CRE
Significance of CRE

Of the CRE, the Carbapenemase producing Enterobacteriaceae (CPE) are the most significant.

Reasons:

- Carbapenemase gene is commonly located on “a mobile genetic element”:
  - gene can move from one bacterial strain and species to another.
  - Plasmid spread is very common in NDMs; clonal outbreaks have been documented.
- Cause nosocomial outbreaks
- Are multi-drug resistant
- Increased # of carbapenemase-producing Enterobacteriaceae being detected in recent years
- Early detection required to prevent and reduce their spread
Laboratory Detection of CRE

- Importance
- Difficulties
- Screening Media
- FH Screen Method
Importance of Detecting CRE

- Clinically: appropriate therapy regimen selection
- For screen: appropriate Infection control measures and management.
Difficulties in Detecting CRE

- Cannot solely rely on antimicrobial resistance profile
- Neither CLSI nor EUCAST have standardized methodology for detecting all carbapenemases
- Which organism/isolate to test?
- MIC level
- Presence of additional resistance mechanism(s)
Specimen types for Detecting CRE

- Rectal swab (fecally stained)
  or
- Stool
And **if required by IC:**
- Urine
- Wounds
- Sputum
- Endo-tracheal aspirates
Screening using routine media

- Tryptic soy broth with 2 μg/mL Imipenem:
  - broth sub-cultured to MacConkey
- MAC with one disk of Ertapenem 10 μg
- CDC method - Trypticase Soy broth (5 mL) with one 10 μg ertapenem or meropenem disc:
  - broth sub-cultured to MacConkey
- Advantages:
  - simple
  - less expensive
- Disadvantages:
  - not very selective (Gram Negative organisms such as Pseudomonads and Enterobacteriaceae will be isolated)
  - higher work load (any growth will require work-up to rule/out CRE)
Screening using Chromogenic media

- Brilliance CRE
- ChromID ESBL
- CHROMagar KPC
- SuperCARBA

Advantages:
- Very selective
- More sensitive than routine screening media: improved limit of detection (ChromID ESBL = $1 \times 10^1$; CHROMagar KPC = $4 \times 10^1$ {does not detect organisms with MIC < 4 $\mu$g/mL})
- Less workload as compared to screening with routine media

Disadvantages:
- Expensive media
- Not all media can detect OXA48:
  - ChromID ESBL detects OXA48 when other ESBLs are also present
  - CHROMagar KPC does not detect OXA48
  - SuperCARBA detects OXA48
FH CRE Screen Method

- MacConkey with one disk of Ertapenem 10 µg
- MacConkey with one disk of Meropenem 10 µg and
- Tryptic soy broth (5mL) with Ertapenem 10 µg

Advantages:
- Simple
- less expensive
- comparable sensitivity to chromogenic media

Disadvantages:
- not very selective
- higher work load (any growth will require work-up to rule out CRE)

Notes:
- FH has started to evaluate the SuperCARBA media; preliminary findings indicate: detection of CRE was comparable to MAC plates/TSB broth, and it reduces the work load.
- FH is looking into availability of SuperCARBA media or recipe to prepare “in-house” SuperCARBA plates
Rapid Phenotypic Confirmation of Carbapenemase Production

- Available Methods
- Method used in Fraser Health
Available Methods

- **MAST discs (Carbapenemase Detection Set D70C):**
  - Meropenem 10µg
  - Meropenem 10µg + MBL inhibitor
  - Meropenem 10µg + KPC inhibitor
  - Meropenem 10µg + AmpC inhibitor

- **ROSCO Disks (Carbapenemases. KPC + MBL Confirm ID kit):**
  - Meropenem 10µg
  - Meropenem 10µg + Boronic Acid (for KPC)
  - Meropenem 10µg + Cloxacillin (for AmpC)
  - Meropenem 10µg + Dipicolinic Acid (for MBL)
  - Temocillin 30µg (for OXA48)
Method used by FH: ROSCO Disks

- **ROSCO Disks (Carbapenemases. KPC + MBL Confirm ID kit):**
  - Meropenem 10µg (MRP10)
  - Meropenem 10µg + Boronic Acid (MRPBO)
  - Meropenem 10µg + Cloxacillin (MRPCX)
  - Meropenem 10µg + Dipicolinic Acid (MRPDP)
  - Temocillin 30µg (TEMOC)

- **Interpretation (as per kit guidelines):** assess synergy between Meropenem and other combination disks; inhibition zone ≥ 5 mm

<table>
<thead>
<tr>
<th>Type</th>
<th>MRPDP</th>
<th>MRPBO</th>
<th>MRPCX</th>
</tr>
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<tbody>
<tr>
<td>MBL</td>
<td>Synergy</td>
<td>No synergy</td>
<td>No synergy</td>
</tr>
<tr>
<td>KPC</td>
<td>No synergy</td>
<td>Synergy</td>
<td>No synergy</td>
</tr>
<tr>
<td>AmpC impermeability</td>
<td>No synergy</td>
<td>Synergy</td>
<td>Synergy</td>
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<tr>
<td>Oxacillinases</td>
<td>No synergy</td>
<td>No synergy</td>
<td>No synergy</td>
</tr>
</tbody>
</table>
Rosco Disks: MBL positive isolate

Rosco Disks results: MRP10=12, MRPCX=11, MRPBO=13, MRPDP=20, TEMOC =10

MIC: Ertapenem, Meropenem, and Imipenem all >32 µg/mL
Rosco Disks: negative isolate

MRP10=20, MRPCX=21, MRPBO=22, MRPDP=19, TEMOC=13; isolate negative for MBL, KPC and OXA
Limitations of Rosco Disks

- Test isolates that are Ertal “R”. Isolates with Ertapenem “S” may test as false + with boronic acid
- Rosco disks:
  - are for interpretation of KPC and MBL
  - do not accurately detect AmpC
  - do not always aid in detection of OXA-48
Notes when Interpreting Rosco Disks results

- Comparing results of MRPCX (Mero + Clox) and MRPBO (Mero + Boronic):
  - Boronic acid inhibits class A (KPC and ESBL are both Class A and AmpC)
  - MRPCX zones are generally bigger than MRPBO

- TEMOC interpretation:
  - If synergy seen between Meropenem and the combination disks: Temoc result is of little value.
  - If no synergy between Meropenem and the combination disks, and TEMOC disc shows no zone: high likelihood of OXA48 carbapenemase
FH Evaluation of ROSCO Disks vs. Molecular Typing

No. of isolates tested = 128

<table>
<thead>
<tr>
<th>Organism ID (#)</th>
<th>NDM (#)</th>
<th>KPC (#)</th>
<th>OXA 48 (#)</th>
<th>Neg or Other ESBL (#)</th>
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<tbody>
<tr>
<td>K. pneumoniae (63)</td>
<td>31</td>
<td>1</td>
<td>3</td>
<td>28</td>
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<tr>
<td>E. coli (28)</td>
<td>9</td>
<td>1</td>
<td>4 (of these 2 had NDM &amp; OXA48)</td>
<td>14</td>
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<tr>
<td>E. cloacae complex (18)</td>
<td>4</td>
<td>3</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>C. freundii (8)</td>
<td>3</td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>K. oxytoca (4)</td>
<td>3</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>C. braakii (1)</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>E. aerogenes (1)</td>
<td></td>
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<td></td>
<td>1</td>
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<tr>
<td>E. amnigenus (1)</td>
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<td></td>
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<tr>
<td>H. alvei (1)</td>
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<td>1</td>
</tr>
<tr>
<td>S. marcescens (1)</td>
<td></td>
<td>1 (class A SME type carbapenemase)</td>
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<td></td>
</tr>
<tr>
<td>S. fonticola (1)</td>
<td></td>
<td></td>
<td></td>
<td>1 (CMY 2)</td>
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<tr>
<td>P. mirabilis (1)</td>
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<td>1</td>
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FH Evaluation of ROSCO Disks vs. Molecular Typing

Method verification results for NDM

<table>
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<th>NDM (BCCDC)</th>
<th>NDM (RD)</th>
<th>Method Verification</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pos</td>
<td>Neg</td>
</tr>
<tr>
<td>NDM (BCCDC)</td>
<td>Pos</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>Neg</td>
<td>0</td>
</tr>
</tbody>
</table>

Sens = sensitivity  
Spec = specificity  
PPV = Positive Predictive Value  
NPV = Negative Predictive Value  
NDM (BCCDC) = tested by molecular typing
FH Evaluation of ROSCO Disks vs. Molecular Typing

Method verification results for KPC

<table>
<thead>
<tr>
<th>KPC (BCCDC)</th>
<th>KPC (RD)</th>
<th>Method Verification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pos</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td>Sens (%)</td>
<td>Spec (%)</td>
</tr>
<tr>
<td>KPC (BCCDC)</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Neg</td>
<td>1</td>
<td>122</td>
</tr>
</tbody>
</table>

Sens = sensitivity
Spec = specificity
PPV = Positive Predictive Value
NPV = Negative Predictive Value
KPC (BCCDC) = tested by molecular typing
FH Evaluation of ROSCO Disks vs. Molecular Typing

Evaluation summary:

- **Phenotypic confirmation of MBLs:** good correlation of the phenotypic ID by Rosco disks vs. molecular typing.
- **Phenotypic confirmation of KPCs:**
  - good correlation of the phenotypic ID by Rosco disks vs. molecular typing.
  - Results must be interpreted carefully - boronic acid inhibits class A (KPC and ESBL are both Class A and false positive results may occur)
- Results are comparable to other evaluations.
Confirmatory Molecular Methods
Confirmatory Molecular Methods

- Reference Laboratory – tests for ESBLs, AmpC cephalosporinases, Metallo β-lactamases (eg. NDM, VIM).
- Commercially available methods:
  - CRE Assay (BD MAX): detects 3 antimicrobial resistance genes – KPC, OXA-48 and/or NDM.
  - Carba-R assay (GeneXpert): detects 5 antimicrobial resistance genes – \( \text{bla}_{\text{KPC}}, \text{bla}_{\text{NDM}}, \text{bla}_{\text{VIM}}, \text{bla}_{\text{OXA-48}} \) and \( \text{bla}_{\text{IMP-1}} \).
Conclusion
Conclusion

- Chromogenic media are best for detection of CRE, but note:
  - it is expensive
  - all CRE types may not be detected from screen media used.
- **Rapid** Phenotypic Identification of MBL and KPC can be obtained using MAST disks or ROSCO disks.
- Molecular identification (confirmation) must be performed.
References


References (ct’d)

- ROSCO Diagnostica Technical Data Sheet 1.5.0: Detection of resistance mechanisms using Neo-Sensitabs™ and Diatabs™ Detection of beta lactamases – Carbapenemases. KPC + MBL Confirm ID kit. 22.03.10 Revision no. 3.
References (ct’d)

- Cepheid Innovation Technical Data Sheet REF RCARBAR-10: GeneXpert® Carba-R. 301-2436, Rev. 2 December.
Acknowledgements

- Dr. Manal Tadros
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Thank you