Issues in the Detection of Multi-Drug Resistant Gram Negative Organisms

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Overview

• Problem MDRO GNB in LTCF
• Prevalence in LTCF
• When to screen
• Who should be screened?
• How screening should be done?
• How to detect MDRO GNB
Antibiotic Resistance in LTCF
ESKAPE Pathogens

- *Enterococcus faecium* (VRE)
- *Staphylococcus aureus* (MRSA, VRSA)
- *Klebsiella pneumoniae* (CRE-KPC)
- *Acinetobacter baumannii* (CRAB-MBL)
- *Pseudomonas aeruginosa* (CRE-MBL)
- *Enterobacter* spp. (CRE-KPC)

# Antibiotic Resistance - GNB

## β-lactamases

<table>
<thead>
<tr>
<th>β-lactamases</th>
<th>Antibiotic Resistance</th>
<th>Enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Broad spectrum</strong></td>
<td>PCN, AMP early cephalosporins</td>
<td>TEM-1, TEM-2, SHV</td>
</tr>
<tr>
<td></td>
<td>As above &amp; Staph PCNs</td>
<td>OXA</td>
</tr>
<tr>
<td><strong>Extended spectrum (ESBL)</strong></td>
<td>PCN, AMP, Staph PCNs &amp; 3&lt;sup&gt;rd&lt;/sup&gt; cephalosporins + monobactams</td>
<td>TEM</td>
</tr>
<tr>
<td>Hospitals 1980s</td>
<td>As above + cefepime [CTX-M]</td>
<td>CTX-M</td>
</tr>
<tr>
<td>Community E. coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Inducible (AmpC)</strong></td>
<td>cephapmics (cefoxitin) β-lactamase inhibitors</td>
<td></td>
</tr>
</tbody>
</table>

**Antibiotic Resistance GNB Carbapenemases**

<table>
<thead>
<tr>
<th>Carbapenemases</th>
<th>Antibiotic Resistance</th>
<th>Enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>K. pneumoniae</em> (KPC)</td>
<td>all β-lactams</td>
<td>KPC 1</td>
</tr>
<tr>
<td>Detected 1996</td>
<td>all carbapenems</td>
<td>KPC 2</td>
</tr>
<tr>
<td>Outbreaks 2001</td>
<td>some aminoglycosides</td>
<td>KPC 3</td>
</tr>
<tr>
<td>Endemic 4 continents</td>
<td></td>
<td>KPC 4</td>
</tr>
<tr>
<td>Found <em>K. pneumoniae</em> first</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Now E. coli, Enterobacter, Salmonella, Citrobacter</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Metallo-β-lactamases (MBL)</strong></td>
<td>As above</td>
<td>IMP VIM SPM-1</td>
</tr>
<tr>
<td>Detected 1991</td>
<td></td>
<td>GIM-1 NDM-1</td>
</tr>
<tr>
<td>Outbreaks 2000 worldwide</td>
<td></td>
<td>OXA</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Community MDR-GNB ESBLs

<table>
<thead>
<tr>
<th>Community onset</th>
<th>Hospital onset</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organism</strong></td>
<td><strong>Klebsiella</strong> spp (and others)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td></td>
</tr>
<tr>
<td><strong>Type of ESBL</strong></td>
<td>SHV (especially SHV2, SHV5) and TEM (especially TEM26, TEM51)</td>
</tr>
<tr>
<td>CTX-M (especially CTX-M15)</td>
<td></td>
</tr>
<tr>
<td><strong>Infection</strong></td>
<td>Respiratory tract, intra-abdominal, and bloodstream infections</td>
</tr>
<tr>
<td>Most often UTIs, but also bacteraemia and gastroenteritis</td>
<td></td>
</tr>
<tr>
<td><strong>Susceptibilities</strong></td>
<td>Resistance to all the penicillins and cephalosporins. High-level resistance to other classes of antibiotics, especially fluoroquinolones and co-trimoxazole</td>
</tr>
<tr>
<td>Most isolates often not clonally related, although clusters have been described in Canada, the UK, Italy, and Spain</td>
<td>Most often clonally related</td>
</tr>
<tr>
<td><strong>Molecular epidemiology</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Risk factors</strong></td>
<td>Longer length of hospital stay; severity of illness (more severe, the higher the risk); longer time in the intensive-care unit; intubations and mechanical ventilation; urinary or arterial catheterisation; previous exposure to antibiotics (especially cephalosporins)</td>
</tr>
<tr>
<td>Repeat UTIs and underlying renal pathology; previous antibiotics including cephalosporins and fluoroquinolones; previous hospitalisation; nursing-home residents; older men and women; diabetes mellitus; underlying liver pathology</td>
<td></td>
</tr>
</tbody>
</table>

*UTI = urinary-tract infection.*

Table 2: Characteristics of infections caused by ESBL-producing bacteria

Pitout JDD et al. Lancet ID 2008;8:159
MDR-GNB in Community
ESBLs – CTX-M

• CTX-M-15 worldwide
  — New Delhi 1999; US 2003
• Originated *Kluyvera* spp.
• True community pathogens
• Associated UTI/BSI
• Most common older adults
• Quinolone resistance common
  — *E. coli, Klebsiella, Proteus*

# ESBL in LTCF Prevalence

<table>
<thead>
<tr>
<th>Author (Date)</th>
<th>Isolates N (%)</th>
<th>E. coli</th>
<th>Where</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muller (02)</td>
<td>200 (16-62)</td>
<td>yes</td>
<td>15 LTCF (Ontario, Canada)</td>
</tr>
<tr>
<td>Rooney (09)</td>
<td>58/294 (49%)</td>
<td>yes CTX-M-15</td>
<td>16 LTCF (N. Ireland)</td>
</tr>
<tr>
<td>March (09)</td>
<td>56/111 (64%)</td>
<td>yes CTX-M</td>
<td>LTCF (Italy)</td>
</tr>
<tr>
<td>Van der Mee-Marquet (10)</td>
<td>9/49 (22%)</td>
<td>CTX-M-15</td>
<td>LTCF (France)</td>
</tr>
<tr>
<td>Arvand (13)</td>
<td>25/240</td>
<td>CTX-M-15</td>
<td>11 LTCF (Germany)</td>
</tr>
<tr>
<td>Cochard (14)</td>
<td>114/1155 (9.9%)</td>
<td>Klebsiella sp (14)</td>
<td>38 LTCF (France)</td>
</tr>
<tr>
<td>Zhao (15)</td>
<td>183/487 (46.9%)</td>
<td>CTX-M (99%)</td>
<td>7 LTCF (Shanghai)</td>
</tr>
<tr>
<td>Willemsen (15)</td>
<td>33/160 (20.6%)</td>
<td>CTX-M-15 (21)</td>
<td>LTCF (Netherlands)</td>
</tr>
</tbody>
</table>
MDR-GNB in LTCF
ESBL

• *E. coli* infection monoclonal outbreaks
  – CTX-M-15, CTX-M-14
  – Ontario (2000-2002) 15 nursing homes
  – UK (2004-2006) 16 nursing homes
  – France (2009)

• Associated with
  – UTI
  – Quinolone, ceftazidime use

• Colonization common
  – urine (22%), rectum (49%)
  – HCW (15%)

• Environmental contamination ~ 0.8% samples

Screening for ESBL Microbiology Definitions

Screen for ESBL

• Disk method
  – cefpodoxime 10 μg
  – ceftazidime 30 μg
  – cefotaxime 30 μg

• Broth
  – cefpodoxime
  – ceftazidime
  – cefotaxime

• Applies only
  – Klebsiella sp
  – E. coli
  – Proteus

Confirm ESBL (+)

• Double disk diffusion
  – ATB alone
  – ATB + β lactamase inh
  – 5 mm zone increase

• Broth
  – ATB alone
  – ATB + β lactamase inh
  – 2-fold reduction MIC

• PCR/molecular typing
  – CTX-M

ESBL Testing

Positive ESBL Double Disk Test

- Susceptible Cefotaxime plus Clavulanic acid
- Resistant Cefotaxime
- Susceptible Ceftazidime plus Clavulanic acid
- Resistant Ceftazidime
ESBL Screening
When, Who, What to Consider?

• Increase infection rates
  — 3rd 4th cephalosporins, monobactams
  — Quinolones
  — *E. coli* ST 131

• No specific risk factors

• Urine and rectum

Carbapenem$^R$ Enterobacteriaceae
What Are They?

- Carbapenems – antibiotics of last resort
- Enterobacteriaceae
  - gram negative bacilli
  - lactose fermenters
  - not *Pseudomonas* or *Acinetobacter*
- Multiple mechanisms of carbapenem$^R$
  - inactivation by key enzymes the main concern
  - not all CRE produce carbapenemases (CPE)
  - many labs can identify CRE but not CPE
Carbapenem Resistance Identification – A Major Issue

• Carbapenemase- producing CRE (CPE)
  — spreading rapidly world wide
  — resistant majority antibiotic classes
  — invasive infections 40-50% mortality
Carbapenem\textsuperscript{R} Enterobacteriaceae (CRE) Identification-Major Issues

• There are many carbapenemases
  – *Klebsiella pneumoniae* carbapenemase (KPC)
  – Verona integron metallo-β-lactamase (VIM)
  – Imipenemase metallo-β-lactamase (IMP)
  – Oxacillinase-48-type carbapenemases (OXA-48)
  – New Delhi metallo-β-lactamase-1 (NDM-1)

• Carbapenemases vary with geography
Carbapenem Resistance (CRE) KPCs

Carbapenem Resistance (CRE)  
Metallo-β-lactamases (MBL)

Metallo-β-lactamases
New Delhi (NDM-1)

Metallo-β-lactamases NDM-1

- **Exposure endemic areas**
  - travelers
  - medical tourists
  - military

- **Now non-endemic acquisition**
  - France, Italy, Canada
  - community, nursing homes, rehabilitation units
  - no travel history
  - evidence transmission
  - spread multiple facilities
  - older patients

**K. Pneumoniae Carbapenemases (KPCs)**

**LTCFs & LTACHs**

- USA, Israel (2008-2011)
- Older adults, co-morbidities, devices
- Most admitted from post-acute care facilities
- Mortality 35-69%
- Mostly related clones
  - *K. pneumoniae* (38/76), ST-258
    - mostly urinary
  - *E. coli* (2)

## CRE in LTCF Prevalence

<table>
<thead>
<tr>
<th>Author (Date)</th>
<th>N (%)</th>
<th>What</th>
<th>Where</th>
</tr>
</thead>
<tbody>
<tr>
<td>Munoz-Price (2010)</td>
<td>8/39 (21%)</td>
<td>KP-CPE</td>
<td>LTACH (US)</td>
</tr>
<tr>
<td>Mills (2011)</td>
<td>7/100 (7%)</td>
<td>KP-CPE</td>
<td>LTCF (US)</td>
</tr>
<tr>
<td>Ben-David (2011)</td>
<td>75/357 (21%)</td>
<td>KP-CRE</td>
<td>LTCF (Israel)</td>
</tr>
<tr>
<td>Marchaim (2012)</td>
<td>42/93 (42.5%)</td>
<td>CRE</td>
<td>Hosp admit LTCF (US)</td>
</tr>
<tr>
<td>Lewis (2013)</td>
<td>20/262 (7.6%)</td>
<td>CPE</td>
<td>LTACH (US)</td>
</tr>
<tr>
<td>Saegeman (2015)</td>
<td>1/257 (0.4%)</td>
<td>CPE</td>
<td>LTCF (Belgium)</td>
</tr>
</tbody>
</table>

**LTACH vs SNF**
- CRE present on admission to hospital
- Proportion of clinical isolates from lab collections
Screening for CRE
Microbiology Definitions

Screen for CRE
• MIC ≥ 8 μg/ml
  – doripenem,
  – imipenem,
  – meropenem
• MIC ≥ 2 μg/ml
  – ertapenem
• Intrinsic imipenem\textsuperscript{R}
  – Morganella, Proteus, Providencia
  – Must be resistant to another carbapenem

Confirm CPE (+)
• Modified Hodge Test
  – disk test, easy
  – false positives
• CARBA NP agar
• MBL inhibition assays
• PCR/molecular tests
  – KPC, VIM, NDM-1
  – IMP, OXA-48

CDC. CRE Tool Kit Update – Nov 2015; Hrbak J CMI 2014;20:839
Modified Hodge Test
CPE Detection

Lawn of *E. coli* ATCC 25922
1:10 dilution of a
0.5 McFarland suspension

Test isolates
Imipenem disk

Described by Lee et al. CMI, 7, 88-102. 2001.
CRE Screening in LTCF
What to Use?

- 3 Belgian SNF & Rehabilitation Center
- Access screening methods for CPE
  - optimal method & site not known
  - swab – visible fecal staining best
  - broth enrichment not helpful
  - MacConkey agar helps-adequate # gnb
  - chromogenic agars no benefit ↑ incubation leads to gpc overgrowth

CRE Screening
Who to Consider?

- Patients at risk
  - Healthcare setting with high rate CPE
    - overnight stay last 6-12 months
  - Foreign countries with CPE
  - ICU patients
  - Transplant patients
  - Immunocompromised

CRE Screening
When to Screen?

- Screen contacts of known CRE (+) pts
  - most important if CPE (+) pts
  - contacts with epidemiological link
    - roommates
    - common HCW
    - wards

- Active surveillance
  - high CPE rates
  - outbreaks CRE
  - control measure

CRE Screening
What Sites to Screen?

• Patients
  – stool, rectum, peri-rectal most often
  – skin, wounds

• Environment
  – seems uncommon

### K. pneumoniae CRE Detection in 6 LTACHs

<table>
<thead>
<tr>
<th>Site</th>
<th>Positive Cultures (N=24)</th>
<th>Sensitivity% (95%C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Skin Sites</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>inguinal</td>
<td>19</td>
<td>79 (58-93)</td>
</tr>
<tr>
<td>axillary</td>
<td>18</td>
<td>75 (53-90)</td>
</tr>
<tr>
<td>upper back</td>
<td>6</td>
<td>25 (10-47)</td>
</tr>
<tr>
<td>antecubital fossa</td>
<td>6</td>
<td>25 (10-47)</td>
</tr>
<tr>
<td><strong>Non-Skin Sites</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rectal</td>
<td>21</td>
<td>88 (68-97)</td>
</tr>
<tr>
<td>urine</td>
<td>10</td>
<td>53 (29-76)</td>
</tr>
<tr>
<td>pharynx/trachea</td>
<td>10</td>
<td>42 (22-63)</td>
</tr>
<tr>
<td><strong>Combined Sites</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rectal &amp; inguinal</td>
<td>24</td>
<td>100 (86-100)</td>
</tr>
<tr>
<td>rectal &amp; axillary</td>
<td>23</td>
<td>96 (79-100)</td>
</tr>
<tr>
<td>axillary &amp; inguinal</td>
<td>22</td>
<td>92 (73-99)</td>
</tr>
</tbody>
</table>

Thurlow CJ et al. ICHE 2013;34:56-61
MDR A. Baumanii in LTCF

Significance

- Outbreaks MDR Acinetobacter reported
- LTCF-LTACH colonization (28-34%)
  - 50% (+) on hospital admission
  - ventilated residents
  - tracheostomy/sputum main site
  - environmental contamination ~10%
  - aerosolization?
  - combat injuries
- Mortality ~ 35%
  - BSI, pneumonia, UTI
A. Baumanii in LTCF Screening Issues

- Acinetobacter – 30 species
  - phenotype not helpful
  - some species not resistant or pathogens
  - A. calcoaceticus-baumannii complex
- Preliminary ID by fermentation (API 20E)
- Speciation difficult
  - MALDI-TOF
- MDR – resistant 3 or more classes
- Not all have carbapenemase
# A. baumannii Complex in LTCF Prevalence

<table>
<thead>
<tr>
<th>Author (Date)</th>
<th>N (%)</th>
<th>What</th>
<th>Where</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stephens (2007)</td>
<td>70/151 (46.4)</td>
<td>MDR</td>
<td>LTACH/Hosp Network (US)</td>
</tr>
<tr>
<td>Furuno (2008)</td>
<td>41/147 (28%)</td>
<td>Not MDR</td>
<td>LTCF (US)</td>
</tr>
<tr>
<td>Stengstock (2010)</td>
<td>153/280 (53)</td>
<td>MDR</td>
<td>17 LTCF (US)</td>
</tr>
<tr>
<td>Perez (2010)</td>
<td>8/39 (20.5)</td>
<td>CRAB OXA-23</td>
<td>LTCAH (US)</td>
</tr>
<tr>
<td>Mortensen (2014)</td>
<td>14/70 (20)</td>
<td>MDR (86%) CRAB (60%)</td>
<td>subacute + vents (US) LTCF</td>
</tr>
<tr>
<td>Mody (2015)</td>
<td>25/168 (14.9)</td>
<td>MDR CRAB</td>
<td>4 LTCF (US)</td>
</tr>
</tbody>
</table>
Screening for CRAB Microbiology Definitions

Screen for CRAB
• CHROMAcinetobacter
• MIC $\geq 8 \, \mu g/ml$
  – doripenem,
  – imipenem,
  – meropenem

Confirm enzyme (+)
• Modified Hodge Test
• PCR/molecular tests
  – PFGE
  – OXA-23, OXA-24/40
  – OXA-58
  – IMP, VIM, SIM

A. baumanii in LTCF
Who to Screen?
## Screening for CRAB
### What Sites?

<table>
<thead>
<tr>
<th>Sites from 129 ICU Patients</th>
<th>CRE colonized N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single</strong></td>
<td></td>
</tr>
<tr>
<td>tracheal aspirate</td>
<td>35 (27)</td>
</tr>
<tr>
<td>rectum</td>
<td>24 (19)</td>
</tr>
<tr>
<td>sternal skin</td>
<td>7 (5)</td>
</tr>
<tr>
<td>urine</td>
<td>4 (3)</td>
</tr>
<tr>
<td><strong>Detection CRE</strong></td>
<td></td>
</tr>
<tr>
<td>any 1 site</td>
<td>70 (54)</td>
</tr>
<tr>
<td>trachea &amp; rectum</td>
<td>97 (75)</td>
</tr>
<tr>
<td>trachea, rectum &amp; sternum</td>
<td>104 (80)</td>
</tr>
<tr>
<td>all 4 sites</td>
<td>108 (85)</td>
</tr>
</tbody>
</table>

MDRO GNB in LTCF
Summary

• MDRO-GPC get more publicity!
• MDRO-GNB an increasing problem
• Transfer resistance between GNB easy
• Confers resistance to all antibiotics
• Serious infections with high mortality
• Detection MDRO-GNB is not simple
• Impact on infection control resources