The CDC big three; challenges from the laboratory perspective

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Disclosures

– PHAC working groups
– Department of Health and Wellness
– Collaborative research grant with GSK for the SOS network and influenza vaccine effectiveness
Outline

• Describe the capabilities and challenges of novel tools used for the detection of carbapenemase-producing organisms.

• Contrast Clostridium difficile testing algorithms.
  – Are labs using the best strategy?

• Describe impact of current laboratory practices as it relates to GC
Call to Action

“is a snapshot of the complex problem of antibiotic resistance today and the potentially catastrophic consequences of inaction.”

• Urgent Threats
  – Significant risk
  – Limited treatment options

• Serious Threats
  – Reduced incidence or more treatment options

• Concerning Threats
Urgent Threats

*Clostridium difficile*

Carbapenem-resistant Enterobacteriaceae

Drug-resistant *Neisseria gonorrhoeae*
CLOSTRIDIUM DIFFICILE

250,000 INFECTIONS PER YEAR
14,000 DEATHS

$1,000,000,000 IN EXCESS MEDICAL COSTS PER YEAR

This bacteria is an immediate public health threat that requires urgent and aggressive action.

Kills normal flora

C diff
C.Diff in Canada

(PHAC)

Rate / 1000 patient days

Year


C.DI Rates

per 1,000 patient admissions

per 10,000 patient-days

Western

Central

Eastern

Overall

Rate / 1000 patient days

2007 2008 2009 2010 2011
Ideal Assay

- Want a rapid, accurate inexpensive test
- Tests of limited sensitivity lead to false negatives and potential for further spread and morbidity
- Tests of low specificity lead to unnecessary isolation (or cohorting that could increase risk of exposure) and treatment
- *No Single test fits these requirements*
<table>
<thead>
<tr>
<th>Assay</th>
<th>Pros</th>
<th>Cons</th>
<th>sens</th>
<th>Spec</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunoassay for Toxin A / B</td>
<td>• Rapid</td>
<td>• Lacks sensitivity</td>
<td>69-99% (as low as 38%)</td>
<td>92-100%</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>• Easy to use</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Not specific</td>
<td>88 – 100%</td>
<td>83-100%</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Positive needs confirmation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>glucose dehydrogenase</td>
<td>• Very high NPV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• batchable</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell Culture Cytotoxic assay</td>
<td>• Identifies presence of the toxin</td>
<td>• Takes 48 hrs for a negative</td>
<td>70-100</td>
<td>90-100</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Requires tissue culture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toxigenic culture</td>
<td>• “gold standard”</td>
<td>• Test takes upto 5 days</td>
<td>90-100</td>
<td>98-100</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Cumbersome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAAT</td>
<td>• Can be rapid</td>
<td>• Expensive</td>
<td>88-91%</td>
<td>96-97%</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>• Very sensitive</td>
<td>• Does not differentiate colonization</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Performance of NAAT: a Systemic Review
(Deshpande et al., 2011. Clin Infect Dis 53:e81-e90)

- Pooled sens – 90%
- Pooled spec – 96%
Multi-Step Algorithms

- Options:
  - NAAT alone
    - How do you confirm
    - What is the batch size
  - Screen with GDH
    - excellent NPV and EIA can be run daily
  - But requires confirmation
    - Confirm with Tox A/B EIA
    - confirm with CCCNA
    - Confirm with NAAT

How do Multi-step Algorithms Perform?

  - Prospective study 432 stool samples (72 pos – prevalence 16.7%)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>EIA only</th>
<th>GDH + EIA</th>
<th>GDH + EIA + cytotoxin</th>
<th>GDH + Xpert</th>
<th>Xpert only</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of specimens</td>
<td>432</td>
<td>432</td>
<td>431</td>
<td>432</td>
<td>428</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>58.3 (42/72)</td>
<td>55.6 (40/72)</td>
<td>83.1 (59/71)</td>
<td>86.1 (62/72)</td>
<td>94.4% (68/72)</td>
</tr>
<tr>
<td>Specificity</td>
<td>94.7 (341/360)</td>
<td>98.3 (354/360)</td>
<td>96.7 (348/360)</td>
<td>97.8 (352/360)</td>
<td>96.3 (343/356)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>90.7 (383/422)</td>
<td>91.2 (391/422)</td>
<td>92.4 (391/431)</td>
<td>95.9 (414/432)</td>
<td>96.9% (411/428)</td>
</tr>
<tr>
<td>PPV</td>
<td>68.9 (42/61)</td>
<td>87.0 (40/46)</td>
<td>83.1 (59/71)</td>
<td>88.6 (62/70)</td>
<td>84.0 (68/81)</td>
</tr>
<tr>
<td>NPV</td>
<td>91.9 (341/371)</td>
<td>91.7 (354/386)</td>
<td>96.7 (348/360)</td>
<td>97.2 (352/362)</td>
<td>98.8 (343/347)</td>
</tr>
</tbody>
</table>

  - Pediatrics n=150 (36% prevalence)

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDH</td>
<td>87</td>
<td>97</td>
</tr>
<tr>
<td>Toxin A/B</td>
<td>29</td>
<td>100</td>
</tr>
<tr>
<td>Illumigen</td>
<td>89</td>
<td>100</td>
</tr>
<tr>
<td>GeneOhm</td>
<td>89</td>
<td>99</td>
</tr>
<tr>
<td>CCNA</td>
<td>33</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 2: Statistics**

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDH + AB</td>
<td>28</td>
<td>97</td>
<td>81</td>
<td>75</td>
</tr>
<tr>
<td>GDH + Illumigen</td>
<td>85</td>
<td>100</td>
<td>100</td>
<td>94</td>
</tr>
<tr>
<td>GDH + GeneOhm</td>
<td>83</td>
<td>99</td>
<td>97</td>
<td>93</td>
</tr>
<tr>
<td>CCFA + CCNA</td>
<td>30</td>
<td>100</td>
<td>100</td>
<td>76</td>
</tr>
</tbody>
</table>
Which is Cost Effective

• **Bartsch et al., 2015** *(Clin Microbiol Infec 21:77e)*
  - Modeled the cost of different algorithms
    • Tox A/B
    • GDH + Tox A/B
    • NAAT
    • GDH/TOX A/B + NAAT
  - Factored in isolation costs, treatment delays, inappropriate treatment, potential for secondary cases
GDH-Tox A/B + NAAT is Cost Effective

- GDH/ToxA/B + NAAT also had fewest unnecessary bed delays
What about antimicrobial resistance?
**GONORRHEA**

**Female**

**Male**

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**Drug-Resistant Neisseria Gonorrhoeae**

*This bacteria is an immediate public health threat that requires urgent and aggressive action.*

**Threat Level: Urgent**

**Drug-Resistant Gonorrhea Infections**

- 246,000

**Resistance to Tetracycline**

- 188,600

**Reduced Susceptibility to Cefixime**

- 11,480

**Reduced Susceptibility to Ceftriaxone**

- 3,280

**Reduced Susceptibility to Azithromycin**

- 2,460

**Gonococcal Infections Per Year**

- 820,000
A) Penicillin first used to treat gonorrhoea infections
B) Penicillinase-producing *N. gonorrhoeae* (PPNG) reported in Africa, South-East Asia, England and the USA
C) PPNG with high level resistance to spectinomycin reported in the Philippines
D) PPNG containing the R-plasmid encoding high-level tetracycline resistance reported in the USA
E) Penicillin and tetracycline are no longer recommended for gonorrhoea treatment
F) Fluoroquinolones recommended to treat gonorrhoea due to high PPNG in the population
G) Fluoroquinolone-resistant *N. gonorrhoeae* detected in Hong Kong and the Philippines
H) Fluoroquinolones no longer recommended to treat gonorrhoea; WHO & CDC now recommend oral cephalosporins
I) Cephalosporin-resistant *N. gonorrhoeae* discovered in Japan and Australia
J) Gonorrhoea with reduced susceptibility to oral cephalosporins detected in the USA
K) Ceftriaxone-resistant *N. gonorrhoeae* found in Japan
L) WHO & CDC revise treatment guidelines to use azithromycin and ceftriaxone combination therapy

Buono et al., 2015 J Antimicrob Chemother 70:374-381
• Selection Pressure allows for Horizontal gene transfer from non GC Neisseria particularly in the throat
• WHO recommends only drugs with >95% efficacy be used as first line rx
• Ideally we could have individualized treatment to ensure narrowest spectrum used
Resistance to Azithromycin and Cephalosporins is a global Problem

Buono et al., 2015 J Antimicrob Chemother 70:374-381

- Resistance to fluorquinolones is global
GC Resistance Rates in Canada are Increasing

Source: Irene Martin NML (National Surveillance of Antimicrobial Susceptibilities of Neisseria gonorrhoeae Annual Summary 2013)
Canadian Data
Molecular testing more common

Source: Irene Martin NML (National Surveillance of Antimicrobial Susceptibilities of Neisseria gonorrhoeae Annual Summary 2013)
Resistance Detection Methods

- Agar dilution methods are CLSI recommended standard
  - Time consuming
  - Labor intensive
- E test / disc diffusion have been used

Images:
- Prajna Sharma and Vishwanath 2012
- www.biomerieux-diagnostics.com/etest
- wikipedia
Molecular identification of resistance

• No commercially available method

• In house methods are available
  – Quinolone – gyrA and parC
  – Azithromycin - 23s rRNA and mtrR mutations
  – Cephalosporin – mosaic penA gene
Challenge

- Rapid evolution
- NAAT requires a known target
- Acquisition of plasmid and chromosomally mediated resistance
- Variability between penA alleles can lead to different MICs
- Wont pick up “unknown” mechanisms like phenotypic testing
- Mechanisms are shared with commensal organisms
Challenges

Multiplexing is possible but not all mechanisms are well characterized

**NAAT Good**
- TEM-1 [penicillin]
- Tet(M) [tetracycline]
- parC/gyrA [quinolone]
- mtrR [azithromycin]

**NAAT not so good**
- Cephalosporins
  - penA – high sequence variability
- Azithromycin
  - 23SrRNA allele availability
Data Starting to Emerge for NAAT from Residual Specimens

• Nicol et al., 2015 (Sex Transm Infect 91:91-93)
  – Three real time assays to detect gyrA, PPNG, and sequence for mosaic penA on residual specimen from Cobas 4800 CT/GC assay
  – 94% of specimens had enough DNA for amplification
Culture Based Surveillance

Canada - Enhanced surveillance of antimicrobial-resistant gonorrhea program (ESAG)

- NS has 3 clinics and callbacks,
- MB has 5 engaged service
- AB has 2 STI clinics (Edmonton and Calgary)
- Other P/Ts are showing interest but not fully participating as yet.

US - Gonoccoccal Isolate Surveillance Project (GISP)
26 sites
• Latest Canadian Data
Global Spread

**THE RESISTANCE MOVEMENT**
Carbapenem-resistant Enterobacteriaceae have been on the move since at least 1996.

1. **2000**: Analysis of a 1996 sample from a North Carolinian hospital finds infectious Klebsiella pneumoniae carrying a gene called KPC that confers resistance to carbapenems.
2. **2003**: KPC-positive bacteria are found spreading rapidly through hospitals across New York City. By 2007, 21% of Klebsiella in the city carry the resistance gene.
3. **2005**: KPC-positive bacteria make their way from New York to several other countries, including Israel. From Israel, the bacteria travel to Italy, Colombia, the United Kingdom and Sweden.
4. **2008**: Doctors in Sweden find a new carbapenem-resistance gene, NDM. Traced back to India, NDM-positive bacteria have moved quickly.

Carbepenem Resistance - Global Spread
### CRE

Variability in Mechanisms

1. **A beta lactamase with a Porin mutation**
2. **Specific Carbapenemase enzymes**

<table>
<thead>
<tr>
<th>Enzyme Class / Characteristics</th>
<th>Different Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class A beta lactamase enzymes</td>
<td><strong>KPC, SME, IMI, NMC, GES</strong></td>
</tr>
<tr>
<td>• Hydrolyze all beta lactams</td>
<td></td>
</tr>
<tr>
<td>• Inhibited by boronic acid and partially by calvulanic acid</td>
<td></td>
</tr>
<tr>
<td>Class B beta lactamase enzymes</td>
<td>Often named by place of origin</td>
</tr>
<tr>
<td>• Highest carbapenemase activity</td>
<td><strong>NDM, IMP, VIM, GIM, SPM, SIM</strong></td>
</tr>
<tr>
<td>• Generally only spare monbactam</td>
<td></td>
</tr>
<tr>
<td>• Not inhibited by BL inhibitors</td>
<td></td>
</tr>
<tr>
<td>• Required Zinc</td>
<td></td>
</tr>
<tr>
<td>Class D beta lactamase enzymes</td>
<td><strong>OXA-48, OXA-181</strong></td>
</tr>
<tr>
<td>• Spares ceftazidime</td>
<td></td>
</tr>
<tr>
<td>• Often require another enzyme (ESBL) for complete resistance</td>
<td>Nordman et al., 2012 Clin Microbiol Infect 18: 432-438</td>
</tr>
</tbody>
</table>
CPE in Canada: CPHLN Data

Source: Mike Mulvey, NML
CRE Detection

- Automated systems may not always be able to detect CRE

- CLSI have lowered breakpoints for better detection

<table>
<thead>
<tr>
<th>MIC (mg/L)</th>
<th>Imipenem</th>
<th>Meropenem</th>
<th>Ertapenem</th>
</tr>
</thead>
<tbody>
<tr>
<td>KPC</td>
<td>0.5 to &gt;32</td>
<td>0.5 to &gt;32</td>
<td>0.5 to &gt;32</td>
</tr>
<tr>
<td>IMP/VIM/NDM</td>
<td>0.5 to &gt;32</td>
<td>0.5 to &gt;64</td>
<td>0.38 to &gt;32</td>
</tr>
<tr>
<td>OXA-48/OXA-181</td>
<td>0.25 to 64</td>
<td>0.38 to 64</td>
<td>0.38 to &gt;32</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CLSI</th>
<th>S (≤)</th>
<th>R (≥)</th>
<th>EUCAST</th>
<th>S (≤)</th>
<th>R (≥)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Meropenem</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Ertapenem</td>
<td>0.5</td>
<td>2</td>
<td>0.5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Doripenem</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

Nordman et al., 2012 Clin Microbiol Infect 18: 432-438

Good Candidate
May lack specificity
Is Confirmation Necessary?
It Depends

• CLSI does not recommend confirmation
  – Breakpoints all that is necessary for treatment decisions
  – But not a lot of treatment data out there
  – Some carbapenemases are susceptible or intermediate to carbapenems (OXAs)
    • How do you screen for theses
  – Only necessary for epidemiology and infection control reasons
The Ideal System

- Rapid (same day results)
- Sensitive and Specific
- Easy to perform
- Easy to interpret results
- Identify the different resistance mechanisms
- Identify the different genetic variants
- In expensive
CRE Confirmation

- Phenotypic tests - None has 100% sensitivity or specificity
- Not good for OXA types

- Modified Hodge Test
  - Good for KPC and OXA
  - Less for NDM
  - Lacks specificity
  - Time consuming
  - Subjective

- Addition of inhibitors
- EDTA / boronic acid

Source: Janet Hindle CLSI webinar update Feb 2015
CARBA NP Test

- Mix suspect colony
- Decrease in pH from hydrolysis of carbapenem
- Reagent must be fresh and takes time to prepare
- False negative for OXA
- Can give invalid results (subjective)

Source: Janet Hindlr CLSI webinar update Feb 2015
MALDI-TOF Identification of CRE

Washed pellet

ertapenem or meropenem

MALDI-TOF Identification of CRE

- **Carvalhaes et al., 2014.** J Antimicrob Chemother 69: 2132-2136
  - Direct detection of CRE from 100 randomly selected blood cultures
  - 21 isolates were CRE
  - All KPCs and one SM1 detected after 4 hours of incubation
  - 3/11 OXA required testing of bacterial colonies in detect carbapenemase activity

  - Addition of NH4HCO3 improved detection of OXA-48
• **MALDI TOF**
  – Can detect CRE independent of the enzyme produced, including novel enzymes
  – rapid
  – Requires molecular to characterize
Molecular Detection (NAAT)

- Biofire (FDA approved)
  - KPC
- Nanosphere (FDA approved)
  - KPC, NDM, OXA, IMP, VIM
- NucliSENS EasyQ VKPC
- Cepheid
  - KPC, NDM, OXA-48, IMP-1, VIM
- BD Max
  - KPC, NDM, OXA-48
- Check-Points
  - KPC, NDM, OXA-48, IMP, VIM
- Amplex - Hyperplex Superbug ID
  - all variants of VIM, IMP, KPC, OXA-48 NDM-1

- Expensive
- Requires molecular expertise
- Sensitivity dependant on amount of DNA
  - may require growth first
- Need to target the gene

Source: Janet Hindle CLSI webinar update Feb 2015
CRE Screening

- Lots of questions that depend on local epidemiology
  - Who, how often etc
- Stools/rectal swabs most common specimen
- None will detect the type of carbapenemase
- Broth enrichment step may increase KPC detection (delays TAT)
- Direct to screening media
  - CRE specific
  - ESBL surrogate screening

www.chromagar.com/clinical-microbiology-chromagar-kpc-focus-on-kpc-resistance-32.html
• Next gen / whole genome sequencing
• Microarray
• MALDI –ToF MS
  – Detect degradation products
# Methods of Detection

<table>
<thead>
<tr>
<th></th>
<th>Modified Hodge Test</th>
<th>Carba NP</th>
<th>Molecular Detection</th>
<th>MALDI-TOF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strengths</strong></td>
<td>Relatively simple</td>
<td>rapid</td>
<td>Determines type of carbapenemase</td>
<td>Rapid Inexpensive Detects variety of MBL</td>
</tr>
<tr>
<td><strong>Weaknesses</strong></td>
<td>• Can be subjective • False positives due to other mechanisms (ESBL or AMPC + porin mutation) • Some false negatives (NDM – can add zinc)</td>
<td>• Can give invalid results (subjective) • Reagent preparation takes time • False negative for OXA</td>
<td>• Expensive • Requires molecular expertise • Need to target the gene (if it is not included it will not be detected)</td>
<td>• Generate own spectral library • Requires molecular differentiation of types of resistance</td>
</tr>
</tbody>
</table>
Potential Algorithm

Hrabak et al., 2014 Clin Micrbiol Infec 20:839-853
Conclusion

• Resistance is a problem
• Many different options for detecting resistance
• Must be tailored to your local context
Questions