Phenotypic detection of ESBLs and carbapenemases

Standardized susceptibility testing – residential workshop 2019

Katie Hopkins, PhD
Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit
Katie.hopkins@phe.gov.uk

©Crown copyright
Gram-negative resistance *ad infinitum*

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Established problems</th>
<th>Emerging threats</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. faecium</em></td>
<td>VRE, HLGR, Amp-R</td>
<td>Lin-R, Dap-R, Tig-R</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>MRSA (ha/ca)</td>
<td>Van-R, Lin-R, Dap-R</td>
</tr>
<tr>
<td><em>Klebsiella</em></td>
<td>ESBLs</td>
<td><strong>Carbapenemases</strong>, Col-R</td>
</tr>
<tr>
<td><em>Acinetobacter</em></td>
<td>MDR, <strong>Carbapenemases</strong></td>
<td>Tig-R, Col-R</td>
</tr>
<tr>
<td><em>Pseudomonas</em></td>
<td>MDR, except Col</td>
<td><strong>Carbapenemases</strong>, Col-R</td>
</tr>
<tr>
<td><em>Enterobacter</em></td>
<td>AmpC, <strong>ESBLs</strong></td>
<td>Carba-R, <strong>Carbapenemases</strong></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Cip-R, <strong>ESBLs</strong></td>
<td><strong>Carbapenemases</strong></td>
</tr>
</tbody>
</table>

- 5 of 7 ‘ESKAPEEs’ are Gram-negative
- Increasing reliance on carbapenems
- detection of ESBLs and carbapenemases required for IPC and public health

*Figure 3.7 Consumption of carbapenem, by prescriber location, expressed as DDD per 1000 inhabitants per day, England, 2010-2015* [ESPAUR report, 2016]
EARS-Net data: Proportion of 3GC-R invasive *E. coli* and *K. pneumoniae*, 2017

**E. coli**
- 87.4% ESBL-positive

**K. pneumoniae**
- 87.8% ESBL-positive
Extended-spectrum β-lactamases

• TEM and SHV mutants
  • the original ESBLs
• CTX-Ms
  • Most clinically relevant
• Occur mostly in Enterobacterales, rarely in NFs
• Minor types, *e.g.* OXA, VEB, PER and GES
  • Rare in the UK and rare in Enterobacterales
• Mobile genes
ESBL resistance profiles

• Resistant to most penicillins and cephalosporins, e.g. cefuroxime, cefotaxime, ceftazidime and ceftriaxone, also aztreonam.

• Not cephamycins (cefoxitin and cefotetan) or carbapenems

• Inhibited by clavulanic acid, sulbactam and tazobactam

• Level of expression and presence of other mechanisms leads to variety of resistance phenotypes
Need to distinguish from:

- Hyperproduced chromosomal AmpC, especially in *Enterobacter* spp.
- Plasmid-mediated AmpC, *e.g.* CMY types in *Klebsiella* spp., *E. coli*, etc.
- Hyperproduced K1 chromosomal β-lactamase in *K. oxytoca*.
- Metallo- (IMP, VIM, NDM) and non-metallo (KPC and OXA-48) carbapenemases.
Detecting ESBL producers

1. Screen for non-susceptibility to an indicator cephalosporin(s)

2. Do confirmatory test based on cephalosporin/clavulanate synergy on those found non-susceptible
## ESBL screening methods for Enterobacterales

<table>
<thead>
<tr>
<th>Method</th>
<th>Antibiotic</th>
<th>Conduct ESBL-testing if</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broth or agar dilution¹</td>
<td>Cefotaxime/ceftriaxone AND Ceftazidime</td>
<td>MIC &gt;1 mg/L for either agent</td>
</tr>
<tr>
<td></td>
<td>Cefpodoxime</td>
<td>MIC &gt;1 mg/L</td>
</tr>
<tr>
<td>Disk diffusion¹</td>
<td>Cefotaxime (5 μg) or Ceftriaxone (30 μg) AND Ceftazidime (10 μg)</td>
<td>Inhibition zone &lt; 21 mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibition zone &lt; 23 mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibition zone &lt; 22 mm</td>
</tr>
<tr>
<td></td>
<td>Cefpodoxime (10 μg)</td>
<td>Inhibition zone &lt; 21 mm</td>
</tr>
</tbody>
</table>

¹ With all methods either test cefotaxime or ceftriaxone AND ceftazidime OR cefpodoxime can be tested alone.
Which cephalosporin?

Ideal indicator cephalosporin is one to which all ESBLs confer resistance, even when production is scanty

- TEM and SHV – obvious CAZ-R, variable CTX-R
- CTX-M – obvious CTX-R, variable CAZ-R
- All ESBLs – CPD-R; but low-level CPD-R common in absence of ESBL (Hope et al. JAC [2007])
- CXM, LEX or RAD unreliable indicators for ESBLs and not recommended
Efficacy of screening methods for detection of cephalosporin-resistant Enterobacterales

<table>
<thead>
<tr>
<th>Resistance</th>
<th>Cephalosporin-S/borderline-R and no clear resistance mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefpodoxime</td>
<td>69%</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>18%</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>15%</td>
</tr>
<tr>
<td>Cefotaxime AND ceftazidime</td>
<td>5%</td>
</tr>
</tbody>
</table>

Hope et al. 2007
Efficacy of screening methods for detection of cephalosporin-resistant Enterobacterales

<table>
<thead>
<tr>
<th>Resistance</th>
<th>Cephalosporin-S/borderline resistance method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefpodoxime</td>
<td>69%</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>18%</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>15%</td>
</tr>
<tr>
<td>Cefotaxime AND ceftazidime</td>
<td>5%</td>
</tr>
</tbody>
</table>

Hope et al. 2007

- Cefpodoxime most sensitive individual indicator but lacks specificity
- Combination of cefotaxime/ceftriaxone AND ceftazidime allows better specificity
EUCAST algorithm for phenotypic detection of ESBLs

ESBL SCREENING:
I/R to one or both of cefotaxime and ceftazidime (or cefpodoxime R)

Yes

No ESBL

Species dependent ESBL confirmation

Group 1:
E. coli, Klebsiella spp., P. mirabilis, Salmonella spp., Shigella spp.

ESBL CONFIRMATION¹
with ceftazidime and cefotaxime +/- clavulanic acid

Negative: No ESBL
Indeterminate
Positive: ESBL

Group 2:
Enterobacteriaceae with inducible chromosomal AmpC:
Enterobacter spp., Citrobacter freundii, Morganella morganii, Providencia stuartii, Serratia spp., Hafnia alvei.

ESBL CONFIRMATION
with cefepime +/- clavulanic acid

Negative: no ESBL
Indeterminate²
Positive: ESBL

¹If cefoxitin has been tested and has an MIC >8 mg/L, perform cefepime +/- clavulanic acid confirmation test
Confirmation tests I: combination disk test (CDT)

- ≥5mm increase in zones around cephalosporin disk vs. cephalosporin + clavulanate
- Cefepime/clav or AmpC inhibitor to detect AmpC activity
Confirmation tests II: double-disk synergy test (DDST)

- Expansion of indicator ceph inhibition zone towards amox/clav disk
- Disk spacing critical - may be reduced or expanded for strains with high or low levels of resistance
- Either use cefepime disc, or add clox to agar for AmpC producers
Confirmation tests III: gradient strips

- ≥8-fold reduction in MIC = ESBL+
- ‘phantom’ zone = ESBL+
- Deformed ellipse = ESBL+
- “non-determinable”. Could be due to AmpC activity, therefore test cefepime/clav or cefotetan/clox
## Confirmatory tests – pros and cons

<table>
<thead>
<tr>
<th></th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Double disc test</td>
<td>cheap</td>
<td>Best disc spacing varies with strain</td>
</tr>
<tr>
<td>Combination disc test</td>
<td>Cheap; do not require critical spacing; sensitive and specific</td>
<td>Batch variation – negative control critical</td>
</tr>
<tr>
<td>Gradient strip</td>
<td>Sensitive; accurate; internally controlled</td>
<td>More expensive</td>
</tr>
</tbody>
</table>

- Performance of confirmation methods differs in different studies
Special considerations in interpretation

• *Klebsiella oxytoca* hyper-producing K1 may give false +ves in ESBL confirmation tests using cefotaxime or cefepime

• Similar phenotype may be seen with *Proteus vulgaris*, *Proteus penneri*, *Citrobacter koseri* (and related spp.) and *Kluyvera* spp. due to inhibition of chromosomal \(\beta\)-lactamase by clavulanate

• Hyper-production of TEM-1, SHV-1 or OXA-1-like broad-spectrum \(\beta\)-lactamases + altered impermeability

• Will not detect OXA ESBLs
Bacteria NOT to screen for ESBLs

- ESBL tests were not developed for *Acinetobacter* spp. and *P. aeruginosa*.

- *Acinetobacter* spp. often susceptible to clavulanate alone

- ESBLs occur in *P. aeruginosa* but are not common and should not be sought routinely
  - Consider in ceftolozane/tazobactam resistant isolates

- *Stenotrophomonas maltophilia*
  - Positive result due to inhibition of L-2 chromosomal β-lactamase ubiquitous in the species
Detection of carbapenemases
EARS-Net data: Proportion of carbapenem non-susceptible (R+I) invasive *E. coli* and *K. pneumoniae*, 2017

- **Most countries <1% non-susceptibility in *E. coli***
- **7 countries reported >5% non-susceptibility in *K. pneumoniae* as judged by surveys**
- **2018 data: blood isolates account for only 3% CPE in UK** [ESPAUR Report, 2019]
Overall European situation regarding occurrence of CPE using an epidemiological scale of nationwide expansion

N.B. epidemiological stage might not represent the true extent of the spread of CPE as it is a subjective judgment by national experts

Albiger et al. 2016
The “big 5” carbapenemases

- **KPC** (44 variants)
- **NDM** (28 variants)
- **OXA-48-like** (>10 variants)
- **VIM** (66 variants)
- **IMP** (80 variants)
Carbapenemases come in many varieties

<table>
<thead>
<tr>
<th>Enzyme Type</th>
<th>Classification by Ambler Class</th>
<th>Activity Spectrum</th>
<th>Organism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KPC</td>
<td>A</td>
<td>All β-lactams</td>
<td>Enterobacterales, P. aeruginosa, A. baumannii</td>
</tr>
<tr>
<td>SME</td>
<td>A</td>
<td>Carbapenems and aztreonam, but not 3rd/4th G cephalosporins</td>
<td>S. marcescens</td>
</tr>
<tr>
<td>NMC–A IMI</td>
<td>A</td>
<td>Carbapenems and aztreonam, but not 3rd/4th G cephalosporins</td>
<td>Enterobacter species and rarely other Enterobacterales</td>
</tr>
<tr>
<td>GES</td>
<td>A</td>
<td>Imipenem and 3rd/4th cephalosporins</td>
<td>P. aeruginosa and Enterobacterales</td>
</tr>
<tr>
<td>IMP VIM NDM</td>
<td>B (metallo-β-lactamases)</td>
<td>All β-lactams except monobactams (aztreonam)</td>
<td>Pseudomonas species Acinetobacter species Enterobacterales</td>
</tr>
<tr>
<td>DIM, SPM, GIM, SIM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIM, TMB, KHM (not detected in the UK yet)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OXA</td>
<td>D</td>
<td>Weakly active against carbapenems</td>
<td>A. baumannii, Enterobacterales and rarely P. aeruginosa</td>
</tr>
</tbody>
</table>
CPE in the UK as referred to AMRHAI

Early cases often imported

“big 5” account for >99% of confirmed CPE in 2018

AMRHAI, unpublished data
Phenotypic detection of ESBLs and carbapenemases - BSAC workshop 2019

Logan & Weinstein 2017
Resistance profiles

- Hydrolyse penicillins, in most cases cephalosporins, and to various degrees carbapenems and aztreonam
- OXA-48-like carbapenemase producers may be fully susceptible to cephalosporins (unless ESBL or AmpC present)
- Metallo-β-lactamase producers are susceptible to aztreonam (unless ESBL or AmpC present)
- Level of expression and association with other β-lactamases, efflux and/or permeability result in a range of resistance phenotypes
- Frequently multi-drug resistant
You don’t need an acquired carbapenemase for carbapenem resistance

- **Intrinsic resistance**
  - Non-fermenters: ertapenem only
  - *Serratia* spp. and Proteeeae: poor susceptibility to imipenem

- **Intrinsic carbapenemases**
  - *Stenotrophomonas maltophilia*, *Aeromonas* spp. and ‘chryseobacteria’
  - *Acinetobacter baumannii*

- **Loss of porin and/or efflux**
  - *Pseudomonas aeruginosa*

- **ESBL or AmpC + impermeability**
  - Mostly *Enterobacter* and *Klebsiella*, rarely *E. coli*
The problem with spotting carbapenemase producers

• Not all carbapenemase producers are resistant to the carbapenems
• ESBL/AmpC + porin loss = not transferable, may have fitness cost → rarely cause outbreaks
Which carbapenem?

- Carbapenemase-producers cannot reliably be detected by CBPs.
- EUCAST have defined screening cut-offs

<table>
<thead>
<tr>
<th>MIC (mg/L)</th>
<th>Zone diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S/I breakpoint</td>
<td>screening cut-off</td>
</tr>
<tr>
<td>MEM</td>
<td>≤2</td>
</tr>
<tr>
<td>ERT</td>
<td>≤0.5</td>
</tr>
</tbody>
</table>

Tängdén & Giske (2015)
EUCAST algorithm for carbapenemase detection

1 Combination of several carbapenemases can also contribute to no synergy – e.g. MBL and KPC in combination. Molecular testing is usually necessary in such cases.

2 High-level temocillin resistance (>128 mg/L, zone diameter <11 mm) is a phenotypic marker of OXA-48.
Confirmation tests

Double disc synergy test

Combination disc tests

Gradient strip tests

- Four discs are tested
  - Carbapenem only
  - Carbapenem + MβL inhibitor
  - Carbapenem + KPC inhibitor
  - Carbapenem + AmpC inhibitor
inhibitor-based tests: pros and cons

Pros:
• Relatively cheap and easy to perform
• Most extensively evaluated, therefore recommended by EUCAST for labs without special expertise in $\beta$-lactamase detection.

Cons:
• Some MBL inhibitors act non-specifically (especially EDTA)
• May lack sensitivity if carbapenemase expressed at low level
• Difficult to interpret when multiple resistance mechanisms present
• No inhibitors for OXA-48
• Overnight incubation required
Modified Hodge test: an oldie but NOT a goldie

• Testing with ERT, IMI, and MEM discs gives maximum sensitivity
• Lacks specificity (AmpC + porin loss…)
  • cloxacillin to inhibit AmpC
• Time-consuming to set up
• Requires overnight incubation
• Results are subjective

*Not recommended by EUCAST*
Carbapenem Inactivation Method (CIM)

[Van der Zwaluw et al. 2015]

- Suspend full loop of bacteria in H₂O
- Add 10 μg meropenem disk
- Incubate for 2 hours 35°C
- Place on Mueller Hinton agar inoculated with E. coli ATCC 25922
- Incubate for at least 6 hours 35°C
- Read presence or absence of inhibition zone

+ Carbapenemase activity

- No carbapenemase activity

- Variable performance
- Requires overnight incubation
Cleavage of β-lactam ring by carbapenemases

Hydrolysis: +18 Da

Decarboxylation: -44 Da

Phenol red
Bromophenol blue

pH change

Phenol red
Bromophenol blue
Detecting hydrolysis: colorimetric tests

- CarbaNP and Blue-Carba test
  - ‘in-house’ (Nordmann et al. [2012], Dortet et al. [2012], Pires et al. [2013])
  - commercial
- β CARBA test
  - Chromogenic substrate
  - Commercial only
- Results ≤2 hrs
- Reported good sensitivity and specificity
- Subjective/non-interpretables
- False-negatives with mucoid strains, some carbapenemases
- Check compatibility of primary culture medium with test
Detecting hydrolysis: MALDI-ToF

- Bacterial isolates + clinical specimens (blood, urine)
- No standardized ‘in house’ protocols and need to change MALDI-ToF settings
- Issues with detecting some carbapenemases
- Bruker MBT STAR®-Carba IVD kit and software [Rapp et al. 2018; Dortet et al. 2018]
Detecting carbapenemase antigens: immunochromatographic tests

- Coris RESIST-4: KPC, OXA-48, NDM and VIM
- NG-Test CARBA 5: KPC, OXA-48, NDM, VIM and IMP
- Result <15 mins
- Some issues with false-negatives [Saleh et al. 2018; Hopkins et al. 2018]
Detecting carbapenemase antigens: immunochromatographic tests

- Coris RESIST-4: KPC, OXA-48, NDM, VIM
- NG-Test CARBA 5: KPC, OXA-48, NDM, VIM and IMP
- Result <15 mins
- Excellent sensitivity/specificity from bacterial colonies [Wareham & Abdul Momin, 2017, Boutal et al., 2018]
- Some issues with false-negatives [Saleh et al., 2018; Hopkins et al., 2018]

Screen your carbapenemase producer for the ‘big 4’ with an immunochromatographic test (or PCR) before referring to AMRHAI and we waive our PCR charge for NHS labs
Summary

• EUCAST has developed practical guidance on detection of specific resistance mechanisms of clinical and/or epidemiological importance

• Following AST inhibitor-based confirmation methods can aid detection of ESBLs and carbapenemases

• Most require overnight incubation

• Faster testing using immunoassays, MALDI-ToF and CarbaNP

  • Testing direct from blood culture or urine → faster implementation of IPC measures
Further guidance

Email: amrhai@phe.gov.uk

http://www.eucast.org/resistance_mechanisms/

https://www.gov.uk/government/collections/standards-for-microbiology-investigations-smi

For information on commercial assays (PCR/LFIA) for detection of carbapenemases: