Multidrug-resistant Gram-negative infections
Laboratory diagnosis

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• Multidrug resistance emerging in Gram-negatives at an alarming rate
  • Extended-spectrum β-lactams and carbapenems
  • Usually combined with resistance to non-β-lactams

• Prompt detection essential
  • Help guide patient treatment
  • Infection prevention and control
  • Plasmid-borne → high rate of transmissibility

• Detection a challenge
  • Few standardised methods
  • Diverse resistance mechanisms

Antibiotic susceptibility testing

• Quantitative methods (MIC, mg/L)
  - agar or broth dilution
  - gradient strips (Etest, MICE)

• Qualitative methods (S/I/R)
  - disk diffusion
  - agar incorporation breakpoint method

• Automated methods

• Data meaningless unless interpretative criteria applied
  - MIC and zone diameter breakpoints indicate likelihood of therapeutic success (S) or failure (R) of antibiotic treatment based on microbiological findings

Adding value to AST...

• Interpretative reading
  - Infer mechanisms from susceptibility patterns (antibiograms)
  - Recognise grossly unusual
  - Edit susceptibilities / identify further drugs to test
  - Tentative surveillance of resistance mechanisms

• Requires isolates to be identified accurately and tested against large batteries of different antibiotics +/- inhibitors
  • It’s not an exact science
    - Multiple mechanisms can lead to confusing/misleading patterns
    - There are always exceptions and anomalies

The problem with spotting carbapenemase producers

Wild-type

Carbapenemase

ESBL / AmpC > porin loss or true carbapenemase?

0.5
16
Carbapenem MIC

- Human experts, subjective - computer algorithms, poor specificity
- "Relative ease": E. coli > K. pneumoniae spp. >> Enterobacter spp.
- Requires isolates to be identified accurately for correct interpretation of mechanisms

Supplemental tests for mechanisms

• Extended-spectrum β-lactamase (ESBL, AmpC)
• Carbapenemase

- Human experts, subjective
- Requires isolates to be identified accurately

A Webber Training Teleclass
www.webbertraining.com
Colorimetric assay: Carba-NP test

- Detection of carbapenemase activity in Enterobacteriaceae and Pseudomonas aeruginosa
- Based on hydrolysis of β-lactam ring of imipenem
- Use of inhibitors → ID of carbapenemase class
- Early detection: <3hrs
- 100% sensitivity
- 100% specificity
- Difficulty if more than one carbapenemase present
- Needs further evaluation by other labs

MALDI-TOF MS

- Positive evaluations for detection of resistance to carbapenem and other β-lactams
- No false-positives or false-negatives
- Potential for detection of other resistance mechanisms if metabolism of antibiotic occurs

DNA-based testing: PCR

- Identifies genes in 2.5 – 4 hrs directly from clinical specimens
- Further evaluations required: issue with detection of diverse IMP genes?

PCR – ELISA: Hyplex® assays

- Multiple targets (TEM, SHV, CTX-M and OXA) or OXA carbapenemases (OXA-23, -40 and -58)
- Can differentiate between non-ESBL and ESBL TEM and SHV
- Assay time 6hr (but req. pure DNA)
- Positive evaluations in:
  - UK (Woodford et al. 2011), France (Baum et al. 2011), USA (Endimiani et al. 2010) and Netherlands (Cohen Stuart et al. 2010)

Chips with everything...

- >100 targets per test:
  - Species identification
  - Resistance genes
  - Virulence genes
  - Epidemicity predictors
  - Strain-specific markers

Check-MDR arrays

- KPC, OXA-48, IMP, VIM, NDM
- Identifies AmpC and CTX-M ESBLs to group level (and beyond…)
- Can differentiate between non-ESBL and ESBL TEM and SHV
- Assay time 8hr (but req. pure DNA)
- Positive evaluations in:
  - UK (Woodford et al. 2011), France (Baum et al. 2011), USA (Endimiani et al. 2010) and Netherlands (Cohen Stuart et al. 2010)
Is there a future for phenotypic AST?

- Rapid
  - faster establishment of appropriate antibiotic therapy
- Confirm precise resistance mechanisms
  - sort out ambiguous phenotypic results
  - good for low-level resistance
  - inform local epidemiology
- Potential for automation

"...use of molecular methods to define the presence or absence of resistance determinants may represent an alternative to phenotypic susceptibility testing..."


Summary

- MDR Gram -ves present an increasing threat to antibiotic therapy
- Interpretative reading can infer major resistance mechanisms
- Phenotypic and genotypic assays ↓ time to confirm resistance
- Platforms becoming more user-friendly
  - MALDI-TOF, commercial RT-PCR assays, NGS...
  - "added value": one platform/assay = multi-purpose
- Confirmation of resistance by diagnostic rather than reference lab
- Confirmation of susceptibility must remain the prime criterion for antibiotic therapy

Molecular detection: the inherent problem

- Molecular methods only detect known mechanisms
  - only as good as available sequence data
  - resistant isolates with known genes identified
    - and new variants, if sufficient homology
    - can't base treatment on a negative molecular result
- Detection not necessarily an accurate predictor of therapeutic failure
  - false-resistance (unexpressed/partial genes)
- Susceptibility must always be confirmed
  - May never (?) replace cheap phenotypic methods