MDRGN – Laboratory Diagnosis
Dr. Katie Hopkins, PhD, HPA Microbiology Services, UK
Broadcast live from the HIS/FIS conjoint conference www.hisconference.org.uk

Multidrug-resistant Gram-negative infections

Laboratory diagnosis

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Challenges

•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 (Etests, 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

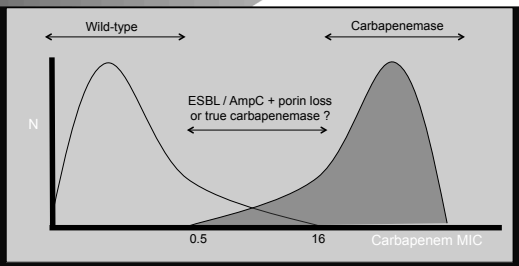
Adding value to AST…

•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

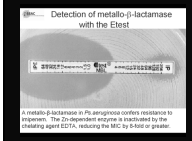
•it's not an exact science
- Multiple mechanisms can lead to confusing/misleading patterns
- There are always exceptions and anomalies

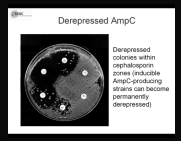
The problem with spotting carbapenemase producers

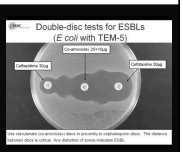


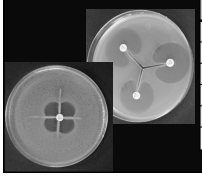
- Human experts, subjective : computer algorithms, poor specificity
- 'relative ease': *E. coli* > *Klebsiella* spp. >> *Enterobacter* spp.
- High index of suspicion: supplemental tests and/or send to Ref. Lab

Supplemental tests for mechanisms









	ESβLs	pAmpC	MβLs	KPC	OXA-48
EDTA			✓		
dipicolinic acid			✓		
boronic acid		✓		✓	
clavulanic acid	✓			weak	
tazobactam	✓			weak	
cloxacillin		✓			

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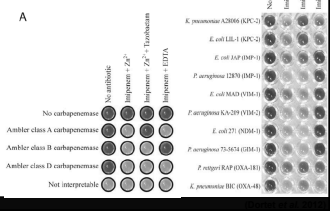
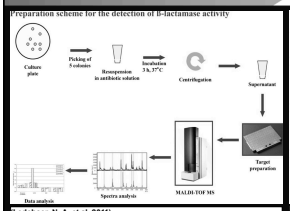
Colorimetric assay: Carba-NP test

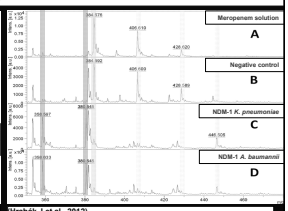
- Detection of carbapenemase activity in Enterobacteriaceae and *Pseudomonas aeruginosa*
- based on hydrolysis of β-lactam ring of imipenem
- Use of inhibitors → ID of carbapenemase class
- Early detection: <3hrs
- 100% sensitivity
- 100% specificity
- Difficulty if more than one carbapenemase present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



MALDI-TOF MS



Ledeboer N. A. et al. 2011)



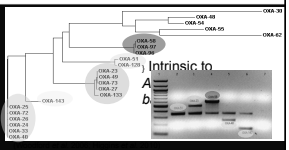
(Hrabák J et al. 2012)

- Positive evaluations for detection of resistance to carbapenems and other β-lactams (Burckhardt & Zimmermann 2011; Hrabák *et al.* 2011; Sparbier *et al.* 2012; Hrabák *et al.* 2012)
- No false-positives or false–negatives
- Potential for detection of other resistance mechanisms if metabolism of antibiotic occurs

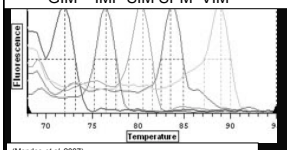
DNA-based testing: PCR

Common TEM and SHV ESBLS, CTX-M groups 1, 2 and 9

All variants of NDM, KPC and OXA-48; common IMP and VIM

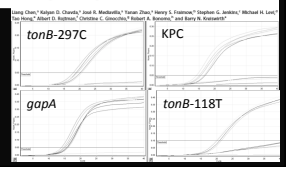


GIM IMP SIM SPM VIM



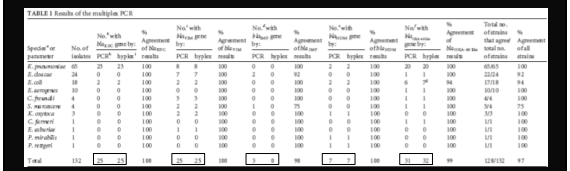
(Mendes *et al.* 2007)

Multiplex Real-Time PCR for Detection of an Epidemic KPC-Producing *Klebsiella pneumoniae* ST258 Clone



PCR – ELISA: Hyplex® assays

- MβLs, ESBLs (TEM, SHV, CTX-M and OXA) or OXA carbapenemases (OXA-23, -40 and -58)
- identifies genes in 2.5 – 4 hrs directly from clinical specimens
- Further evaluations required: issue with detection of diverse IMP genes? (Kaase *et al.* 2012)



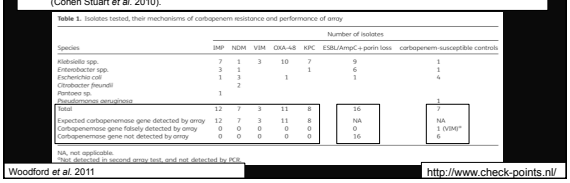
Chips with everything…

Total profiling:
more cost-effective than PCR

>100 targets per test:
- species identification
- resistance genes
- virulence genes
- epidemicity predictors
- strain-specific markers

Check-MDR arrays

- KPC, OXA-48, IMP, VIM, NDM
- Plasmidic AmpC and CTX-M ESBLs to group level (and beyond…)
- 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 (Naas *et al.* 2011), USA (Endimiani *et al.* 2010) and Netherlands (Cohen Stuart *et al.* 2010).



Woodford *et al.* 2011

http://www.check-points.nl/

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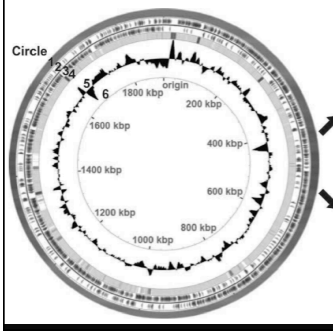
Next generation sequencing



Molecular Pathogenesis
strain genotype-disease phenotype
genetic basis of virulence traits
intrahost variation
antibiotic resistance

Phylogenetic Analysis
population genetics
strain emergence
outbreak investigation
pathogen discovery

New Clinical Tools
vaccines
diagnostics
therapeutics
infection control

(Olsen *et al.* 2012)

NGS: Hospital-acquired Infection Biodetection System

- Detects and types 12 most common HAIs
  - Multiple bacterial spp. per sample
- Also detects common resistance genes
- Equivalent to 144 PCRs in one assay
- Data automatically analysed by software
- "extraction to result" in <12 hours

Acinetobacter baumannii	Enterobacter cloacae
Clostridium difficile	Klebsiella pneumoniae
Escherichia coli	Proteus mirabilis
Enterococcus faecalis	Pseudomonas aeruginosa
Enterococcus faecium	Coagulase-negative Staph (epidermidis, saprophyticus)
Enterobacter aerogenes	Staphylococcus aureus

CARB	PER	ermA
CMY	SHV	vanA
CTX-M	VEB	vanB
GES	VIM	mecA
IMP	NDM	mexA
KPC	OXA	TEM

http://www.pathogenica.com/

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..."
Doern, JCM suppl. Sept 2011.

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

Summary

- MDR Gram -ves present an increasing threat to antibiotic therapy
- Interpretative reading can infer major resistance mechanisms
- Pheno- 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

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