


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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www.webbertraining.com November 19, 2012

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 \rightarrow high rate of transmissibility
- Detection a challenge
 - Few standardised methods
 - Diverse resistance mechanisms

Antibiotic susceptibility testing

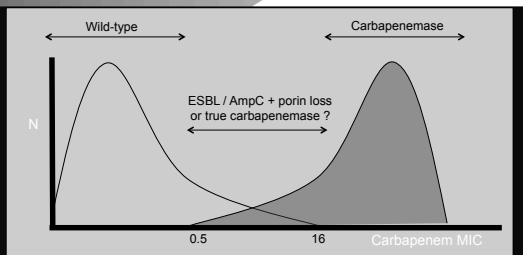
- Quantitative methods (MIC, mg/L)
 - agar or broth dilution
 - gradient strips (Etests, MICE)
- Qualitative methods (S/I/R)
 - disk diffusion
 - agar incorporation breakpoint method
- Automated methods
- Data meaningless unless interpretative criteria applied
 - MIC and zone diameter breakpoints indicate 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

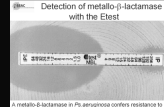
The problem with spotting carbapenemase producers



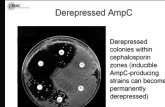
- Human experts, subjective : computer algorithms, poor specificity
- 'relative ease': *E. coli* > *Klebsiella* spp. >> *Enterobacter* spp.
- High index of suspicion: supplemental tests and/or send to Ref. Lab

Supplemental tests for mechanisms

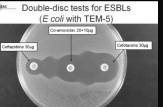
EDTA - Detection of metallo- β -lactamase with the EDTA



Derepressed AmpC



Double-disc tests for ESBLs (E coli with TEM-5)



	ES β Ls	pAmpC	M β Ls	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 \rightarrow ID of carbapenemase class
- Early detection: <3hrs
- 100% sensitivity
- 100% specificity
- Difficulty if more than one carbapenemase present
- Needs further evaluation by other labs

Figure A: Colorimetric assay results for various *P. aeruginosa* strains.

Strain	No carbapenemase	Amhler class A carbapenemase	Amhler class B carbapenemase	Amhler class D carbapenemase	Not interpretable
<i>K. pneumoniae</i> A20865 (KPC-2)	Blue	Yellow	Yellow	Yellow	Blue
<i>E. coli</i> LB-1 (KPC-2)	Blue	Yellow	Yellow	Yellow	Blue
<i>E. coli</i> J33P (IMP-1)	Blue	Blue	Blue	Blue	Blue
<i>P. aeruginosa</i> 12879 (IMP-1)	Blue	Blue	Blue	Blue	Blue
<i>E. coli</i> M43D (IMP-1)	Blue	Blue	Blue	Blue	Blue
<i>P. aeruginosa</i> K1291 (IMP-1)	Blue	Blue	Blue	Blue	Blue
<i>E. coli</i> J271 (NDM-1)	Blue	Blue	Blue	Blue	Blue
<i>P. aeruginosa</i> 75104 (OXA-48)	Blue	Blue	Blue	Blue	Blue
<i>P. vulgaris</i> KAP (OXA-181)	Blue	Blue	Blue	Blue	Blue
<i>K. pneumoniae</i> 18C (OXA-48)	Blue	Blue	Blue	Blue	Blue

MALDI-TOF MS

Preparation scheme for the detection of β -lactamase activity

- Positive evaluations for detection of resistance to carbapenems and other β -lactams (Burckhardt & Zimmermann 2011; Hrabak et al. 2011; Spatler et al. 2012; Hrabak 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

Check-MDR arrays

- Common TEM and SHV ESBLs, CTX-M groups 1, 2 and 9
- All variants of NDM, KPC and OXA-48
- Common IMP and VIM

Intrinsic to

- tonB-297C
- gapA
- tonB-118T

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

PCR – ELISA: Hyplex® assays

- MpLs, 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)

Species/Strain	No. of isolates	% with KPC ⁺ by PCR	% Agreement of PCR	No. with KPC ⁺ by PCR	% Agreement of PCR	No. with KPC ⁺ by PCR	% Agreement of PCR	Total no. of isolates	% Agreement of PCR
<i>P. aeruginosa</i>	20	23	100	8	8	100	0	100	100
<i>E. coli</i>	18	0	100	7	7	100	2	100	100
<i>E. coli</i>	18	2	100	2	2	100	2	100	100
<i>E. coli</i>	18	0	100	0	0	100	0	100	100
<i>C. freundii</i>	4	0	100	3	3	100	0	100	100
<i>S. pneumoniae</i>	1	0	100	2	2	100	1	100	100
<i>K. pneumoniae</i>	3	0	100	2	2	100	1	100	100
<i>C. difficile</i>	1	0	100	0	0	100	1	100	100
<i>E. coli</i>	1	0	100	1	1	100	0	100	100
<i>P. mirabilis</i>	1	0	100	0	0	100	1	100	100
<i>P. vulgaris</i>	1	0	100	0	0	100	1	100	100
Total	132	23	100	23	23	100	7	100	100

Chips with everything...

Total profiling: more cost-effective than PCR

>100 targets per test:

- species identification
- resistance genes
- virulence genes
- epidemic 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).

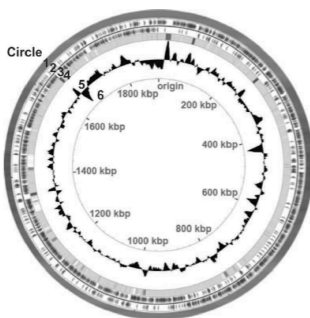
Species	IMP	NDM	VIM	OXA-48	KPC	ESBL/AmpC + pen loss	carbapenem susceptible controls
<i>Klebsiella</i> spp.	7	1	3	10	7	9	1
Enterobacter spp.	3	1	1	1	4	1	1
Enterobacter coli	1	3	1	1	1	4	4
Enterobacter faecalis	1	2	1	1	1	1	1
Total	12	7	5	11	8	16	7
Expected carbapenemase gene detected by array	12	7	3	11	8	NA	NA
Carbapenemase gene falsely detected by array	0	0	0	0	0	0	2 (OXA*)
Carbapenemase gene not detected by array	0	0	0	0	0	16	6

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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 HAI's
 - 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	CARB	PER	ermA
Clostridium difficile	Klebsiella pneumoniae	CMY	SHV	vanA
Escherichia coli	Proteus mirabilis	CTX-M	VEB	vanB
Enterococcus faecalis	Pseudomonas aeruginosa	GES	VIM	mecA
Enterococcus faecium	Coagulase-negative Staph (epidermidis, saprophyticus)	IMP	NDM	mexA
Enterobacter aerogenes	Staphylococcus aureus	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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 For more information on the individual Federation of Infection Societies visit their websites by clicking on the logos below:

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