NONFERMENTING GRAM NEGATIVE RODS

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OBJECTIVES

- Discuss basic limitations to assessing carbapenem resistance in nonfermenting GNRs
- Discuss antimicrobial susceptibility testing and reporting strategies
  - *P. aeruginosa*
  - *Acinetobacter baumannii-calcoaceticus* complex

BACKGROUND

- *P. aeruginosa* and *Acinetobacter baumannii* are common nosocomial pathogens
- Can be extremely drug-resistant
- Same mechanisms responsible for carbapenem resistance in *Enterobacteriaceae* may be present in nonfermenting GNRs (e.g. plasmid beta-lactamase) however resistance is typically combinatorial
- Quick to become multidrug-resistant given arsenal of intrinsic resistance mechanisms

BETA-LACTAM RESISTANCE IN P. AERUGINOSA

- Main contributors:
  - Chromosomal AmpC
  - **Loss of porin OprD**
  - Hyperexpression of efflux pump MexAB-OprM
- In a recent study of isolates with reduced to no susceptibility to ceftazidime, a total of 21 different combinations of resistance mechanisms were found
- Chromosomal and acquired beta-lactamases
- Metallo-beta-lactamase most common carbapenemase

Castanheira M et. al., AAC, 2014

BETA-LACTAM RESISTANCE IN ACINETOBACTER

- Main contributors:
  - Chromosomal AmpC-type beta-lactamase
  - **Oxacillinases**
  - Metallo-beta-lactamases
- Carbapenem resistance is most often linked to a carbapenemase
- Carbapenemases (e.g. OXA) pose additional threat because many are located on mobile genetic elements

Potron A et. al., Int J Antimicrob Agents, 2015

MAIN MECHANISMS OF CARBAPENEM RESISTANCE

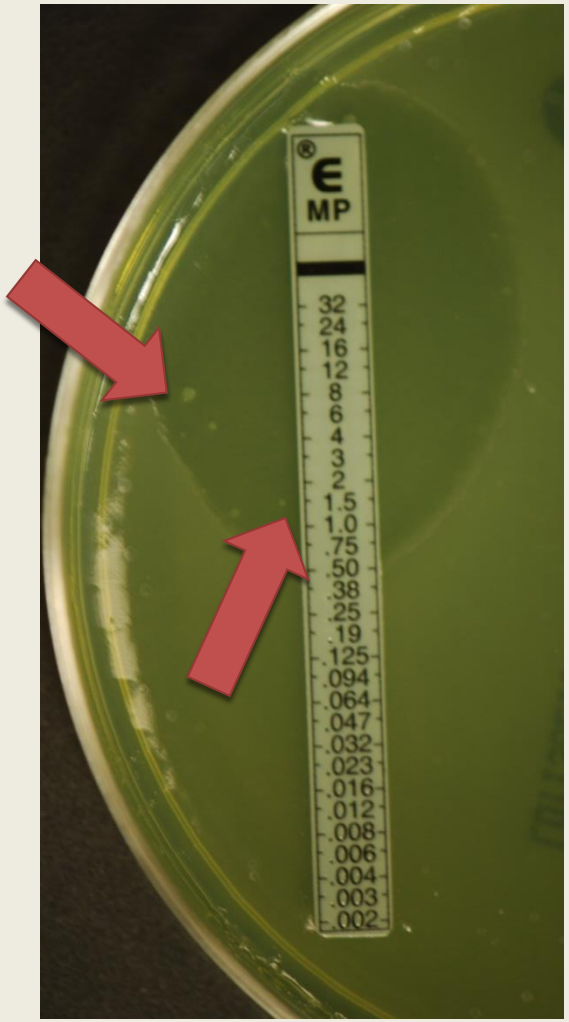
Enterobacteriaceae	Cephalosporinase + porin loss +/- ESBL Carbapenemase
P. aeruginosa	Porin loss Up-regulated efflux Carbapenemase
Acinetobacter spp.	Cephalosporinase + porin loss Carbapenemase

DIFFICULTIES WITH PHENOTYPIC TESTING FOR DETECTION OF CARBAPENEM RESISTANCE

- Accuracy of automated systems for detection of carbapenem resistance is varied
- For *Acinetobacter baumannii/calcoaceticus* complex, reported very major error rates against imipenem
  - Vitek2 – 0.7-4% VME
  - MicroScan – 2.8-25% VME
  - Phoenix - 1.9% VME
- Disk diffusion and Etest are typically reliable when compared against broth microdilution

Markelz AE et. al., AAC, 2011

DIFFICULTIES WITH PHENOTYPIC TESTING



- Disk diffusion and Etest may be difficult to interpret (fuzzy zones or inner colonies)
- *P. aeruginosa* may be mucoid hindering inoculum density measurement and zone definition

TESTS FOR CARBAPENEMASES

- Traditional phenotypic tests (e.g. MHT) demonstrate poor performance for detection of carbapenemases in *P. aeruginosa* and *A. baumannii*
- Is determination of carbapenem resistance mechanisms in nonfermenting GNRs be important?

TESTS FOR CARBAPENEMASES

- Is determination of carbapenem resistance mechanisms in nonfermenting GNRs be important?
  - Infection prevention and control
    - Many mechanisms are chromosomal (and/or combinatorial)
    - Would not want MDR Acinetobacter to become endemic even if no plasmid-mediated resistance mechanisms absent
  - Epidemiology – better tracking of transmission events
  - Unlikely to affect treatment decisions

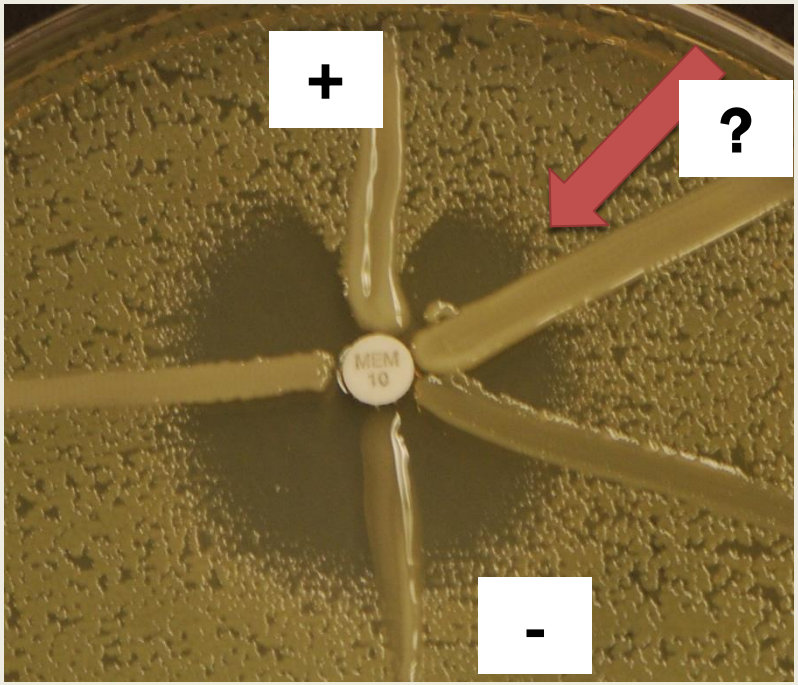
Prior to initiating a carbapenemase testing protocol (especially for nonfermenting GNRs), determine how (and if) mechanistic information will be used by infection control and prevention and pharmacy

M100-S26 (TABLE 3)

	Tests Used for Epidemiological or Infection Control–Related Testing		
	MHT (Table 3B)	**Carba NP (Table 3C)**	**Other (eg, molecular assays)**
Organisms	*Enterobacteriaceae* that are nonsusceptible to one or more carbapenems	*Enterobacteriaceae*, *P. aeruginosa*, and *Acinetobacter* spp. that are nonsusceptible to one or more carbapenems	*Enterobacteriaceae*, *P. aeruginosa*, and *Acinetobacter* spp. that are nonsusceptible to one or more carbapenems to determine the presence of a carbapenemase, or to determine carbapenemase type in isolates positive by MHT or Carba NP
Strengths	Simple to perform No special reagents or media necessary	Rapid	Determines type of carbapenemase in addition to absence or presence of the enzyme
Limitations	False-positive results can occur in isolates that produce ESBL or AmpC enzymes coupled with porin loss. False-negative results are occasionally noted (eg, some isolates producing NDM carbapenemase). Only applies to *Enterobacteriaceae*.	Special reagents are needed, some of which necessitate in-house preparation (and have a short shelf life). Invalid results occur with some isolates. Certain carbapenemase types (eg, OXA-type, chromosomally encoded) are not consistently detected.	Special reagents and equipment are needed. Specific to targeted genes; false-negative result if specific carbapenemase gene present is not targeted.

Abbreviations: ESBL, extended-spectrum β-lactamase; MIC, minimal inhibitory concentration; MHT, modified Hodge test; NDM, New Delhi metallo-β-lactamase.

MODIFIED HODGE TEST



- Results in a large number of indeterminate and falsely negative results
- Not recommended by CLSI for organisms other than *Enterobacteriaceae*
- Performance is less than desirable for MBLs, which may be present in *Pseudomonas* > *Acinetobacter*

CARBA NP

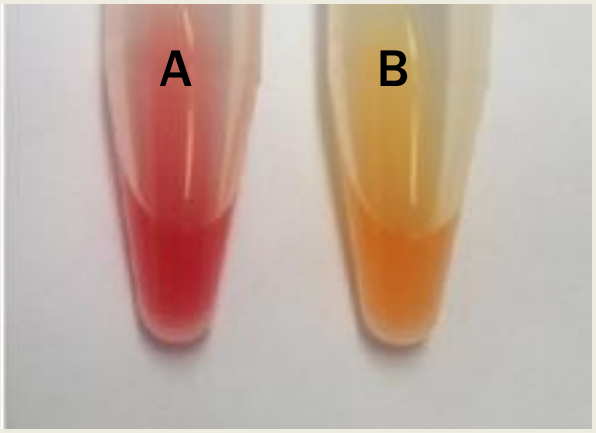
- Based on *in vitro* hydrolysis of imipenem by a bacterial lysate (See Dr. Humphries' slides for description)
- Endorsed by CLSI for *Enterobacteriaceae, P. aeruginosa*, and *Acinetobacter* spp.
- High level of sensitivity and specificity (>90% for both) in early reports, reevaluation indicates my be less for sensitive depending upon user
- Poor sensitivity for OXA-48-type carbapenemases (can adjust method), GES in *P. aeruginosa*
- Labor intensive (requires imipenem reagent preparation at time of use)

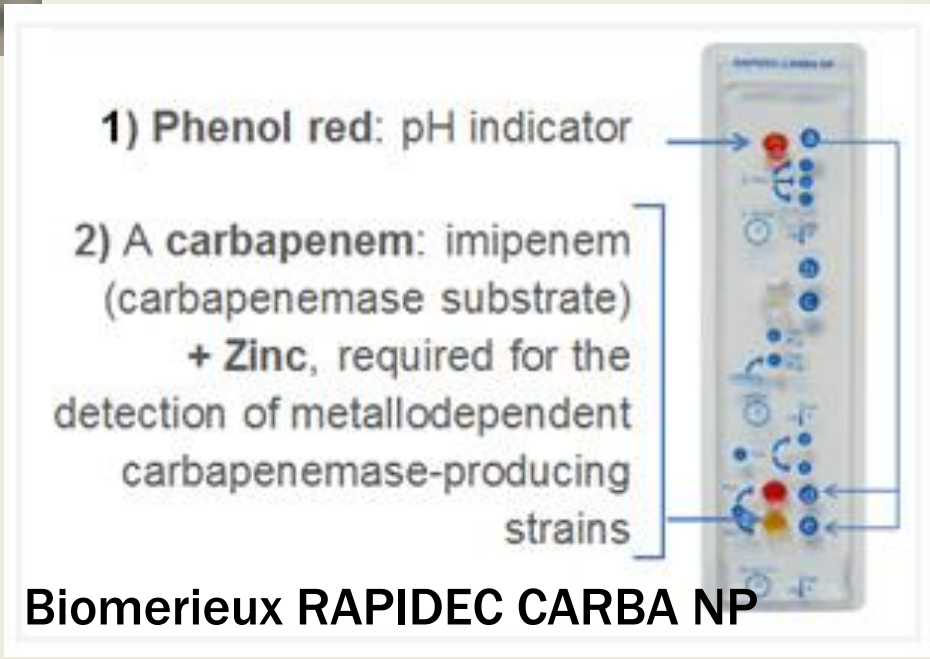
CARBA-NP: COMMERCIAL VERSIONS

Rosco Rapid CARB Screen

- Few studies of commercial assay performance
- CARBA NP method outperforms CARB Screen

CLSI M100-S25 Method

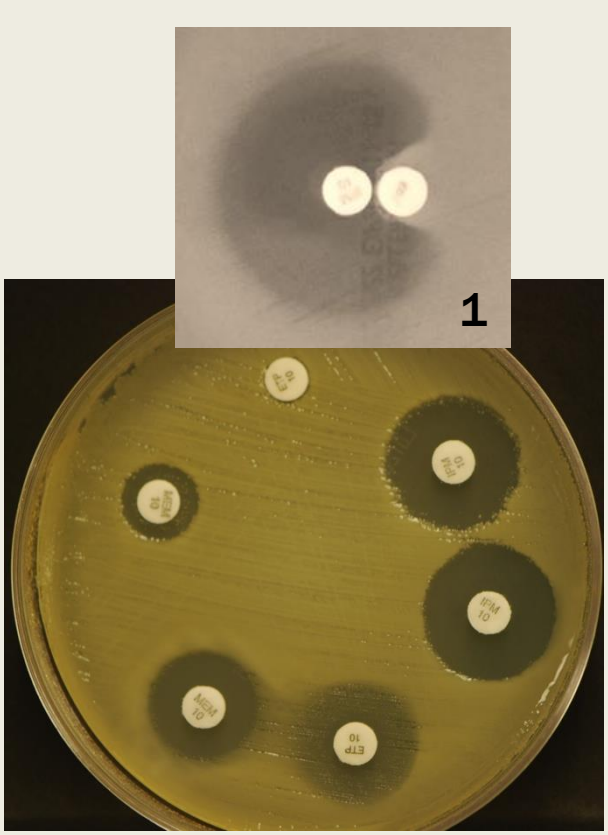




Biomerieux RAPIDEC CARBA NP

DOUBLE DISK/INDIRECT/COMBINED/ INHIBITION TESTS

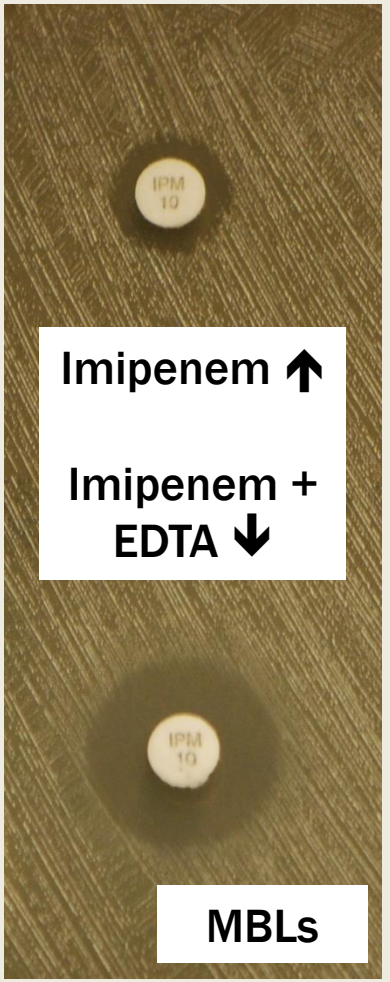
- Several tests utilize inhibitors that allow for detection and differentiation of carbapenemases



[1] Mathers AJ et. al. , JCM 2013

COMBINED DISK TEST

- Imipenem disk compared to imipenem + EDTA disk (commonly look for ≥5 mm difference in zone of inhibition with addition of EDTA)
- Inhibition by EDTA is a characteristic used to distinguish MBLs from other beta-lactamases
- Carbapenemases other than MBLs may be responsible for carbapenem resistance – this is not a standalone test



PHENOTYPIC TESTS

- Take home message: no phenotypic test can be used as a standalone test for detection and differentiation of carbapenemases in nonfermenting GNRs

So what about molecular tests…

MOLECULAR METHODS

- As with all molecular methods, dependent upon knowledge about target sequence
- Carbapenem resistance may result from mechanisms other than carbapenemases
  - Would correctly indicate no carbapenemases present
  - Stewardship: could use result and initiate use of carbapenem while awaiting susceptibility results
  - Knowledge of local mechanisms are beneficial

MOLECULAR METHODS

- Oxacillinase genes in *Acinetobacter* may be detected but may not result in phenotypic resistance
  - Probably one of the best methods for detection of oxacillinases in *Acinetobacter*
- Commercially-available assays are a great method for detection of some carbapenemases (NDM, VIM, IMP, OXA, KPC) but many others would be missed
  - *P. aeruginosa* may be challenging

FREQUENCY OF SUSCEPTIBILITY TESTING

- Emergence of resistance in *P. aeruginosa* against imipenem is common, especially with prolonged treatment
  - 0.3% of the genome is devoted to antimicrobial resistance genes
  - 10% genes organized in pathogenicity islands that can be easily mobilized
  - Genes may be up or downregulated based on antibiotic pressure
- Differing opinions on frequency of susceptibility testing for GNRs (range 1 – 3 days)

Mesaros N et. al., Clin Microbiol Infect, 2006

REPORTING STRATEGY

- Plug in intrinsic resistance first
- Check for updates

Appendix B. (Continued)

B2. Non-*Enterobacteriaceae*

Organism \ Antimicrobial Agent	Ampicillin, Amoxicillin	Piperacillin	Ticarcillin	Ampicillin-sulbactam	Amoxicillin- clavulanate	Piperacillin-tazobactam	Cefotaxime	Ceftriaxone	Ceftazidime	Cefepime	Aztreonam	Imipenem	Meropenem	Ertapenem	Polymyxin B Colistin	Aminoglycosides	Tetracyclines/ Tigecycline	Trimethoprim	Trimethoprim-sulfamethoxazole	Chloramphenicol	Fosfomycin
Acinetobacter baumannii/ Acinetobacter calcoaceticus complex	R			·	R						R			R				R		R	R
Burkholderia cepacia complex	R	R	R	R	R	R	R	R		R	R	R		R	R	R		R			R
Pseudomonas aeruginosa	R			R	R		R	R						R			R	R	R	R	R
Stenotrophomonas maltophilia	R	R	R	R	R	R	R	R			R	R	R	R		R	†	R			R

P. AERUGINOSA

Table 2B-1
Pseudomonas aeruginosa
M02 and M07

aztreonam	>32	R
ceftazidime	<8	S
imipenem	≤2	S
ciprofloxacin	>4	R
gentamicin	>16	R
pip-tazo	≤ 8	S
ertapenem		R

Consider reporting some intrinsically resistant agents (e.g. ertapenem)

ACINETOBACTER

Table 2B-2
Acinetobacter spp.
M02 and M07

amp/sulbact	<8S*
ceftazidime	<8 S
imipenem	≤2 S
ciprofloxacin	>4 R
gentamicin	>16 R
pip-tazo	≤ 16 S
ertapenem	R

Consider reporting some intrinsically resistant agents (e.g. ertapenem)

*sulbactam is the active component

REPORTING STRATEGY

- Current commercial panels should have appropriate concentrations to differentiate susceptibility and resistance
- Detection of mechanism(s) responsible for carbapenem resistance is not necessary for treatment but may aid in identification of resident resistance mechanisms

REPORTING CARBAPENEM RESISTANCE IN NON-ENTEROBACTERIACEAE

- No standard definition for reporting of carbapenem resistance in nonfermenting GNRs (e.g. carbapenem resistant organism, multi-drug resistant organism)
- Many public health laboratories focus on CRE, so guidance is somewhat lacking
- Develop protocol with infection control for notification of carbapenem-resistant organisms