Identification of CRE Using Techniques That Every Laboratory Can Perform

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Objectives

- Participants will be able to:
  - Identify mechanisms of carbabenemase resistance in members of the Enterobacteriaceae
  - Modify antibiotic interpretations, based on type(s) of resistance observed
  - Add appropriate interpretative comments to susceptibility reports to aid in physician understanding

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CLSI Guidance on KPC Testing After Implementing New Breakpoints

- Will Tests for carbapenemases (e.g. Modified Hodge Test) be needed with new carbapenem breakpoints for Enterobacteriaceae?
  - CLSI says **No**. For patient management, tests for carbapenemases are not necessary
  - If requested, tests for carbapenemases may be done for Infection Control purposes
- I believe that detecting resistance mechanisms is important and necessary for patient reporting and infection control purposes even in the Community Hospital Setting

(CLSI Jan 2011 M100-S21, p. 55)

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Carbapenems

- In order to demonstrate this point I will use the example of carbapenem resistance and show how various mechanisms can mediate resistance to carbapenems
- Some of these mechanisms require that patient reports be modified and some require infection control interventions
- Laboratories should know which mechanism require intervention and which do not

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Carbapenemases in the U.S.

Molecular Class	Carbapenemase	Found in:	Some Key Features
A	KPC	*K. pneumoniae* and other Enterobacteriaceae	Some are chromosomal (NmcA, Sme, IMI-1, SFC-1) others are plasmid encoded (KPC, IMI-2, GES). All hydrolyze carbapenems and are partially inhibited by clavulanic acid
	SME	*S. marcescens*	
	also IMI, NMCA, GES	Enterobacteriaceae	
B	Metallo beta-lactamases (IMP, VIM, GIM, SPM, NDM-1)	*P. aeruginosa, Enterobacteriaceae, Acinetobacter, S. maltophilia*	Hydrolyze all ß-lactams except aztreonam. Activity inhibited by EDTA but not by clavulanic acid
D	OXA	*Acinetobacter baumannii, Enterobacteriaceae*	OXA-48 first reported in Turkey in 2003. Not inhibited by EDTA or clavulanic acid

Adapted from Queenan & Bush. 2007. Clin Microbiol Rev. 20:440.

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Need to Distinguish Between Mechanisms of Carbapenem Resistance – Why?

- Carbapenemase
  - Isolate likely to be resistant to all carbapenems and other β-lactam agents
  - May need to change susceptible reports to resistant for β-lactam drugs
  - Need to implement infection control measures such as contact precautions and possibly active surveillance testing
  - **These are an Infection Control Emergency**

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Need to Distinguish Between Mechanisms of Carbapenem Resistance – Why?

- Cephalosporins combined with porin-loss
  - Class A ESBL's (CTX-M) + reduced permeability
  - Class C High AmpC + reduced permeability
- These hydrolyze ertapenem more than meropenem or imipenem
  - Not necessarily resistant to all carbapenems (i.e., would not need to change susceptible results to resistant reports for β-lactam drugs
- These isolates are MDRO and infection control measures are recommended. However, Healthcare institutions may reserve more aggressive measures for carbapenemase-producing isolates

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Why labs should continue to perform Modified Hodge Test and EDTA Inhibition Test on isolates that test non-susceptible to carbapenems

- Knowing the resistance mechanism is important
- The following cases demonstrate 5 different mechanisms of carbapenem resistance. Some require changes in antibiotic reporting, some require infection control notification and some require no action
- Can you tell the difference between them by MIC alone?

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Patient History Case 1

- 58 y/o male, morbidly obese (>500 lbs)
- Presented to ER with episode of hypoxia and hypotension during dialysis
- PMH
  - Pt has trach for hypercapnea (COPD and OSA), currently vent dependent
  - Chronic foley catheter
  - Diabetes mellitus type 2
  - ESRD
- Exam:
  - Afebrile
  - Multiple decubitus ulcers (sacrum, spine, right leg)
  - Urine is grossly dirty

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Patient History

- Concerned that septic => Pan-cultures
  - Urine: *Klebsiella*…

- Spot Indole Neg

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Vitek ID: [redacted] Oxidase -
Type: Gram Negative General Susceptibility 143 (GNS-143)
Status: Final
Elapsed Time: 13 hours
Organism: Klebsiella pneumoniae
Source: Manual
Demographics: [redacted]

	MIC	Instrument	Expert
Ampicillin	>=32	R	
Ampicillin/Sulbactam	>=32	R	
Piperacillin/Tazobactam	>=128	R	
Cefazolin	>=32	R	
Ceftriaxone	>=64	R	
Ceftazidime	>=32	R	
Cefepime	8	S	
Aztreonam	>=32	R	
Imipenem	<=4	S	
Gentamicin	4	S	
Tobramycin	>=16	R	
Ciprofloxacin	>=4	R	
Levofloxacin	>=8	R	
Trimeth-Sulfa	>=320	R	
Nitrofurantoin	64	I	
ESBL		Negative	

MIC values in mcg/ml (MI) Wait for All
The presence of other Beta-lactamases (e.g. AmpC, IRT) may mask ESBL production.

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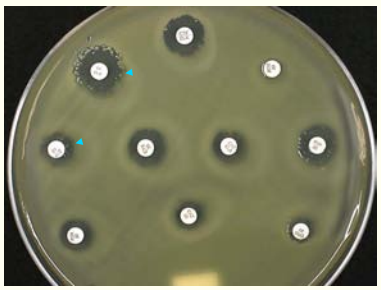
Double Disk Potentiation Method – *Case 1*



Imipenem - S
Ertapenem - R

Suggests possible KPC which should be confirmed with Hodge test or sent to reference lab for confirmation

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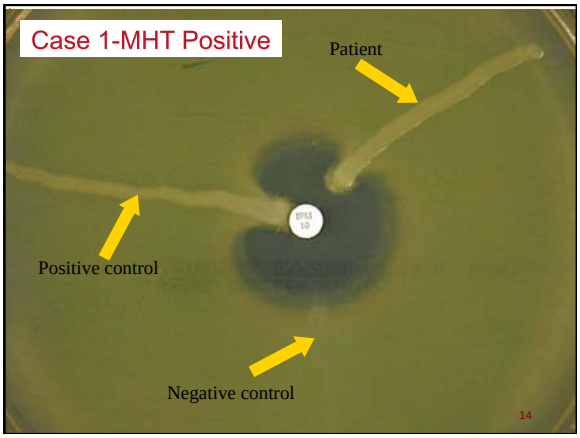
Case 1-MHT Positive



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And the Answer is …….

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Carbapenemases in the U.S.

Molecular Class	Carbapenemase	Found in:
A	KPC	*K. pneumoniae* and other Enterobacteriaceae
	SME	*S. marcescens*
	also IMI, NMCA, GES	Enterobacteriaceae
B	Metallo beta-lactamases (IMP, VIM, GIM, SPM, NDM-1)	*P. aeruginosa, Enterobacteriaceae, Acinetobacter, S. maltophilia*
D	OXA	*Acinetobacter baumannii, Enterobacteriaceae*

Adapted from Queenan & Bush. 2007. Clin Microbiol Rev. 20:440.

Modified from Janet Hindler 16

Patient Report Case 1

If using FDA breakpoints change all carbapenems to resistant and add following statement to report:

"Multiple drug resistant organism, KPC identified. Treatment with any beta lactam drug including carbapenems is not reliable, Patient requires contact isolation."

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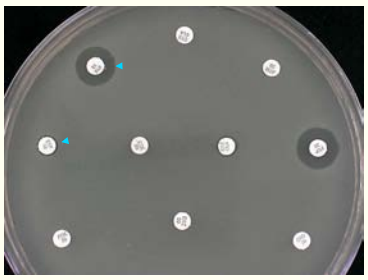
Double Disk Potentiation Method – *Case 2 Blood Culture with Enterobacter cloacae*



Imipenem - S
Ertapenem - R

Suggests possible KPC which should be confirmed with Hodge test or sent to reference lab for confirmation

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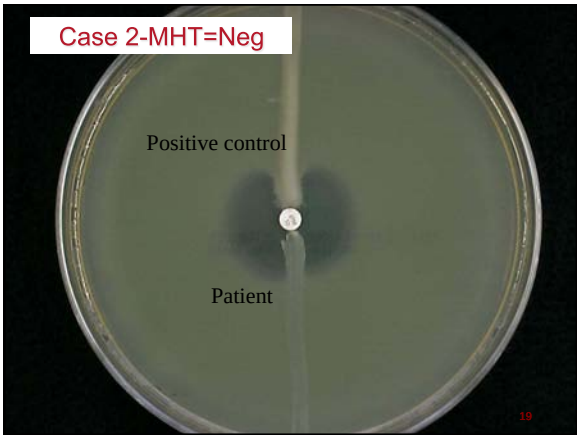

And the Answer is ……..

Chromosomal AmpC (Derepressed mutant) + Porin mutation

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Patient Report Case 2

Note the susceptibility pattern in Case 2 is identical to susceptibility pattern seen in Case 1, except in this case we have a chromosomal AmpC that is not MDRO, is not an infection control risk, and does not require modification of the susceptibility report. The following comment is added to our patient report:

"This organism is known to possess an inducible ß-lactamase. Isolates may become resistant to all cephalosporins after initiation of therapy. Avoid ß -lactam-inhibitor drugs"

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Case 3 #227-1 (9-27-10)

- 88 Y.O. female, bed ridden with Alzheimer's
- Urinary incontinence for >10 years
- Foley cath for 1 year
- Gastrostomy tube since 2001
- Admitted for gastrostomy tube replacement
- Patient pulled out foley catheter
- PMH UTI including MRSA
- Urine culture grew >100,000 Serratia marcescens

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MicroScan Report – Case 3

Panel Data

Biotype: 70405346
Organism: S. marcescens

Biochemical Results: (Biochemicals that are bolded and underlined are atypical for the stored organism)

GLU	+	RAF	-	ING	+	URE	-	LYS	+	TDA	-	CIT	+	CL4	+	ACE	-	K4	-	P4	+
SUC	+	RHA	-	ADO	-	H2S	-	ARG	+	ESC	+	MAL	-	CF8	+	CET	-	NIT	-	TAR	-
SOR	+	ARA	-	MEL	-	IND	-	ORN	+	VP	+	ONPG	-	OXI	-	FD64	+	OFIG	+	TO4	-

MIC Results: (Antimicrobics marked with "Ø" are suppressed from Long and Short Format Patient Reports)

AM	A/S	P/T	CFZ	CAX	CAZ	CPE	MER	GM	Ø TE	TO	CP	TIS	FD	Ø AK
>16	>16/8	<=16	>16	<=8	<=1	<=4	<=1	<=4	8	<=4	<=1	<=2/38	>64	<=16
R	R	S	R	S	S	S		S	I	S	S	S	R	S

CAZ/CA	CFT	CFT/CA	ETP	Ø IMP	Ø AUG	Ø CRM	Ø LVX	Ø MXF	Ø TIM
<=0.25	<=2	4	<=2	4	>16/8	>16	<=2	<=2	<=16
	S		S	S	R	R	S		S

Extra Tests: ESBL ...

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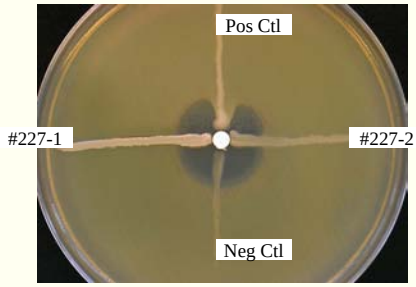
Case 3 #227-1
Modified Hodge Test



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And the Answer is …….

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Carbapenemases in the U.S.

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	SME	*S. marcescens*
	also IMI, NMCA, GES	Enterobacteriaceae
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D	OXA	*Acinetobacter baumannii, Enterobacteriaceae*

Adapted from Queenan & Bush. 2007. Clin Microbiol Rev. 20:440.

Modified from Janet Hindler 27

What is unique about SME as compared to KPC?

- Gene located on chromosome (vs. KPC on plasmid)
- Less hydrolysis of ceftriaxone, cefotaxime, ceftazidime, cefepime than other carbapenemases (*S. marcescens* "S" to these in vitro; ? activity in vivo)
  - Majiduddin et al. 2005. Antimicrob Agents Chemother. 59:3421.
  - Queenan et al. 1992. Antimicrob Agents Chemother. 44:3035.

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Specimen: Urine
Diagnosis: UTI
Serratia marcescens

Case 3 Final report

	MIC (µg/ml)	
amikacin	1 S	
ampicillin	>32 R	
cefazolin	>32 R	
ceftriaxone	≤0.5 S	
ciprofloxacin	≤0.25 S	
gentamicin	≤0.5 S	
imipenem	>16 R	
piper-tazobactam	≤8 S	
tobramycin	1 S	
trimeth-sulfa	≤1/19 S	

Report comment:
"Imipenem-R is due to carbapanemase production (but not KPC). The effectiveness of other β-lactams (that test "S) in treating infections due to carbapenemase -producing *S. marcescens* has not been established. Infectious Disease consult suggested."

Courtesy Janet Hindler

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Case 4. (9-9-10)

- Patient is a 65 Y.O. female with history of lymphoma. Complaint of diarrhea, fevers, neutropenia. Started empirically on Flagyl and imipenem.
- Developed sinusitis with swelling of left face, redness and CT consistent with sinusitis.
- Blood culture grew Enterobacter cloacae. Urine culture also ordered and grew E. cloacae <10,000 cfu/ml.

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MicroScan Report – Case 4

Panel Data

Biotype: 77101372
Organism: E. cloacae

Biochemical Results: (Biochemicals that are bolded and underlined are atypical for the stored organism)

GLU	+	RAF	+	INO	-	URE	-	LYS	-	TDA	-	CIT	+	CL4	-	ACE	-	K4	-	P4	+
SUC	+	RHA	+	ADO	-	H2S	-	ARG	-	ESC	+	MAL	+	CF8	+	CET	-	NIT	+	TAR	-
SOR	+	ARA	+	MEL	+	IND	-	ORN	+	VP	+	ONPG	+	OXI	-	FD64	-	OFG	+	TO4	-

MIC Results: (Antimicrobics marked with "Ø" are suppressed from Long and Short Format Patient Reports)

AM	A/S	P/T	CFZ	CAX	CAZ	CPE	MER	GM	Ø TE	TO	CP	T/S	Ø FD	Ø AK						
>16	>16/8	<=16	>16	<=8	<=1	<=4	>8	<=4	<=4	<=4	<=1	<=2/38	<=32	<=16						
R	R	S	R	S	S	S	R	S	S	S	S	S		S						
CAZ/CA	CFT	CFT/CA	ETP	IMP	Ø AUG	Ø CRM	Ø LVX	Ø MXF	Ø TIM											
<=0.25	<=2	<=0.5	>4	>8	>16/8	16	<=2	<=2	64											
	S		R	R	R	I	S	S	I											

Extra Tests: ESBL ...

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ID Consult Case 4. (9-9-10)

- Impression: Enterobacter bacteremia, likely related to sinusitis. It is susceptible to all other antibiotics, but imipenem is being rechecked. In light of this we will switch her to levaquin

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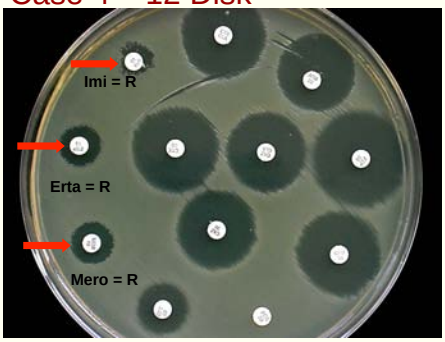
Case 4 – 12 Disk



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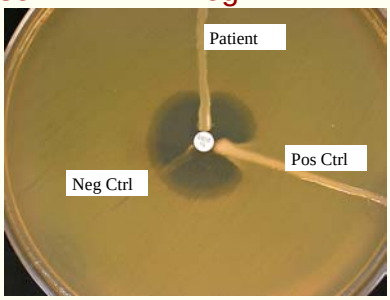
Case 4 – MHT=Neg



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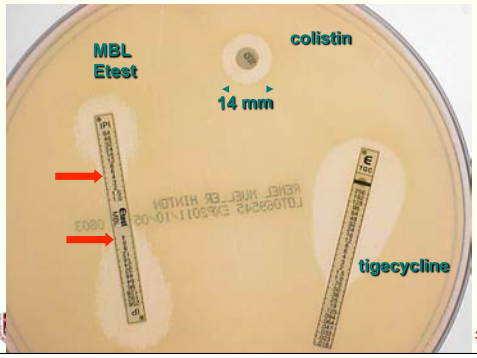
Case 4 – *Enterobacter cloacae*



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And the Answer is …….

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D	OXA	*Acinetobacter baumannii, Enterobacteriaceae*

Adapted from Queenan & Bush. 2007. Clin Microbiol Rev. 20:440.

Modified from Janet Hindler 37

IMI/NMC-A Enzymes

- Class A imipenemase/non-metallocarbapenemase
- Forms two subgroups; IMI and NMC-A
- Found sporadically in clinical isolates of Enterobacter cloacae and environmental isolates from rivers in USA
- NMC-A enzyme is inducible by cefoxitin and imipenem; and the expression of NMC-A is co-regulated with AmpC by the AmpD gene

Pottumarthy S et al. Emerg Infect Dis. 2003 Aug;9(8):999-1002

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Patient Report Case 4

In this case resistance to carbapenems is due to a chromosomal carbapenemase. The organism is not MDRO, is not an infection control risk, and does not require modification of the susceptibility report. The following comment is added to report:

"Imipenem-R is due to a chromosomal carbapenemase production but not KPC. The effectiveness of other β-lactams (that test "S) in treating infections due to carbapenemase-producing *E. cloacae* has not been established. Infectious Disease consult is recommended"

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Case 5. (5-12-10)

- Patient is a 40 Y.O. male paraplegic who traveled to New Dehli India for a surgical procedure. 3-4 months after returning to the U.S. patient presents to outpatient center in Chicago with multiple decubitus ulcers and urinary tract infection. Urine collected from foley cath is submitted for culture.

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MicroScan Report – Case 5

Panel Data

Biotype: 73115012

Organism Identification:

	Organism	% Probability	Footnotes	Special Characteristics
1	E. coli	99.99		

Biochemical Results: (Biochemicals that are bolded and underlined are atypical for the first choice organism)

GLU	+	RAF	-	INO	-	URE	-	LYS	+	TDA	-	CIT	-	CL4	-	ACE	-	K4	+ P4	+
SUC	+	RHA	+	ADO	-	H2S	-	ARG	-	ESC	-	MAL	-	CF8	+	CET	-	NIT	+ TAR	-
SOR	+	ARA	+	MEL	+	IND	+	ORN	+	VP	-	ONPG	+	CXI		FD64	-	OFIG	+ TO4	+

MIC Results: (Antimicrobics marked with "Ø" are suppressed from Long and Short Format Patient Reports)

AM	A/S	P/T	CFZ	CAX	CAZ	CPE	MER	QM	Ø TE	TO	CP	T/S	Ø FD	AK
>16	>16/8	>64	>16	>32	>16	>16	>8	>8	>8	>8	>4	>2/38	<=32	>32
R	R	R	R	R	R	R	R	R	R	R	R	R		R

CAZ/CA	CFT	CFT/CA	ETP	IMP	Ø AUG	Ø CRM	Ø LVX	Ø MXF	Ø TIM
>2	>32	>4	>4	4	>16/8	>16	>4	>4	>64
	R		R	S	R	R	R	R	R

Extra Tests: ESBL -

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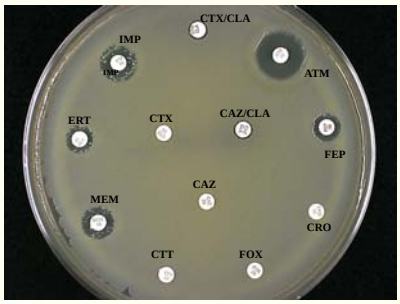
Case 5. 12 Disk



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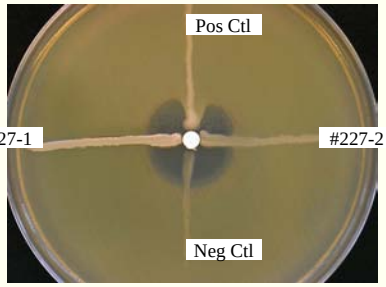
Case 5 #227-2
Modified Hodge Test



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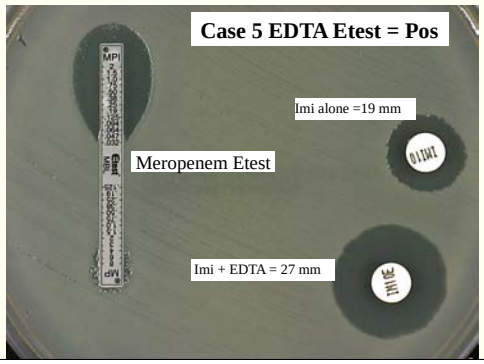
Rosco Diagnostica IMI/EDTA Disks
(Available from Key Scientific)



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D	OXA	*Acinetobacter baumannii, Enterobacteriaceae*

Adapted from Queenan & Bush. 2007. Clin Microbiol Rev. 20:440.

Modified from Janet Hindler 46

NDM-1
New Class B: Metallo-β-Lactamases

- MBLs hydrolyze all β-lactams, including carbapenems, penicillins, extended-spectrum cephalosporins, but not aztreonam
- MBLs pose a serious threat in terms of infection control because of their high mobility
- MBLs require zinc for enzymatic activity which is not diminished by serine β-lactamase inhibitors but is inhibited by EDTA and other chelators of divalent cations

Antimicrobial Agents and Chemotherapy. December, 2009. 53:5046-5054.

Courtesy Brandi Limbago, CDC 47

MicroScan Report

Panel Data

Biotype: 73115012

Organism Identification:

	Organism	% Probability	Footnotes	Special Characteristics
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Biochemical Results: (Biochemicals that are bolded and underlined are atypical for the first choice organism)

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SUC	+	RHA	+	ADO	-	H2S	-	ARG	-	ESC	-	MAL	-	CF8	+	CET	-	NIT	+	TAR	-
SOR	+	ARA	+	MEL	+	IND	+	ORN	+	VP	-	ONPG	+	OXI		FD64	-	OFIG	+	TO4	+

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>16	>16/8	>64	>16	>32	>16	>16	>8	>8	>8	>8	>4	>2/38	<=32	>32
R	R	R	R	R	R	R	R	R	R	R	R	R		R

CAZ/CA	CFT	CFT/CA	ETP	IMP	Ø AUG	Ø CRM	Ø LVX	Ø MXF	Ø TIM
>2	>32	>4	>4	4	>16/8	>16	>4	>4	>64
	R		R	S	R	R	R	R	R

Extra Tests: ESBL -

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Enterobacteriaceae - Revised Carbapenem Breakpoints (MIC µg.ml)

Agent	CLSI M100-S19 (2009)			CLSI M100-S20 (2010) Supplement		
	Susc	Int	Res	Susc	Int	Res
Doripenem	-	-	-	≤1	2	≥4
Ertapenem	≤2	4	≥8	≤0.5	1	≥2
Imipenem	≤4	8	≥16	≤1	2	≥4
Meropenem	≤4	8	≥16	≤1	2	≥4

CLSI. Performance Standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement (June 2010 Update). CLSI document M100-S20-U. Wayne, PA; 2010

CLSI M100-S20-U. Table 2A

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Patient Report Case 5

- If using FDA breakpoints change all carbapenems to resistant and add following statement to report:
- "Multiple drug resistant organism, NDM-1 identified. Treatment with any beta lactam drug including carbapenems is not reliable, Patient requires contact isolation."

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Recommendations

- Apply new (lower) breakpoints to clinical isolates as soon as testing capability becomes available
- Perform Hodge Test and MBL Etest on all Enterobacteriaceae with carbapenem MIC > 1 mg/ml (except Proteus, Providencia and Morganella)
- If not using new breakpoints, Report MIC and Change AST result to I or R when resistant mechanism detected
- Report Resistant mechanism to clinicians and infection control practitioners

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QUESTIONS?

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