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Microbiology and Stewardship

THE ESSENTIAL ROLE OF CLINICAL MICROBIOLOGY IN ANTIMICROBIAL STEWARDSHIP

Disclosures

- Consultant: Accelerate Diagnostics, ThermoFisher, GI Scientific, QPex
- Stocks: Accelerate Diagnostics

Objectives

1. Identify core elements of laboratory testing that promote antimicrobial stewardship
2. Understand MIC-driven therapeutic decisions
3. Describe antimicrobial therapy challenges in critically ill patients

Vanderbilt Univ. Medical Center

Case 1.

- 57 year old man
- Suboccipital craniectomy for 4th ventricular ependymoma
- Post-surgery, develops altered mental status
- Febrile, leukocytosis to 29.6
- CSF: 60 WBC, Gram stain = GNR
- Meningitis/ventriculitis

CSF Culture: *Enterobacter (Klebsiella) aerogenes*

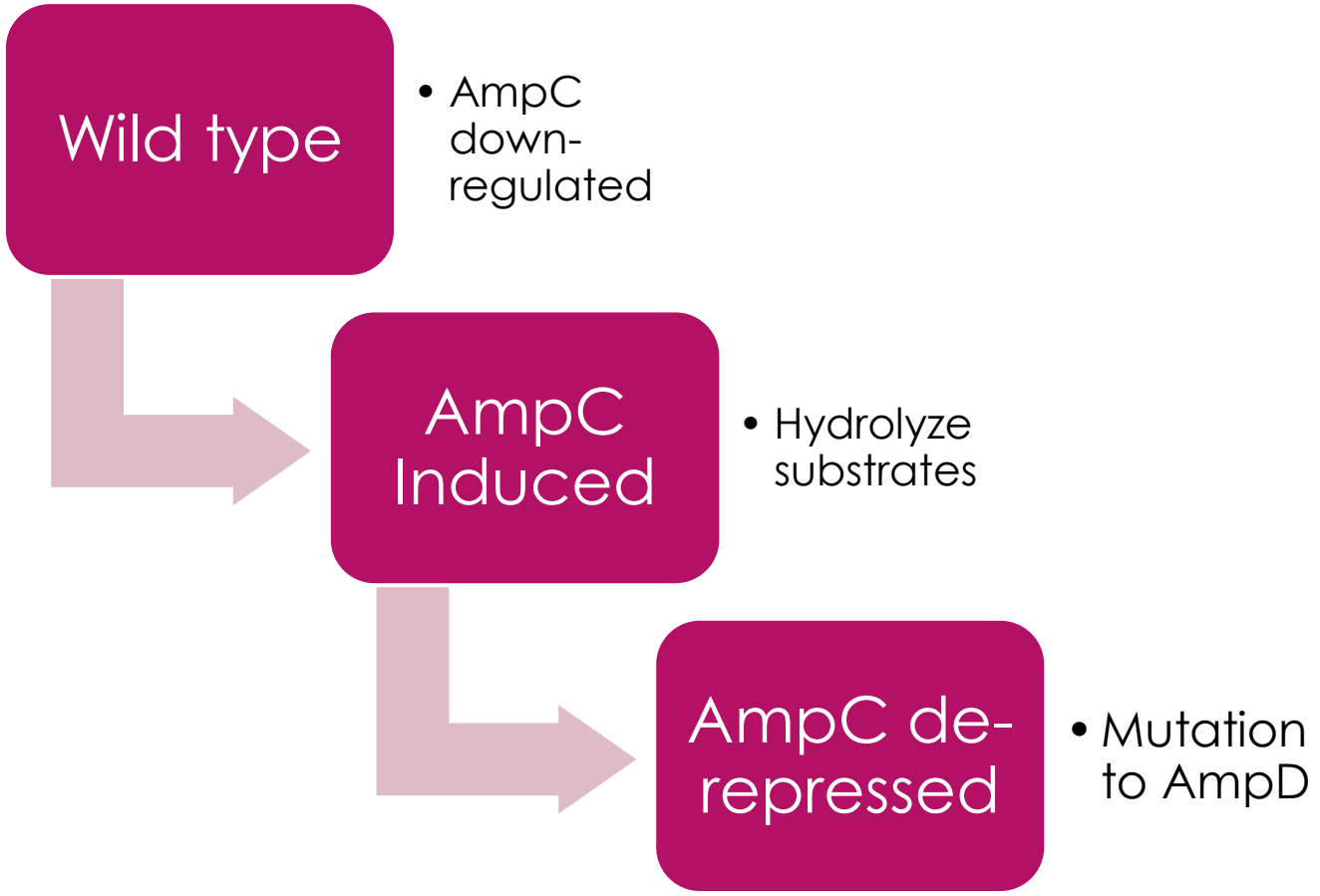
Antimicrobial	MIC (mcg/mL)	
Ampicillin	>16	R
Aztreonam	≤2	S
Cefepime	≤1	S
Ceftriaxone	≤1	S
Pip-tazo	4	S

Case 1, continued

- Patient started empirically on cefepime, but changed to ceftriaxone due to seizures (toxicity?)
- Patient continues to have high WBC in CSF
- Repeat isolates recovered, but AST not performed
  - Laboratory policy is to repeat AST only every 5 days
- Finally, day 6, AST performed: "R" to ceftriaxone

Antimicrobial	Isolate #1 Day 1	Isolate #2 Day 6
Ampicillin	>16, R	>16 R
Aztreonam	≤2, S	16, R
Cefepime	≤1, S	≤1, S
Ceftriaxone	**≤1, S**	**32, R**
Pip-tazo	**4, S**	**64, R**
Meropenem	S	S

AmpC Induction Overview



Jacoby. CMR. 2009. 161-182

AmpC de-repressed mutants: Impact on AST results

Species	TZP	CTX	CAZ	FEP
E. cloacae	<5%	0%	0%	35%
E. aerogenes	10%	<5%	0%	98%
C. freundii	29%	0%	0%	80%
P. rettgeri	12%	5%	0%	81%
S. marcescens	55%	0%	75%	88%
Typically	**S or R**	**R**	**R**	**S**

0-25 %
25-50%
50-75%
>75%

Kohlmann, Bahr and Gatermann. 2018 JAC 73: 1530
Kohlmann, Bahr and Gatermann. 2019. CMI. 25:1158

Review of AmpC VUMC: *Enterobacter, Citrobacter, Serratia* in Blood

N= 37 patients YTD in 2020
Initial culture results:
81% ceftriaxone – S
89% pip-tazo - S

	Total	%
Patients with repeat testing	28	76%
Repeat + culture grew on repeat testing Median duration of bacteremia	8 36 h	29% 24-168 h
AST performed on repeat	3	37.5%
Number de-repressed AmpC	2	66.7%

50% of patients treated with ceftriaxone (n=4) suspected of de-repression due to clinical decompensation

CLSI guidance

Enterobacter, *Klebsiella aerogenes*, *Citrobacter*, and *Serratia* may develop resistance during prolonged therapy with 3rd-generation cephalosporins as a result of derepression of AmpC beta-lactase. Therefore, isolate that are initially susceptible may become resistant within 3-4 days after initiation of therapy. Testing repeat isolates may be warranted." – M100 S30, page 34

Opportunities for the lab

1. Provide guidance on frequency of repeating cultures
2. Repeat AST more often than every 5 days
3. "warning" re: use of ceftriaxone for AmpC organisms:
    1. Suppress ceftriaxone / ceftazidime results
    2. Provide a comment re: risk of AmpC de-repression

Cascade / selective reporting

	Cascade Reporting	Selective reporting
Definition	Reporting broader antimicrobials only if more narrow spectrum agents are "R"	Suppressing select agent results from the laboratory reports based on ASP needs (e.g., formulary, select suppressions etc)
Example	Only report ertapenem if ceftriaxone is "R"	Suppress fluoroquinolone results from urine cultures to support ASP initiative to decrease their use in treatment of cystitis

Example; cascade & selective reporting

BLUE = selective

- Drives providers to make the right choice
- Hides 'harmful' choices
- Supports ASP

RED = cascade

- Drives provider to pick narrow-spectrum
- Supports ASP

Test	Pan "S" *E. coli*	SPICE organism pan-S	ESBL type E. coli	ESBL type E. coli (urine)
Amikacin				
Gentamicin	Gentamicin	Gentamicin	Gentamicin	Gentamicin
Tobramycin				
Ampicillin	Ampicillin	Ampicillin (R)	Ampicillin (R)	Ampicillin (R)
Cefazolin	Cefazolin	Cefazolin (R)	Cefazolin (R)	urine breakpoints (R)
Aztreonam				
Ceftriaxone	Ceftriaxone		Ceftriaxone (R)	Ceftriaxone (R)
Ceftazidime				
Cefepime		Cefepime		
Ertapenem			Ertapenem	Ertapenem
Meropenem			Meropenem	Meropenem
Imipenem				
Pip-Tazo	Pip-tazo	Pip-tazo	Do not report (MERINO)	Do not report
Amp-sulb				Amp-Sulbactam
SXT	SXT	SXT	SXT	SXT
Nitrofurantoin				Nitrofurantoin
Ciprofloxacin	Ciprofloxacin	Ciprofloxacin	Ciprofloxacin	Ciprofloxacin
Levofloxacin				Levofloxacin
Ceftolo-tazo				
Ceftaz-avi				

Treatment options

Cefepime

Or

Pip-tazo

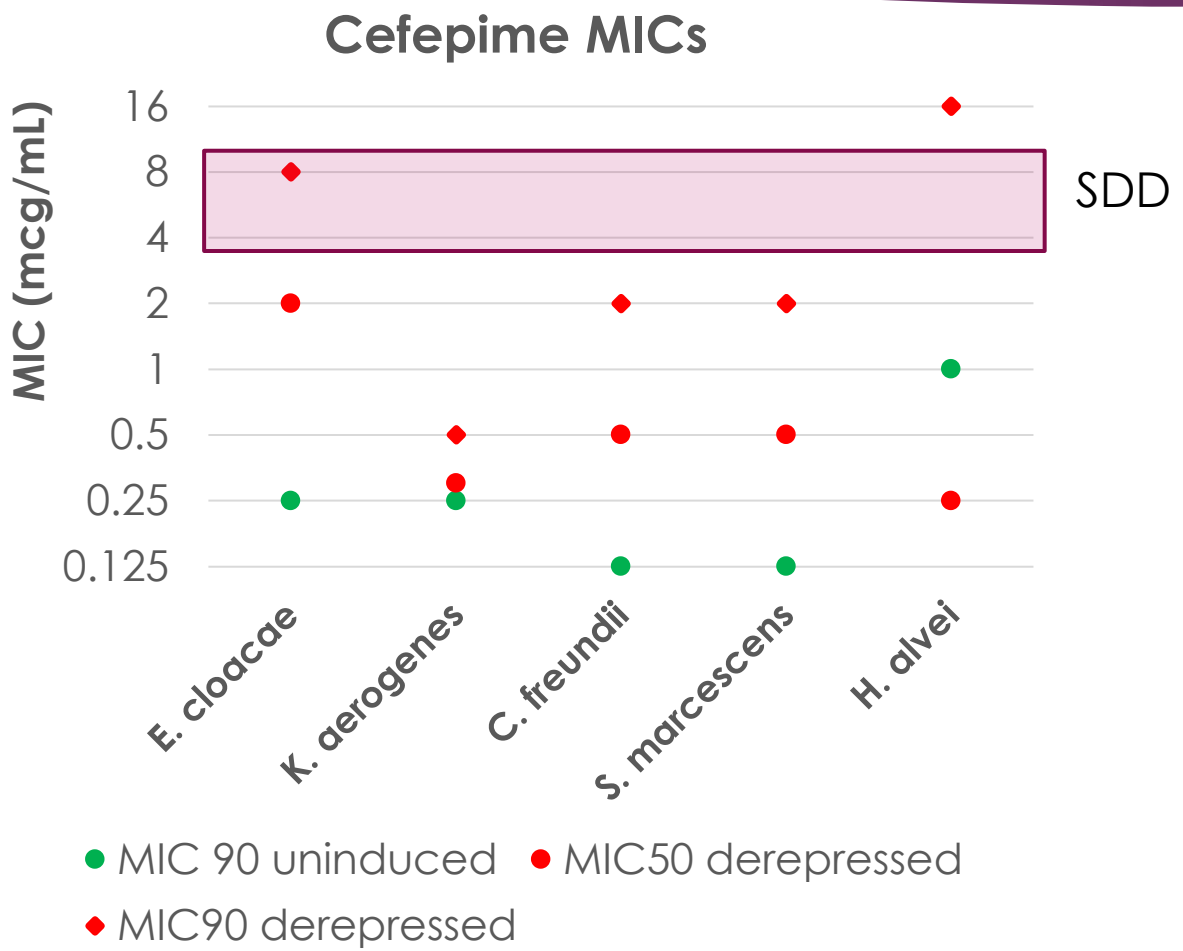
Carbapenem

Very little clinical data!

- Only retrospective studies
- Small "N"
- Significant bias in who gets a carbapenem vs. not (disease severity)

- Nice review by P. Tamma @ IDWeek 2020

MIC matters



Interpretation	Dose
S	1 g q 12
SDD (4 mcg/mL)	1 g q 8 or 2 g q 12
SDD (8 mcg/mL)	2 g q 8

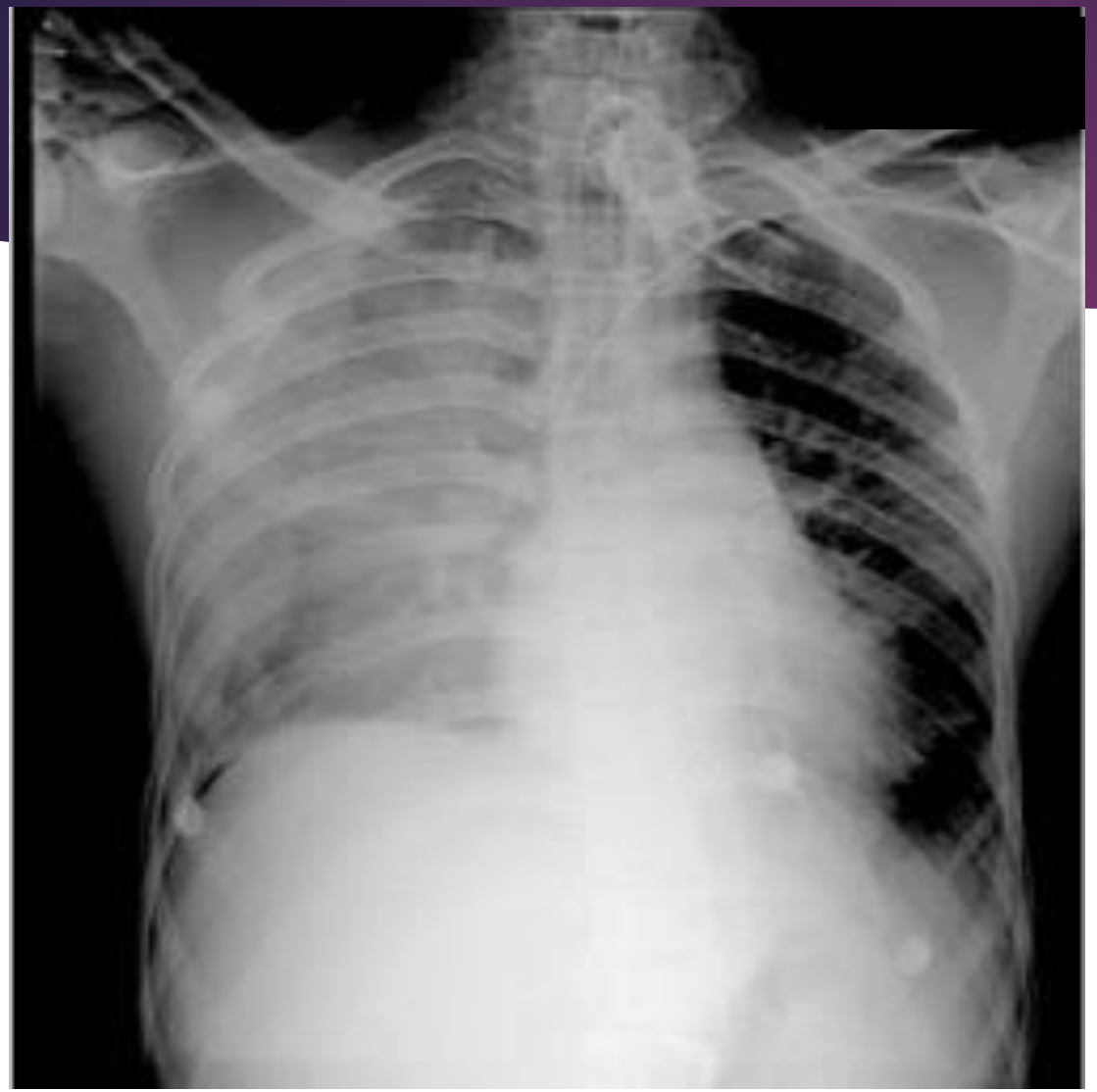
Combined with source control, disease severity and patient risk

Kohlmann, Bahr and Gatermann. 2019. CMI. 25:1158

Case 2: COVID-19 Secondary bacterial pneumonia

- 65 YO man
- Diagnosed with COVID-19, late August
- intubated, high ventilation settings, deep sedation, paralysis
- Completed dexamethaxone, remdesivir, vancomycin & pip-tazo

- Transferred to VUMC at family's request
- Arrives septic, sputum produced with deep in-line suctioning



Blood and respiratory cultures

HEAVY GROWTH OF *KLEBSIELLA PNEUMONIAE*

AST RESULTS

ANTIMICROBIAL	MIC	Interpretation
Ampicillin	>16	R
Amp/sulb	>16	R
Aztreonam	>16	R
Cefepime	>16	R
Ceftriaxone	>32	R
Ertapenem	>4	R
Meropenem	8	R
Ceftaz-avibactam	>16	R
Ceftolozane-tazobactam	>32	R

Rapid blood culture test:

"CTX-M" detected

Do these make sense?

ANTIMICROBIAL	MIC	Interpretation
Amikacin	≤8	S
Ampicillin	>16	R
Amp/sulb	>16	R
Aztreonam	>16	R
Cefepime	>16	R
Ceftriaxone	>32	R
Ertapenem	>4	R
Meropenem	8	R
Ceftaz-avibactam	16	R
Ceftolozane-tazobactam	>32	R

"CTX-M detected"

Gram negative resistance prediction

Appendix H, Table H4

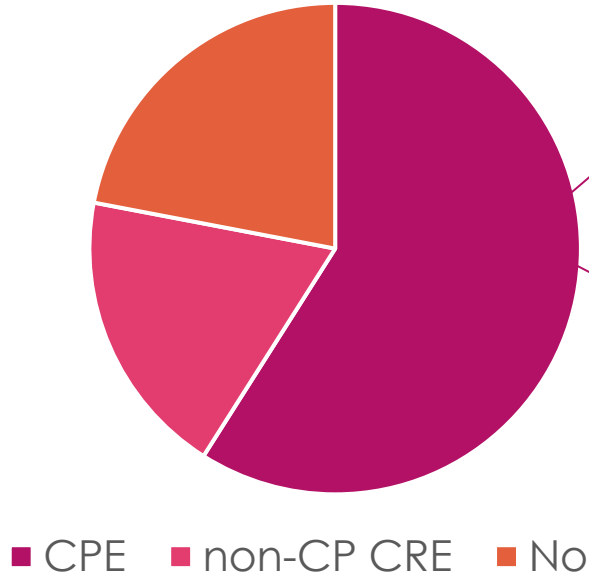
Detection of carbapenem resistance in **Enterobacterales**	KPC, OXA-48-like, VIM, NDM, or IMP	NAAT, microarray	Colony, blood culture	Detection of any tested carbapenemase target	Resistance to all carbapenems (eg, meropenem R, imipenem R, doripenem R, ertapenem R)	N/A	Report phenotypic results as found (if available); consider reporting presence of molecular target per institutional protocol.	1–4, 12–14
				Detection of any tested carbapenemase target	Susceptible to all carbapenems except ertapenem (variable) (eg, meropenem S, imipenem S, doripenem S, ertapenem R or S)	Repeat molecular and phenotypic tests. If blood culture, check for mixed culture. If mixed, test isolates individually and report phenotypic results as found; consider a phenotypic test for carbapenemase activity (such as CarbaNP or mCIM).	If the discrepancy is not resolved, repeat AST should be performed using a reference method and the conflicting genotypic and phenotypic testing results should both be reported along with a comment advising caution; current clinical and laboratory evidence is insufficient to conclude whether carbapenem monotherapy of carbapenemase-carrying strains with an MIC in the S range will be effective, or whether the molecular assays are completely accurate.	1–4, 12–15

Addresses carbapenems only…
Our isolate was "R" to the carbapenems
All tests repeat – same results.

So… are we good?

Incidence of resistance mechanism: carbapenems

Among carbapenem-R Enterobacterales (CRACKLE-2)



KPC = 93%
NDM= 3%
OXA-48-like = 3%
Other = 1% (VIM, IMI, SME, NMC-A)

CPE, carbapenemase
non-CP: no carbapenemase
Not CR: unconfirmed as carbapenem-R by central lab

Van Duin et al. Lancet Infect Dis.2020 20:731-41

What about other beta-lactams?

Enterobacterales

Agent on test system	No carb'ase	With carbapenemase		
		Class A	Class B	Class D
Ceftazidime-avibactam				
Cefotolozane-tazobactam				
Meropenem-vaborbactam				
Aztreonam			*	
Cefepime				

Intrinsic R or %S <30%	%S 30-80%	%S >80%

* If no ESBL present

Typically expect "S" to ceftazidime avibactam…

- ✓ Always make sure the identification, genotype and phenotype match
- ✓ Troubleshoot discrepancies: re-ID, re-AST, purity plates
- ✓ When in doubt… report more resistant result
- ✓ Keep an eye out for trends & escalate

Adapted from Tamma P & Hsu AJ. 2019. J Ped Infect Dis. 8:251.

Should we perform tests for carbapenemase?

- Help troubleshoot AST results
- Of value to predict activity of new antimicrobials
- Valuable for epidemiological studies

- Best predictor of outcome = MIC
- May be confusing in light of other results
- Extra labor, QC, etc

Resolution…

- Performed mCIM test: negative
- Performed PCR for NDM, IMP, VIM, KPC, OXA-48: negative
- Performed Sensititre for ceftazidime-avibactam: MIC of 16 mcg/mL, resistant

Reported results as found…

Discussed case with team, agreed to investigate cefiderocol

Whole genome sequencing = plasmid AmpC in this isolate

Clinical Evolution of AmpC–Mediated Ceftazidime–Avibactam and Cefiderocol Resistance in *Enterobacter cloacae* Complex Following Exposure to Cefepime

Ryan K Shields, Alina Iovleva, Ellen G Kline, Akito Kawai, Christi L McElheny, Yohei Doi ✉

Clinical Infectious Diseases, ciaa355, https://doi.org/10.1093/cid/ciaa355

Published: 01 April 2020 **Article history** ▾

"2-amino acid deletion in the R2 loop of AmpC beta-lactamase, which caused resistance to ceftazidime-avibactam, and reduced susceptibility to cefiderocol"

Testing newer agents: GNRs

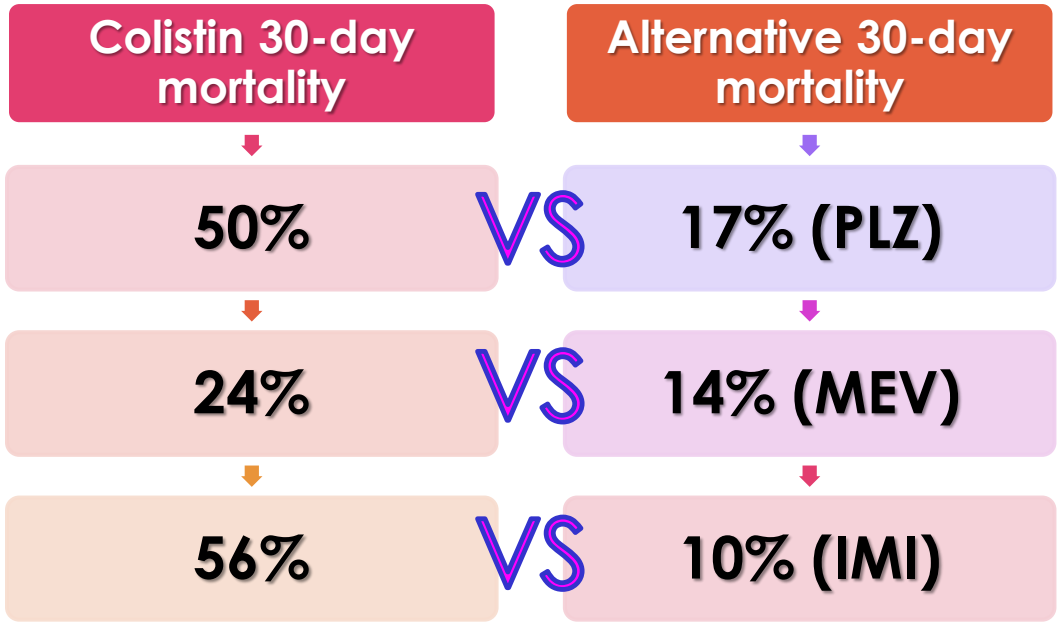
Agent	Test			
	Disk	Gradient strip	Automated Systems	Manual MIC
Ceftazidime-avibactam	✓	✓	✓	✓
Cefotolozane-tazobactam	✓	✓	✓	✓
Imipenem-relebactam	Hardy	Etest, MTS	Sensititre, Vitek 2	Sensititre
Meropenem-vaborbactam	✓	✓	✓	✓
Cefiderocol	Hardy	-	Sensititre	Sensititre
Eravacycline	Hardy	Etest, MTS	Mscan, Sensititre, Vitek 2	Sensititre
Plazomicin	Hardy	Etest, MTS	Sensititre	Sensititre
Colistin	No	No	RUO on some	RUO on some

✓ Available on most platforms

*based on 510(k) summary search on fda.gov Oct 26 2020

A note about colistin…

More patients die 30 days after therapy with colistin than with comparator agents



Randomized controlled trials comparing colistin + 2nd drug (eg, imipenem) to newer agents for treatment of CRE.

Abbreviations: PLZ, plazomicin; MEV, meropenem-vaborbactam; IMI, imipenem-relebactam

Satlin et al. 2020. Clin Infect Dis. In press.

Infectious Diseases Society of America Guidance on the Treatment of Antimicrobial Resistant Gram-Negative Infections

Published by IDSA, 9/8/2020

A Focus on Extended-Spectrum β-lactamase Producing Enterobacterales (ESBL-E), Carbapenem-Resistant Enterobacterales (CRE), and *Pseudomonas aeruginosa* with Difficult-to-Treat Resistance (DTR-*P. aeruginosa*)

Pranita D. Tamma*, Samuel L. Aitken, Robert A. Bonomo, Amy J. Mathers, David van Duin, Cornelius J. Clancy

**Corresponding Author*

IDSA guidance: CRE* treatment

If carbapenemase test not done or negative

Ertapenem	Meropenem	Preferred treatment	Alternative
R	S	Extended infusion meropenem	Ceftazidime-avibactam
R	R	Ceftazidime-avibactam Meropenem-vaborbactam Imipenem-relebactam	Cefiderocol Tigecycline, eravacycline

* CDC definition: “R” to any of ertapenem, doripenem, meropenem or imipenem

Review > Expert Rev Anti Infect Ther. 2019 Oct;17(10):819-827.
doi: 10.1080/14787210.2019.1673731. Epub 2019 Oct 8.

Use of meropenem in treating carbapenem-resistant Enterobacteriaceae infections

Renato Pascale [1], Maddalena Giannella [1], Michele Bartoletti [1], Pierluigi Viale [1], Federico Pea [2 3]

Affiliations + expand

PMID: 31559876 DOI: 10.1080/14787210.2019.1673731

Abstract

Introduction: The epidemiology of carbapenem-resistant Enterobacterales (CRE) is increasingly worldwide. Production of carbapenemases is the most common and efficient mechanism of carbapenem resistance, and could theoretically be overcome by optimizing the pharmacokinetic/pharmacodynamic (PK/PD) behavior of meropenem. **Areas covered**: This article overviews the available literature concerning the potential role that meropenem may still have in the treatment carbapenem-resistant Enterobacteriaceae infections. Clinical studies published in English language until June 2019 were searched on PubMed database. **Expert commentary**: High-dose continuous infusion meropenem-based combination regimens could still represent a valuable option for treating CRE infections in specific circumstances. Knowledge of the local prevalent mechanisms of carbapenem resistance, of patient clinical severity, of the site of infection, of an accurate minimum inhibitory concentration (MIC) value, coupled with the possibility of carrying-out a real-time therapeutic drug monitoring (TDM)-based PK/PD optimization of drug exposure must all be considered as fundamental for properly pursuing this goal.

Keywords: CRE treatment; Combination therapy; Meropenem; PK/PD optimization; continuous infusion; therapeutic drug monitoring.

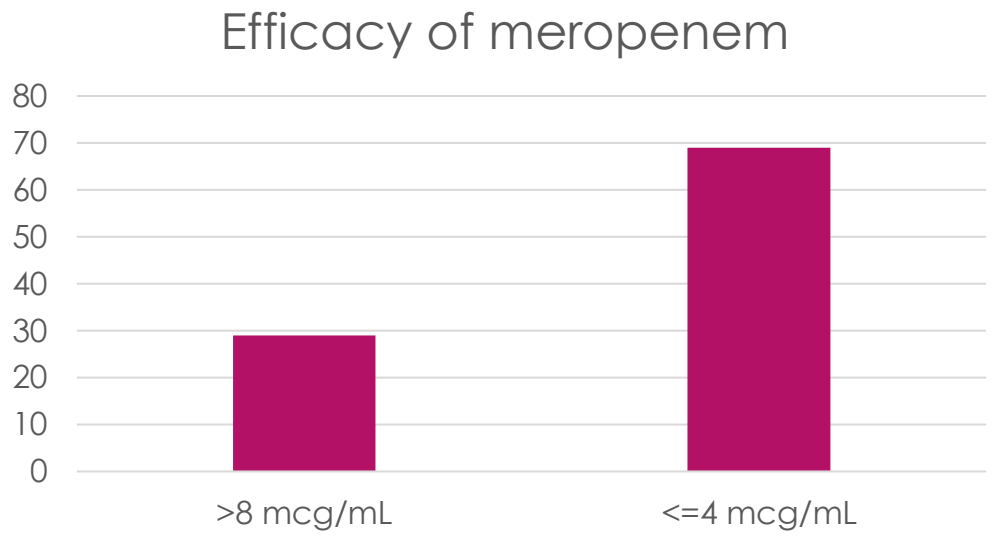
Knowledge of:
- Local resistance mechanisms
- Clinical severity
- **Accurate MIC**

How does the MIC come into play?

- Meropenem, 2g q8 h by prolonged infusion, is recommended by some to treat isolates with MICs of 2- 8 mcg/mL.

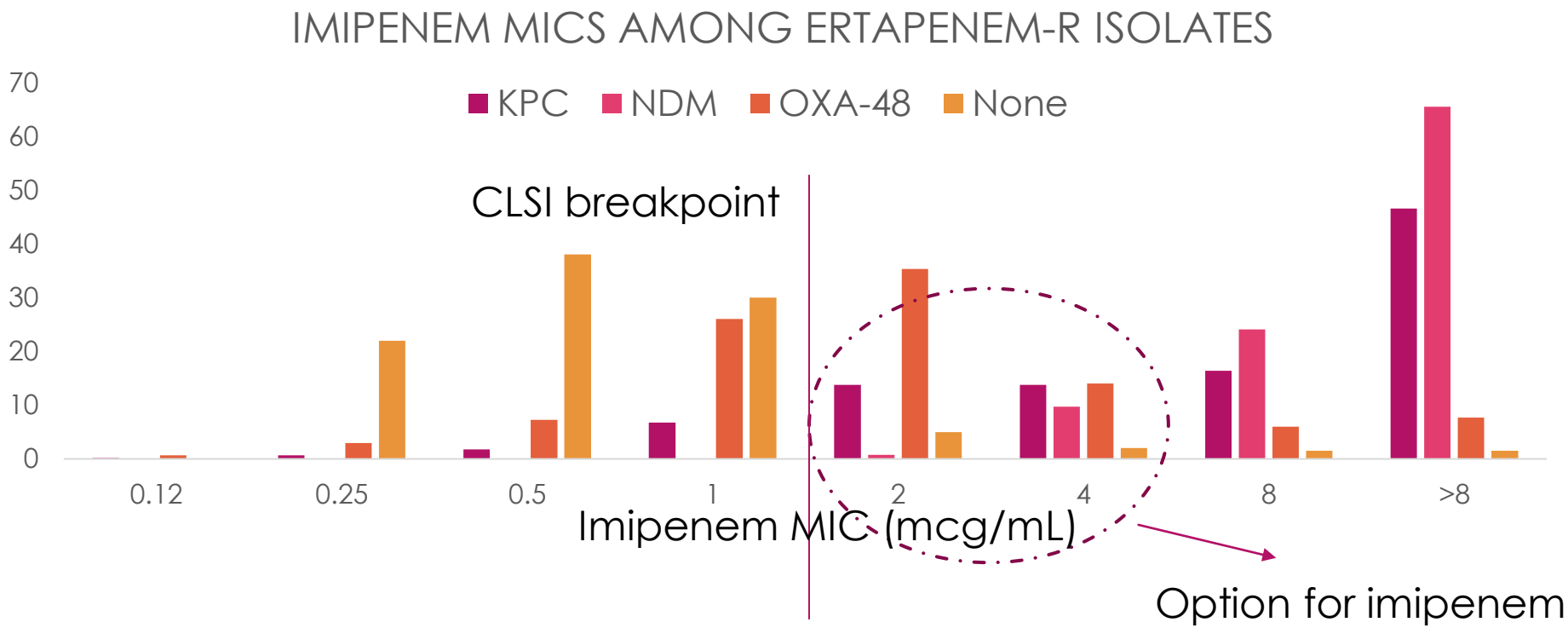
Carbapenem	EUCAST		CLSI	
	S	R	S	R
Ertapenem	≤0.5	>0.5	≤0.5	>1
Meropenem	≤2	>8	≤1	>2
Imipenem	≤2	>4	≤1	>2

EUCAST: MIC OF 4 (mero/imi) or 8 (mero) can be treated with elevated exposures (dose, frequency etc)



Sheu et al. 2019. Front Microbiol

Imipenem MIC by carbapenemase type



Option for imipenem treatment?
"S" or "I" by EUCAST breakpoints

Karlowskey et al. JCM 2017. 55: 1638
Jean et al. J Glob Antimicrob Resis. 2020. 21:91

IDSA Guidance: CPE

Carbapenemase	Preferred	Alternative
KPC	Ceftazidime-avibactam Meropenem-vaborbactam Imipenem-relebactam	Cefiderocol, Tigecycline, Eravacycline
NDM, VIM, IMP	Ceftazidime-avibactam + Aztreonam Cefiderocol	Tigecycline, Eravacycline
OXA-48-like	Ceftazidime-avibactam	Cefiderocol Tigecycline Eravacycline

CPE, Carbapenemase producers

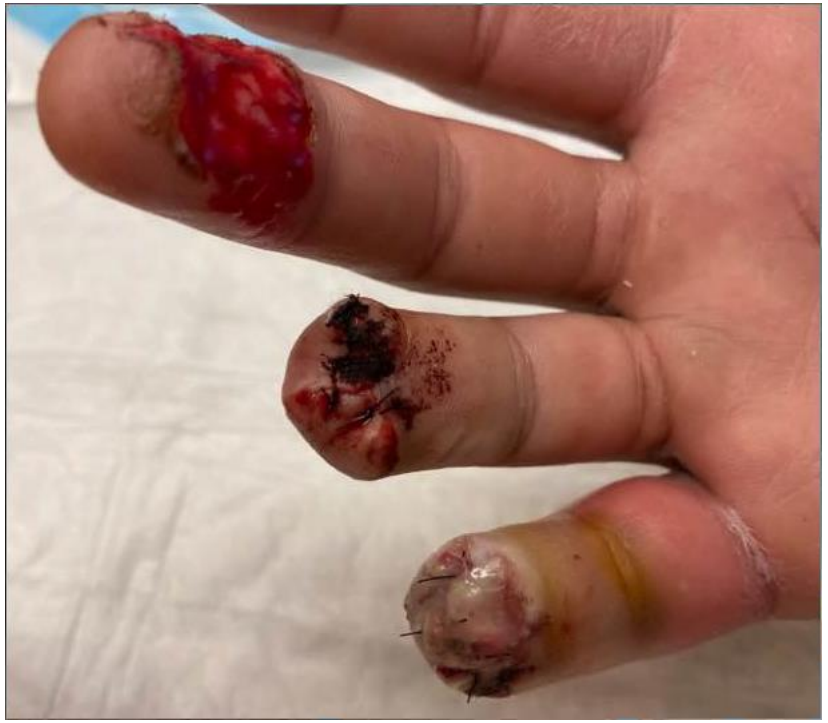
IDSA Rx Options: carbapenemase producers

Agent	Drug class	IDSA indication	Resistance?
Ceftaz-avib	Beta-lactam combo	KPC OXA-48	Mutation to KPC gene
Mero-vabor	Beta-lactam combo	KPC	? Porin loss/efflux (rare)
Imi-relebactam	Beta-lactam combo	KPC	? Not documented
Cefiderocol	Siderophore cephalosporin	MBL 2nd line, KPC, OXA-48	~20% are "R", due to modest enzymatic activity vs. cefiderocol
Eravacycline	Fluorocycline	2nd line, any	Often R, must test
Tigecycline	Glycylcycline	2nd line, any	May be R, must test

Mushtaq et al. AAC. 2020 In press; Simner and Patel. JCM2020 in press.
Livermore et al. AAC 2020. in press

Case 3: Surgical infection

- 48 YO man
- Crush injury to hand
- Amputation to 2 fingers: small finger and ring finger
- Postoperatively given cephalexin
- Returns to ED 1 week later due to pain, foul odor
- Surgical revision, irrigation and debridement, infection of flexor tendon sheath of small finger



Culture results

K. pneumoniae	MIC (mcg/mL)	
Amox-clav	>16	R
Amp-sulbactam	8	S
Aztreonam	≤2	S
Cefoxitin	≤4	S
Cefepime	4	SDD
Ceftazidime	≤2	S
Ceftriaxone	>32	R
Ciprofloxacin	≤0.25	S
SXT	≤0.5	S

"ESBL detected"

Laboratory:
"Should we trust these results?"

Back to M100 Appendix H

Table H3. Reporting Results From Extended-Spectrum β-Lactamase Resistance and Carbapenemase Molecular Tests for Enterobacterales

Indication	Target(s)	Method	Specimen Type	Results		Suggestions for Resolution	Report as:	Comments[a]
				Molecular Target Results	Observed Phenotype (if tested)			
Detection of ESBL resistance in **Enterobacterales** (in an isolate susceptible to all carbapenems)	ESBL type CTX-M, SHV, TEM	NAAT, microarray	Colony, blood culture	Detection of any ESBL target	R to all 3rd- and 4th-generation cephalosporins tested (eg, ceftriaxone R, cefotaxime R, ceftazidime R, cefepime R)	N/A	Report phenotypic results as found (if available); consider reporting molecular [illegible] institution	1–12
				Detection of any ESBL target	S to all 3rd- and 4th-generation cephalosporins tested (eg, ceftriaxone S, cefotaxime S, ceftazidime S, cefepime S)	Repeat molecular and phenotypic tests. If blood culture, check for mixed culture. If mixed, test isolates individually and report phenotypic results as found.	If the disc resolved, should be using a re method, a conflicting phenotypi results sh reported.	
				Detection of *CTX-M* ESBL target	Variable resistance to 3rd- and 4th-generation cephalosporins (eg, ceftriaxone R, cefotaxime R, ceftazidime R or S, cefepime R or S)	Expected phenotype for some *CTX-M* strains. Check cefepime using a reference method if S.	Report ph results as including reference cefepime result; consider reporting presence of molecular target per institutional protocol.	
				Detection of *TEM* or *SHV* ESBL target	Variable resistance to 3rd- and 4th-generation cephalosporins (eg, ceftriaxone R or S, cefotaxime R or S, ceftazidime R or S, cefepime R or S).	Expected phenotype for some *TEM/SHV* strains. Check cefepime using a reference method if S.	Report phenotypic results as found, including reference cefepime result; consider reporting presence of molecular target per institutional protocol.	1–12

Variable resistance to 3rd and 4th- generation cephalosporins

Expected phenotype

Report phenotype as found

M100
Appendix H

Types of ESBLs in the US (common)

	Prevalence	**Observed susceptibility (2020 breakpoints)**			
		Ceftriaxone	Ceftazidime	Cefepime	Pip-tazo
SHV type	~25%				
TEM type	<5%				
CMT	Low				
CTX-M-14	~10%				
CTX-M-15	~43%				

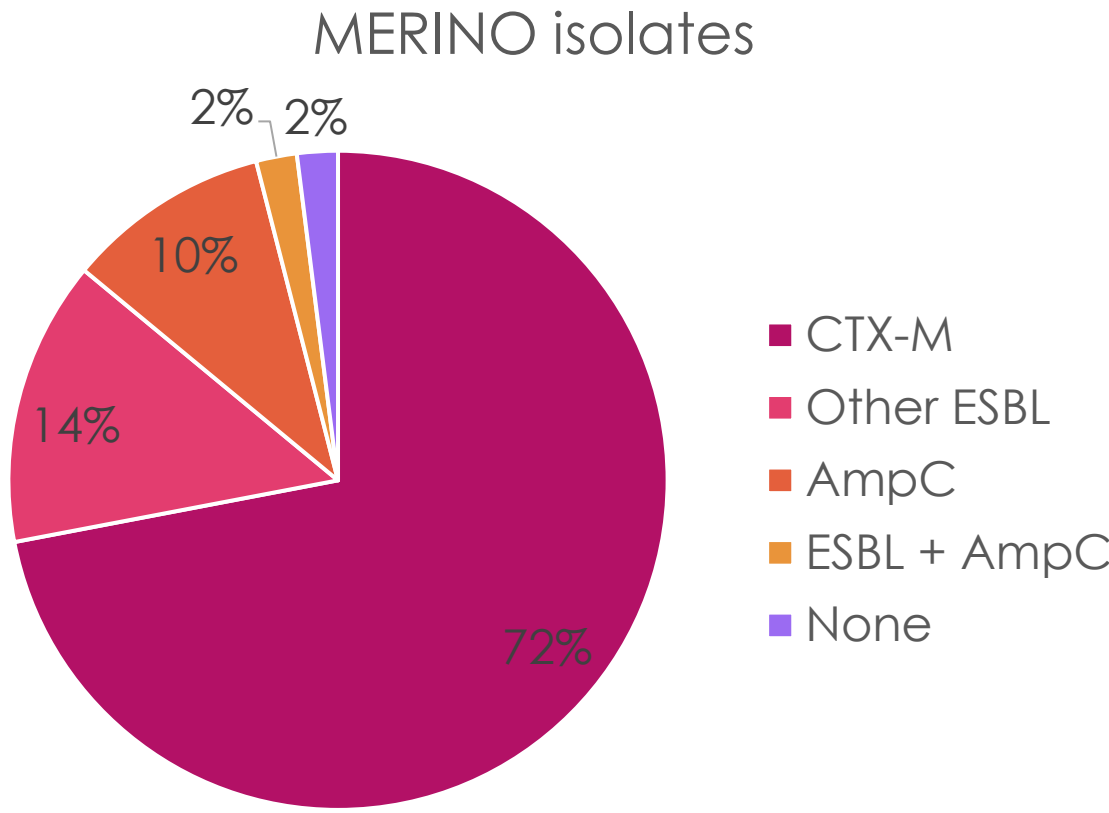
>90% R
>75% R
~50% R
<10% R

Paterson and Bonomo. 2005. CMR. 18:657
Wang et al. JCM. 2011. 49:3127
Castanheira et al. 2014. AAC. 58:838

MERINO Trial

- Study of "ceftriaxone not susceptible" isolates
- Patient with bacteremia treated with:

	Mortality
- Pip-tazo	12.3%
- Meropenem	3.7%



Should we do ESBL testing again?

- MERINO was trial of ceftriaxone I or R, not ESBL (many did not have ESBL)
  - Ceftriaxone NS can be due to: hyperexpression of TEM/SHV, AmpC, porin mutations, etc
- CLSI (and commercial tests) vary in ability to detect ESBL:

CLSI double disk[1]	E. coli	K. pneumoniae
PPV	97.6%	81.8%
NPV	75.9%	95.2%

- "Ceftriaxone not- susceptible *E. coli* and *Klebsiella* spp (I or R) are associated with an increased risk for treatment failure with piperacillin-tazobactam. Meropenem therapy preferred."

1. Morrissey et al. JMM. 2014. 63: 556-61

IDSA Guidelines: ESBL treatment

Infection	First-line	Second-line
Uncomplicated UTI	Nitrofurantoin Trim-sulfamethoxazole	Amox-clavulanate Aminoglycoside Oral Fosfomycin
Complicated UTI	Carbapenem Fluoroquinolone SXT	-
Other	Carbapenem	Oral step-down: - Fluoroquinolone - Trimeth-sulfa

"Piperacillin-tazobactam should be avoided, even if "S".
If started empirically for uUTI, and clinically improving, no need to change.

Example: Ceftriaxone-R *E. coli*

Typical GN AST panel

Antimicrobial	Result	Report?
Ampicillin	R	Yes
Amox-clavulanate	S	If urine
Aztreonam	R	Yes
Cefazolin	R	Yes
Ceftriaxone	R	Yes
Cefepime	**SDD**	**No**
Pip-Tazo	**S**	**No**
Ertapenem	S	Yes
Meropenem	S	Yes
Trim-sulfa	S	Yes
Amikacin	**S**	**No**
Tobramycin	**S**	**No**
Gentamicin	S	Yes
Tetracycline	**S**	**No**
Ciprofloxacin	R	Yes
Levofloxacin	R	Yes
Nitrofurantoin	S	If urine

Final reports: urine

Antimicrobial	Result
Ampicillin	R
Amox-clavulanate	S
Aztreonam	R
Cefazolin	R
Ceftriaxone	R
Ertapenem	S
Meropenem	S
Trim-sulfa	S
Gentamicin	S
Ciprofloxacin	R
Levofloxacin	R
Nitrofurantoin	S

Optional comments:

- Ceftriaxone resistance is consistent with the presence of an ESBL in this isolate
- Nitrofurantoin or trimethoprim-sulfamethoxazole are preferred options for uncomplicated cystitis, if tolerated
- Nitrofurantoin should not be used in patients with renal insufficiency
- Nitrofurantoin should not be used for cases of complicated UTI, including pyelonephritis.

Final reports: not urine

Antimicrobial	Result
Ampicillin	R
Aztreonam	R
Cefazolin	R
Ceftriaxone	R
Ertapenem	S
Meropenem	S
Trim-sulfa	S
Gentamicin	S
Ciprofloxacin	R
Levofloxacin	R

- Ceftriaxone resistance is consistent with the presence of an ESBL in this isolate
- Carbapenems are preferred therapy of infections outside of the urinary tract

ESBL: options

- Check cephalosporin vs. ESBL call (or CTX-M detection from molecular test)
- Aware ceftazidime may be S or R, expect ceftriaxone and cefepime are R
- If ceftriaxone I or R, do not report pip-tazo (regardless of MIC). Report meropenem

Summary

- Genotype: phenotype interpretations can be complex
- Maximize value of AST testing by doing both, but only if:
  - Laboratory has protocols for how to compare genotype with phenotype
  - Laboratory has protocols for troubleshooting results
- Knowing the MIC can be of value: troubleshooting and also for treatment
- Lots of new drug options! But… must test most.

Thank you!

- Question?
- Email me: romney.humphries@vumc.org