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Stewardship of Difficult-to-Treat Gram Negative Infections: Metallo-β-Lactamases

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Disclosures

- Advisory boards
  - Shionogi
  - bioMerieux
  - Meitheal
  - Wockhardt
  - Beckman Coulter
  - Melinta (now Cormedix)
  - GSK
- Scientific Congress speaker
  - GSK
  - bioMerieux

- I will discuss off-label use of antimicrobials
- I will discuss investigational use of antimicrobials
- I will present my own evidence-based opinions

Objectives

- Describe the different types of metallo-β-lactamases (MBL) found in Enterobacterales infections
- Compare evidence-based antibiotic treatment options for infections caused by MBL-producing Enterobacterales, including advantages, limitations, and appropriate clinical scenarios for newer therapies
- Determine mechanisms of resistance of MBL-producing Enterobacterales to novel antibiotics

Last Update: [redacted]
Collected:
Specimen Desc: Urine

URINE CULTURE

Accession Num: [redacted]
Special Request:
Status: Modified

Culture: >=100,000 colonies/ml Escherichia coli [redacted]
Observation date/time changed from [redacted]

ESCHERICHIA COLI

	MIC (mcg/mL)	MIC Interpretation
Amox/K Clavulanate	>16/8	Resistant
Amp/Sulbactam	>16/8	Resistant
Ampicillin	>16	Resistant
Aztreonam	>16	Resistant
CEFTAZIDIME/AVIBACTAM	>16	Resistant
Cefazolin	>16	Resistant

When cefazolin is used for therapy of UNCOMPLICATED UTIs due to E.coli, K.pneumoniae or P.mirabilis. Breakpoints are based on a dosage regimen of 1g every 12h.

Cefepime	>16	Resistant
Ceftazidime	>16	Resistant
Ceftriaxone	>32	Resistant
Cefuroxime	>16	Resistant

When oral cefuroxime is used for therapy of UNCOMPLICATED UTIs due to E.coli, Proteus mirabilis or Klebsiella pneumoniae.

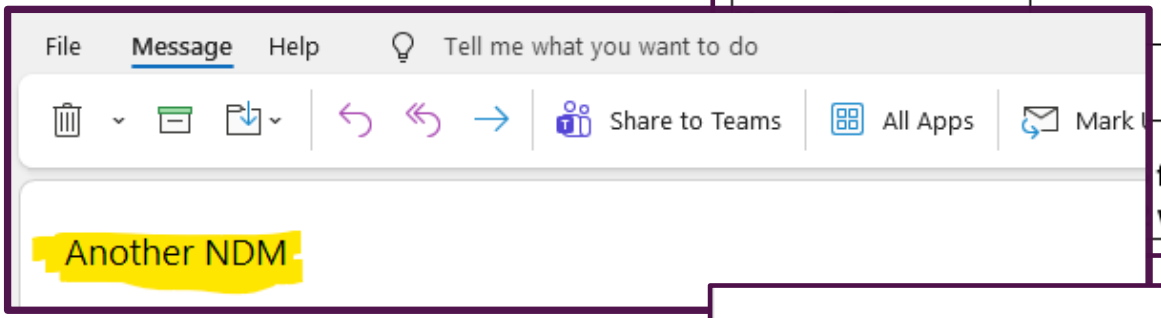
Ciprofloxacin	>2	Resistant
Ertapenem	>1	Resistant
Gentamicin	>8	Resistant
Levofloxacin	>4	Resistant
Meropenem	>8	Resistant
Meropenem/Vaborbactam	>16	Resistant
Nitrofurantoin	<=32	Sensitive
Piperacillin/Tazobactam	>64	Resistant
Sulfa/Trimethoprim	>2/38	Resistant
Tetracycline	>8	Resistant
Tobramycin	>8	Resistant

To: Carba5 <Carba5@upmc.edu>
Subject: CRO isolated

Good morning, Altoona,

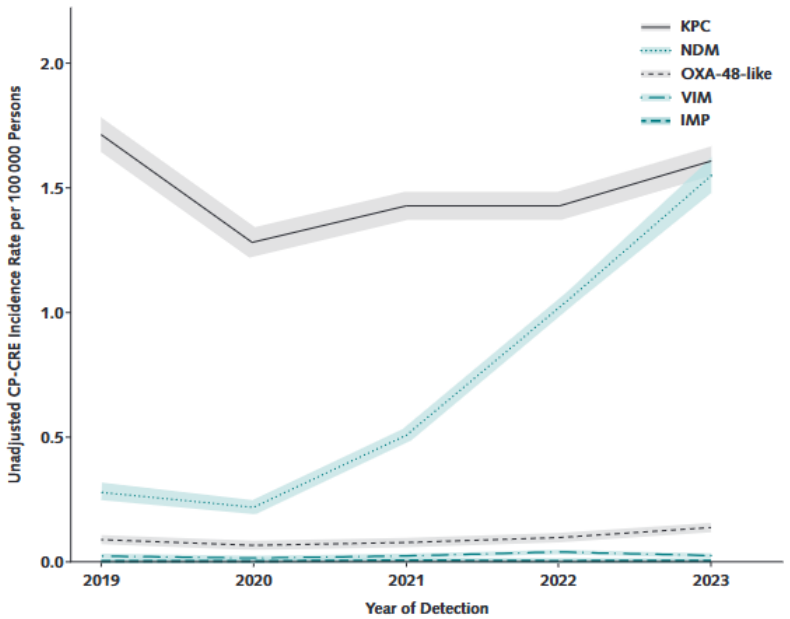
Attached please find documentation of CRO isolated from an outpatient. Additional susceptibility testing is pending.

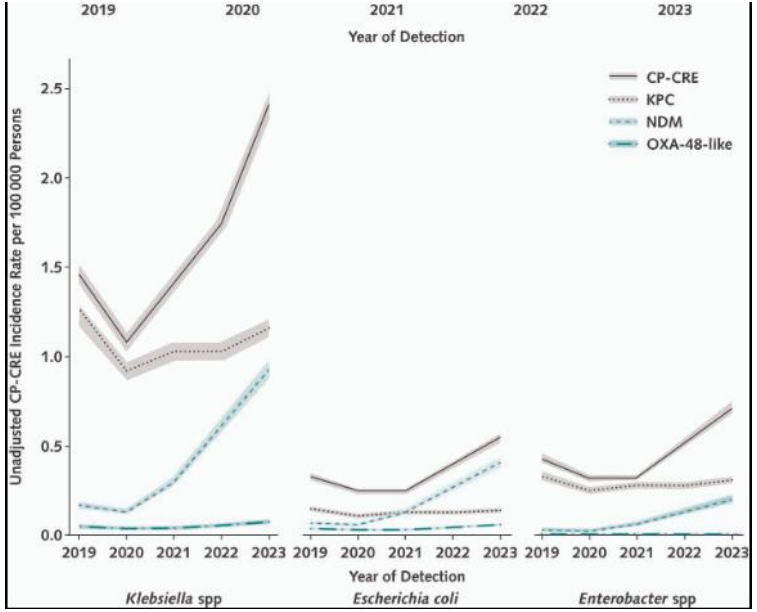
Organism	**Ecoli 1 and 2**
	for morph 1 was positive for NDM. Carba 5 for was positive for NDM and OXA.



Patient current hospital location: Chautauqua 5D
Patient Name: [redacted]
Patient MRN: [redacted]
Specimen Accession: 26CHA-[redacted]
Specimen type: Sputum, Transtracheal Aspirate
Organism name: Klebsiella pneumoniae
Carbapenemase Detected (CARBA 5 result): Control Positive, NDM Positive

It’s the season of NDMs





- NDM incidence increased by >400% between 2019 and 2023
- Now most common carbapenemase identified in NYC
- From CDC data, NDM increased from 5.4% (2016) to 39.8% (2023)

Devinney K, et al. *MMWR* 2025;74(23):401-3
Rankin DA, et al. *Ann Intern Med* 2025;178(12):1818-21

We go way back…

Biochem. J. (1966) **98**, 11c

Zinc as a Cofactor for Cephalosporinase from *Bacillus cereus* 569

By L. D. Sabath* and E. P. Abraham
Sir William Dunn School of Pathology, University of Oxford

(*Received* 25 *October* 1965)

Crude preparations of β-lactamase from *Bacillus cereus* 569 show a selective loss of cephalosporinase activity on purification (Abraham & Newton, 1956) and during prolonged action on substrate (Sabath & Abraham, 1965). They also show a much greater loss of penicillinase (EC 3.5.2.6) activity than cephalosporinase activity on keeping at 60° (Crompton, Jago, Crawford, Newton & Abraham, 1962). During attempts to purify the crude preparation cephalosporinase activity was repeatedly lost although penicillinase activity was retained. when measurements were made with enzyme that had been treated with EDTA (at concentrations down to 2·7 μM) but not dialysed.

Cephalosporinase activity was restored to solutions of crude enzyme that had been subjected to treatment with EDTA and dialysis by the addition of $ZnSO_4$. When the latter was added in optimum amount (final concn. 1 mM) the cephalosporinase activity rose to 4–5 times that of the original crude enzyme (Table 1). Addition of Zn^{2+} to the original crude enzyme, or to the dialysed enzyme, also

Metallo-β-Lactamases (MBLs)

B1 are plasmid-borne MBLs and include **IMP**, **VIM** and **NDM**
Broad-spectrum substrate profile which include penicillins, cephalosporins, and carbapenem
Chromosomal B1 enzymes include BcII (*B. cereus*), IND (*C. indologenes*), BlaB (*E. meningoseptica*)

IMP: First identified in 1988 in *P. aeruginosa* (Japan) – characterized as IMP-1 from a *S. marcescens* clinical isolate in 1994. Now endemic in Australia, Japan and SE Asia

VIM: First reported in 1999 in *P. aeruginosa* (Verona, Italy) – endemic in China, Middle East, Mexico, Columbia. More conserved (less diverse) than IMP – found mostly in *P. aeruginosa* clinically

NDM: First reported in 2009 in *K. pneumoniae* (Sweden) from patient of Indian origin

B2 are **chromosomal enzymes** such as **CphA** from *Aeromonas* and **Shf-1** from *Serratia fonticola*
Narrow-spectrum substrate profile limited to carbapenems

B3 are **chromosomal enzymes** such as **L1** from *Stenotrophomonas maltophilia*
Broad-spectrum substrate profile which include penicillins, cephalosporins, and carbapenems

Boyd SE, AAC 2020; Bahr G, et al. Chem Rev 2021;121(31):7957-8094

Slide modified from Maria Mojica, PhD

MBLs are everywhere

VIM enzyme	First report of variant discovery
VIM-1	Italy
VIM-2	France
VIM-3	Taiwan
VIM-4	Greece
VIM-5	Turkey
VIM-6	Singapore
VIM-7	United States
VIM-8	Colombia
VIM-10	United Kingdom, India

Bonnin et al. Clin Microbio Reviews. 2025.

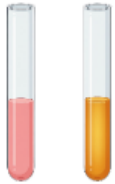
How do we find them?

Modified carbapenem inactivation method (mCIM)

- Incubate isolate with a meropenem disk,
- Positive test means disk is inactivated on a susceptible *E.coli* lawn
- Low cost, low complexity, uses standard micro supplies, turnaround time 18-24h

Carba NP Test

- Rapid colorimetric assay that detects pH changes caused by hydrolysis of imipenem
- Fast turnaround time, color change can be subjective

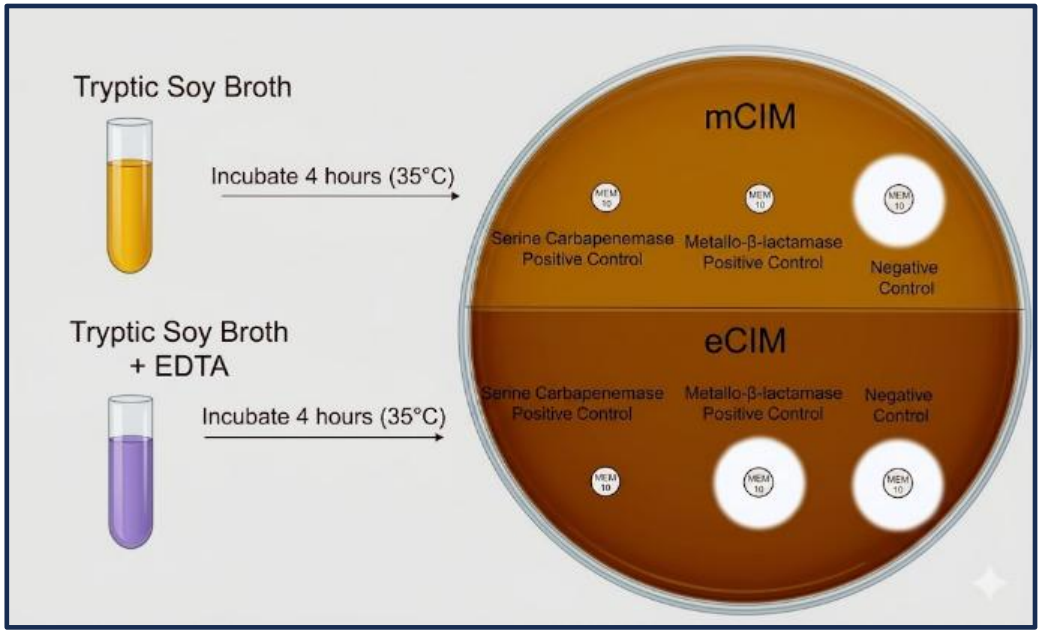


Modified Hodge Test

- No longer recommended by CLSI
- The test isolate and a carbapenem susceptible isolate are plated with a carbapenem containing disk – positive tests give a cloverleaf-life indention

Tsai YM et al. BMC Microbiol 2020;20(1):315.
Cunningham SA, et al. J Clin Microbiol 2017;55(6):1954-60.
Simner PJ. J Clin Micro 2026 Mar 18:e0045125.
Images courtesy of University of Maryland Medical Center Microbiology Laboratory

EDTA-modified carbapenem inactivation method (eCIM)



Simner PJ. J Clin Micro 2026 Mar 18:e0045125.
Sfeir M et al. J Clin Microbiol 2019;57(5):e01751-18.
Image created by Google Gemini

Carbapenemase Differentiation Tests

Immunochromatographic lateral flow assays

- Detects 5 common carbapenemase families (KPC, OXA-48-like, VIM, IMP, NDM)
- Fast turnaround time (5-15 min)
- Low complexity
- Low to moderate cost
- Can be set up simultaneously with the mCIM, as reflex only for mCIM positive, or independently off phenotype of standard AST

Jenkins S, et al. J Clin Microbiol 2020;58(7):e00344-20.
Simner PJ. J Clin Micro 2026 Mar 18:e0045125.
Image generated by Google Gemini
slide modified from Ryan Shields and Emily Heil

Molecular Detection Methods (i.e., PCR)

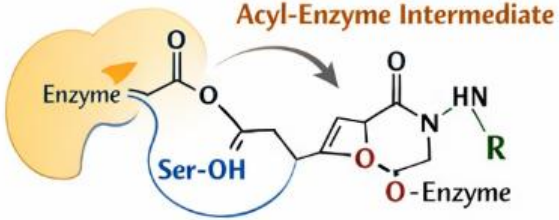
- Typically focus on detection of the ‘big five’ - *bla*$_{KPC}$ *bla*$_{NDM}$ *bla*$_{VIM}$ *bla*$_{IMP}$ and *bla*$_{OXA\text{-}48\text{-}like}$
- Numerous tests and panels available; most are for positive blood cultures (i.e., built into your Verigene, Biofire, EPLEX, etc…)
- Some available for isolates from other non-blood sources
- Turnaround times are generally <2 hours
- High sensitivity and specificity compared to other methods
- Higher cost and complexity

Simner TJ, et al. Clin Micro Reviews 2024;37(4):e00054-22.
slide modified from Ryan Shields and Emily Heil

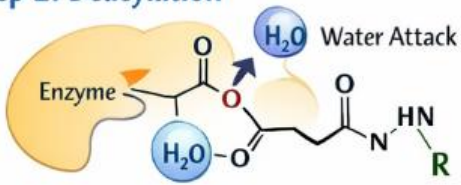
β-Lactamase Hydrolysis Mechanisms

Serine β-Lactamase (Serine-based)

Step 1: Acylation



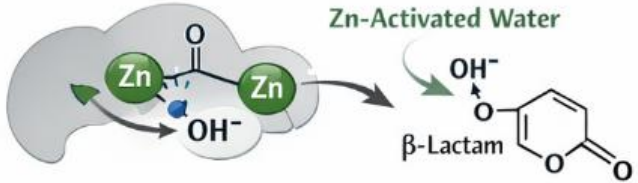
Step 2: Deacylation



- Covalent Acyl-Enzyme
- Water for Deacylation
- Two-Step Process
- Ser-OH Attack

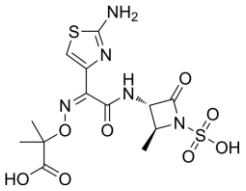
Metallo-β-Lactamase (Zinc-based)

One-Step Hydrolysis



Direct Attack on β-Lactam

- Zn-Activated Hydroxide
- Water as Nucleophile
- One-Step Process
- Direct Attack



Aztreonam

Non-productive binding

Image created using ChatGPT, slide from Ryan Shields and Emily Heil

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How do we treat MBL-producing Enterobacterales?

Ceftazidime-avibactam plus aztreonam

- ATM+AVI development began in 2012 → we got creative in the meantime!
- NDM-9 *E. coli* at MD Anderson → compassionate use **CZA** combined with ATM in 2015[1]
- CZA+ATM to treat recurrent S. maltophilia bacteremia in a pediatric patient[2]

1. Aitken SL, et al. CID. 2016; 2. Mojica MF, et al. AAC. 2016

C. freundii at UPMC in 2018 harboring blaNDM

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Culture: >100,000 col/mL Citrobacter freundii Confirmed as a carbapenemase producer and is RESISTANT TO ALL CARBAPENEMS
>100,000 col/mL Citrobacter freundii complex No.2 Confirmed as a carbapenemase producer and is RESISTANT TO ALL CARBAPENEMS

CITROBACTER FREUNDII COMPLEX

	MIC (mcg/mL)	MIC Interpretation
Amikacin	<=16	Sensitive
Amp/Sulbactam	>16/8	Resistant
Ampicillin	>16	Resistant
Aztreonam	>16	Resistant
Cefepime	>16	Resistant
Ceftazidime	>16	Resistant
Ceftriaxone	>32	Resistant
Ciprofloxacin	<=1	Sensitive
Ertapenem	>1	Resistant
Gentamicin	>8	Resistant
Imipenem	8	Resistant
Levofloxacin	0.5	Sensitive
Meropenem	8	Resistant
Nitrofurantoin	<=32	Sensitive
Piperacillin/Tazobactam	>64	Resistant
Sulfa/Trimethoprim	>2/38	Resistant
Tetracycline	>8	Resistant
Ticarcillin/Clavulanic Acid	>64	Resistant
Tobramycin	>8	Resistant

CITROBACTER FREUNDII

	MIC (mcg/mL)	MIC Interpretation
Amikacin	<=8	Sensitive
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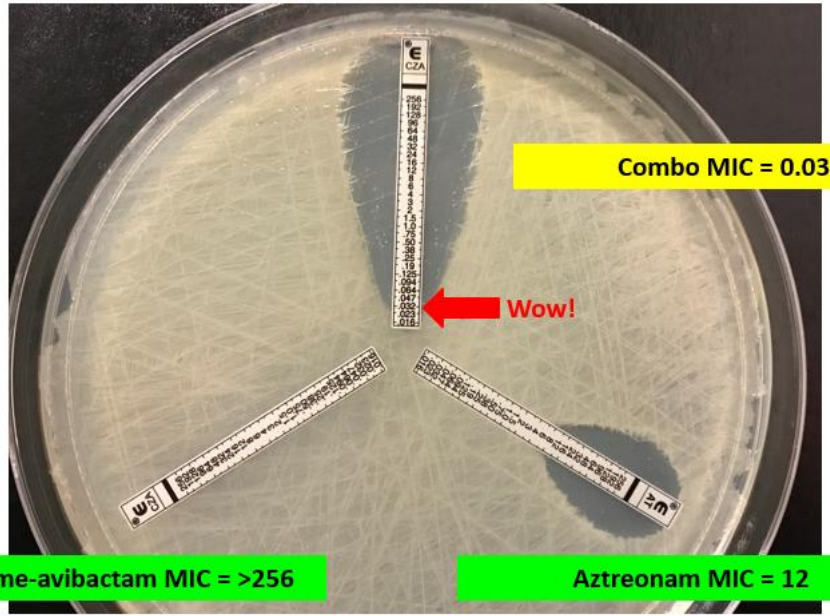
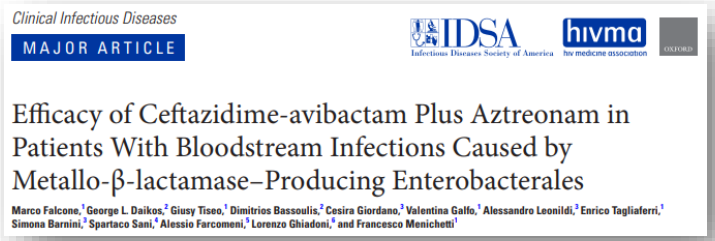
Cefepime	>16	Resistant
Ceftazidime	>16	Resistant
Ceftriaxone	>32	Resistant
Ciprofloxacin	<=1	Sensitive
Ertapenem	>1	Resistant
Gentamicin	>8	Resistant
Meropenem	>8	Resistant
Nitrofurantoin	<=32	Sensitive
Piperacillin/Tazobactam	>64	Resistant
Sulfa/Trimethoprim	>2/38	Resistant
Tobramycin	>8	Resistant

Synergy with ceftaz-avi + aztreonam



The first large, observational clinical data set for CZA+ATM for Enterobacterales



- 102 MBL-producing bloodstream infections; treatment with ≥1 antimicrobial showing in vitro activity for at least 48 hours
- Mortality
  - **19.2%** CZA-ATM vs **59.3%** Colistin-containing vs **26.1%** Non-colistin therapy

Factor	HR (95% CI)	P Value
Cardiovascular disease	6.62 (2.77–15.78)	<.001
Solid organ transplantation	3.52 (1.42–8.69)	.006
SOFA score (each 1 point increment)	1.21 (1.1–1.32)	<.001
CZA+ATM (vs OAA)	**0.17 (.07–.41)**	**<.001**

Falcone M, et al. CID. 2021.

3-year follow up data for CZA+ATM for Enterobacterales

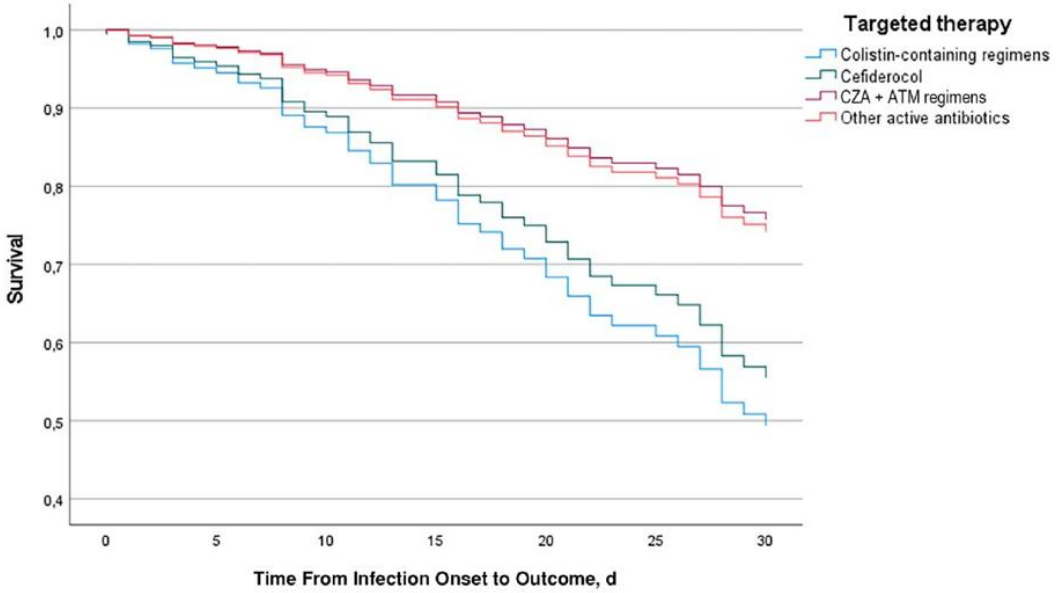


Figure 3. Sensitivity analysis (after exclusion of patients who did not receive any active antibiotic therapy), showing Kaplan-Meier survival curves according to targeted antibiotic regimens (adjusted for age, Charlson comorbidity index, septic shock, source control, active therapy within 48 hours, and respiratory tract as source of infection). Abbreviations: ATM, aztreonam; CZA, ceftazidime-avibactam, $P < .05$.

Falcone M, et al. CID. 2024

What about *P. aeruginosa?*

- 8 patients with VIM-producing *P. aeruginosa*
- 7/8 achieved cure; 2/8 died within 28 days
- **ATM and ATM-AVI MICs are the same**

Table 1. Antibiotic susceptibility profiles, sequence type and WGS resistome of the VIM-producing *Pseudomonas aeruginosa* isolates

		MIC (mg/L)															WGS resistome	
Patient	TZP	CAZ	CZA	FEP	C	ATM	ATA	I	IMR	MEM	MVB	AMK	TOB	CIP	CST	FDC	ST	Acquired resistome	Mutational resistome
1	≥128	16	32	≥32	≥	**16**	**16**	≥	>32	**8**	16	≥64	≥16	2	**2**	**0.25**	235	VIM-2, *aac(6')-Ib3*, *aac(6')-II*, *aac(3)-I*, *aadA6*	*mexR* (nt424Δ11), *mexB* (S1041G, V1042A), *mexZ* (E149K), *gyrA* (T83I)
2	≥128	16	32	16	≥	**4**	**4**	≥	>32	16	**8**	**4**	≥16	≥4	**2**	**0.12**	253	VIM-2, *aac(6')-Ib-Hangzhou*	*mexZ* (aa102-105Δ), *mexD* (S915A), *pbpA*-PBP2 (A481T), *gyrA* (T83I), *parC* (S87W)
3	≥128	16	32	16	≥	**8**	**8**	≥	>32	**8**	**8**	**4**	≥16	≥4	**≤0.5**	**0.25**	253	VIM-2, *aac(6')-Ib-Hangzhou*	*mexZ* (aa102-105Δ), *mexD* (S915A), *pbpA*-PBP2 (A481T), *gyrA* (T83I), *parC* (S87W)
4	≥128	16	16	16	≥	**4**	**4**	≥	>32	**4**	**8**	**4**	≥16	≥4	**≤0.5**	**0.25**	253	VIM-2, *aac(6')-Ib-Hangzhou*	*mexZ* (aa102-105Δ), *mexD* (S915A), *pbpA*-PBP2 (A481T), *gyrA* (T83I), *parC* (S87W)
5	≥128	16	32	16	≥	**8**	**8**	≥	>32	**8**	**8**	**4**	≥16	≥4	**≤0.5**	**0.25**	253	VIM-2, *aac(6')-Ib-Hangzhou*	*mexZ* (aa102-105Δ), *mexD* (S915A), *pbpA*-PBP2 (A481T), *gyrA* (T83I), *parC* (S87W)
6	≥128	≥64	>256	≥32	≥	**8**	**4**	≥	>32	≥16	128	**4**	≥16	1	**2**	**0.5**	309	VIM-1, CARB-4, *aac(6')-Ib3*, *aadA1*, *qnrVC1*	*mexA* (K76Q), *mexZ* (A61P), *mexE* (D533E), *oprD* (nt606insIS256-like), pbpC-PBP3a (T335S), gyrA (T83I)
7	≥128	16	16	16	≥	**4**	**8**	≥	>32	16	**8**	**4**	≥16	≥4	**1**	**0.5**	253	VIM-2, *aac(6')-Ib-Hangzhou*	*mexZ* (aa102-105Δ), *mexD* (S915A), *pbpA*-PBP2 (A481T), *gyrA* (T83I), *parC* (S87W)
8	≥128	16	16	16	≥	**4**	**4**	≥	>32	**8**	**8**	**4**	≥16	≥4	**2**	**0.12**	253	VIM-2, *aac(6')-Ib-Hangzhou*	*mexZ* (aa102-105Δ), *mexD* (S915A), *pbpA*-PBP2 (A481T), *gyrA* (T83I), *parC* (S87W)

AMK, amikacin; ATA, aztreonam-avibactam; ATM, aztreonam; CAZ, ceftazidime; CIP, ciprofloxacin; CST, colistin; CZA, ceftazidime-avibactam; CLT, ceftolozane-tazobactam; FEP, cefepime; FDC, cefiderocol; IMP, imipenem; IMR, imipenem-relebactam; MEM, meropenem; MVB, meropenem-vaborbactam; TOB, tobramycin; TZP, piperacillin-tazobactam.
Bold values illustrate susceptible MICs.

Pina-Sanchez M, et al. J Antimicrob Chemther. 2025.

CZA+ATM Dosing

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COMBINE Study

8g/day aztreonam monotherapy continuous infusion stopped early by data safety monitoring board due to LFT abnormalities

168-hour hollow-fiber model against known NDM-producing *E. coli* and *K. pneumoniae* isolates with simulated human dosing regimens

1. Administer simultaneously – staggered administration had inferior kill
2. Prolonged infusion associated with greater bacterial killing, 30-min infusions resulted in no bacterial killing
3. Both agents given every 8 hours as 2-hour infusions simultaneously resulted in >1 log kill at 168 hours and multi-log kill at 48 hours

CZA 2.5g IV every 8 hours over 3-hour infusion PLUS ATM 2g IV every 8 hours over 3-hour infusion administered simultaneously

Lodise. J Antimicrob Chemother. 2020;75:2622.
Lodise T, et al. Antimicrob Agent Chemother 2022;66(12):e00935-22.

Aztreonam-avibactam

- Approved by the European Medicines Agency in 2024; FDA in 2025
- EUCAST, CLSI, and FDA-approved susceptibility breakpoints are ≤4/4 mg/L for Enterobacterales
- Formulated as a fixed-dose product containing a 3:1 ratio of ATM to avibactam
- 2.67g loading dose followed by 2g (1.5g ATM/0.5g AVI) every 6 hours, all 3-hour infusions

ATM+AVI Trial Data

- **REVISIT**
  - Phase 3, randomized, global trial of 442 patients
  - ATM-AVI (± metronidazole) versus meropenem (± colistin) in patients with complicated intraabdominal infection or pneumonia
  - ATM+AVI non-inferior to MEM
  - Only 80 isolates tested for carbapenemases; only 10 MBLs
  - Clinical response for MBLs: 28.6% (2/7) ATM-AVI vs 66.7% (2/3) meropenem
- **ASSEMBLE**
  - 18 patient RCT, randomized 2:1 to ATM-AVI or BAT for confirmed MBL
  - Clinical cure rates at test of cure were 5/12 (42%) for ATM-AVI and 0/3 (0%) for BAT
  - 1 patient died in each group

Cornely O et al. J Antimicrob Chemother 2020;75(3):618-27.
Xie R et al. Antimicrob Agents Chemother 2025;aac.01950-24
Carmeli Y et al. Lancet ID 2025;25(2):218-30
Heil EL, McCreary EK. Lancet Infect Dis. 2025
Daikos GL, et al. JAC Antimicrob Resist 2025;7(4):dlaf131

What about *S. maltophilia?*

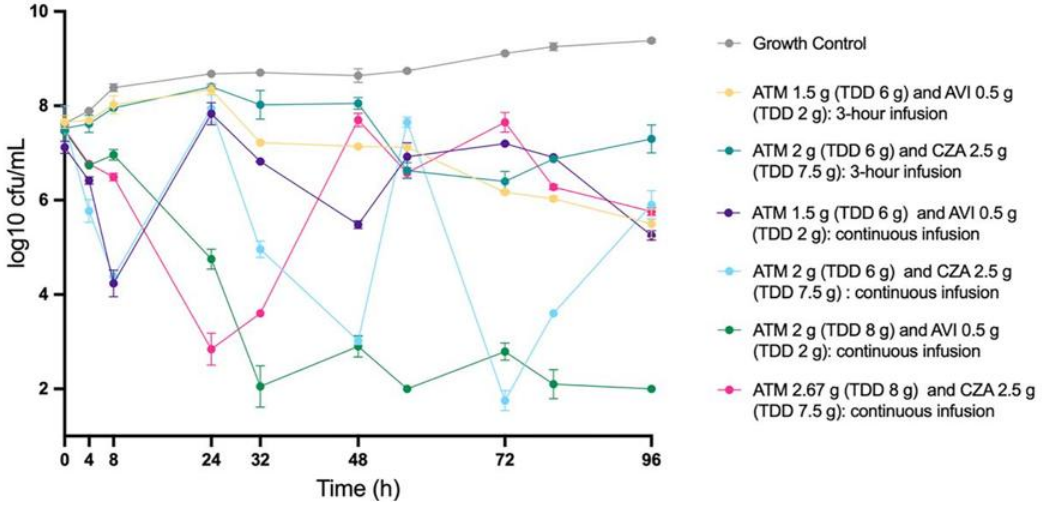
J Antimicrob Chemother 2026; **81**: dkag177
https://doi.org/10.1093/jac/dkag177

Journal of Antimicrobial Chemotherapy

Durability and mechanistic performance of aztreonam/avibactam versus aztreonam plus ceftazidime/avibactam against *Stenotrophomonas maltophilia* complex

Ashlan J. Kunz Coyne[1]*, Rachel Gray [1], Pavani Gonnabathula[1], Elizabeth S. May[1], Paul Puccio[2] and Christine Wu[2]

"Under human-simulated conditions, only continuous-infusion aztreonam/avibactam with intensified aztreonam exposure (8 g/day) achieved durable bactericidal activity against S. maltophilia complex, whereas adding ceftazidime did not improve pharmacodynamic outcomes and was associated with greater β-lactamase induction"



	CZA+ATM	ATM-AVI
Dose	2.5g IV q8h CZA + 2g IV q8h ATM	2.67g loading dose once, followed by 2g (1.5g ATM, 0.5g AVI) every 6 hours
Administration	• Package insert states 30-minute infusion for ATM, 2 hours for CZA • Clinical studies prefer 3-hour infusions; some studies suggest 8-hour infusions • More complex preparation, administration, and stewardship coordination • Consider simultaneous infusion of both drugs to optimize early bacterial killing • More operational burden (extra IV access, compatibility/logistics, pharmacy complexity)	• Standard 3-hour infusion • Less pharmacy and nursing time • Less plastic waste • Less fluid per day

	CZA+ATM	ATM-AVI
Susceptibility testing	Provisional broth disk elution method	Gradient diffusion strip Disc diffusion (30/20 µg ATM/AVI) Broth microdilution
Adverse reactions	Higher doses of ATM are associated with greater likelihood of hepatic injury; both options utilize 6g ATM/day. Definitions of hepatic impairment and hepatic injury are inconsistent across published studies. Side effect profiles are similar and include: infusion reactions, rash, hypokalemia, diarrhea, nausea, anemia, neutropenia The additive toxicity of including ceftazidime is unknown and there are no direct comparative safety data between the two options.	
Extended infusion stability	Stable up to 12 hours at room temperature; up to 72 hours refrigerated. Not stable for 24 hours at body temperature	Stable up to 12 hours at room temperature One case report of administering total daily dose continuously as two, 12-hour infusions for OPAT

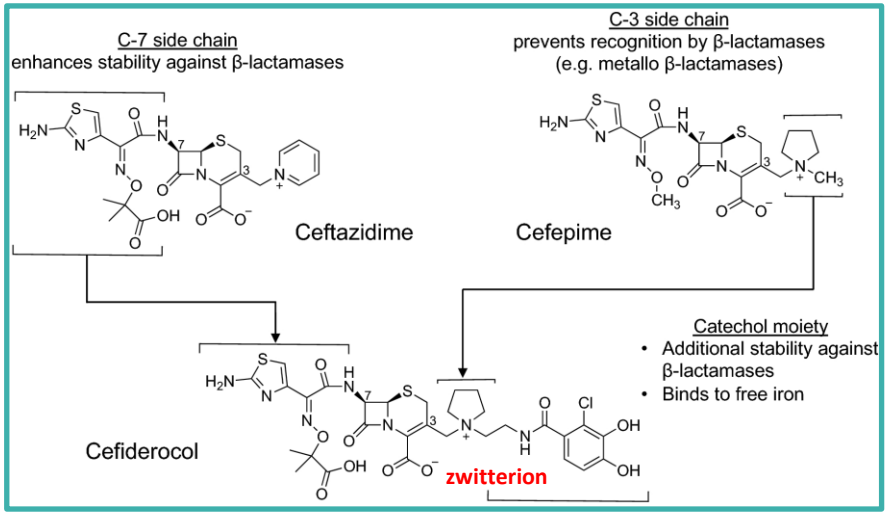
	CZA+ATM	ATM-AVI
Cost	Varies by country and institution contracts but generally cost neutral between the two options	
In vitro activity against MBL-producing pathogens	No clinically relevant differences have been described to date Enterobacterales – yes Pseudomonas – no Steno – maybe Acinetobacter – no	
Other considerations	More published clinical data and real-world experience for MBL-producing Enterobacterales "Target redundancy"	More avibactam exposure and PK/PD optimized Availability globally is more limited that CZA+ATM Supported by randomized clinical data, although limited published use in MBL-producing infections

What does the guidance say?

IDSA 2026 Guidance	ESCMID 2022 Guidance
Enterobacterales ▪ ATM-AVI or Cefiderocol Pseudomonas • Cefiderocol Alternative ▪ Tigecycline or eravacycline – Omadacycline is not recommended – Generally reserved for IA infections – Not recommended for treatment of UTIs or BSIs	Cefiderocol If cefiderocol resistant, then aztreonam plus ceftazidime/avibactam For cUTI → aminoglycosides

Cefiderocol (Fetroja, FDC)

- FDA approved: November 14, 2019 (first available in Spring 2020)
  - 3 RCTs – UTI, pathogen-directed, PNA
- Siderophore cephalosporin
  - Evades efflux pumps and porin mutations by entering cell through iron transporter channels
- Ceftazidime + cefepime structure
  - Stable against hydrolysis by most beta-lactamases (but not all!!)
- *In vitro* activity against gram-negative pathogens is everything we hoped for
  - No appreciable *in vitro* activity against gram-positive or anaerobic pathogens



Zhanel GC, et al. Drugs. 2018.
Microbiol Res. 2018 Jul - Aug;212-213:103-111.

Too good to be true?

In vitro, at 2 µg/mL (EUCAST breakpoint), cefiderocol inhibited 80.6% of VIM-producing *Enterobacterales* **but only 41% of NDM-producing *Enterobacterales***

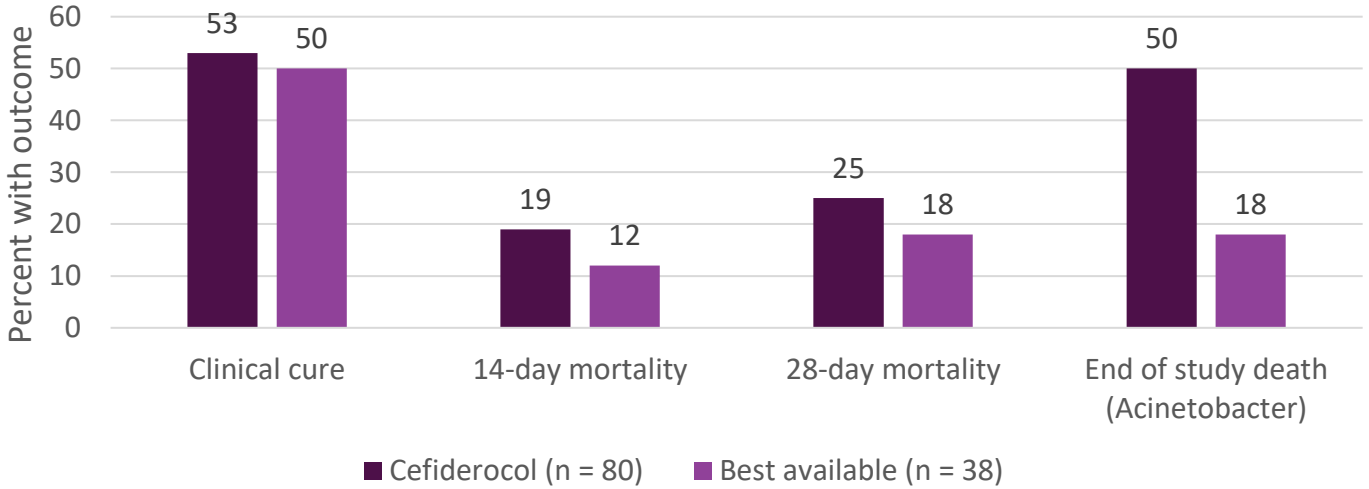
Table 1 Steady-state kinetic parameters for FDC hydrolysis by NDM-1, NDM-5, VIM-2, and IMP-1. These results are compared to the CAZ hydrolysis parameters for the same enzymes reported in the literature, with CAZ hydrolysis data for NDM-1 determined in this work

Enzyme/substrate	K_M (µM)	k_{cat} (s^{-1})	k_{cat}/K_M (M^{-1} s^{-1})
NDM-1			
FDC	443 ± 59	84 ± 11	1.91×10^5
CAZ	45 ± 12	187 ± 13	4.16×10^6
NDM-5			
FDC	405 ± 40	75 ± 3	1.85×10^5
CAZ[45]	86 ± 11	15	1.8×10^5
VIM-2			
FDC	250 ± 80	1.10 ± 0.14	4.4×10^3
CAZ[46a]	72	3.6	5×10^4
IMP-1			
FDC	300 ± 120	1.25 ± 0.23	4.1×10^3
CAZ[47]	44 ± 3	8 ± 1	1.8×10^5

[a] Standard deviations were <10%, as reported by the authors.

Warecki B, et al. Chem Sci. 2025.
Mushtaq S et al.Antimicrob Agents Chemother 2020; 64:e01582–20
Warecki BA et al. Chem Sci 2025;16:12519-12533.

Mortality increased with cefiderocol for carbapenem-resistant GNRs



- No clear explanation for mortality imbalance
- Mortality differences limited exclusively to *Acinetobacter*

Bassetti M, et al. *Lancet Infect Dis* 2021;21:226 – 40
Slide modified from Sam Aitken, PharmD

Cefiderocol *seemed* to work fine against MBL Enterobacterales

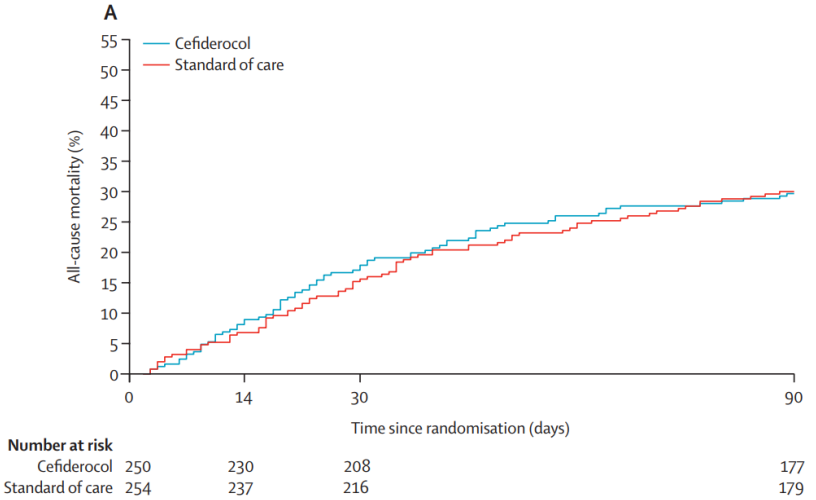
	Clinical Cure at TOC		Eradication at EOT		ACM Day 28	
CREDIBLE-CR + APEKS-NP[e]	Cefiderocol (N = 24)	All Comparators[f] (N = 10)[a]	Cefiderocol (N = 24)	All Comparators[f] (N = 10)[a]	Cefiderocol (N = 24)	All Comparators[f] (N = 10)[a]
Overall	70.8 (17/24)	40.0 (4/10)	58.3 (14/24)	30.0 (3/10)	12.5 (3/24)	50.0 (5/10)
Type of infection						
Pneumonia	71.4 (10/14)	50.0 (3/6)	42.9 (6/14)	33.3 (2/6)	21.4 (3/14)	33.3 (2/6)
Other diagnoses[b]	70.0 (7/10)	25.0 (1/4)	80.0 (8/10)	25.0 (1/4)	0 (0/10)	75.0 (3/4)
MBL type						
NDM	56.3 (9/16)	33.3 (2/6[a])	62.5 (10/16)	16.7 (1/6[a])	18.8 (3/16)	50.0 (3/6[a])
Non-NDM	100 (8/8)	40.0 (2/5[a])	50.0 (4/8)	40.0 (2/5[a])	0 (0/8)	40.0 (2/5[a])
Pathogen type						
Enterobacterales	73.3 (11/15)	20.0 (1/5)	66.7 (10/15)	20.0 (1/5)	13.3 (2/15)	60.0 (3/5)
Non-fermenters	66.7 (6/9)	60.0 (3/5)	44.4 (4/9)	40.0 (2/5)	11.1 (1/9)	40.0 (2/5)

- Reduced mortality relative to comparator agents in licensing RCTs
- No published head-to-head comparisons with aztreonam plus ceftazidime-avibactam

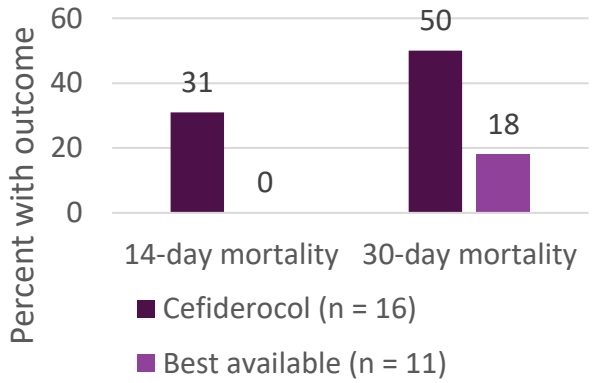
Timsit JF, et al. *Clin Infect Dis* 2022;75(6):1081-4

Slide modified from Sam Aitken

The GAME CHANGER trial



Mortality in MBL+ Enterobacterales



- Open label RCT of cefiderocol versus local standard of care in ~500 patients with Gram-negative bloodstream infections; overall non-inferior for 14, 30, 90-day mortality
- Increased mortality *not* seen in the *Acinetobacter* subset but was seen in MBL+ CRE!
- ~50% NDM+ CRE with MIC >2 to cefiderocol (resistant by EUCAST standards)

Paterson DL, et al. *Lancet Infect Dis* 2026;26:148-59; McCreary EK, et al. *CMI Communications.* 2026.

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LIFE CHANGING MEDICINE

Where does that leave us?

Resistance to ATM-AVI

PBP3 insertions (YRIN, YRIK)

- Most common in *E. coli*
- Fitness cost in *K. pneumoniae*
- PBP3 is the primary binding target for ceftazidime, cefiderocol, and aztreonam
- PBP2 mutations emerging

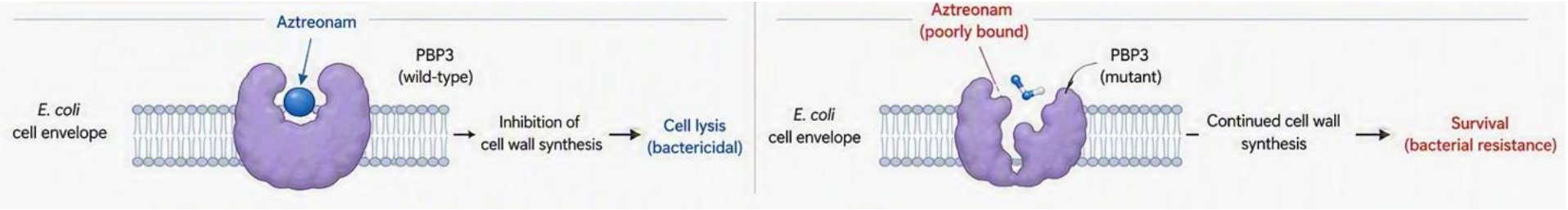


Image created with Microsoft Copilot

PBP3 mutants may not always cause in vitro resistance

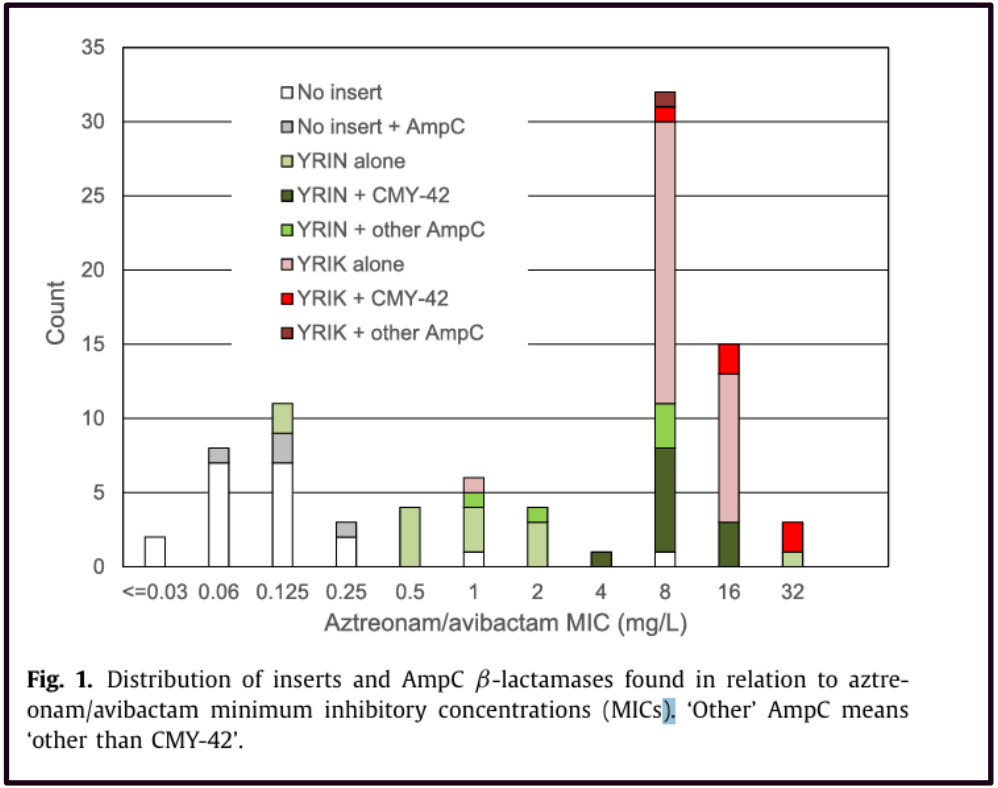
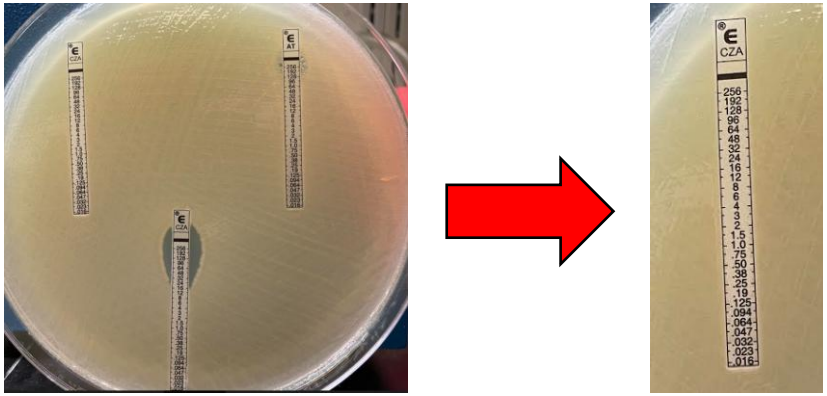


Fig. 1. Distribution of inserts and AmpC β-lactamases found in relation to aztreonam/avibactam minimum inhibitory concentrations (MICs). 'Other' AmpC means 'other than CMY-42'.

Livermore DM, et al. IJAA. 2023.

But they certainly can

- 61 y/o male with **NDM-5** *E. coli* bacteremia (YRIN insertion)
- CAZ-AVI + ATM MIC increased from 16 to >256 after therapy
- WGS of both isolates revealed an additional mutation in PBP3 (A417V) that may block binding through steric hindrance



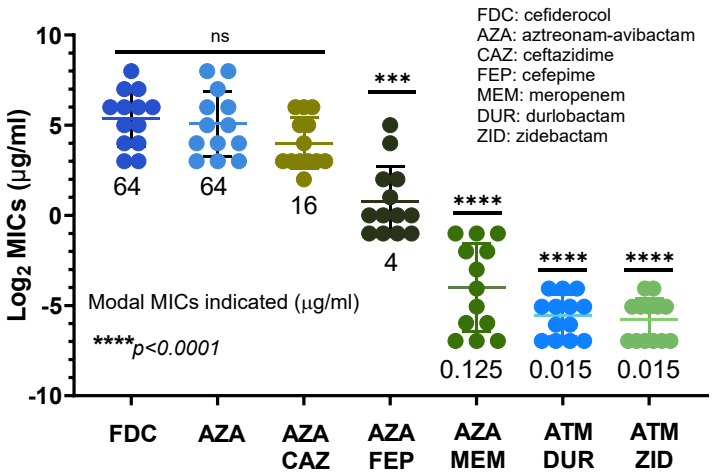
Slide developed with Ryan Shields

Resistance to Cefiderocol

- PBP3 insertions
- Mutations in iron transporter genes
  - *piuA, piuD,* and *pirA* in *P. aeruginosa*
  - c*irA, fiu,* and *piuA* in Enterobacterales
  - *piuA, pirA* in *A. baumannii*
- Porin deficiencies (e.g., *ompK35*/*ompK36*) that slow drug transport across the cell membrane
- **NDM-1 and NDM-5 efficiently hydrolyze cefiderocol**

What do we do?!
Target multiple PBPs, “enhancers”

Log_2 MICs of multiple-PBP-targeting β-lactam combinations against representative clinical NDM-producing *E. coli* strains



Mean MIC fold change relative to AZA		↓4x	↓16x	↓512x	↓2134x	↓2134x
PBP affinity	PBP3	PBP3	PBP3, PBP1a, 1b	PBP3, PBP2, 1a/b,4	PBP3, PBP2, 4,1a/b	PBP3, PBP2	PBP3, PBP2

Green= strong affinity (IC_{50} <0.1μg/ml), Blue= moderate affinity (IC_{50} <1μg/ml)

Fonseca IY, et al (ASM Microbe 2026); Khan – data on file

Durlobactam

- Diazabicyclooctane β-lactamase inhibitor that has independent *in vitro* activity mediation by PBP2 inhibition against Enterobacterales
- Not meaningfully degraded by β-lactamase enzymes
- Not an MBL inhibitor

Table 3 | *In vitro* characterization of ETX2514.

Class or species	Enzyme	k_{inact}/K_i ($M^{-1}\,s^{-1}$)*
Class A	CTX-M-15	$7\ (\pm2) \times 10^6$
	TEM-1	$1.4\ (\pm0.6) \times 10^7$
	KPC-2	$9.3\ (\pm0.6) \times 10^5$
	SHV-5	$6.4\ (\pm0.5) \times 10^6$
Class C	AmpC	$9\ (\pm5) \times 10^5$
	P99	$2.3\ (\pm0.4) \times 10^6$
Class D	OXA-10	$9\ (\pm2) \times 10^3$
	OXA-23	$5.1\ (\pm0.2) \times 10^3$
	OXA-24	$9\ (\pm2) \times 10^3$
	OXA-48	$8\ (\pm2) \times 10^5$
A. baumannii	PBP1a	$18\ (\pm1) \times 10^1$
	PBP2	$1.8\ (\pm0.6) \times 10^3$
	PBP3	3.37 ± 0.06
P. aeruginosa	PBP1a	12 ± 7
	PBP2	24.3 ± 0.6
	PBP3	$6\ (\pm1) \times 10^1$
E. coli	PBP1a	$12\ (\pm2) \times 10^1$
	PBP2	$1.7\ (\pm0.3) \times 10^4$
	PBP3	2.3 ± 0.6

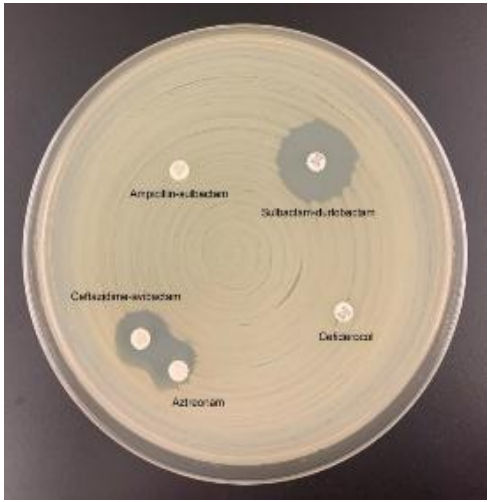
*Data expressed as mean ± s.d.; $n = 3$, except $n = 2$ for TEM-1, AmpC and *E. coli* PBP1a. Acylation by ETX2514 of representative class A, C and D β-lactamases or Gram-negative bacterial PBPs.

Durand-Reville TF, et al. Nature Microbiology. 2017.

Durlobactam

- UPMC and Michigan tested isolates from 5 patients with NDM *E. coli* and PBP3 insertions

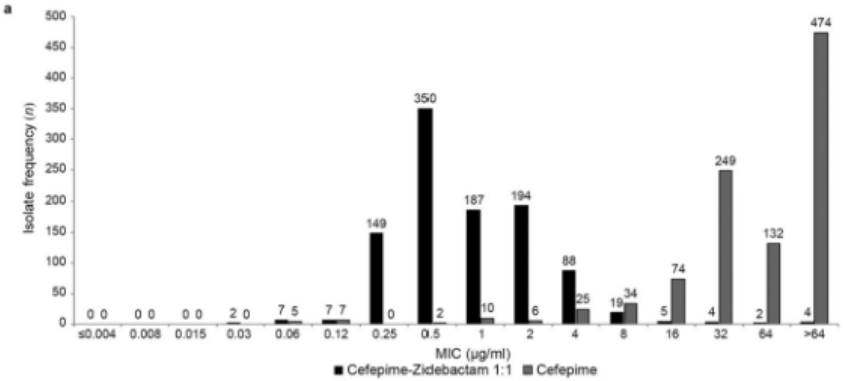
Patient	ATM-AVI	Cefiderocol	Durlobactam	ATM + Durlo
1	4	>32	2	≤0.25
2	2	2	0.5	≤0.25
3	8	2	1	≤0.25
4	16	8	2	≤0.25
5	16	1	2	≤0.25



Aitken SL et al. Clin Infect Dis 2024;79(4):834-7.

Cefepime-zidebactam

- Bicyclodiazabicyclooctane class (BCH) that was developed to enhance PBP2 binding
  - Inhibits class A and C β-lactamases
  - Enhancer effect when combined with a PBP3-targeting β-lactam like cefepime for Enterobacterales and non-fermenters



CRE Category	MIC50	MIC90	% Susceptible
MBL (n=191)	0.5	4	94.8%
KPC (n=561)	0.5	2	100%
OXA-48 (n=111)	0.5	2	100%
Non-CP CRE (n=104)	4	8	96.4%

Karlowsky JA, et al. *AAC* 2020;64(12):e01432-20

Cefepime-zidebactam

- FDA-approved breakpoint for FEP-ZID is ≤ 8 mg/L for Enterobacterales and *P. aeruginosa*
- CLSI established a provisional susceptibility breakpoint of ≤64 mg/L
  - Based on the dose used in Phase 3 studies (2g cefepime, 1g zidebactam every 8 hours over a 1 hour infusion)
  - Cefepime and zidebactam are tested at a 1:1 ratio
  - Pharmacodynamic target is unlike any prior agent due to potent killing at sub-MIC concentrations (*f*T>subMIC of FEP/ZID)

Genov P, et al. *OFID* 2026;13(Supple 1):P-1209

Complexity of PK-PD

- Concurrent PBP binding of cefepime and zidebactam demonstrated bacterial killing at concentrations below the MIC for *K. pneumoniae, P. aeruginosa,* and *A. baumannii*
- Cefepime PBP3 binding at low concentrations resulting in cell elongation
- Zidebactam PBP2 binding at low concentrations results in spheroplast formation
- PBP2 and PBP3 simultaneous inhibition causing a cell morphology that is lysis-prone

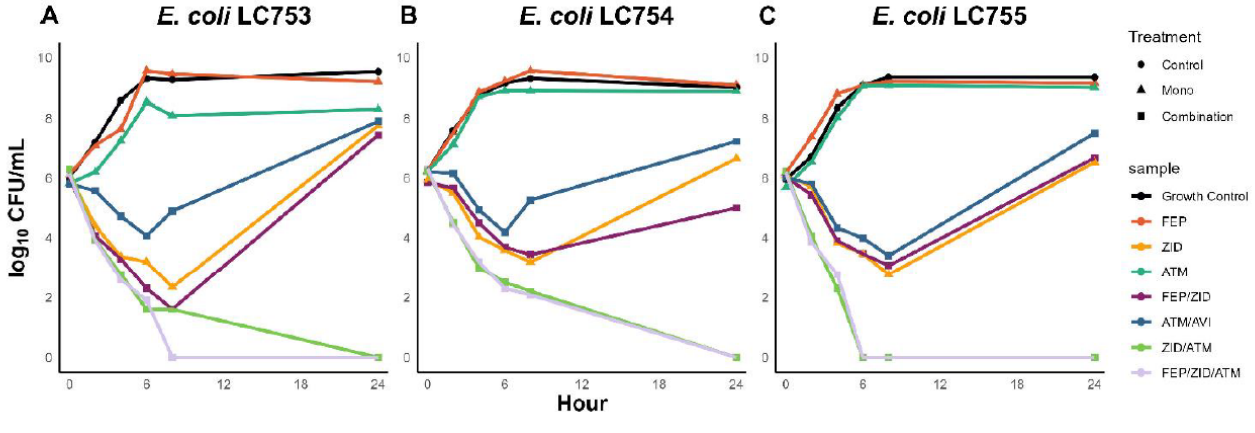
Avery et al. AAC. 2018.

Cefepime-zidebactam

- Several case studies have reported compassionate use of cefepime-zidebactam for MBL-producing bacterial infections (>85 cases)
- Phase III efficacy data (ENHANCE-1) have not yet been fully reported
  - Composite success rates in MITT 89% vs 68% (Difference 20.6; 95% CI: 12.3 – 29.5)
- Elevated MICs due to
  - Altered AmpC, efflux (P. aeruginosa)
  - PBP2 mutants, PBP3 mutants (Enterobacterales)

This seems like magic. Does that magic hold against PBP3 mutants?

Figure 3. Combinations inhibiting PBP2 and PBP3 rapidly kill NDM-producing *E. coli* isolates with insertion mutations in PBP3. Activities of cefepime (FEP), zidebactam (ZID), aztreonam (ATM), and aztreonam/avibactam (ATM/AVI) alone and in combination against NDM-producing *E. coli* isolates with insertion mutations in PBP3 in time-kill assays LC753 (**A**), LC754 (**B**), and LC755 (**C**). Cefepime and zidebactam were tested at concentrations of 16 and 8 mg/L, respectively. Aztreonam was tested at a concentration of 1× of the aztreonam/avibactam MIC (LC753: 0.5 mg/L; LC754: 8 mg/L; LC755: 4 mg/L).



Watkins JV, et al. npj Antimicrob Resist. 2026.

Le Terrier C, et al. JAC. 2023.

Pathogen	MBL	Preferred	Alt	Considerations
E. coli	NDM	ATM-AVI FEP-ZID ATM + SUL-DUR ATM + FEP-ZID	CZA+ATM	The authors avoid cefiderocol for NDM-harboring Enterobacterales due to elevated MICs, ability to be hydrolyzed, and suboptimal outcomes in clinical trials. Multiple options are listed for NDM E. coli due to rapid global expansion of isolates harboring PBP3 insertions. Cefepime-zidebactam may have more potent in vitro activity against these isolates compared to ATM-AVI; however, time-kill assays show similar antibacterial activity of ATM-AVI and FEP-ZID against these mutants. There are no comparative clinical data and extremely limited clinical data for all available antibiotics. Aztreonam plus zidebactam or aztreonam plus durlobactam may be in vitro active and have demonstrated potency against isolates with PBP3 insertions.
Other Enterobacterales	NDM	ATM-AVI	CZA+ATM FEP-ZID	
	IMP, VIM	ATM-AVI	CZA+ATM FEP-ZID FDC	
P. aeruginosa	NDM	FEP-ZID	FDC* Polymyxins*	*In combination with other in vitro active agent(s)
	IMP, VIM	Cefiderocol (+/- another in vitro active agent)	FEP-ZID Polymyxins*	

Pipeline

- Cefepime-nacubactam, aztreonam-nacubactam
  - Integral-1, phase 3, RCT for cUTI
  - Integral-2 = RCT for CRE
    - FEP-NAC if serine carbapenemase, ATM-NAC if MBL
  - Doesn’t appear to be active against non-fermenters like zidebactam
- Xeruborbactam (with cefiderocol? meropenem?)
  - Oral and IV!

Other treatment notes for completeness

- **IV fosfomycin** (new for the U.S., tons of experience globally)
  - Large sodium load – hard to tolerate in many patients
  - Probably needs to be used as combination therapy for any serious infection – what's the best combination?
  - Susceptibility testing remains a challenge in most labs
- **Aztreonam, ciprofloxacin, levofloxacin**, or **TMP/SMX** monotherapy, if in vitro susceptible, is reasonable for MBL-producing infections
- **Tigecycline** or **eravacycline** is reasonable for intraabdominal, skin and soft tissue, or bone/joint infections
- **Nitrofurantoin** or **oral fosfomycin** are reasonable for uncomplicated urinary tract infections due to *E. coli*
- **Aminoglycosides**, if in vitro susceptible, are reasonable for urinary tract infections due to Enterobacterales or *P. aeruginosa*
- Antimicrobial susceptibility testing should always be obtained for any antibiotic being used for definitive

Assessment Question

What is the most common metallo-B-lactamase found in Enterobacterales globally?

A. VIM
B. NDM
C. IMP
D. CphA

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Assessment Question

What antibiotic is currently recommended for treatment of NDM-producing E. coli?

a. Aztreonam-avibactam

b. Ceftazidime-avibactam

c. Meropenem-vaborbactam

d. Imipenem-relebactam

Assessment Question

What antibiotic is currently recommended for treatment of NDM-producing E. coli?

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Assessment Question

What is the most common mechanism of resistance in MBL-producing E. coli to aztreonam-avibactam?

a. CTX-M production

b. VIM production

c. PBP2 mutation

d. PBP3 mutation

Assessment Question

What is the most common mechanism of resistance in MBL-producing E. coli to aztreonam-avibactam?

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Stewardship of Difficult-to-Treat Gram Negative Infections: Metallo-β-Lactamases

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