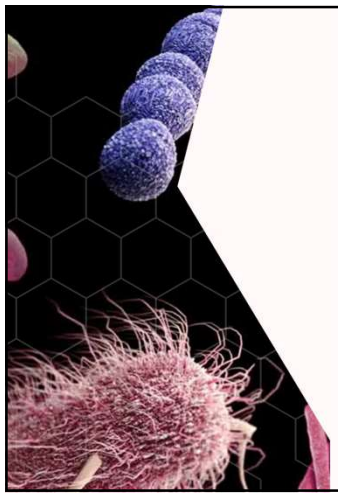


Antimicrobial Pipeline: Understanding Gram-Negative Resistance

Presented by Tony Anderson, PharmD, BCPS, BCIDP

Clinical Pharmacist - Infectious Diseases and Antimicrobial Stewardship

1

Conflict of Interest Declaration

- I have no actual or potential conflicts of interest

2

Off-label Indications

- I will be discussing off-label indications and currently unapproved agents

3

Learning Objectives

- Define Multidrug-Resistant (MDR) and identify the most common pathogens
- Describe the mechanisms behind multi-drug resistance (MDR)
- Compare available antimicrobial agents for MDR pathogens
- Evaluate the impact of MDR on patient care and public health

4

Impact of Gram-negative Infections?

- Gram-negative resistance continues to increase
  - Somewhat driven by antibiotic use
- Morbidity and mortality due to Gram-negative infections continue to increase
- Treatment can be challenging
  - Few antibiotics available to treatment but has been increasing
  - Multiple resistance mechanisms present in one organism
  - Resistance can develop rapidly

5

Gram-negative Resistant Pathogens

- Extended-spectrum □-lactamase (ESBL)-producing *Enterobacterales*
- AmpC □-lactamase-producing *Enterobacterales*
- Carbapenem-resistant *Enterobacterales* (CRE)
- Difficult-to-Treat (DTR) *Pseudomonas aeruginosa*
- Carbapenem-resistant *Acinetobacter baumannii* (CRAB)
- Multidrug-resistant *Stenotrophomonas maltophilia*

6

Impact of Gram-negative Resistance

Organism	Infections/Year	Deaths/Year	CDC Category
ESBL-producing Enterobacterales	197,400	9,100	Serious
AmpC-producing Enterobacterales	Not Reported	Not Reported	Not Classified
CRE	13,100	1,100	Urgent
DTR P. aeruginosa*	32,600	2,700	Serious
CRAB	8,500	700	Urgent

* Reported as the broader MDR P. aeruginosa

7

Gram-negative Resistance Mechanisms

- Minimization
  - Porin mutations
  - Efflux pumps upregulated
- Modification
  - Replace target
  - Mutate target
  - Protect target
- Inactivation
  - Modify antibiotics
  - Enzymatically hydrolyze antibiotics

8

Minimization: Efflux Pumps

- Complex transcriptional response to antibiotic exposure that leads to upregulating transporters
- 6 families of efflux pumps
  - Major Facilitator Superfamily (MFS)
  - ATP-Binding Cassette (ABC)
  - Multidrug And Toxic compound Extrusion (MATE)
  - Small Multidrug Resistance (SMR)
  - Resistance-Nodulation-Division (RND)
  - Proteobacterial Antimicrobial Compound Efflux (PACE)

9

Minimization: Outer Membrane Porins

Defense mechanism restricting antimicrobial access across Gram-negative bacterial membranes

Loss of Porins

Elimination of specific membrane proteins blocks antibiotic penetration into periplasmic space

Mutation of Porins

Structural alterations selectively block antibiotics while allowing essential nutrient uptake

Decreased Expression

Reduced porin synthesis limits channels available for antibiotic entry

Clinically Significant Porin-Mediated Resistance

E. coli

Porins: OmpC, OmpF

Resistance: β-lactams, including extended-spectrum cephalosporins

K. pneumoniae

Porin: OmpK35, OmpK36

Resistance: Cephalosporins, carbapenems, fluoroquinolones

P. aeruginosa

Porin: OprD

Resistance: Primarily imipenem and meropenem (carbapenems)

10

Modification: Replace

Normal Target

Standard antibiotic binding sites (PBPs, ribosomal subunits, DNA gyrases) vulnerable to antimicrobials

Replace Target

Alternative proteins that perform identical functions with minimal antibiotic binding

Result

MRSA produces low-affinity PBP2a; VRE alters peptidoglycan structure; S. pneumoniae modifies PBPs to evade β-lactams

11

Modification: Modify

Modification resistance mechanisms alter the bacterial targets that antibiotics attack, rendering treatments ineffective. These sophisticated molecular adaptations are particularly concerning in Gram-negative pathogens:

Mutation Modification

Genetic mutations in *gyrA* and *parC* genes alter DNA gyrase and topoisomerase IV binding sites in *Enterobacterales*, conferring fluoroquinolone resistance while preserving enzyme function

Enzymatic Modification

Bacteria modify their cellular targets to prevent antibiotic binding without compromising essential functions, creating a molecular shield against antimicrobial agents

12

Modification: Protect

Bacteria develop protection mechanisms that shield antibiotic targets, rendering treatments ineffective. These adaptations are especially concerning in Gram-negative pathogens:

- **Protect target:** *Pseudomonas aeruginosa* produces Qnr proteins to shield DNA gyrase from quinolones, while *Acinetobacter* species use TetM proteins to prevent tetracycline binding to ribosomes

13

ESBL (Ambler Class A)

Class A Extended-Spectrum β-Lactamases

- *TEM, SHV, CTX-M* families (*CTX-M-15* most common)
- Serine active site enzymes inhibited by β-lactamase inhibitors*

Substrate Profile

- Hydrolyze penicillins, all generation cephalosporins, and aztreonam
- Spares cephamycins (cefoxitin, cefotetan) and carbapenems

Epidemiology

- Plasmid-encoded, spread through horizontal transfer
- Defined by elevated MICs to 3rd generation cephalosporins (≥4 mcg/mL)

14

ESBL Detection

Disk Diffusion

Test ceftazidime and cefotaxime with and without clavulanate

ESBL: ≥5mm zone diameter increase with clavulanate

Broth Microdilution

Test ceftazidime and cefotaxime with and without clavulanate

ESBL: ≥3× 2-fold MIC reduction with clavulanate

15

ESBL Detection (cont)

Detection Methods

- **Molecular**: Multiplex PCR identifies specific ESBL genes (*TEM, SHV, CTX-M*)
- **Automated Testing Systems**: Use ESBL screening algorithms (>95% sensitivity)

Laboratory Reporting Protocol

Report resistance to all penicillins, cephalosporins, and aztreonam, even if in vitro susceptible, as these drugs may not be effective.

- Include: "ESBL-producing organism detected. Treatment with these agents may result in clinical failure."

16

MERINO-1

Key findings from this important clinical study:

Study Design

Multicenter, open-label, parallel group, randomized, controlled trial

Primary Outcome: 30-day mortality

12.3% vs 3.7% (risk difference 8.6%; 97.5% CI negative infinity to 14.5%)

Study Population

378 pts with *E. coli* or *K. pneumoniae* bacteremia susceptible to piperacillin-tazobactam and meropenem but not ceftriaxone

Resistance Genes

- CTX-M group: 273
- Narrow spectrum OXA: 102
- SHV ESBL: 12
- AmpC: 36

17

MERINO-1: Post-Hoc Analysis

E. coli Sequence Types (ST)

Most common: ST131 (n=143 isolates)

- Co-harboring OXA genes (45%) – Higher rate than other STs combined (21%)
- Harboring both OXA and ESBLs = Significantly higher modal PTZ MICs 8 vs 2 mcg/mL; P <0.001)

30-day Mortality Outcomes

Primary outcome: 30-day mortality

- PTZ NS breakpoint (MIC >16 mcg/mL) (OR, 14.9; 95% CI, 2.8–87.2)
  - Mortality difference increased 9% (95% CI, 3–15%)
  - Post-hoc microbiologic assessable populations – mortality difference increased 8% (95% CI, 2–15%)

18

Example Susceptibility Report: *ESBL*

Antibiotic	MIC (mcg/mL)	Interpretation
Ampicillin	64	Resistant
Ampicillin/sulbactam	32/16	Resistant
Cefazolin	16	Resistant
Cefepime	64	Resistant
Cefoxitin	1	Susceptible
Ceftriaxone	8	Resistant
Ciprofloxacin	0.5	Susceptible
Gentamicin	2	Susceptible
Meropenem	0.25	Susceptible
Piperacillin/tazobactam	128	Resistant

19

Treatment

- Treatment of choice: **carbapenems**
- Potential options
  - Ceftazidime/avibactam
  - Ceftolozane/tazobactam
  - Polymyxins
  - Tigecycline

20

Treatment Options for ESBL Pathogens

Comparing key characteristics of alternative antimicrobial agents

Ceftazidime/avibactam

Spectrum of Activity:

- Effective against ESBLs
- Active against *P. aeruginosa*[a]
- Covers AmpCs
- Effective against *K. pneumoniae* carbapenemases (KPCs)

Dosing:

- 2.5 g IV every 8 hr over 2 hr
- Renally adjust as needed

Ceftolozane/tazobactam

Spectrum of Activity:

- Variable against ESBLs
- Effective against *P. aeruginosa*
- No activity against carbapenemases

Dosing:

- cIAI/cUTI: 1.5 g IV every 8 hr over 1 hr
- HAP/VAP: 3 g IV every 8 hr over 1 hr

Polymyxins

Spectrum of Activity:

- Effective against ESBLs
- Active against *P. aeruginosa*[a]
- Covers AmpCs
- Effective against *K. pneumoniae* carbapenemases (KPCs)

Dosing:

- 2.5 g IV every 8 hr over 2 hr
- Renally adjust as needed

Tigecycline

Spectrum of Activity:

- Effective against ESBLs
- *P. aeruginosa*[a]
- AmpCs
- *K. pneumoniae* carbapenemases (KPCs)

Dosing:

- 2.5 g IV every 8 hr over 2 hr
- Renally adjust as needed

cIAI = complicated intraabdominal infection; cUTI = complicated urinary tract infection; HAP = hospital-acquired pneumonia; VAP = ventilator-associated pneumonia

21

Clinical Controversies

Cephamycins

- Limited clinical evidence in comparative studies
- Reduced efficacy when additional resistance mechanisms are present

Aminoglycosides

- Often compromised by co-occurring resistance determinants
- May serve as adjunctive option in empiric combination therapy regimens

Fluoroquinolones

- Consider only when susceptibility is definitively confirmed by laboratory testing
- Monitor for emergence of resistance during therapy

22

Do NOT use

- Cephalosporins other than cephamycins, cefiderocol, future others?
  - Avoid 1st – 3rd generation
  - Cefepime may be considered with MIC <= 2 mcg/mL when high doses are used
- Piperacillin/tazobactam
  - Treatment failures have been reported
  - Higher mortality compared with meropenem in GN infections resistant to 3rd generation

23

ESBL Risk Factors

Prior antibiotic exposure (within 3 months)

3rd generation cephalosporins

Fluoroquinolones

Prolonged therapy (>7 days)

Healthcare exposures

Indwelling devices (central/urinary catheters >72 hours)

Mechanical ventilation

Recent surgery, especially GI procedures

Prolonged hospitalization (>14 days, ICU stay)

Comorbid conditions

Uncontrolled diabetes (HbA1c >8%)

Malignancies (solid/hematological)

Chronic renal failure (GFR <50 mL/min)

Community-acquired UTI risk factors

Females (>65 years)

Recurrent UTIs (≥3/year)

24

ESBL Clinical Pearls

- Carbapenems are drugs of choice
  - Use extended infusion over intermittent infusions if able
- May consider carbapenem-sparing therapy but likely more expensive
- Avoid polymyxins or tigecycline monotherapy for sever infections
- Optimize dosing in severe infections

25

Which bacterial resistance mechanism is represented by this susceptibility profile?

Antibiotic	Interpretation
Ampicillin	Resistant (R)
Ampicillin/sulbactam	Resistant (R)
Cefazolin	Resistant (R)
Cefepime	Resistant (R)
Cefoxitin	Susceptible (S)
Ceftriaxone	Resistant (R)
Meropenem	Susceptible (S)
Piperacillin/tazobactam	Resistant (R)
Ceftazidime/avibactam	Susceptible (S)
Meropenem/vaborbactam	Susceptible (S)

Note: Resistance to carbapenems with susceptibility to newer β-lactam/β-lactamase inhibitor combinations is the key diagnostic pattern.

Select the most likely mechanism:

1. AmpC β-lactamase
2. Extended-spectrum β-lactamase (ESBL)
3. Klebsiella pneumoniae carbapenemase (KPC)
4. New Delhi metallo-β-lactamase (NDM)
5. Oxacillinase-48 (OXA-48)

26

AmpC □-lactamase (Class C)

Two major types with distinct clinical implications

Chromosomal AmpC

Constitutively expressed in certain *Enterobacterales*

Can be inducible with certain □-lactams, leading to treatment failure

Plasmid-Mediated AmpC

Transferable resistance mechanism found in *K. pneumoniae* and *E. coli*

Not inducible but confers resistance to cephalosporins and penicillin/BLI combinations

Both types hydrolyze penicillins, cephalosporins (including cephamycins), and are resistant to □-lactamase inhibitors like clavulanate and tazobactam

27

AmpC Inducible chromosomal resistance

AmpC □-lactamase production regulation pathway:

- AmpG: Membrane permease transporting muropeptides, activates signaling
- AmpD: Amidase cleaving anhydromuropeptides, reduces AmpR binding
- UDP-N-acetylmuramic acid: Enables AmpR-DNA binding, repressing AmpC (**Repressed** state)
- □-lactam exposure (cefoxitin, imipenem) disrupts pathway, accumulates anhydromuropeptides, activates AmpR

Mutations in AmpD, AmpR, or AmpG can cause hyperproduction and resistance without antibiotic exposure

28

Chromosomal AmpC

- Chromosomal gene in HECK-Yes organisms
  - H: *Hafnia alvei*
  - E: *Enterobacter cloacae complex (ECC)*
  - C: *Citrobacter freundii complex*
  - K: *Klebsiella aerogenes*
  - Y: *Yersinia enterocolitica*

29

Chromosomal AmpC

What about MYSPPACE/SPACE/SPICE/ESKAPE?

- HECK-Yes pathogens:
  - 15-fold higher than *Serratia liquefaciens*
  - 50- to 150- fold higher than *Providencia spp.* and *Serratia marcescens*
  - 600-fold higher than *Morganella morganii*

30

AmpC □-lactamases

AmpC enzymes are cephalosporinases that hydrolyze penicillins, cephalosporins, and occasionally carbapenems.

- Resistant to traditional□-lactamase inhibitors:
  - Tazobactam*
  - Sulbactam
  - Clavulanate
- Notable exceptions:
  - *Morganella morganii* AmpC: uniquely inhibited by tazobactam
  - Hyperproduced AmpC can overcome tazobactam
- Effectively inhibited by newer agents:
  - Avibactam
  - Vaborbactam
- Clinical impact: Confers resistance to:
  - 3rd gen cephalosporins
  - BL/BLI

31

Chromosomal AmpC: Induction

Strong Inducers AND Hydrolyzed

- Aminopenicillins
- 1st gen cephalosporins
- Cephamycins
- Clavulanate

DO NOT USE

Strong Inducer NOT Hydrolyzed

- Carbapenems

POSSIBLE OPTION

Weak Inducer AND Hydrolyzed

- Ureidopenicillins
- 3rd gen cephalosporins
- Aztreonam

COMPLICATED

32

AmpC: Weak Inducer AND Hydrolyzed

- Weak inducers select for derepressed mutatnts
- Initially appear susceptible but develop resistance on therapy
- Should avoid use in suspected AmpC infections

33

AmpC Clinical Data

Cefepime for AmpC-producing infections:

- Tamma et al. (2016): No mortality difference between cefepime and meropenem
- Caution: 89% of *E. cloacae* with cefepime MICs 4-8 mcg/mL were ESBL producers
- High-dose extended infusion improves outcomes

Piperacillin-tazobactam vs. carbapenems:

- Harris (2018): Higher failure rates with piperacillin-tazobactam
- MERINO-2 trial: Ongoing comparison
- Tazobactam exhibits variable AmpC inhibition by organism

Guidelines: Carbapenems preferred for serious AmpC infections; cefepime if susceptible and optimally dosed.

34

Plasmid-Mediated AmpC

Constitutively expressed at high levels without induction (unlike chromosomal AmpC)

Gene Families

- CMY (≈70% of plasmid AmpC), DHA, FOX, ACC, ACT, MOX
- CMY-2: >90% of plasmid AmpC in the US

Prevalence in *Enterobacterales*

- *E. coli*: primary reservoir (2-8% of clinical isolates)
- *K. pneumoniae*: concerning in healthcare settings (1-5%)
- *P. mirabilis*: naturally AmpC-negative (acquisition problematic)

Clinical Impact

- 30-day mortality: 15-25% in bloodstream infections
- 3-fold higher treatment failure when misidentified as ESBL
- Often co-harbored with ESBLs and carbapenemases

35

AmpC Take Home Points

1. Moderate to High Risk AmpC (*E. cloacae, K. aerogenes, C. freundii*)
   - Use cefepime for MIC ≤ 2 mcg/mL
   - Switch to carbapenem if MIC ≥ 4 mcg/mL (ESBL co-production risk)
   - Optimal dosing: cefepime 2g q8h (3-4 hour infusion)
   - Consider carbapenem for critical illness or high-inoculum infections
2. Lower Risk AmpC Organisms (*M. morganii, P. stuartii, P. rettgeri*)
   - Ceftriaxone or piperacillin/tazobactam suitable for UTI and uncomplicated infections
   - Monitor for plasmid-mediated AmpC in K. pneumoniae and E. coli
   - Cefoxitin MIC ≥ 16 mcg/mL suggests AmpC production
   - Cefepime susceptibility indicates minimal ESBL co-production
   - Piperacillin-tazobactam: higher failure rates than carbapenems
3. Empiric Therapy Guidance
   - Assess risk factors: prior antibiotics, healthcare exposure
   - Use institutional antibiogram data
   - De-escalate with susceptibilities and clinical improvement
4. Clinical Considerations
   - Heightened risk: bacteremia, pneumonia, high-inoculum infections
   - Match therapy to source control and infection severity

36

AmpC Detection

- No CLSI standardized methods or criteria for routine AmpC identification
- Detection challenging due to variable sensitivity/specificity of phenotypic tests
- Antimicrobial susceptibility patterns provide clues for presumptive identification
- Definitive molecular testing primarily available at reference laboratories

37

AmpC Detection

Phenotypic disk-based methods

- Cloxacillin disk: ≥4 mm zone increase indicates AmpC
- Boronic acid disk: 80-95% sensitivity, 85-90% specificity
- Cefoxitin-cloxacillin test: Detects plasmid-mediated variants

Broth microdilution methods

- ≥4-fold cefoxitin MIC reduction with cloxacillin confirms AmpC
- Commercial panels: Available in Europe, not FDA-approved in US

Molecular diagnostic techniques

- Multiplex PCR: Detects AmpC genes (2-4 hours)
- MALDI-TOF: Identifies □-lactam hydrolysis (93-97% accuracy)
- Whole genome sequencing: Reference standard, research use only

38

AmpC Detection: Susceptibility Reports

- Chromosomal AmpC
  - May appear susceptible to 3rd gen cephalosporins when induction hasn't been observed
  - Resistance can develop upon antibiotic inducer exposure
- Plasmid-mediated AmpC
  - Susceptibility reports show resistance to 3rd generation cephalosporins

39

AmpC Susceptibility Reports: *Enterobacter* spp.

Antibiotic	MIC (mcg/mL)	Interpretation
Ampicillin	16	Resistant
Ampicillin/sulbactam	32/16	Resistant
Cefazolin	≥64	Resistant
Cefepime	2	Susceptible
Cefoxitin	64	Resistant
Ceftriaxone	0.5	Susceptible*
Ciprofloxacin	0.5	Susceptible
Gentamicin	4	Susceptible
Meropenem	0.5	Susceptible
Piperacillin/tazobactam	16/4	Susceptible*

40

AmpC Susceptibility Reports: *E. coli*

Antibiotic	MIC (mcg/mL)	Interpretation
Ampicillin	16	Resistant
Ampicillin/sulbactam	32/16	Resistant
Cefazolin	≥64	Resistant
Cefepime	2	Susceptible
Cefoxitin	64	Resistant
Ceftriaxone	8	Resistant
Ciprofloxacin	0.5	Susceptible
Gentamicin	4	Susceptible
Meropenem	0.5	Susceptible
Piperacillin/tazobactam	128	Resistant

41

AmpC: Treatment Strategies

Treatment selection should be guided by susceptibility testing, infection site, and patient factors.

Severe Infections

- Carbapenems (meropenem, imipenem) - First-line for serious infections
- New BL/BLI combinations - Should be reserved
  - Reserve for those cases that are also carbapenem resistant

Mild-to-Moderate Infections

- Cefepime - Stable against AmpC hydrolysis at appropriate dosing
- Fluoroquinolones - When susceptibility is confirmed
- Trimethoprim-sulfamethoxazole - For susceptible urinary and soft tissue infections
- Nitrofurantoin - Reserved for uncomplicated lower UTIs only

42

AmpC: Clinical Controversies

- **Cefepime Considerations:**
  - Optimize dosing (2g IV q8h) for maximum efficacy
  - Suitable for isolates with MIC ≤ 2 mcg/mL
  - Higher treatment failure risk with concomitant ESBL
  - Better for non-severe than critical infections
- **Piperacillin/tazobactam Challenges:**
  - Inconsistent clinical efficacy data
  - Outcomes vary by infection type
  - Evaluate patient factors and local resistance patterns

43

AmpC: Risk Factors

- Risk profile overlaps significantly with **ESBL-producing** organisms
- Prior antibiotic exposure
  - Particularly 3rd generation cephalosporins (ceftriaxone, cefotaxime)
  - Extended and repeated courses increase risk
- Severe infections requiring intensive care admission
- Significant comorbidities
  - Hematologic malignancies and solid tumors
  - Immunosuppressive therapy (transplant, biologics)
  - Chronic renal or hepatic dysfunction
- Recent hospitalization or healthcare facility exposure

44

AmpC: Clinical Pearls

- Standard labs rarely identify AmpC producers; rely on susceptibility patterns and clinical suspicion
- Recognize AmpC-producing organisms (*Enterobacter*, *Citrobacter*, *Serratia*) and adjust therapy accordingly
- Be aware of inducible AmpC when using certain β-lactams
- Treatment options:
  - Severe infections: Carbapenems
  - Moderate infections: Cefepime (appropriate dosing) or non-β-lactams when susceptible
- Avoid third-generation cephalosporins and ampicillin-sulbactam regardless of reported susceptibility

45

Which bacterial resistance mechanism is represented by this susceptibility profile?

Antibiotic	Interpretation
Ampicillin	Resistant (R)
Ampicillin/sulbactam	Resistant (R)
Cefazolin	Resistant (R)
Cefepime	Susceptible (S)
Cefoxitin	Resistant (R)
Ceftriaxone	Resistant (R)
Meropenem	Resistant (R)
Piperacillin/tazobactam	Resistant (R)
Ceftazidime/avibactam	Susceptible (S)
Meropenem/vaborbactam	Susceptible (S)

Note: Resistance to carbapenems with susceptibility to newer β-lactam/β-lactamase inhibitor combinations is the key diagnostic pattern.

Select the most likely mechanism:

1. AmpC β-lactamase
2. Extended-spectrum β-lactamase (ESBL)
3. Klebsiella pneumoniae carbapenemase (KPC)
4. New Delhi metallo-β-lactamase (NDM)
5. Oxacillinase-48 (OXA-48)

46

Carbapenem-Resistant *Enterobacterales* (CRE) Epidemiology

- Carbapenem resistance methods:
  - Carbapenemases
  - Non-enzymatic
- Carbapenemases
  - Carried on plasmids
  - Easily transmissible
- 7% of hospital-acquired Gram-negative infections in the U.S. from 2010–2014 were attributed to CRE-producing organisms
  - *Klebsiella pneumoniae* carbapenemases (KPCs) are the most common carbapenemase in the U.S.

47

Non-Carbapenemase CRE

Resistance mechanisms without carbapenemase production:

- **AmpC β-lactamases + OMP changes:** AmpC overexpression with outer membrane protein mutations (OmpK35/36 in *Klebsiella*, OmpF/C in *E. coli*) limiting carbapenem entry
- **AmpC + Efflux pumps:** AmpC hyperproduction with enhanced efflux systems (RND family) removing carbapenems from periplasmic space
- **ESBL + Reduced permeability:** Extended-spectrum β-lactamases (CTX-M, SHV, TEM variants) with porin alterations preventing carbapenem accumulation

Susceptibility pattern: Meropenem shows lower MICs (1–8 mcg/mL) while ertapenem shows higher MICs (≥ 2 mcg/mL) due to greater vulnerability to these mechanisms

Unlike KPC-producing strains common in U.S., these strains lack plasmid-mediated transmission

48

Carbapenemase Producing - CRE

Resistance via carbapenemase enzyme production:

- **Klebsiella pneumoniae carbapenemase (KPC):** Dominant in U.S., found in *K. pneumoniae*, spreading to other Enterobacterales
- **New Delhi metallo-β-lactamase (NDM):** Global emergence, linked to medical tourism
- **Verona integron-encoded metallo-β-lactamase (VIM):** Prevalent in Mediterranean, spares only aztreonam
- **Imipenemase (IMP):** Rare, mainly in *Enterobacter* species
- **OXA-48-like:** Detection challenges, weaker activity but clinically significant

Susceptibility pattern: High meropenem MICs (≥16 mcg/mL), resistant to most β-lactams. Plasmid-mediated transmission enables rapid spread.

49

Carbapenemases

Critical enzymes driving carbapenem resistance in bacteria

Enzyme Classification and Distribution

Properties	KPCs	Metallo-β-lactamases	OXA-48-like
Hydrolyzes	All β-lactams	All except aztreonam	All β-lactams
Found in	Enterobacterales	Enterobacterales & non-fermenters	Enterobacterales

β-Lactamase Inhibitor Susceptibility

Properties	KPCs	Metallo-β-lactamases	OXA-48-like
Traditional BLI*	Minimal effect*	No effect	No effect
Avibactam	**Inhibits**	No effect	**Inhibits**
Vaborbactam/relebactam	**Inhibits**	No effect	No effect

*Traditional β-lactamase inhibitors (BLIs) include clavulanic acid, sulbactam, and tazobactam.

50

CRE Detection

- Traditionally was based on breakpoint-based susceptibility testing
  - Enzymatic vs other mechanisms?
  - What about inefficient carbapenemases?
- Rapid spread of plasmid carbapenemases

51

Carbapenemase Detection Methods

Laboratory techniques for identifying carbapenemase production

Modified Hodge Test (MHT)

Traditional phenotypic assay

Colorimetric assays (e.g., Carba-NP)

Rapid pH-based detection

Modified carbapenem inactivation method (mCIM)

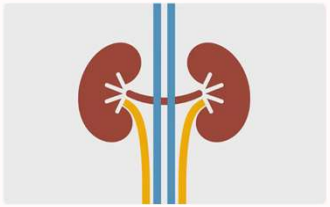
Improved sensitivity test

Rapid molecular diagnostic platforms

- Polymerase chain reaction (PCR) for specific genes (*KPC, NDM, VIM, IMP, OXA*)
- Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) – enzyme activity detection

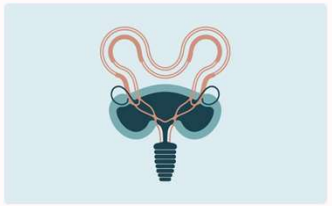
52

Treatment: Urinary CRE



Upper/Complicated UTI (UUTI/cUTI)

- 1st Line: Co-trimoxazole, Quinolone
- 2nd Line: Aminoglycoside, New BL/BLIs
- Don't Use: Nitrofurantoin, PO Fosfomycin, Colistin



Lower UTI (LUTI)

- 1st Line: Nitrofurantoin, Co-trimoxazole, Quinolone
- 2nd Line: Aminoglycoside, PO Fosfomycin (E. coli only), Colistin, New BL/BLIs

Note: Treatment selection should be guided by susceptibility testing. New □-lactam/□-lactamase inhibitors (BL/BLIs) should be reserved as second-line options.

53

Treatment: Systemic CRE (non-CPE)

Treatment options vary based on carbapenem susceptibility patterns

Antimicrobial Agent	Ertapenem Resistant but Meropenem/Imipenem Susceptible	Resistant to All Carbapenems
Meropenem or Imipenem (extended infusion)	1st Line Therapy	Not Recommended
Ceftazidime/avibactam	2nd Line Therapy	1st Line Therapy
Meropenem/vaborbactam	2nd Line Therapy	1st Line Therapy
Imipenem/relebactam	2nd Line Therapy	1st Line Therapy
Tigecycline/Eravacycline (Avoid for bloodstream or urinary infections)	2nd Line Therapy	2nd Line Therapy
Carbapenem + additional agent combination	Not Recommended	Not Recommended

Note: CRE = Carbapenem-Resistant Enterobacterales; CPE = Carbapenemase-Producing Enterobacterales; BSI = Bloodstream Infection; UTI = Urinary Tract Infection

54

Treatment: Systemic CRE (KPC Confirmed)

1st Line Options
- **#1 Choice:** Meropenem/vaborbactam
- **#2 Choice:** Ceftazidime/avibactam**
- **#3 Choice:** Imipenem/relebactam

2nd Line Option
- Cefiderocol (avoid for UTI)

3rd Line Options
- Tigecycline/Eravacycline (avoid for BSI or UTI)

Not Recommended
- Aztreonam combinations
- Polymyxins
- Carbapenem combination therapy

***CZA - Lower barrier to resistance with continued use*

55

Treatment: Systemic CRE (OXA-48 Confirmed)

1st Line Therapy
- Ceftazidime/avibactam

2nd Line Therapy
- Cefiderocol

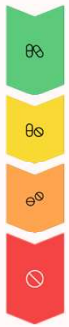
3rd Line Therapy
- Tigecycline/Eravacycline (avoid for BSI or UTI)

Not Recommended
- Vaborbactam/Relebactam combinations
- Polymyxins
- Carbapenem combination therapy

56

Treatment: Systemic CRE (MBL Confirmed)

Treatment options for Class B: Metallo-β-lactamases (NDM, VIM, IMP)



1st Line Options

- **#1 Choice:** Aztreonam + ceftazidime/avibactam
- **#2 Choice:** Cefiderocol

2nd Line Option

- Aztreonam + other new BL/BLI

3rd Line Option

- Tigecycline/Eravacycline (avoid for BSI or UTI)

Not Recommended

- Polymyxins
- Carbapenem combination therapy

57

Treatment: Systemic CRE (CPE Confirmed) - Overview

Antimicrobial Agent	Class A: *KPC* Carbapenemase	Class B: MBLs (*NDM, VIM, IMP*)	Class D: *OXA-48* Carbapenemase
Meropenem/vaborbactam	1st Line; Preferred Choice	Not Recommended	Not Recommended
Ceftazidime/avibactam	1st Line; Alternative	Not Recommended	1st Line
Imipenem/relebactam	1st Line; Alternative	Not Recommended	Not Recommended
Cefiderocol	2nd Line (avoid for UTI)	1st Line; Alternative	2nd Line
Aztreonam + ceftazidime/avibactam	Not Recommended	1st Line	Not Recommended
Aztreonam + other BL/BLI combinations	Not Recommended	2nd Line	Not Recommended
Tigecycline/Eravacycline (Avoid for BSI and UTI)	3rd Line	3rd Line	3rd Line
Polymyxins (Colistin/Polymyxin B)	Not Recommended	Not Recommended	Not Recommended
Carbapenem + additional agent combination therapy	Not Recommended	Not Recommended	Not Recommended

Note: CRE = Carbapenem-Resistant Enterobacterales; CPE = Carbapenemase-Producing Enterobacterales; BSI = Bloodstream Infection; UTI = Urinary Tract Infection; BL/BLI = β-lactam/β-lactamase Inhibitor; MBL = Metallo-β-lactamase

58

Risk Factors: *CRE*

- **Previous antibiotic exposure**, particularly:
  - Carbapenems (imipenem, meropenem, ertapenem)
  - Polymyxins (colistin, polymyxin B)
  - Fluoroquinolones and extended-spectrum cephalosporins
- **Healthcare exposure**:
  - Intensive care unit (ICU) admission
  - Prolonged hospitalization (>2 weeks)
  - Residence in long-term care facilities
- **Patient factors**:
  - Severe underlying illness (APACHE II score >15)
  - Immunocompromised state (transplantation, biologics, steroids)
  - Multiple comorbidities (diabetes, renal failure, liver disease)
- **Invasive procedures**:
  - Mechanical ventilation
  - Indwelling devices (central lines, urinary catheters)
  - Recent surgical procedures

59

Clinical Pearls: *CRE*

- **Mortality impact:** CRE infections carry 30-50% mortality rates, requiring rapid identification and intervention
- **No standardized treatment:** Therapy varies based on infection site, carbapenemase type, comorbidities, and local resistance patterns
- **Optimize PK/PD parameters:** Use aggressive dosing with extended infusions to maximize therapeutic efficacy

60

Which bacterial resistance mechanism is represented by this susceptibility profile?

Antibiotic	Interpretation
Ampicillin	Resistant (R)
Ampicillin/sulbactam	Resistant (R)
Cefazolin	Resistant (R)
Cefepime	Resistant (R)
Cefoxitin	Resistant (R)
Ceftriaxone	Resistant (R)
Meropenem	Resistant (R)
Piperacillin/tazobactam	Resistant (R)
Ceftazidime/avibactam	Susceptible (S)
Meropenem/vaborbactam	Susceptible (S)

Note: Resistance to carbapenems with susceptibility to newer □-lactam/□-lactamase inhibitor combinations is the key diagnostic pattern.

Select the most likely mechanism:

1. AmpC □-lactamase
2. Extended-spectrum □-lactamase (ESBL)
3. Klebsiella pneumoniae carbapenemase (KPC)
4. New Delhi metallo-□-lactamase (NDM)
5. Oxacillinase-48 (OXA-48)

61

Background: DTR *P. aeruginosa*

- Non-fermenting Gram-negative bacilli
- Multidrug resistant (MDR) and Difficult to Treat Resistant (DTR) *P. aeruginosa* is increasing in prevalence
  - MDR = not susceptible to at least one antibiotic in at least three antibiotic classes for which *P. aeruginosa* susceptibility is generally expected: penicillins, cephalosporins, fluoroquinolones, aminoglycosides, and carbapenems
  - DTR = non-susceptibility to all of the following: piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, meropenem, imipenem-cilastatin, ciprofloxacin, and levofloxacin

62

DTR *PsA* - Mechanisms of Resistance

Multiple mechanisms of intrinsic resistance:

- Porin channel loss - Loss of OprD leads to low-level carbapenem resistance
- Efflux pump overexpression - RND pump upregulation including:
  - MexAB-OprM
  - MexCD-OprJ
- AmpC β-lactamase overproduction - Chromosomally encoded
- Target site modifications:
  - DNA gyrase/topoisomerase IV mutations
  - Ribosomal modifications
  - Penicillin binding protein alterations

63

DTR *PsA* - Mechanisms of Resistance

Also acquires plasmid-mediated resistance:

Metallo-β-lactamases (MBLs)

IMP, VIM, NDM families; resist β-lactamase inhibitors

OXA enzymes

Carbapenemases affecting penicillins and carbapenems

Extended-Spectrum β-lactamases (ESBLs)

PER, VEB, GES families conferring resistance to extended-spectrum cephalosporins

Combined intrinsic and acquired resistance creates the DTR phenotype with extremely limited treatment options.

64

Treatment: DTR *P. aeruginosa*

Non-UTI Infection Treatment Options

First-Line Therapies

- Ceftolozane/tazobactam (3g q8h)
- Ceftazidime/avibactam (2.5g q8h)
- Imipenem/relebactam (1.25g q6h)

Second-Line Therapy

- Cefiderocol (2g q8h) - preferred for MBL-producing strains

Limited Efficacy Options

- Tobramycin/Amikacin (dosing based on TDM)
- Colistin (high toxicity profile)

65

Treatment: DTR *P. aeruginosa*

Complicated and Upper Urinary Tract Infection (cUTI, UUTI) Treatment Options

First-Line Options

- Ceftolozane/tazobactam (1.5g q8h)
- Ceftazidime/avibactam (2.5g q8h)
- Imipenem/relebactam (1.25g q6h)
- Cefiderocol (2g q8h) - preferred for MBL-producing strains
- Tobramycin/Amikacin (dosing based on TDM)

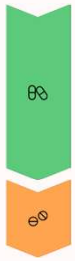
Not Recommended

- Colistin (high toxicity profile)

66

Treatment: DTR *P. aeruginosa*

Lower Urinary Tract Infection (LUTI) Treatment Options



First-Line Options

- Ceftolozane/tazobactam (1.5g q8h)
- Ceftazidime/avibactam (2.5g q8h)
- Imipenem/relebactam (1.25g q6h)
- Cefiderocol (2g q8h) - preferred for MBL-producing strains
- Tobramycin/Amikacin (dosing based on TDM)

Third-Line Option (Use with caution)

- Colistin (high toxicity profile)

67

Ceftolozane/tazobactam characteristics

- Advanced BL/BLI with optimized pharmacokinetics
- Superior anti-DTR *P. aeruginosa* activity via:
  - Enhanced PBP binding (4–8× lower MICs vs. ceftazidime)
  - Resistance to MexAB-OprM efflux (85% effective)
  - AmpC β-lactamase stability (91% active)
  - Superior ESBL coverage vs. piperacillin/tazobactam (4×) and ceftazidime (8×)
  - Effective against OprD loss mutations (82%)
- Optimized 2:1 inhibitor ratio for broader coverage
- First-line therapy: 3g q8h (pneumonia/BSI), 1.5g q8h (UTIs)
- >85% clinical efficacy for DTR *P. aeruginosa*

68

Which bacterial resistance mechanism is represented by this susceptibility profile?

Antibiotic	Interpretation
Cefepime	Susceptible (S)
Meropenem	Resistant (R)
Piperacillin/tazobactam	Susceptible (S)
Ceftolozane/tazobactam	Susceptible (S)
Ceftazidime/avibactam	Susceptible (S)
Meropenem/vaborbactam	Resistant (R)

Note: Resistance to carbapenems with susceptibility to newer β-lactam/β-lactamase inhibitor combinations is the key diagnostic pattern.

Select the most likely mechanism:

1. AmpC β-lactamase
2. OprD Porin Loss
3. New Delhi metallo-β-lactamase (NDM)
4. Oxacillinase-48 (OXA-48)

69

Background: CRAB/Stenotrophomonas

- Carbapenem-resistant *Acinetobacter baumannii* (CRAB)
  - Nosocomial pathogen with 50-80% ICU mortality
  - Survives 30 days on surfaces
  - Causes severe pneumonia, bloodstream, and wound infections
- *Stenotrophomonas maltophilia*
  - Opportunistic pathogen with intrinsic resistance to carbapenems, β-lactams, and aminoglycosides
  - Affects immunocompromised patients, those with malignancies, and extended hospitalizations (>14 days)
  - 30-day mortality: 20-60% in critical illness

70

CRAB: Mechanisms of Resistance

Enzymatic Mechanisms

OXA Carbapenemases

Primary resistance mechanism in *A. baumannii*, these enzymes hydrolyze the β-lactam ring in carbapenems

- OXA-23 (prevalent in Asia, Europe)
- OXA-24/40 (common in Mediterranean regions)
- OXA-58 (widespread globally)

Cephalosporinases

Overexpression of chromosomal Acinetobacter-derived cephalosporinases (ADCs) contributing to β-lactam resistance, especially ADC-7 and ADC-25 variants that confer resistance to extended-spectrum cephalosporins

These enzymatic mechanisms work synergistically with other resistance mechanisms to create highly resistant CRAB strains.

71

CRAB: Mechanisms of Resistance

Non-Enzymatic Mechanisms

1. **PBP modifications**
   Alterations in penicillin-binding proteins (primarily PBP2a) that reduce affinity for carbapenems by up to 10-fold
2. **Efflux pump overexpression**
   Upregulation of RND-type systems (AdeABC, AdeIJK, AdeFGH) that actively expel multiple antibiotics
3. **Porin channel reduction**
   Decreased expression of outer membrane proteins (CarO and Omp33-36) limiting carbapenem entry into the cell

These mechanisms frequently co-occur, resulting in high-level carbapenem resistance. Isolates with porin reduction often show meropenem MICs ≥16 mcg/mL.

72

Steno: Mechanisms of Resistance

Enzymatic Mechanisms

L1 β-lactamase

- Zn^{2}□-dependent metallo-□-lactamase that efficiently hydrolyzes all ϕ-lactams except monobactams (aztreonam)
- Exhibits extraordinary carbapenem hydrolysis capacity (100–1000× higher than other □-lactamases)
- Expression increases 5–10 fold during antibiotic exposure, leading to rapid resistance development

L2 β-lactamase

- Inducible Ambler class A cephalosporinase that neutralizes □-lactam/inhibitor combinations (clavulanate, tazobactam, sulbactam)
- Expression amplifies 4–8 fold when exposed to imipenem
- Acts synergistically with efflux systems to enhance multidrug resistance profiles

73

Steno: Mechanisms of Resistance

Non-Enzymatic Mechanisms

- **Efflux pump overexpression**
  - SmeABC, SmeDEF, SmeIJK, SmeVWX systems expel fluoroquinolones, tetracyclines, chloramphenicol
  - SmeDEF: increases levofloxacin MICs 4–16×
  - SmeABC: confers aminoglycoside and ϕ-lactam resistance
  - SmeVWX: affects quinolones and tetracyclines
- **Porin Alterations**
  - Reduced porins (SmeC, OmpATb, MspA) limit antibiotic entry
  - Reduces aminoglycoside susceptibility 4–16×; polymyxins 2–8×
  - Found in 35–45% of isolates; enhances treatment failure with ϕ-lactamase production

74

Treatment Regimens

Organism	First-line Therapy	Second-line Therapy	Third-line Therapy	Not Recommended
CRAB Infections	Sulbactam-durlobactam + carbapenem	High-dose ampicillin-sulbactam† + second agent*	Combination therapy with polymyxin, tetracycline derivative, and/or cefiderocol	• Carbapenems monotherapy • Rifamycins
Stenotrophomonas Infections **(Bold = preferred options)**	Any two of: **cefiderocol**, minocycline, TMP-SMX, or levofloxacin			Ceftazidime monotherapy
	Aztreonam + Ceftazidime/avibactam			

† 9g/day sulbactam component

* Second agent options: tetracycline derivative, polymyxin B, or cefiderocol

75

Risk Factors:

Antibiotic Exposure

- Carbapenems (particularly for CRAB)
- Fluoroquinolones (both CRAB and Stenotrophomonas)
- 3rd generation cephalosporins (CRAB)
- Antipseudomonal □-lactams (Stenotrophomonas)

Healthcare-Associated Factors

- ICU admission and critical illness severity
- Mechanical ventilation >72 hours
- Invasive procedures (central lines, urinary catheters)
- Prolonged hospitalization (>14 days)

Host Factors

- Immunocompromised state (malignancy, neutropenia)
- Parenteral nutrition dependence
- Advanced age and multiple comorbidities

76

Clinical Pearls:

- CRAB infections require combination therapy targeting multiple resistance mechanisms
- MDR *Stenotrophomonas* responds better to dual therapy (TMP-SMX, minocycline, cefiderocol)
- Optimize PK/PD parameters with extended infusions and higher doses when appropriate
- Effective source control is critical for both pathogens
- Seek early infectious disease consultation for these challenging infections

77

Overall ASP Considerations:

- Optimize dosing strategies to maximize therapeutic efficacy while accounting for pathogen susceptibility, site of infection, and patient-specific factors
- Implement de-escalation protocols to minimize toxicity, prevent emergence of resistance, and reduce collateral damage to patient microbiome
- Conduct regular audits of antimicrobial prescribing patterns to identify opportunities for intervention and education
- Collaborate with microbiology laboratory to ensure timely reporting of susceptibility data for guided therapy decisions

78

Conclusions

- Gram-negative bacteria employ sophisticated resistance mechanisms including target modifications, protective strategies, and efflux pumps to simultaneously evade multiple antibiotic classes.
- Species like *P. aeruginosa*, *E. coli*, and *Acinetobacter* create formidable treatment barriers through multi-layered defense strategies
- Understanding these resistance pathways is essential for developing effective next-generation antimicrobials against multidrug-resistant Gram-negative infections.

79

References

80

References: ESBL

1. Paterson DL, et al. (2005). Clin Microbiol Rev. 18(4):657–686.
2. Tamma PD, et al. (2024). https://www.idsociety.org
3. CDC. (2024) About ESBL-producing Enterobacterales. Available at: https://www.cdc.gov
4. Tamma PD, et al. (2019). Open Forum Infect Dis. 6:ofz353.
5. Tamma PD, et al. (2021). J Clin Microbiol. 59:e00127-21.
6. Castenheira M, et al. (2023). Open Forum Infect Dis. 10:ofad038.
7. Tamma PD, et al. (2021). JAC Antimicrob Resist. 3:dlab019.
8. Mathers AJ, et al. (2021). JAC Antimicrob Resist. 3:dlab020.
9. Marchaim D, et al. (2012). Infect Control Hosp Epidemiol. 33:817-30.
10. Harris PNA, et al. (2018). JAMA. 320:984-94.
11. Henderson A, et al. (2020). Clin Infect Dis. 73:e3842.50.
12. Harada Y, et al. (2014). 20:O831-9.
13. Monogue ML, et al. (2021). Pharmacotherapy. 41:864-80.
14. Crass RL, et al. (2019). Pharmacotherapy. 39:182-95.
15. Seo YB, et al. (2017). BMC Infect Dis. 17:404.

81

References: ESBL (cont)

16. Kusumoto M, et al. (2023). J Vet Med Sci. 85:653-6.
17. Stewart AG, et al. (2021). Microbiol Spectr. 9:e0022621.
18. Bush K, et al. (2019). Nat Rev Microbiol. 17:295-306.
19. Jacobs MR, et al. (2019). Antimicrob Agents Chemother. 63:e01801-18.
20. Karlowsky JA, et al. (2019). Int J Antimicrob Agents. 53:456-66.
21. Sutherland CA, et al. (2015). Clin Ther. 37:1564-71.
22. CLSI. (2024). M100:34 ed.

82

References: AmpC

1. Tamma PD, et al. (2024). https://www.idsociety.org
2. Lupo, et al. (2013). Diag Microbiol Infect Dis. 77(3):179-94
3. MacDougall C, et al. (2011). J Pediatr Pharmacol Ther. 16(1):23-30
4. Kohlmann R, et al. (2018). J Antimicrob Chemother. 73(6):1530-6
5. Tamma PD, et al. (2019). Clin Infect Dis. 69(8):1446-1455
6. Jacoby GA. (2009). Clin Microbiol Rev. 22:161-82.
7. Jacobson KL, et al. (1995). Clin Infect Dis. 21:1107-13.
8. Honore N, et al. (1986). EMBO J. 5:3709-14.
9. Philippon A, et al. (2002). Antimicrob Agents Chemother. 46:1-11.
10. Eliopoulos GM. (1988). J Antimicrob Chemother. 22(Suppl A):37-44.
11. Bennett PM, et al. (1993). Antimicrob Agents Chemother. 37:153-8.
12. Jacobson KL, et al. (1995). Clin Infect Dis. 21:1107-13.
13. Tamma PD, et al. (2013). Clin Infect Dis. 57:781-8.
14. Kaye KS, et al. (2001). Antimicrob Agents Chemother. 45:2628-30.
15. CLSI. (2024). M100:34 ed.

83

References: AmpC (cont)

16. Hardy ME, et al. (2023). Antimicrob Agents Chemother. 67:e0082623.
17. Sanders CC, et al. (1997). Antimicrob Agents Chemother. 41:2013-5.
18. Hancock RE, et al. (1996). J Chemother. 8(Suppl 2):31-6.
19. Harris PN, (2016). J Antimicrob Chemother. 71:296-306.
20. Lee NY, et al. (2015). Antimicrob Agents Chemother. 59:7558-63.
21. Cheng L, et al. (2017). Antimicrob Agents Chemother. 61:e00276-17.
22. Lu B, et al. (2023). J Antimicrob Chemother. 78:1009-14.
23. Stewart AG, et al. (2021). Open Forum infect Dis. 8:ofab387.
24. Karlowsky JA, et al. (2016). Antimicrob Agents Chemother. 60:2849-57.
25. Shields RK, et al. (2020). Clin Infect Dis. 71:2713-16.
26. Stewart AG, et al. (2021). Trials. 22:301.

84

References: CRE

1. CDC. (2024) About ESBL-producing Enterobacterales. Available at: https://www.cdc.gov
2. Tamma PD, et al. (2024). https://www.idsociety.org
3. Sabour S, et al. (2021). Antimicrob Agents Chemother. 65:e0110521.
4. van Duin D, et al. (2020). Lancet Infect Dis. 20:731-41.
5. Sader HS, et al. (2023). Open Forum Infect Dis. 10:ofad046.
6. Senchyna F, et al. (2019). Diagn Microbiol Infect Dis. 93:250-7.
7. Mojica MF, et al. (2022). Lancet Infect Dis. 22:e28-34.
8. CLSI. (2024). M100:34 ed.
9. Tamma PD, et al. (2018). J Clin Microbiol. 56:e01140-18.
10. Carmeli Y, et al. (2016). Lancet Infect Dis. 16:661-73.
11. Kaye KS, et al. (2018). JAMA. 319:788-99.
12. Bassetti M, et al. (2021). Lancet Infect Dis. 21:226-40.
13. Sims M, et al. (2017). J Antimicrob Chemother. 72:2616-26.
14. Portsmouth S, et al. (2018). Lancet Infect Dis. 18:1319-28.
15. USCAST. (2024).

85

References: CRE (cont)

16. Adelman MW, et al. (2022). Open Forum Infect Dis. 9:ofad643.
17. Shields RK, et al. (2017). Antimicrob Agents Chemother. 61:e00883-17.
18. Bonnin RA, et al. (2023). Antibiotics (Basel). 12:102.
19. Di Bella S, et al. (2021). J Glob Antimicrob Resist. 25:268-81.
20. Ackley R, et al. (2020). Antimicrob Agents Chemother. 64:e02313-19.
21. McCreary EK, et al. (2021). Antimicrob Agents Chemother. 64:e0217120.
22. Ito A, et al. (2018). Antimicrob Agetns Chemother. 62:e01454-17.
23. Wise MG, et al. (2023). Microb Drug Resist. 29:360-70.
24. Harris H, et al. (2023). J Clin Microbiol. 61:e0164722.
25. Lodise TP, et al. (2022). Antimicrob Agents Chemother. 66:e0093522.
26. Takemura M, et al. (2023). J Antimicrob Chemother. 78:2019-27.
27. Lima O, et al. (2022). Eur J Clin Microbiol Infect Dis. 41:1173-82.
28. Tamma PD, et al. (2012). Clin Microbiol Rev. 25:450-70.
29. Tumbarello M, et al. (2021). Clin Infect Dis. 73:1664-76.

86

References: DTR *PsA*

1. Tamma PD, et al. (2024). https://www.idsociety.org.
2. Kadri SM, et al. (2018). Clin Infect Dis, 67(10):1662–70.
3. Pang Z, et al. (2019). Biotechnol Adv. 37:177–92.
4. Glen KA, et al. (2021). Pathogens. 10:1638.
5. Reyes J, et al. (2023). Lancet Microbe. 4:e159–70.
6. Karlowsky JA, et al. (2022). Antimicrob Agents Chemother. 66:e0018922.
7. Castanheira M, et al. (2021). JAC Antimicrob Resist. 3:dlab092.
8. Gajdaxs M. (2020). Antibiotics (Basel). 9:153.
9. Bauer KA, et al. (2013). Antimicrob Agents Chemother. 57:2907–12.
10. Khalili Y, et al. (2019). Acta Microbiol Immunol Hung. 66:529–40.
11. Babich T, et al. (2020). Clin Infect Dis. 70:2270–80.
12. Wise MG, et al. (2023). Microb Drug Resist. 29:360–70.
13. Murano K, et al. (2008). Bioorg Med Chem. 16:2261–75.
14. Castanheira M, et al. (2014). Antimicrob Agents Chemother. 58:6844–50.
15. CLSI. (2024). M100:34 ed.

87

References: DTR *PsA* (cont)

16. Carmeli Y, et al. (2016). Lancet Infect Dis. 16:661–73.
17. Bassetti M, et al. (2021). Lancet Infect Dis. 21:226–40.
18. Torres A, et al. (2018). Lancet Infect Dis. 18:285–95.
19. Kollef MH, et al. (2019). Lancet Infect Dis. 19:1299–311.
20. Motsch J, et al. (2020). Clin Infect Dis. 70:1799–808.
21. Stone GG, et al. (2018). J Antimicrob Chemother. 73:2519–23.
22. Almangour TA, et al. (2023). Antimicrob Agents Chemother. 67:e0040523.
23. Mauri, et al. (2021). Antibiotics (Basel). 10:1012.
24. Lee M, et al. (2021). Diagn Microbiol Infect Dis. 99:115227.
25. Fraile-Ribot PA, et al. (2018). J Antimicrob Chemother. 73:658–63.
26. Tamma PD, et al. (2020). Clin Infect Dis. 73:e4599–606.
27. Tamma PD, et al. (2012). Clin Microbiol Rev. 25:450–70.

88

References: CRAB/Steno

1. Tamma PD, et al. (2024). https://www.idsociety.org.
2. CDC. (2019) Carbapenem-Resistant *Acinetobacter baumannii.*
3. Karakonstantis S, et al. (2022) Antibiotics (Basel). 11:723.
4. O□Donnell JN, et al. (2021) Pharmacotherapy. 41:762-80.
5. Butler DA, et al. (2019) Curr Infect Dis Rep. 21:46.
6. Penwell WF, et al. (2015) Antimicrob Agents Chemother. 59:1680-9.
7. Bonomo RA, et al. (2006). Clin Infect Dis. 43(Suppl 2):S49-56.
8. CLSI Breakpoint Working Group Presentation. (2024).
9. CLSI. (2024). M100:34 ed.
10. Jung SY, et al. (2017). Crit Care. 21:319.

89

References: Stenotrophomonas

1. Tamma PD, et al. (2024). https://www.idsociety.org
2. Mojica MF, et al. (2022). JAC Antimicrob Resist. 4:dlac040.
3. Mojica MF, et al. (2019). mBio. 10:e00405-19
4. Crossman LC, et al. (2008). Genome Biol. 9:R74.
5. Isom CM, et al. (2022). J Bacteriol. 204:e0039821.
6. Brooke JS, et al. (2012). Clin Microbiol Rev. 25:2-41.
7. Guerci P, et al. (2019). Crit Care. 23:371.
8. CLSI. (2024). M100:34 ed.
9. Zelenitsky SA, et al. (2005). Diagn Microbiol Infect Dis. 51:39-43
10. Biagi M, et al. (2020). Antimicrob Agents Chemother. 64:e00559-20

90