AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

MINIREVIEW

The Ins and Outs of Susceptibility Testing for New β-Lactam/β-Lactamase Inhibitor Combinations for Gram-Negative Organisms

Tanis C. Dingle,[a,b] Johann Pitout[a,b,c]

[a]Alberta Precision Laboratories, Calgary, Alberta, Canada
[b]Department of Pathology and Laboratory Medicine, University of Calgary, Calgary, Alberta, Canada
[c]Department of Medical Microbiology, University of Pretoria, Pretoria, South Africa

ABSTRACT Ceftazidime-avibactam, meropenem-vaborbactam, and imipenem-relebactam are among the newest β-lactam/β-lactamase inhibitors (BL/BLIs) introduced to the North American antibiotic market. All have broad Gram-negative activity, including against certain carbapenemases. Despite this, susceptibility testing is warranted due to variable activity against certain β-lactamases (e.g., oxacillinases) and the presence of acquired resistance mechanisms in some isolates. Here, we discuss what we know about these new antimicrobial agents and how to navigate implementation of susceptibility testing and reporting of these agents in clinical laboratories.

KEYWORDS antimicrobial activity, antimicrobial resistance, antimicrobial susceptibility testing, β-lactamases, β-lactams, carbapenemase

Inpatient data from U.S. hospitals suggest that 3.4% of infections caused by Gram-negative pathogens are not susceptible to carbapenems (1). Carbapenem-resistant *Enterobacterales* (CRE), carbapenem-resistant *Acinetobacter baumannii* (CRAB), and multidrug-resistant *Pseudomonas aeruginosa* are listed among the urgent and serious threats in the Centers for Disease Control and Prevention (CDC)'s 2019 "Antibiotic Threats Report" (2). These organisms are resistant to most available antibiotics, making treatment of these infections very challenging. Since 2015, there have been a number of new β-lactam/β-lactamase inhibitor combination antibiotics approved by the U.S. Food and Drug Administration (FDA) for the treatment of resistant bacterial infections, including ceftazidime-avibactam (CZA), meropenem-vaborbactam (MEV), and imipenem-cilastatin-relebactam (IMR). In particular for resistant Gram-negative organisms, these new agents have been a welcome addition to a market with few options available.

The Infectious Diseases Society of America (IDSA) guidelines now recommend the use of these three agents as first-line treatment for infections caused by CRE and *P. aeruginosa* with difficult-to-treat resistance (DTR-*P. aeruginosa*; resistant to piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, meropenem, imipenem, ciprofloxacin, and levofloxacin) when they test susceptible (refer to reference 3 for detailed recommendations). Though resistance to these agents is not widespread, there is still a need for laboratories to be able to offer susceptibility testing on a routine basis depending on local prevalence of resistant Gram-negative organisms. Here, we provide overviews of CZA, MEV, and IMR and detail what laboratories should know about susceptibility testing of these new antimicrobial agents.

CEFTAZIDIME-AVIBACTAM

Mechanism of action. CZA is composed of the 3rd-generation cephalosporin, ceftazidime, and the β-lactamase inhibitor, avibactam. Ceftazidime binds to and inhibits

Editor Romney M. Humphries, Vanderbilt University Medical Center

Address correspondence to Tanis C. Dingle, tanis.dingle@ucalgary.ca.

The authors declare no conflict of interest.

Published 7 April 2022

TABLE 1 Activity of newer β-lactam/β-lactamase inhibitor combinations against different β-lactamases[a]

Resistant mechanism type	Ceftazidime	Ceftazidime-avibactam	Meropenem	Meropenem-vaborbactam	Imipenem	Imipenem-relebactam
ESBL	X	✓	✓	✓	✓	✓
AmpC cephalosporinase	X	✓	✓	✓	✓	✓
Class A carbapenemase (e.g., KPC)	X	✓	X	✓	X	✓
Class B carbapenemase (e.g., NDM)	X	X	X	X	X	X
Class D carbapenemase (e.g., OXA-48)	X	✓	X	X	X	X

[a]X, no activity; ✓, activity (susceptibility testing is still required as resistance is documented).

penicillin-binding proteins (PBPs), leading to an unstable peptidoglycan cell wall (4). Inhibition of PBP cross-linking by ceftazidime compromises the structural integrity of the bacterial cell wall and leads to aberrant cellular morphology and insufficient concentrations, cell lysis, and death. Avibactam, a diazacyclooctane (DBO) inhibitor (5), binds covalently to β-lactamases through the formation of a carbamate bond between avibactam's position 7 carbonyl carbon and active-site serine that participates in acyl bonding with β-lactam substrates (6). Avibactam forms a carbamate linkage when transitioning to the enzyme intermediate upon opening of the diazabicyclooctane ring structure. Avibactam diminishes the availability of active β-lactamases for hydrolysis and decreases inactivation of the β-lactam antibiotic (6).

Spectrum of activity. Ceftazidime is hydrolyzed by extended-spectrum class A β-lactamases (ESBLs), the AmpC class C cephalosporinases, and most of the carbapenemases (including the class A *Klebsiella pneumoniae* carbapenemases [KPCs], class B metallo-β-lactamases [MBLs], and some of the class D OXA β-lactamases) (6). The addition of avibactam allows CZA to inhibit class A (e.g., KPCs) KPCs), class C (AmpCs), and some of the class D OXA β-lactamases (OXA-48 and variants), providing CZA with a broad Gram-negative coverage (6) (Table 1), including members of *Enterobacterales* and *P. aeruginosa*. Overall, CZA's spectrum of activity is limited against class B MBLs, *Acinetobacter* OXA-type carbapenemases, Gram-positive bacteria, and anaerobes (6, 7).

Mechanisms of resistance. The overall global resistance rates of CZA against *Enterobacterales* are less than 5% and against *P. aeruginosa*, less than 20% (8, 9). *Enterobacterales a*nd *P. aeruginosa* develop resistance against CZA through amino acid substitutions and overexpression of certain β-lactamases as well as the restriction of access to PBP targets (via porin mutation and efflux pumps) (9). Several KPC-3, KPC-2, CTX-M-14, CTX-M-15, OXA-48, CMY-6, CMY-10, and SHV point mutations (most often in the enzyme's Ω-loop) have been published. Such mutations enable enzymes to more efficiently hydrolyze ceftazidime and also strongly bind avibactam. Mutations in OmpK35/36 porins (*Klebsiella pneumoniae*), enhanced expression of efflux pumps (*P. aeruginosa*), and overexpression of bla_{KPC}s (*K. pneumoniae*) and *Pseudomonas*-derived cephalosporinases (PDC; *P. aeruginosa*) are rare causes of resistance to CZA (8, 9).

Which organisms should be tested for ceftazidime-avibactam susceptibilities? CLSI classifies CZA as a group B antimicrobial for testing and reporting for the *Enterobacterales* and *Pseudomonas aeruginosa* (10). As such, it may warrant primary testing for the *Enterobacterales* and *Pseudomonas aeruginosa*, but it should be reported only selectively, such as when a narrower-spectrum agent of the same class (ceftazidime, for example) is resistant (10). Alternatively, if CZA is not tested with a primary testing panel, testing can be performed at a physician's request for *Enterobacterales* or *P. aeruginosa* when resistance to narrower-spectrum β-lactams or carbapenems is observed.

If carbapenemase testing is performed by the laboratory, CZA susceptibility testing is warranted for a CRE that tests positive for KPC or OXA-48-like carbapenemases or CRE isolates that are carbapenemase negative. Since CZA does not inhibit the MBLs, there is limited value in testing MBL-producing CPE to this agent. For DTR-*P. aeruginosa*, CZA susceptibility testing should be performed on non-MBL-producing isolates, including those that are carbapenemase negative.

TABLE 2 Practical challenges and solutions for CZA, MEV, and IMR susceptibility testing

Challenge	Solution
Requests for susceptibility testing of multidrug-resistant organisms with no clinical breakpoints (e.g., *Acinetobacter baumannii* complex and CZA).	Refer to the literature to determine if the request is reasonable. If so, perform the testing in-house or at a reference laboratory. Report the MIC and add a comment indicating that there are no established standardized methodology or clinical breakpoints for interpretation. MIC distributions and published literature may provide some guidance on interpretation.
Disk diffusion zone diameters that must be confirmed by an MIC method (e.g., *Escherichia coli* and CZA with a disk diffusion zone diameter of 21 mm and interpreted with CLSI breakpoints).	Confirm susceptibility result in-house by a verified MIC method (e.g., broth microdilution, gradient testing) or refer the isolate to a reference laboratory for testing by a verified MIC method.
Disk diffusion zone diameters within the area of technical uncertainty (e.g., *Pseudomonas aeruginosa* and CZA with a disk diffusion zone diameter of 16 mm interpreted with EUCAST breakpoints).	1) Repeat the test. OR 2) Confirm the susceptibility result in-house by a verified method (e.g. broth microdilution, gradient testing) or refre the isolate to a reference laboratory for testing by a verified MIC method. OR 3) Report no result with an interpretive comment. OR 4) Downgrade the result to R. Refer to reference 12 for further guidance.
Discrepant results when labs test by multiple methods (e.g., MEV on automated AST system, S, and MEV by disk, R).	Repeat results by both methods. If discrepancy persists after repeat testing, refer isolate to a reference laboratory for testing by a third method.
Finding of unexpected resistance (e.g., ceftazidime, S, and CZA, R).	Repeat testing for both antimicrobials. If discrepancy persists after repeat testing, refer isolate to a reference laboratory for testing of the isolate against both antimicrobials.
Requests for IMR testing for organisms in the *Morganellaceae* family.	Do not test. Explain that relebactam confers no additional activity to imipenem for members of this group. Provide references to support, if needed.
Requests for CZA, IMR, and MEV when narrower-spectrum agents (i.e., ceftazidime, imipenem, and meropenem, respectively) have tested susceptible.	Do not test. Explain that susceptibility to the narrower-spectrum agents infers susceptibility to the BL/BLI combination. Note that the opposite is not necessarily true: isolates susceptible to the BL/BLI combination are not necessarily susceptible to the narrower-spectrum agent.
High error rates when verifying a CZA susceptibility testing method.	Limit selection of isolates near the clinical breakpoint. Allow more leniency for errors occurring near the clinical breakpoint but within essential agreement.
Finding resistant isolates for verification of a susceptibility testing method for a new BL/BLI inhibitor.	Select isolates which are known to be resistant to the agent in question (e.g. MBL producer for CZA). Obtain characterized isolates from another laboratory (e.g., CDC and FDA Antibiotic Resistance Isolate Bank; https://wwwn.cdc.gov/ARIsolateBank/).

An important consideration for CZA susceptibility testing is that organisms that test susceptible to ceftazidime alone will also be susceptible to CZA, and therefore, susceptibility testing in this scenario is not necessary (Table 2). However, the opposite is not true; isolates susceptible to CZA are not necessarily susceptible to ceftazidime (10). This principle is also true for meropenem and MEV and imipenem and IMR, which are discussed in later sections (Table 2) (10).

Susceptibility testing. Broth microdilution (BMD) using standard cation-adjusted Mueller-Hinton broth and disk diffusion using standard Mueller-Hinton agar have been developed to determine *in vitro* activity of CZA against *Enterobacterales* and *P. aeruginosa*. Interpretive criteria have been published by CLSI and EUCAST (10–12) (Table 3). The FDA has recognized the CLSI M100 breakpoints.

For BMD, the concentration of avibactam is fixed at 4 μg/mL by both the CLSI and EUCAST methods (10, 12, 13). For disk diffusion, CLSI recommends 30/20-μg disks, while EUCAST recommended 10/4-μg disks. For *Enterobacterales*, the 30/20-μg CZA disks overestimate resistance (14), and CLSI recommends performing confirmatory MIC testing (e.g., gradient diffusion or BMD) for isolates with zone sizes of 20 to 22 mm to avoid reporting false-susceptible or false-resistant results (Tables 2 and 3). EUCAST guidelines recommend an area of technical uncertainty (ATU) for *P. aeruginosa* isolates with zones sizes of 16 to 17 mm (Table 3). For details on how to interpret and report ATUs, please refer to the EUCAST website (12) and Table 2.

TABLE 3 CLSI and EUCAST interpretive criteria for newer β-lactam/β-lactamase inhibitor combinations (10, 12)[a]

Breakpoint	Test type	Data for drug: Ceftazidime-avibactam	Meropenem-vaborbactam	Imipenem-relebactam
Enterobacterales[b]				
CLSI (FDA)	MIC (ug/mL)	S ≤ 8/4, R ≥ 16/4	S ≤ 4/8, I = 8/8, R ≥ 16/8	S ≤ 1/4, I = 2/4, R ≥ 4/4
	DD (mm)	S ≥ 21, R ≤ 20 mm[c]	S ≥ 18, I = 15–17, R ≤ 14 mm	S ≥ 25, I = 21–24, R ≤ 20
EUCAST	MIC (ug/mL)	S ≤ 8/4, R > 8/4	S ≤ 8/8, R > 8/8	S ≤ 2/4, R > 2/4
	DD (mm)	S ≥ 13, R < 13	—	S ≥ 22, R < 22
Pseudomonas aeruginosa				
CLSI (FDA)	MIC (ug/mL)	S ≤ 8/4, R ≥ 16/4	—	S ≤ 2/4, I = 4/4, R ≥ 8/4
	DD (mm)	S ≥ 21, R ≤ 20	—	S ≥ 23, I = 20–22, R ≤ 19
EUCAST	MIC (ug/mL)	S ≤ 8/4, R > 8/4	S ≤ 8/8, R > 8/8	S ≤ 2/4, R > 2/4
	DD (mm)	S > 17, R ≤ 17, ATU = 16–17	—	S ≥ 22, R < 22
Acinetobacter calcoaceticus-A. baumannii complex[d]				
FDA	MIC (ug/mL)	—	—	S ≤ 2/4, I = 4/4, R ≥ 8/4
	DD (mm)	—	—	—
EUCAST	MIC (ug/mL)	—	—	S ≤ 2/4, R > 2/4
	DD (mm)	—	—	S ≥ 24, R < 24 mm
Haemophilus influenzae				
FDA	MIC (ug/mL)	—	—	S ≤ 4/4
	DD (mm)	—	—	—
Anaerobes (Gram positive and Gram negative)[d]				
CLSI	MIC (ug/mL)	—	—	S ≤ 2/4, I = 4/4, R ≥ 8/4
	DD (mm)	—	—	—
EUCAST	MIC (ug/mL)	—	—	S ≤ 2/4, R > 2/4
	DD (mm)	—	—	—

[a]Disk contents as follows: for CLSI/FDA, 30/20 μg CZA, 20/10 μg MEV, and 10/25 μg IMR; for EUCAST, 10/4 μg CZA and 10/25 μg IMR. DD, disk diffusion; ATU, area of technical uncertainty; S, susceptible; R, resistant; I, indeterminate; —, interpretive criteria not established.
[b]Breakpoints for imipenem/relebactam for the Enterobacterales exclude members of the *Morganellaceae* family.
[c]Zones of 20 to 22 mm should be confirmed by the MIC method.
[d]Despite interpretive criteria being established for IMR and these organisms, the addition of relebactam does not confer additional benefit compared to imipenem alone. Testing is reasonable in cases of mixed infection where IMR will be used.

There are a number of FDA-approved commercial antibiotic susceptibility testing (AST) systems for CZA, including disk diffusion (Hardy Diagnostics; Becton Dickenson), gradient strips (Etest, bioMérieux; MIC test strip [MTS], Liofilchem), and automated/semiautomated systems (Vitek-2 [bioMérieux], MicroScan [Beckman Coulter], Phoenix [Becton, Dickinson], Sensititre [Thermo Fisher Scientific]). The package inserts of any CZA method considered for implementation by the clinical laboratory should be examined carefully, as all methods have one or more limitations, including, but not limited to, lack of performance criteria for resistant isolates or recommendations to retest certain species that test at specific MICs by an alternate method to prevent major errors (MEs). In the literature, CZA Etest (15, 16) and an automated AST panel (i.e., Vitek 2 [17]) for *Enterobacterales* and *P. aeruginosa* met or exceeded the FDA and ISO performance criteria and are reliable alternatives to the BMD reference method for routine susceptibility testing. Laboratories should be aware, however, that CZA disk diffusion performs variably depending on manufacturer (15, 16), and this should be considered when selecting a method to verify.

There are currently no CLSI or EUCAST CZA intermediate breakpoints for BMD or disk diffusion, which created difficulties for manufacturers during the FDA approval process and could create difficulties for clinical laboratories when verifying susceptibility testing of this agent due to potential for high very major error (VME) and ME rates. As such, laboratories may wish to select only limited susceptible and resistant isolates near the clinical breakpoint. In addition, the laboratory may wish to allow more

leniency for errors occurring near the clinical breakpoint but within essential agreement to the reference method.

For serious infections with CREs that test positive for class B MBLs, the IDSA recommends combination therapy of CZA with aztreonam (3). However, synergy testing guidance has not been published by CLSI, and the interpretation of synergy testing results remains questionable.

MEROPENEM-VABORBACTAM

Mechanism of action. Meropenem-vaborbactam (MEV) is a combination of a carbapenem, meropenem, and a cyclic boronic acid-based β-lactamase inhibitor, vaborbactam. Meropenem is a broad-spectrum carbapenem that inhibits cell wall synthesis through binding to PBPs. It is stable to most β-lactamases and cephalosporinases, but not carbapenem-hydrolyzing enzymes (carbapenemases). Vaborbactam is a novel cyclic boronate-based β-lactamase inhibitor which forms stable enzyme-inhibitor complexes with class A serine β-lactamases (5). Although it has no intrinsic antibacterial activity, its addition protects meropenem from degradation by class A serine β-lactamases (e.g., KPCs).

Spectrum of activity. Meropenem, on its own, has been used for many decades in the treatment of resistant Gram-negative infections caused by *Enterobacterales*, *P. aeruginosa*, and *Acinetobacter baumannii*. It is active against ESBLs and the AmpC class C cephalosporinases. It is hydrolyzed by most carbapenemases, including class A serine carbapenemases, class B MBLs, and class D OXA-type carbapenemases. The addition of vaborbactam extends the spectrum of activity of MEV to organisms producing class A KPC carbapenemases (Table 1). MEV does not, however, have activity against the MBLs (e.g. NDM, IMP, VIM) or the class D OXA-type carbapenemases (e.g., OXA-48) (18) (Table 1). MEV retains activity to some *Enterobacterales* isolates that are CZA resistant due to bla_{KPC} mutations (19). MEV does not have improved activity against DTR-*P. aeruginosa* and CRAB due to noncarbapenemase mechanisms of resistance for which MEV has no activity and the presence of class B and D carbapenemases, respectively (20).

Mechanisms of resistance. In large *in vitro* surveillance studies $\geq$ 99% of KPC-producing *Enterobacterales* are susceptible to meropenem-vaborbactam (21, 22). Despite excellent activity against class A carbapenemase-producing *Enterobacterales*, resistance has been reported. The membrane porins, OmpK35 and, in particular, OmpK36, allow entry of meropenem and vaborbactam into the organism. Porin alteration via mutation in *ompK35* and *ompK36* is the primary means of resistance to MEV, as this causes reduced membrane permeability (23). KPC overexpression due to increased bla_{KPC} gene copy number has also been reported as a mechanism of resistance in KPC-producing *Enterobacterales* (24). There have not yet been reports of KPC enzyme mutation conferring resistance to MEV, unlike for CZA (19).

Which organisms should be tested for meropenem-vaborbactam susceptibilities? Given that resistance has been reported even among KPC-producing *Enterobacterales*, laboratories should consider providing susceptibility testing results for MEV in appropriate settings. CLSI classifies MEV as a group B antimicrobial for testing and reporting for the *Enterobacterales*, and so, it is reasonable to test this agent when *Enterobacterales* isolates are resistant to meropenem (3, 10). When carbapenemase testing is available for CRE and can differentiate carbapenemase type, MEV testing and reporting are also reasonable when a class A carbapenemase, such as a KPC, is identified or when no carbapenemase is detected. There is no utility in testing MEV for organisms harboring class B or class D carbapenemases. Routine testing for Gram-negative organisms other than the *Enterobacterales*, including *P. aeruginosa* and *Acinetobacter baumannii*, is not warranted since vaborbactam does not confer additional activity compared to meropenem alone. Despite this, EUCAST has published a clinical breakpoint for *P. aeruginosa*, and laboratories may elect to test and report MEV using EUCAST breakpoints on request from physicians when meropenem has not already been tested (as susceptibility is inferred from meropenem if already tested for this organism). This is an unlikely scenario given most laboratories will test meropenem for *P. aeruginosa*.

Susceptibility testing. CLSI provides guidelines for BMD, agar dilution, and disk diffusion for MEV susceptibility testing. MIC-based methods such as BMD, agar dilution, and gradient strips are performed with a fixed concentration of vaborbactam at 8 μg/mL (CLSI and EUCAST). Disk diffusion by the CLSI method is performed with 20/10-μg disks. Both CLSI MIC and disk diffusion interpretive criteria are available for MEV for the *Enterobacterales* (Table 3). The FDA recognizes the CLSI M100 breakpoints. EUCAST provides MIC interpretive criteria for both the *Enterobacterales* and *P. aeruginosa* (Table 3). The susceptible CLSI MIC breakpoint for the *Enterobacterales* is one dilution lower (susceptibility [S] $\leq$ 4 μg/mL) than EUCAST (S $\leq$ 8 μg/mL). There are no CLSI interpretive criteria for MEV and *P. aeruginosa* or *Acinetobacter baumannii* (Table 3), and testing for these organisms is generally not necessary.

There are now several commercially available, FDA-approved methods for MEV testing for clinical laboratories to consider implementing. Automated/semiautomated testing is available through the Vitek-2, MicroScan, Phoenix, and Sensititre systems. Manual methods available include Etests and MIC test strips and disk diffusion (Hardy Diagnostics and Oxoid). All submissions to the FDA did not show performance data for resistant isolates of certain species due to lack of resistant strains at the time of testing. Unless a verification study is performed with sufficient numbers of resistant isolates, it is suggested that isolates yielding a resistant result (for the species denoted in the method package insert) be submitted to a reference laboratory for testing. Additionally, BD recommends confirming MEV MICs around the breakpoints at 4/8, 8/8, and 16/8 μg/mL on the Phoenix system (25). In the literature, a recent study of MEV Etests found accurate results for the *Enterobacterales* other than *Proteus mirabilis* when using CLSI breakpoints compared to reference BMD, but not when using EUCAST breakpoints (26). This limitation for *P. mirabilis* and MEV Etest was also observed in the FDA submission study (27). Evaluations of other MEV testing methods have yet to be published.

IMIPENEM-RELEBACTAM

Mechanism of action. Imipenem-cilastatin-relebactam (IMR) is a combination of carbapenem, imipenem, a renal dihydropeptidase inhibitor, cilastatin, and the β-lactamase inhibitor, relebactam (28). Imipenem and relebactam form the microbial-active components of IMR. Imipenem, like meropenem, is a broad-spectrum carbapenem that inhibits cell wall synthesis through binding to penicillin-binding proteins (29). Similar to avibactam, relebactam is a novel DBO β-lactamase inhibitor that protects imipenem from degradation by binding to the active site of class A (e.g., SHV, TEM, KPC) and class C (i.e., AmpC) β-lactamases (29). In doing so, it allows imipenem to retain activity against a broad array of aerobic and anaerobic Gram-positive and Gram-negative bacterial pathogens.

Spectrum of activity. Given that relebactam and avibactam are DBO inhibitors (5), they have a similar spectrum of activity (Table 1). IMR has activity against ESBL-, AmpC cephalosporinase-, and class A carbapenemase (i.e., KPC)-producing *Enterobacterales* (Table 1). One important exception within the *Enterobacterales* order is the organisms within the *Morganellaceae* family (*Proteus* spp., *Providencia* spp., and *Morganella* spp.) for which imipenem alone and IMR have poor activity due to permeability issues and not carbapenemase production. Relebactam confers no additional activity against these organisms compared to imipenem alone because the mechanism of resistance is not carbapenemase mediated.

IMR retains activity against non-MBL-producing DTR-*P. aeruginosa*, and neither imipenem nor relebactam is susceptible to efflux in this organism. IMR reduces the MICs of imipenem 2- to 128-fold in *Enterobacterales* and *P. aeruginosa* isolates (30). Global *in vitro* surveys have found that the *Enterobacterales* (non-*Morganellaceae*) and *P. aeruginosa* are >99% and >94% susceptible to IMR, respectively (31, 32). Among KPC-producing *Enterobacterales*, IMR susceptibility *in vitro* is greater than 96% (31). In imipenem-resistant *P. aeruginosa* isolates, IMR susceptibility is 80 to 92% (31, 33). Notably, IMR may be active in isolates that are CZA resistant (34). Imipenem resistant *A. baumannii* complex isolates are also resistant to IMR. (32).

Mechanisms of resistance. The most common mechanism of resistance to IMR is the production of class B (e.g., NDM, IMP, VIM) or class D OXA-type carbapenemases

(e.g., OXA-48), as relebactam is not active against these enzymes. In addition, IMR is not active against the Guiana extended-spectrum (GES) carbapenemase, which is most often carried by *P. aeruginosa* (35). Other mechanisms of resistance are likely, but not yet well defined, and include altered permeability and efflux pump expression (36).

Which organisms should be tested for imipenem-relebactam susceptibility? It is most reasonable to report IMR against CRE and DTR-*P. aeruginosa*. IMR falls in group B antimicrobial for testing and reporting by CLSI for the *Enterobacterales* and *P. aeruginosa*. For laboratories that do not perform carbapenemase testing, it would be reasonable to test IMR when imipenem tests resistant for these organisms. If carbapenemase testing is performed for CRE or DTR-*P. aeruginosa*, testing is warranted when a class A carbapenemase (e.g., KPC) is identified or when carbapenemase testing is negative. On the other hand, if an MBL (class B) or OXA-type (class D) carbapenemase is identified, there is no utility is performing IMR testing (Table 1). Within the *Enterobacterales*, there is also no utility in testing IMR against the *Morganellaceae* family (i.e., *Proteus* spp., *Providencia* spp., and *Morganella* spp.), even when a class A carbapenemase is identified. There are no interpretive criteria for the organisms in this family, as relebactam does not provide additional activity compared to imipenem alone.

Despite IMR having excellent activity against many aerobic and anaerobic Gram-negative organisms, the addition of relebactam does not confer additional activity compared to imipenem alone except for the *Enterobacterales* and *P. aeruginosa* (35, 37). It is therefore reasonable to test imipenem alone for non-*Enterobacterales* and non-*P. aeruginosa* Gram-negative organisms with established imipenem interpretive criteria to infer susceptibility to IMR. For organisms outside the *Enterobacterales* and *P. aeruginosa* for which there are established interpretive criteria for IMR (i.e., *A. baumannii* complex, *H. influenzae*, and anaerobes), it is reasonable to test IMR on physician request if imipenem is not already tested (since imipenem infers susceptibility for these organisms).

Susceptibility testing. Recommendations for susceptibility testing of IMR are available from CLSI and EUCAST. MIC-based methods such as BMD, agar dilution, and gradient strips are performed with a fixed concentration of relebactam at 4 μg/mL. Disk diffusion is performed with 10/25-μg disks. CLSI interpretive criteria have been established for the *Enterobacterales*, *P. aeruginosa*, and anaerobic Gram-negative bacteria (Table 3). The CLSI interpretive criteria for these organisms are recognized by the FDA. The FDA has additionally established MIC interpretive criteria for *A. baumannii* complex and *Haemophilus influenzae* (Table 3). Organisms for which EUCAST has defined breakpoints are listed in Table 3. For *A. baumannii* complex, *Haemophilus influenzae*, and anaerobes, relebactam does not provide additional activity to imipenem, and therefore, routine testing of IMR is not needed unless requested and imipenem has not already been tested.

Both Vitek-2 and Sensititre have FDA-approved Gram-negative panels that include IMR. Manual methods with FDA approval include MTS and Etest gradient strips, and disks (Hardy Diagnostics). The package insert of any IMR method implemented in the laboratory should be read carefully, as some common species did not produce acceptable performance criteria during the submission study (e.g., *Proteus mirabilis* for the MTS method) (38), and/or resistant isolates of some species were lacking for the data submission. Additionally, it is recommended that IMR tested by Sensititre for *P. aeruginosa* be retested by alternate method when an MIC of 2/4 μg/mL is obtained (39). A recent study found that the MTS and Etest gradient strips performed acceptably well compared to BMD for essential agreement (85.2% and 90.0%, respectively) and categorical agreement (96.2% and 96.6%, respectively) for the *Enterobacterales* and *P. aeruginosa* (40). Both methods tended to have MICs 1 to 2 dilutions higher than the BMD MICs but were still within acceptable categorical agreement to BMD. To our knowledge, the other available methods have yet to be evaluated in the literature.

RESOURCES FOR CLINICAL LABORATORIES

It is important for laboratories to perform a verification study, such as that outlined in CLSI M52 (41), prior to implementing FDA-approved testing methods for the new β-lactam/β-lactamase inhibitor combinations. The literature and FDA trial data can

provide valuable evaluation data to help guide method selection. For U.S. laboratories looking for characterized isolates with known MICs to these agents, the CDC and FDA Antibiotic Resistance Isolate Bank provides verification panels to laboratories at no cost (https://wwwn.cdc.gov/ARIsolateBank/). Alternatively, if the volume of testing required is minimal or it is not feasible to implement testing of these agents in your laboratory, many reference laboratories now offer testing, or testing can be performed at one of the CDC's Antibiotic Resistance Laboratory Network (ARLN) laboratories (https://www.cdc.gov/drugresistance/laboratories.html). In Canada, the National Microbiology Laboratory accepts isolates for testing, and some local and provincial laboratories also have testing available.

CONCLUSION

With the introduction of new antimicrobial agents, the onus falls on clinical microbiology laboratories to deliver accurate and reproducible antimicrobial susceptibility results to treating physicians. A summary of the challenges clinical laboratories may face when implementing, testing, and reporting these agents and possible solutions are summarized in Table 2. CZA, MEV, and IMR have broad-spectrum Gram-negative activity and are especially useful for the treatment of resistant infections due to *Enterobacterales* and DTR-*P. aeruginosa*. The decision to test and report these agents is at the discretion of the laboratory director in partnership with antimicrobial stewardship. Laboratories may wish to test these agents routinely yet only report based on cascading rules or algorithms based on carbapenemase testing results. Alternatively, labs may elect to test these agents on request only. In all cases, it is most useful to test and report results for organisms with CLSI, FDA, or EUCAST interpretive criteria. There are now multiple FDA-approved manual and automated AST methods available for testing of these agents and resources available for laboratories to perform the required verification studies. Once susceptibility testing methods and reporting criteria are in place for these new β-lactam/β-lactamase inhibitors (BL/BLIs), laboratories will provide essential treatment guidance to better manage patients with multidrug-resistant Gram-negative infections.

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