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β-LACTAM-β-LACTAMASE INHIBITOR COMBINATIONS AND NOVEL β-LACTAMS TARGETING GRAM-NEGATIVE BACTERIA

This document aims to guide diagnostic laboratories in the expected activity of approved and phase 3 clinical trial β-lactam-β-lactamase inhibitor (BLBLI) combinations as well as cefiderocol against various β-lactamases that impact β-lactam susceptibility in Gram-negative bacteria. It does not provide clinical treatment guidelines or replace antimicrobial susceptibility testing. The predicted activities are based on *in vitro* data, experimental data in animal models, and evidence from clinical trials (references 1-15).

Tables 1.1-3 summarize the expected activity of BLBLI combinations and cefiderocol against Enterobacterales, *Pseudomonas* spp., and *Acinetobacter baumannii* complex, respectively. The sulbactam-durlobactam combination is only approved by FDA. Several novel BLBLI combinations have recently completed clinical trials. Of those, only cefepime-zidebactam has an ongoing regulatory application process (12,16). If more than one β-lactamase are present, the expected activity will be dependent on the specific combination. As a rule, only agents active against bacteria harboring each individual β-lactamase will also be active against bacteria harboring the combination.

Table 2 highlights the expected activity of β-lactamase inhibitors (BLIs) alone on specific groups of β-lactamases. Note that the activity of inhibitors may vary within and between variants in each β-lactamase group.

Notably, some recently developed β-lactamase inhibitors (BLIs) also exhibit intrinsic antibacterial activity through their ability to inhibit penicillin-binding proteins (PBPs). This property drives synergistic bactericidal activity when combined with β-lactam antibiotics targeting alternative PBPs. Specifically, durlobactam and zidebactam have been shown to inhibit PBP2 activity in many Gram-negative pathogens (11, 13). Consequently, the activity of β-lactam/β-lactamase inhibitor (BLBLI) combinations against carbapenemase-producing organisms may result from the restoration of β-lactam activity through β-lactamase inhibition and/or the synergistic bactericidal effect arising from complementary PBP inhibition by the BLI and the β-lactam.

1. EXPECTED ACTIVITY OF BLBLIs AND CEFIDEROCOL ON β-LACTAMASE-PRODUCING GRAM-NEGATIVE BACTERIA

Tables 1.1-1.3 describe the expected activity of BLBLIs and cefiderocol relevant for Enterobacterales, *Pseudomonas* spp., and *A. baumannii* complex, respectively, expressing clinically important β-lactamases. The activity must be examined by EUCAST recommended antimicrobial susceptibility testing methods.

Table 1.1. Enterobacterales – expected activity of BLBLIs and cefiderocol [1]

Antimicrobial	ESBL (CTX-M)	Chromosomal/ plasmid-mediated AmpC (CMY, DHA)	Class A carbapenemases (KPC, IMI, GES)	Class B carbapenemases (NDM, VIM, IMP)	Class D carbapenemases (OXA-48-like)
Amoxicillin-clavulanic acid[2]	+	-	-	-	-
Ampicillin-sulbactam[2]	+	-	-	-	-
Aztreonam-avibactam	+	+	+	+[3]	+
Cefepime-enmetazobactam	+	+[4]	-	-	+[4]
Cefepime-zidebactam	+	+	+	+[5]	+[5]
Cefiderocol	+	+	+	+/-	+
Ceftazidime-avibactam	+	+	+	-	+
Ceftolozane-tazobactam	+	-	-	-	-
Imipenem-relebactam[6]	+	+	+	-	-
Meropenem-vaborbactam	+	+	+	-	-
Piperacillin-tazobactam	+	-	-	-	-

[1] +: activity to be expected; -: no activity to be expected; +/-: variable activity to be expected.
[2] Relevant for *E. coli, Klebsiella* spp. (except *K. aerogenes*), *Raoultella* spp. and *P. mirabilis*. Not relevant for AmpC-producing Enterobacterales.
[3] The activity of the combination is due to the lack of class B carbapenemase (metallo-β-lactamases – MBLs) hydrolytic activity against aztreonam and avibactam's inhibitory activity against ESBLs/AmpCs that are frequently found in class B carbapenemase-producing isolates. Avibactam has no inhibitory activity against class B carbapenemases.
[4] The activity of the combination is due to the limited OXA-48/AmpC-hydrolytic activity against cefepime and enmetazobactam's inhibitory activity against ESBLs that are frequently found in OXA-48-like-producing isolates (7, 10).
[5] The activity of the combination is mainly due to the synergistic bactericidal effect arising from complementary PBP inhibition by zidebactam and cefepime, rather than restoration of cefepime activity through β-lactamase inhibition. Direct inhibitory activity of zidebactam against class B/OXA-48-like carbapenemases is not expected.
[6] BLBLI relevant for Enterobacterales except *Morganellaceae* which has reduced susceptibility to imipenem.

Table 1.2. *Pseudomonas* spp. – expected activity of BLBLIs and cefiderocol[1]

Antimicrobial resistance in *Pseudomonas aeruginosa* is driven by a combination of chromosomal mutations, transferable resistance determinants, and the global spread of multidrug-resistant (MDR) and extensively drug-resistant (XDR) epidemic lineages (17). The remarkable ability of *P. aeruginosa* to develop mutation-driven resistance against newer β-lactams, β-lactamase inhibitors, and siderophore-conjugated antibiotics (e.g., cefiderocol) introduces greater uncertainty in the expected activity profile of β-lactam/β-lactamase inhibitor (BLBLI) combinations compared to Enterobacterales. This uncertainty is particularly associated with mutations affecting the chromosomal AmpC β-lactamase, prevalent OXA-2/10 β-lactamases, penicillin-binding protein 3 (PBP3), the MexAB-OprM efflux pump, and iron transporters (17).

Antimicrobial	ESBL (CTX-M, GES, PER)	Chromosomal AmpC	Class A carbapenemases (KPC, GES-variants)	Class B carbapenemases (NDM, VIM, IMP)	Class D[2] carbapenemases (OXA-48-like, OXA-23-like, OXA-58-like)
Aztreonam-avibactam[3]	(+)	(+)	(+)	(+)	(+)
Cefepime-zidebactam	+	+	+	+[4]	+[4]
Cefiderocol	+[5]	+	+	+/-	+
Ceftazidime-avibactam	+	+	+	-	-
Ceftolozane-tazobactam	+	+[6]	-	-	-
Imipenem-relebactam	+	+	+/-[7]	-	-
Meropenem-vaborbactam	+	+	+/-[7]	-	-
Piperacillin-tazobactam	+	-	-	-	-

[1] +: activity to be expected; -: no activity to be expected; +/-: variable activity to be expected; (+): potential activity in combination therapy
[2] Class D carbapenemases are rare in *Pseudomonas* spp. and the clinical efficacy of relevant BLBLIs is uncertain (15).
[3] EUCAST has no clinical breakpoints for aztreonam-avibactam against *Pseudomonas* spp. due to high MICs compared to the potential exposure.
[4] The activity of the combination is mainly due to the synergistic bactericidal effect arising from complementary PBP inhibition by zidebactam and cefepime, rather than restoration of cefepime activity through β-lactamase inhibition. Direct inhibitory activity of zidebactam against class B/OXA-48 carbapenemases is not expected.
[5] Cefiderocol activity can be compromised by the presence of SHV- (SHV-12), PER-, VEB-, and BEL-like β-lactamases (8).
[6] The activity of the combination is mainly due to the lack of AmpC-hydrolytic activity against ceftolozane and stability against mutation-driven resistance (17).
[7] Recent *in vitro* studies have shown that relebactam and vaborbactam resulted in poor augmentation of the partner carbapenem activity, imipenem and meropenem, respectively, in class A carbapenemase-producing *P. aeruginosa* (9, 17).

Table 1.3. *A. baumannii* complex – expected activity of BLBLIs and cefiderocol[1]

Cephalosporins and monobactams, even when combined with β-lactamase inhibitors (BLIs), except cefiderocol and cefepime-zidebactam, are not deemed clinically relevant due to insufficient evidence supporting their efficacy in treating infections caused by the *A. baumannii* complex.

Antimicrobial	Class A carbapenemases (KPC)[2]	Class B carbapenemases (NDM, VIM, IMP)	Class D carbapenemases (OXA-23-like, OXA-24/40-like, OXA-58-like)
Cefepime-zidebactam	Uncertain		
Cefiderocol[3]	(+)	+/-	+
Imipenem-relebactam	(+)	-	-
Meropenem-vaborbactam	(+)	-	-
Sulbactam-durlobactam	(+)	-	+

[1] +: activity to be expected; -: no activity to be expected; +/-: variable activity to be expected; (+): potential activity in combination therapy.
[2] Class A carbapenemases are rare in *A. baumannii* complex and the clinical efficacy of relevant BLBLIs is uncertain (14).
[3] EUCAST has not established clinical breakpoints (only a note) for cefiderocol against the *A. baumannii* complex. The EUCAST note reads: *Isolates with MIC values ≤0.5 mg/L (zone diameter ≥21 mm) are mostly devoid of resistance mechanisms and are likely to be a target for treatment with this agent. Isolates with MICs 1-2 mg/L have some acquired resistance mechanisms. Little clinical data exists regarding clinical outcome for these isolates; however, they may still be a target for treatment with this agent if there are limited treatment options. Isolates with MIC values >2 mg/L (zone diameter <17 mm) have acquired resistance mechanisms and are likely to be resistant to this agent.*

2. EXPECTED ACTIVITY OF β-LACTAMASE INHIBITORS (BLIs) ON CLINICALLY RELEVANT β-LACTAMASES

Several novel BLIs have recently completed clinical trials in various BL-combinations, but only zidebactam has an ongoing regulatory application process in combination with cefepime (12,16). Some of the new BLIs (durlobactam and zidebactam) exhibit intrinsic antibacterial activity due to PBP-2 inhibition (6, 11, 13). Durlobactam has also a unique class D carbapenemase inhibitor activity including the common OXA-carbapenemases (OXA-23-/24-/58-like) in *A. baumannii* (11).

Note that the activity of inhibitors may vary within and between variants in each β-lactamase group.

Table 2. Expected inhibitor activity of BLIs on clinically relevant β-lactamases[1]

β-lactamase inhibitor	ESBL (CTX-M)	Chromosomal/ plasmid-mediated AmpC (CMY, DHA)	Class A carbapenemases (KPC, IMI)	Class B carbapenemases (NDM, VIM, IMP)	Class D carbapenemases[2] (OXA-48, OXA-181, OXA-244)	Class D carbapenemases[3] (OXA-23-/24-/58-like)
Avibactam	+	+	+	-[4]	+	(+)
Clavulanic acid	+	-	-	-	-	-
Durlobactam	+	+	+	-	+	+
Enmetazobactam	+	-	(+)	-	-	-
Relebactam	+	+	+	-	-	-
Sulbactam	+	-	-	-	-	-
Tazobactam	+	-	-	-	-	-
Vaborbactam	+	+	+	-	-	-
Zidebactam	+	+	+	-[5]	-[5]	-

[1] +: activity to be expected; -: no activity to be expected; (+) uncertain or insufficient activity.

[2] Class D carbapenemases mainly associated with Enterobacterales but also found occasionally in *P. aeruginosa*.

[3] Class D carbapenemases mainly associated with *A. baumannii* complex. These are rare among Enterobacterales, except for some reports mainly in *P. mirabilis*. The activity of avibactam and taniborbactam is uncertain against these β-lactamases.

[4] Activity is to be expected against MBL-producing Enterobacterales in combination with aztreonam, as aztreonam is not a target for MBL.

[5] Activity is to be expected against Enterobacterales and *P. aeruginosa* in combination with cefepime due to the synergistic bactericidal effect arising from complementary PBP inhibition by zidebactam and cefepime.

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