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In Vitro Activities of Ceftazidime-Avibactam, Aztreonam-Avibactam, and a Panel of Older and Contemporary Antimicrobial Agents against Carbapenemase-Producing Gram-Negative Bacilli

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Among 177 carbapenemase-producing Gram-negative bacilli (108 KPC, 32 NDM, 11 IMP, 8 OXA-48, 4 OXA-181, 2 OXA-232, 5 IMI, 4 VIM, and 3 SME producers), aztreonam-avibactam was active against all isolates except two NDM producers with elevated MICs of 8/4 and 16/4 mg/liter; ceftazidime-avibactam was active against all KPC-, IMI-, SME-, and most OXA-48 group-producing isolates (93%) but not metallo-β-lactamase producers. Among older and contemporary antimicrobials, the most active were colistin, tigecycline, and fosfomycin, with overall susceptibilities of 88%, 79%, and 78%, respectively.

The recent emergence and global dissemination of carbapenemase-producing Gram-negative bacilli (CP-GNB) pose a significant therapeutic challenge. Avibactam is a diazabicyclooctane non-β-lactam β-lactamase inhibitor with broad activity against Ambler class A and C β-lactamases and certain class D β-lactamases by covalent acylation of the β-lactamase active site serine residue. It restores susceptibility of *Enterobacteriaceae* harboring extended-spectrum β-lactamases (ESBLs), AmpC cephalosporinases, and class A carbapenemases to ceftazidime or ceftaroline (1). *In vitro* studies of avibactam in combination with aztreonam have also demonstrated activity against *Enterobacteriaceae* harboring NDM (a class B metallo-β-lactamase); however, there are scant data for the other less commonly encountered carbapenemases (2–4).

The aim of this study was to examine the activities of ceftazidime and aztreonam with and without avibactam against a large, contemporary, international collection of CP-GNB with diverse resistance mechanisms, with MICs determined using agar dilution as recommended by the Clinical and Laboratory Standards Institute (CLSI) (5, 6). A secondary aim was to evaluate the activity of antimicrobials commonly used to treat CP-GNB infections, including the "legacy antibiotics" colistin, amdinocillin (mecillinam), and fosfomycin. A total of 177 CP-GNB were studied (Table 1), comprising 122 and 53 clinical isolates from the United States and Singapore, respectively, and 2 NCTC (National Collection of Type Cultures, United Kingdom) reference isolates. These consisted of 172 *Enterobacteriaceae* isolates (107 KPC, 32 NDM, 8 OXA-48, 4 OXA-181, 2 OXA-232, 5 IMI, 3 SME, and 11 IMP producers) and 5 *Pseudomonas aeruginosa* isolates (4 VIM producers and 1 KPC producer). Genotypic characterization was performed using PCR/sequencing as previously described (7–15). All CP-GNB isolates tested positive by the CarbaNP test (16), except for one isolate each of OXA-181 and OXA-232, which were CarbaNP negative, and one OXA-48-producing isolate, which was CarbaNP indeterminate. In addition, as a control/comparator group, we studied 29 *Enterobacteriaceae* (11 *Klebsiella pneumoniae* and 18 *Escherichia coli* isolates), including 18 ESBL producers (10 with porin loss), 6 plasmid-mediated AmpC producers (1 with porin loss and another coproducing an ESBL), and 5 derepressed AmpC mutants (2 with porin loss).

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Antimicrobial susceptibility testing was performed by agar dilution for ceftazidime and aztreonam (with or without avibactam at a fixed concentration of 4 mg/liter), cefepime, meropenem, piperacillin-tazobactam, levofloxacin, ciprofloxacin, colistin, gentamicin, tobramycin, amikacin, and, for non-*Pseudomonas aeruginosa* isolates, ceftriaxone, ertapenem, fosfomycin, amdinocillin, nitrofurantoin, and trimethoprim-sulfamethoxazole (6). Due to intrinsic resistance, nitrofurantoin and colistin were not evaluated against *Serratia*, *Proteus*, or *Providencia* species. Tigecycline MICs were determined by gradient diffusion (bioMérieux, France, or Liofiochem, Italy) on cation-adjusted Mueller-Hinton agar (BBL, Becton, Dickinson and Company, Franklin Lakes, NJ) for all isolates, except those with intrinsic resistance (*P. aeruginosa*, *Proteus* species, and *Providencia* species). CLSI interpretive breakpoints were applied with the following exceptions: aztreonam-avibactam activity was extrapolated from the aztreonam FDA breakpoints (≤4 mg/liter or ≤8 mg/liter for *Enterobacteriaceae* and *P. aeruginosa* isolates, respectively) as there are currently no interpretive criteria. FDA breakpoints were used for ceftazidime-avibactam (susceptible, ≤8/4 mg/liter) and tigecycline (sus-

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TABLE 1 CP-GNB isolates studied

Origin of CP-GNB isolates	Ambler class	Resistance mechanism (*n*)	Species (*n*)
Singapore	A	IMI-1 (4)	*Enterobacter cloacae* complex (4)
		IMI (unspecified) (1)	*E. cloacae* complex (1)
	B	NDM-1 (19)	*Klebsiella pneumoniae* complex (9), *Escherichia coli* (5), *E. cloacae* complex (4), *Citrobacter sedlakii* (1)
		NDM-7 (1)	*E. coli* (1)
		NDM (unspecified) (6)	*K. pneumoniae* complex (4), *E. cloacae* complex (1), *Citrobacter freundii* (1)
		IMP-1 (7)	*K. pneumoniae* complex (4), *E. cloacae* complex (2), *Citrobacter freundii* (1)
		IMP-4 (1)	*K. pneumoniae* complex (1)
		IMP (unspecified) (3)	*E. cloacae* complex (2), *E. coli* (1)
	D	OXA-48 (5)	*K. pneumoniae* complex (3), *E. coli* (1), *Citrobacter koseri* (1)
		OXA-181[a] (4)	*K. pneumoniae* complex (4)
		OXA-232[a] (2)	*K. pneumoniae* complex (2)
U.S.	A	KPC, unspecified (108)	*K. pneumoniae* complex (84), *E. cloacae* (6), *E. coli* (5), *Enterobacter aerogenes* (3), *C. freundii* (2), *C. koseri* (2), *Proteus stuartii* (2), *Serratia marcescens* (2), *Proteus mirabilis* (1), *Pseudomonas aeruginosa* (1)
		SME (unspecified) (3)	*S. marcescens* (3)
	B	NDM-1 (1)	*E. coli* (1)
		NDM-7 (2)	*E. coli* (1), *K. pneumoniae* complex (1)
		NDM (unspecified) (2)	*K. pneumoniae* complex (2)
		VIM-2 (4)	*P. aeruginosa* (4)
	D	OXA-48 (2)	*K. pneumoniae* complex (2)
Reference NCTC[b] isolates	B	NDM-1 (NCTC 13443) (1)	*K. pneumoniae* complex (1)
	D	OXA-48 (NCTC 13442) (1)	*K. pneumoniae* complex (1)

[a] OXA-181 and OXA-232 are part of the OXA-48 group.
[b] NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, United Kingdom.

ceptible, ≤2 mg/liter), and EUCAST breakpoints for colistin were used for *Enterobacteriaceae* (susceptible, ≤2 mg/liter). Finally, susceptibility to amdinocillin (MIC of ≤8 mg/liter) for non-*E. coli Enterobacteriaceae* was extrapolated from the EUCAST urinary breakpoint for *E. coli*. American Type Culture Collection (ATCC) strains *E. coli* 25922 and 35218, *P. aeruginosa* 27853, *Staphylococcus aureus* 29213, and *K. pneumoniae* 700603 were used as quality control (QC) organisms.

Aztreonam-avibactam was highly active against CP-GNB of all resistance types tested, all with MICs of ≤4/4 mg/liter (*Enterobacteriaceae*) and ≤8/4 mg/liter (*P. aeruginosa*), except for 2 NDM-positive *E. coli* isolates from Singapore: these were NDM-1 and NDM-7 positive, with MICs of 8/4 and 16/4 mg/liter, respectively. Ceftazidime-avibactam was active against all KPC-, IMI-, and SME-producing isolates and the majority of OXA-48 group CP-GNB (93%), but not against class B CP-GNB, which is not unexpected given the mechanism of action of avibactam (Table 2). In comparison, the overall susceptibilities of CP-GNB to the other antimicrobials tested were as follows: colistin, 88%; tigecycline, 79%; fosfomycin, 78%; amikacin, 51%; gentamicin, 48%; tobramycin, 15%; trimethoprim-sulfamethoxazole, 23%; nitrofurantoin, 16%; amdinocillin, 11%; levofloxacin, 17%; and ciprofloxacin, 11%. Colistin, which is often resorted to for treatment of serious CP-GNB infections, had only 91% and 87% susceptibilities for NDM- and KPC-positive isolates, respectively (Table 3). All ESBL- and AmpC-producing GNB tested were susceptible to aztreonam-avibactam and ceftazidime-avibactam, except for one ESBL (SHV)- and plasmid-mediated AmpC (DHA)-positive *E. coli* isolate with a MIC of 8/4 mg/liter to aztreonam-avibactam; the ceftazidime-avibactam MIC was 2/4 mg/liter.

Our findings are consistent with the reported and expected activities of aztreonam-avibactam and ceftazidime-avibactam, except for a few isolates with elevated MICs to aztreonam-avibactam. While breakpoints have not been established for aztreonam-avibactam, a single carbapenemase-negative, but SHV- and DHA-positive, *E. coli* isolate and one NDM-positive isolate had an aztreonam-avibactam MIC of 8/4 mg/liter, and a single NDM-positive isolate had an aztreonam-avibactam MIC of 16/4 mg/liter. Aztreonam has a specific affinity to penicillin-binding protein-3 (PBP3); we postulate that in the isolates with elevated aztreonam-avibactam MICs, there may have been altered PBP3 affinity, as has been recently described in NDM-positive *E. coli* isolates (17). Resistance to avibactam may also result from changes in its β-lactamase binding site—e.g., from single amino acid substitutions or Ω-loop mutations in coproduced class A and C β-lactamases (18–20). Also, porin loss and overexpression of efflux pumps may be contributory, although we did not assess our isolates for these. As β-lactam–avibactam combinations are intro-

TABLE 2 Susceptibility of CP-GNB to ceftazidime and aztreonam with or without avibactam

CP-GNB resistance genotype (*n*)	Antibiotic[a]	No. (cumulative %) of isolates inhibited at ceftazidime or aztreonam concn (mg/liter) shown with or without 4 mg/liter avibactam															MIC(μg/ml)[b]		
		≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	>512	50%	90%	% S
KPC (108)[c]	ATM			1 (1)				1 (2)		1 (3)	2 (5)	7 (11)	11 (21)	17 (37)	28 (63)	40 (100)	512	>512	2
	AZA	20 (19)	35 (51)	38 (86)	9 (94)	3 (97)	2 (99)		1 (100)								0.12/4	0.5/4	100
	CAZ			1 (1)					3 (4)	3 (7)	6 (12)	12 (23)	23 (44)	26 (69)	31 (97)	3 (100)	256	512	2
	CZA	7 (6)	3 (9)	9 (18)	22 (38)	44 (79)	15 (92)	5 (97)	3 (100)								1/4	2/4	100
NDM (32)	ATM		2 (6)		1 (9)				1 (13)		2 (19)	6 (38)	1 (41)	7 (63)	3 (72)	9 (100)	256	>512	9
	AZA	6 (19)	11 (53)	4 (66)	4 (78)	4 (91)		1 (94)	1 (97)	1 (100)							0.12/4	1/4	94
	CAZ															32 (100)	>512	>512	0
	CZA															32 (100)	>512/4	>512/4	0
OXA-48 group (14)	ATM	1 (7)	1 (14)								1 (21)	3 (43)	4 (71)	1 (79)		3 (100)	128	>512	14
	AZA	4 (29)	4 (57)	2 (71)	2 (86)	2 (100)											0.12/4	1/4	100
	CAZ		1 (7)	0 (7)	1 (14)						5 (50)	1 (57)	2 (71)	1 (79)	2 (93)	1 (100)	32	512	14
	CZA	1 (7)	1 (14)	2 (28)	3 (50)	5 (86)		1 (93)								1 (100)	0.5/4	4/4	93
IMP (11)	ATM	1 (9)		1 (18)							1 (27)	1 (36)	2 (55)	2 (72.7)	1 (82)	2 (100)	128	>512	18
	AZA	2 (18)		5 (46)	2 (82)	2 (100)											0.25/4	1/4	100
	CAZ												2 (18)	3 (46)	3 (73)	3 (100)	>512	>512	0
	CZA					1 (9)						1 (18)	3 (46)	2 (64)		4 (100)	256/4	>512/4	9
IMI (5)	ATM				1 (25)	4 (100)													100
	AZA	3 (60)	2 (100)																100
	CAZ		1 (20)	1 (40)	1 (60)	1 (80)			1 (100)										80
	CZA		1 (20)	4 (100)															100
VIM (4)[c]	ATM								5 (100)										100
	AZA							1 (25)	4 (100)										100
	CAZ										2 (50)	2 (100)							0
	CZA										2 (50)	2 (100)							0
SME (3)	ATM									2 (67)			1 (100)						0
	AZA		1 (33)	1 (67)	1 (100)														100
	CAZ	1 (33)					1 (67)					1 (100)							67
	CZA	1 (33)			1 (67)		1 (100)												100

[a] ATM, aztreonam; AZA, aztreonam-avibactam; CAZ, ceftazidime; CZA, ceftazidime-avibactam. Because no CLSI breakpoints exist for AZA, *Enterobacteriaceae* and *Pseudomonas aeruginosa* isolates with MICs of ≤4 mg/liter and ≤8 mg/liter, respectively, to the aztreonam component were considered susceptible (S). For CAZ and CZA, FDA breakpoints were used: MICs of ≤8 mg/liter and ≤8/4 mg/liter, respectively, were considered susceptible.

[b] 50% and 90%, MIC_{50} and MIC_{90}, respectively.

[c] All KPC-producing CP-GNB were *Enterobacteriaceae* with MICs to AZA of ≤4/4 mg/liter, except for one *P. aeruginosa* isolate with a MIC of 8/4 mg/liter (susceptible). All VIM CP-GNB were *P. aeruginosa*.

TABLE 3 Comparative susceptibilities of carbapenemase-producing Gram-negative bacilli to various antimicrobials

Antimicrobial[a]	% susceptibility with resistance mechanism shown (total no. of isolates)						
	KPC (108)	NDM (32)	OXA-48 group (14)	IMP (11)	IMI (5)	VIM (4)	SME (3)
β-Lactams and β-lactam–β-lactamase inhibitor combinations							
ATM	2	9	14	18	100	100	0
AZA	100	94	100	100	100	100	100
CAZ	2	0	14	0	80	0	67
CZA	100	0	93	9	100	0	100
CRO[b]	1	0	14	0	100		67
FEP[c]	2	0	14	0	80	0	33
ETP[b]	2	0	7	0	0		0
MEM	5	0	43	36	20	0	0
TZP	1	0	0	27	100	0	67
MEC[b]	0	16	43	18	100		33
Aminoglycosides							
GEN	63	16	36	0	80	0	100
TOB	9	9	36	9	80	0	100
AMK	51	41	43	82	100	0	100
Fluoroquinolones							
LVX	11	13	14	36	100	0	100
CIP	10	0	7	18	80	0	67
Other antimicrobial agents							
SXT[b]	21	9	14	45	80		100
NIT[b]	14	25	7	27	20		
FOF[b]	78	78	71	73	100		100
CST[d]	87	91	100	91	40	75	
TGC[b,d]	81	69	93	55	100		100

[a] Antimicrobial abbreviations: ATM, aztreonam; AZA, aztreonam-avibactam; CAZ, ceftazidime; CZA, ceftazidime-avibactam; CRO, ceftriaxone; FEP, cefepime; ETP, ertapenem; MEM, meropenem; TZP, piperacillin-tazobactam; MEC, amdinocillin; GEN, gentamicin; TOB, tobramycin; AMK, amikacin; LVX, levofloxacin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; NIT, nitrofurantoin; FOF, fosfomycin; CST, colistin; TGC, tigecycline.

[b] Excluding *Pseudomonas aeruginosa* isolates for the antimicrobial agents with no inherent antipseudomonal activity and/or without CLSI breakpoints (CRO, ETP, MEC, SXT, NIT, FOF, and TGC). Additionally, for NIT, *Proteus*, *Providencia*, and *Serratia* species were excluded due to intrinsic resistance. An amdinocillin (MEC) breakpoint of ≤8 mg/liter was applied to *Enterobacteriaceae*.

[c] Susceptibility to FEP was defined as ≤2 mg/liter, per CLSI M100-S25 (6). Susceptible dose-dependent isolates (MICs of 4 to 8 mg/liter) were not included in this category.

[d] EUCAST breakpoints were used for CST for *Enterobacteriaceae* and FDA breakpoints for TGC. Due to intrinsic resistance, for CST, *Proteus*, *Providencia*, and *Serratia* species were excluded, and for TGC, *Pseudomonas*, *Proteus*, and *Providencia* species were excluded.

duced into clinical use, it will be important to monitor for and further characterize nonsusceptible isolates and correlate clinical outcomes with the various resistance genotypes.

Interestingly, among the β-lactams, amdinocillin, an old extended-spectrum penicillin antibiotic, demonstrated the most activity against NDM (16%) and OXA-48 (43%) CP-GNB, after ceftazidime-avibactam (0% and 93% activities against NDM and the OXA-48 group, respectively) and aztreonam-avibactam (94% and 100% activities against NDM and the OXA-48 group, respectively). The interpretive criteria used, however, were for urinary isolates as this agent is currently mostly used in an oral formulation (pivmecillinam) in Nordic countries for the treatment of urinary tract infections. Although an inoculum effect has been demonstrated (21), amdinocillin achieves high urinary concentrations and is relatively stable to ESBLs and AmpCs. An intravenous formulation is available in Denmark (22). A previous study found that amdinocillin with avibactam showed a modest reduction in MICs in ESBL and AmpC producers (23). While we did not specifically study this combination, further studies of avibactam in combination with older penicillins such as amdinocillin are warranted.

Overall, we found that aztreonam-avibactam showed excellent activity against CP-GNB from all Ambler classes tested (A, B, and D). The ceftazidime-avibactam combination showed activity against class A CP-GNB and the majority of OXA-48 group CP-GNB tested, but not against class B CP-GNB. The evaluated β-lactam–avibactam combinations may provide an important advance in the treatment of CP-GNB infections.

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REFERENCES

1. **Drawz SM, Papp-Wallace KM, Bonomo RA.** 2014. New beta-lactamase inhibitors: a therapeutic renaissance in an MDR world. Antimicrob Agents Chemother **58:**1835–1846. http://dx.doi.org/10.1128/AAC.00826-13.
2. **Castanheira M, Farrell SE, Krause KM, Jones RN, Sader HS.** 2014. Contemporary diversity of beta-lactamases among *Enterobacteriaceae* in

the nine U.S. census regions and ceftazidime-avibactam activity tested against isolates producing the most prevalent beta-lactamase groups. Antimicrob Agents Chemother **58:**833–838. http://dx.doi.org/10.1128/AAC.01896-13.

3. **Livermore DM, Mushtaq S, Warner M, Zhang J, Maharjan S, Doumith M, Woodford N.** 2011. Activities of NXL104 combinations with ceftazidime and aztreonam against carbapenemase-producing *Enterobacteriaceae*. Antimicrob Agents Chemotherapy **55:**390–394. http://dx.doi.org/10.1128/AAC.00756-10.
4. **Wang X, Zhang F, Zhao C, Wang Z, Nichols WW, Testa R, Li H, Chen H, He W, Wang Q, Wang H.** 2014. In vitro activities of ceftazidime-avibactam and aztreonam-avibactam against 372 Gram-negative bacilli collected in 2011 and 2012 from 11 teaching hospitals in China. Antimicrob Agents Chemother **58:**1774–1778. http://dx.doi.org/10.1128/AAC.02123-13.
5. **CLSI.** 2015. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard—10th ed. CLSI document M07-A10. Clinical and Laboratory Standards Institute, Wayne, PA.
6. **CLSI.** 2015. Performance standards for antimicrobial susceptibility testing; 25th informational supplement. CLSI document M100-S25. Clinical and Laboratory Standards Institute, Wayne, PA.
7. **Balm MN, La MV, Krishnan P, Jureen R, Lin RT, Teo JW.** 2013. Emergence of *Klebsiella pneumoniae* co-producing NDM-type and OXA-181 carbapenemases. Clin Microbiol Infect **19:**E421–E423. http://dx.doi.org/10.1111/1469-0691.12247.
8. **Cunningham SA, Noorie T, Meunier D, Woodford N, Patel R.** 2013. Rapid and simultaneous detection of genes encoding *Klebsiella pneumoniae* carbapenemase (bla_{KPC}) and New Delhi metallo-beta-lactamase (bla_{NDM}) in Gram-negative bacilli. J Clin Microbiol **51:**1269–1271. http://dx.doi.org/10.1128/JCM.03062-12.
9. **Cunningham SA, Johnston B, Vasoo S, Johnson JR, Patel R.** 2014. Evaluation of Checkpoints Check-MDR CT103 PCR-microarray kit for detection and classification of ESBL, AmpC and carbapenemase genes, abstr C-149. Abstr 54th Intersci Conf Antimicrob Agents Chemother.
10. **Koh TH, Cao D, Shan QY, Bacon A, Hsu LY, Ooi EE.** 2013. Acquired carbapenemases in *Enterobacteriaceae* in Singapore, 1996-2012. Pathology **45:**600–603. http://dx.doi.org/10.1097/PAT.0b013e3283650b1e.
11. **Poirel L, Heritier C, Tolun V, Nordmann P.** 2004. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. Antimicrob Agents Chemother **48:**15–22. http://dx.doi.org/10.1128/AAC.48.1.15-22.2004.
12. **Poirel L, Naas T, Nicolas D, Collet L, Bellais S, Cavallo JD, Nordmann P.** 2000. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-beta-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. Antimicrob Agents Chemother **44:**891–897. http://dx.doi.org/10.1128/AAC.44.4.891-897.2000.
13. **Teo J, Ngan G, Balm M, Jureen R, Krishnan P, Lin R.** 2012. Molecular characterization of NDM-1 producing *Enterobacteriaceae* isolates in Singapore hospitals. West Pac Surveill Response J **3:**19–24. http://dx.doi.org/10.5365/wpsar.2011.2.4.010.
14. **Teo JW, Kurup A, Lin RT, Hsien KT.** 2013. Emergence of clinical *Klebsiella pneumoniae* producing OXA-232 carbapenemase in Singapore. New Microbes New Infect **1:**13–15. http://dx.doi.org/10.1002/2052-2975.4.
15. **Teo JW, La MV, Krishnan P, Ang B, Jureen R, Lin RT.** 2013. *Enterobacter cloacae* producing an uncommon class A carbapenemase, IMI-1, from Singapore. J Med Microbiol **62:**1086–1088. http://dx.doi.org/10.1099/jmm.0.053363-0.
16. **Vasoo S, Cunningham SA, Kohner PC, Simner PJ, Mandrekar JN, Lolans K, Hayden MK, Patel R.** 2013. Comparison of a novel, rapid chromogenic biochemical assay, the Carba NP test, with the modified Hodge test for detection of carbapenemase-producing Gram-negative bacilli. J Clin Microbiol **51:**3097–3101. http://dx.doi.org/10.1128/JCM.00965-13.
17. **Alm RA, Johnstone MR, Lahiri SD.** 2015. Characterization of *Escherichia coli* NDM isolates with decreased susceptibility to aztreonam/avibactam: role of a novel insertion in PBP3. J Antimicrob Chemother **70:**1420–1428. http://dx.doi.org/10.1093/jac/dku568.
18. **Winkler ML, Papp-Wallace KM, Taracila MA, Bonomo RA.** 2015. Avibactam and inhibitor-resistant SHV β-lactamases. Antimicrob Agents Chemother **59:**3700–3709. http://dx.doi.org/10.1128/AAC.04405-14.
19. **Lahiri SD, Johnstone MR, Ross PL, McLaughlin RE, Olivier NB, Alm RA.** 2014. Avibactam and class C β-lactamases: mechanism of inhibition, conservation of the binding pocket, and implications for resistance. Antimicrob Agents Chemother **58:**5704–5713. http://dx.doi.org/10.1128/AAC.03057-14.
20. **Lahiri SD, Walkup GK, Whiteaker JD, Palmer T, McCormack K, Tanudra MA, Nash TJ, Thresher J, Johnstone MR, Hajec L, Livchak S, McLaughlin RE, Alm RA.** 2015. Selection and molecular characterization of ceftazidime/avibactam-resistant mutants in *Pseudomonas aeruginosa* strains containing derepressed AmpC. J Antimicrob Chemother **70:**1650–1658. http://dx.doi.org/10.1093/jac/dkv004.
21. **Thomas K, Weinbren MJ, Warner M, Woodford N, Livermore D.** 2006. Activity of mecillinam against ESBL producers in vitro. J Antimicrob Chemother **57:**367–368. http://dx.doi.org/10.1093/jac/dki451.
22. **Jensen KS, Henriksen AS, Frimodt-Møller N.** 2009. Estimation of MIC breakpoints for various intravenous dosage regimens of mecillinam, abstr P1458. Abstr 19th Eur Congr Clin Microbiol Infect Dis.
23. **Lagacé-Wiens PR, Tailor F, Simner P, DeCorby M, Karlowsky JA, Walkty A, Hoban DJ, Zhanel GG.** 2011. Activity of NXL104 in combination with beta-lactams against genetically characterized *Escherichia coli* and *Klebsiella pneumoniae* isolates producing class A extended-spectrum beta-lactamases and class C beta-lactamases. Antimicrob Agents Chemother **55:**2434–2437. http://dx.doi.org/10.1128/AAC.01722-10.