AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

Association between the Presence of Aminoglycoside-Modifying Enzymes and *In Vitro* Activity of Gentamicin, Tobramycin, Amikacin, and Plazomicin against *Klebsiella pneumoniae* Carbapenemase- and Extended-Spectrum-β-Lactamase-Producing *Enterobacter* Species

Ghady Haidar,[a] Ammar Alkroud,[b] Shaoji Cheng,[b] Travis M. Churilla,[b] Bryce M. Churilla,[b] Ryan K. Shields,[b,c] Yohei Doi,[a,b] Cornelius J. Clancy,[b,c,d] M. Hong Nguyen[a,b,c]

University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA[a]; University of Pittsburgh, Pittsburgh, Pennsylvania, USA[b]; XDR Pathogen Laboratory, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA[c]; Pittsburgh VA Healthcare System, Pittsburgh, Pennsylvania, USA[d]

We compared the *in vitro* activities of gentamicin (GEN), tobramycin (TOB), amikacin (AMK), and plazomicin (PLZ) against 13 *Enterobacter* isolates possessing both *Klebsiella pneumoniae* carbapenemase and extended-spectrum β-lactamase (KPC+/ESBL+) with activity against 8 KPC+/ESBL−, 6 KPC−/ESBL+, and 38 KPC−/ESBL− isolates. The rates of resistance to GEN and TOB were higher for KPC+/ESBL+ (100% for both) than for KPC+/ESBL− (25% and 38%, respectively), KPC−/ESBL+ (50% and 17%, respectively), and KPC−/ESBL− (0% and 3%, respectively) isolates. KPC+/ESBL+ isolates were more likely than others to possess an aminoglycoside-modifying enzyme (AME) (100% versus 38%, 67%, and 5%; $P = 0.007$, 0.06, and <0.0001, respectively) or multiple AMEs (100% versus 13%, 33%, and 0%, respectively; $P < 0.01$ for all). KPC+/ESBL+ isolates also had a greater number of AMEs (mean of 4.6 versus 1.5, 0.9, and 0.05, respectively; $P < 0.01$ for all). GEN and TOB MICs were higher against isolates with >1 AME than with ≤1 AME. The presence of at least 2/3 of KPC, SHV, and TEM predicted the presence of AMEs. PLZ MICs against all isolates were ≤4 μg/ml, regardless of KPC/ESBL pattern or the presence of AMEs. In conclusion, GEN and TOB are limited as treatment options against KPC+ and ESBL+ *Enterobacter*. PLZ may represent a valuable addition to the antimicrobial armamentarium. A full understanding of AMEs and other aminoglycoside resistance mechanisms will allow clinicians to incorporate PLZ rationally into treatment regimens. The development of molecular assays that accurately and rapidly predict antimicrobial responses among KPC- and ESBL-producing *Enterobacter* spp. should be a top research priority.

Enterobacter aerogenes and *Enterobacter cloacae* are important nosocomial pathogens (1), which collectively constitute the 8th most common cause of health care-associated infections (2). *Enterobacter* spp. are well recognized for their capacity to develop acquired β-lactam resistance via inducible or derepressed production of AmpC β-lactamases (3, 4). In addition, *Enterobacter* isolates often manifest multiple antibiotic resistance mechanisms, including coproduction of extended-spectrum β-lactamases (ESBLs), upregulation of efflux pumps, and deficiency of outer membrane porins, which may confer multidrug resistance (MDR) phenotypes (5–8). More recently, carbapenem-resistant *Enterobacter* spp. have emerged worldwide (6, 9–15). As with other carbapenem-resistant *Enterobacteriaceae* (CRE), carbapenem resistance in *Enterobacter* spp. is most commonly conferred through production of *Klebsiella pneumoniae* carbapenemases (KPCs) or metallo-β-lactamases (MBLs) (6, 16). These resistance determinants are commonly located on plasmids that carry other genes attenuating susceptibility to multiple antibiotic classes. As a result, therapeutic options against carbapenem-resistant *Enterobacter* infections are limited, and optimal treatment regimens are yet to be established.

Aminoglycosides retain potent bactericidal activity against some, but not all, CRE (17). The most common determinants of aminoglycoside resistance are aminoglycoside-modifying enzymes (AMEs) (17–21), which are typically found on plasmids and are often present in various combinations in a given strain. Plazomicin (PLZ), a semisynthetic, next-generation aminoglycoside derived from sisomicin, is currently being evaluated in clinical trials for the treatment of complicated urinary tract infections and CRE infections (21, 22; see also registration numbers NCT01970371 and NCT02486627 at ClinicalTrials.gov). Specifically designed to evade modification by all AMEs with the exception of AAC(2′)-Ia, -Ib, and -Ic (found only on the chromosomes of *Providencia* spp.), PLZ has excellent activity *in vitro* against most CRE. PLZ resistance among CRE is due to the production of 16S rRNA methyltransferases, which are principally associated with New Delhi MBLs (NDM-1) and confer resistance to all aminoglycosides (21, 23, 24). The vast majority of CRE in the United States produce KPCs rather than NDM-1, suggesting that PLZ should be broadly active. In a previous study, we demonstrated that PLZ was highly active against KPC-producing *K. pneumoniae*

Received 20 April 2016 **Returned for modification** 22 May 2016
Accepted 8 June 2016
Accepted manuscript posted online 13 June 2016
Citation Haidar G, Alkroud A, Cheng S, Churilla TM, Churilla BM, Shields RK, Doi Y, Clancy CJ, Nguyen MH. 2016. Association between the presence of aminoglycoside-modifying enzymes and *in vitro* activity of gentamicin, tobramycin, amikacin, and plazomicin against *Klebsiella pneumoniae* carbapenemase- and extended-spectrum-β-lactamase-producing *Enterobacter* species. Antimicrob Agents Chemother 60:5208–5214. doi:10.1128/AAC.00869-16.
Address correspondence to M. Hong Nguyen, mhn5@pitt.edu.

TABLE 1 AME primers used in this study

PCR[a]	Gene	Forward primer	Reverse primer	Amplicon size (bp)
Multiplex 1	*aac(3)-II*	5′-CCCTACGAGGAGACTCTGAATG-3′	5′-CGAGAATGCCGTTTGAATCGTA-3′	442
	aac(6′)-II	5′-AAGAGTCCGTAACACCGTACAT-3′	5′-CCAGAGACTGGTCTATTCCTCG-3′	141
Multiplex 2	*aac(6′)-Ib*	5′-ACACAATACACAGCATCGTGAC-3′	5′-TGTATGGAGTGACGGACTCTTG-3′	207
	aadA1[b]	5′-ACGATCGACATTGATCTGGCTA-3′	5′-CCAAGCGATCTTCTTCTTGTCC-3′	343
Multiplex 3	*aph(3′)-VI*	5′-TAAAATTGGTCAGTCGCCATCG-3′	5′-AAAGCGCTGAAATTGGTTTTGC-3′	239
	armA	5′-GACGAATGAAAGAGTCGCAACA-3′	5′-GCTGTTTTAGCACAGGAAGCAT-3′	308
	rmtB	5′-CATAAATCCCCCAAACAGACCG-3′	5′-AATCGTACAGGGTATCCAGCTC-3′	187
Multiplex 4	*aadA2*[b]	5′-CCGGTTCCTGAACAGGATCTAT-3′	5′-CAACTGACTTGATGATCTCGCC-3′	319
	aadB[b]	5′-CGCAGGTCACATTGATACACAA-3′	5′-ATAGTCCAACTCCTCCATGACG-3′	212
Uniplex	*aac(3)-IV*	5′-GTGCAATACGAATGGCGAAAAG-3′	5′-TAGGGAACCTTTGCCATCAACT-3′	491

[a] Amplification was done in a 20-μl final volume using the following protocol: denaturation at 95°C for 30 s, followed by 30 cycles consisting of 30 s at 95°C for denaturation, 30 s at 52°C for annealing, and 60 s at 68°C for extension.
[b] Both *aadA1* and *aadA2* encode ANT(3″)-Ia, and *aadB* encodes ANT(2″)-Ia (19, 20).

isolates from two U.S. centers, including those that produced diverse AMEs (17).

In this study, we evaluated the *in vitro* activities of gentamicin (GEN), tobramycin (TOB), amikacin (AMK), and PLZ against 65 *Enterobacter* clinical isolates, including those producing KPC and ESBL. We screened isolates for the presence of eight common AMEs to identify genetic markers for diminished susceptibility to one or more aminoglycosides.

MATERIALS AND METHODS

Enterobacter isolates from unique patients at the University of Pittsburgh Medical Center (UPMC) were identified from our biorepository. We included 21 *Enterobacter* isolates that harbored KPC (12 *E. aerogenes* and 9 *E. cloacae* isolates), as determined by PCR, and 44 randomly selected KPC− isolates (12 *E. aerogenes* and 32 *E. cloacae* isolates). Isolates were subcultured at least twice on Mueller-Hinton agar before testing. GEN, TOB, and AMK were purchased from the UPMC pharmacy. PLZ was supplied by Achaogen, Inc. (South San Francisco, CA). Stock solutions of antimicrobial agents were prepared and stored at −80°C. MICs were determined by standard broth microdilution methods (25). Quality control (QC) was performed with *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. Susceptibility to GEN, TOB, and AMK was defined using Clinical and Laboratory Standards Institute (CLSI) MIC breakpoints of ≤4, ≤4, and ≤16 μg/ml, respectively (25). Isolates were considered resistant to these agents if MICs were classified as intermediate or resistant by CLSI breakpoints (≥8, ≥8, and ≥32 μg/ml, respectively). At present, susceptibility breakpoints for PLZ are not established. ESBL production was tested using a modified CLSI method with boronic acid supplementation (26). Detection of KPC and β-lactamase genes was performed using multiplex PCR (27). All isolates were examined by multiplex PCR for genes encoding the following common AMEs: AAC(6′)-Ib, AAC(6′)-II, AAC(3)-II, AAC(3)-IV, APH(3′)-Ia, APH(3′)-VI, ANT(3″)-Ia (encoded by *aadA1* and *aadA2*), and ANT(2″)-Ia (encoded by *aadB*) (19). AME primers are listed in Table 1. PCR for *aph(3′)-Ia* was performed as previously described (28). *Enterobacter* isolates with PLZ MICs of ≥4 μg/ml were screened for the presence of 16S methyltransferase genes *armA and rmtB* by PCR.

Graphics and statistical analyses were performed using Microsoft Excel (Redmond, WA) and GraphPad Instat and Prism software (La Jolla, CA). Comparisons were made by Fisher's exact test for categorical variables and the Mann-Whitney test for continuous variables. Correlations between pairs of variables were assessed by calculating Spearman's rank correlation coefficient. Significance was defined as a P value of <0.05 (two tailed).

RESULTS

We began by classifying 65 *Enterobacter* isolates into four groups differentiated by KPC and ESBL patterns (Table 2): 13 had both KPC and ESBLs (KPC+/ESBL+), 8 had KPC only (KPC+/ESBL−), 6 had ESBLs only (KPC−/ESBL+), and 38 had neither KPC nor ESBLs (KPC−/ESBL−). TEM was the most common β-lactamase (34% [22/65]), followed by SHV (31% [20/65]); 11 isolates had both TEM and SHV. CTX-M was present in 14 isolates; all but 4 CTX-M+ isolates also had at least one other β-lactamase (TEM or SHV).

***In vitro* susceptibility.** Median MICs of GEN, TOB, and AMK were higher against KPC+/ESBL+ isolates than against KPC+/ESBL−, KPC−/ESBL+, and KPC−/ESBL− isolates (Fig. 1). Likewise, GEN and TOB resistance rates were higher against the KPC+/ESBL+ group than the other groups (Table 3). Only 3% (2/65) of isolates were resistant to AMK; one was KPC+/ESBL+, and the other was KPC+/ESBL−. PLZ MICs were ≤4 μg/ml against all isolates and did not differ based on the presence of KPC or ESBLs.

Distribution of AMEs. Thirty-four percent (22/65) of the isolates possessed at least one AME. AAC(6′)-Ib, AAC(6′)-II, and ANT(3″)-Ia (encoded by *aadA2*) were the most common, found in 73% (16/22), 64% (14/22), and 64% (14/22) of the isolates possessing AMEs, respectively (Fig. 2A). Seventy-three percent (16/22) of AME+ isolates possessed more than one AME (Fig. 2B). There was a total of 10 different AME gene combinations, the most common of which was *aac(6′)-II*, *aac(6′)-Ib*, *aadA1*, *aac(3)-IV*, and *aadA2* (n = 5 isolates). Two isolates carried *aac(6′)-II*, *aac(6′)-Ib*, *aadA1*, *aadB*, and *aadA2*, and an additional two carried

TABLE 2 Classification of *Enterobacter* isolates according to presence of absence of KPC and ESBL enzymes

Organism	No. of isolates			
	KPC+/ESBL+	KPC+/ESBL−	KPC−/ESBL+	KPC−/ESBL−
E. aerogenes	6	2	5	11
E. cloacae	7	6	1	27
Total	13	8	6	38

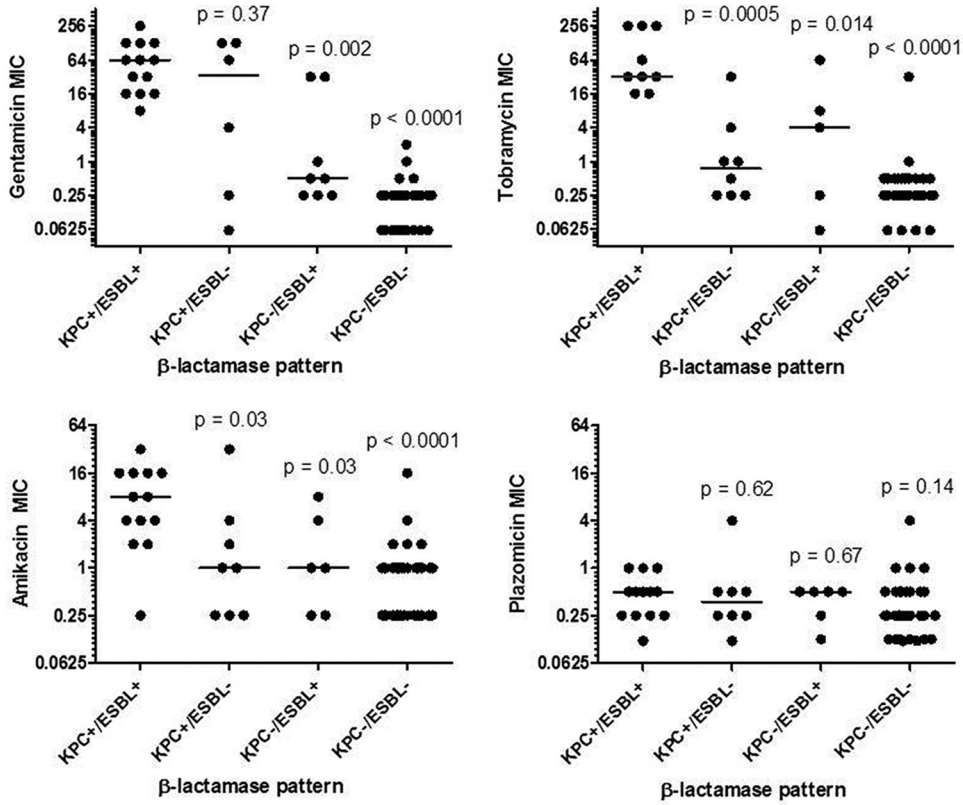

FIG 1 Aminoglycoside MICs (in micrograms per milliliter), stratified by KPC and ESBL pattern. Solid lines designate median MICs. *P* values were calculated with KPC+/ESBL+ as the reference.

aac(6′)-*II*, *aac*(3)-*II*, *aac*(6′)-*Ib*, *aadA1*, and *aadA2*. The 7 other AME gene combinations were found in 1 isolate each (data not shown). The two isolates with PLZ MICs of 4 μg/ml were negative for 16S rRNA methyltransferase genes *armA* and *rmtB*.

We next evaluated the association between the presence of KPC, ESBLs, and AMEs. KPC+/ESBL+ isolates were significantly more likely to harbor at least one AME (100% [13/13]) than KPC+/ESBL− (38% [3/8]; $P = 0.007$), KPC−/ESBL+ (67% [4/6]; $P = 0.06$), and KPC−/ESBL− isolates (5% [2/38]; $P < 0.0001$). In addition, KPC+/ESBL+ isolates harbored more AMEs than KPC+/ESBL−, KPC−/ESBL+, or KPC−/ESBL− isolates (mean numbers of AMEs: 4.6, 1.5, 0.9, and 0.05, respectively; $P < 0.05$ for all) (Fig. 3). One hundred percent (13/13) of KPC+/ESBL+ isolates had 3 or more AME genes, compared to 13% of KPC+/ESBL− isolates (1/18), 33% of KPC−/ESBL+ isolates (2/6), and 0/38 of KPC−/ESBL− isolates ($P < 0.01$ for all). When ESBL+ isolates were compared to ESBL− isolates without regard for KPC, they were found to be more likely to carry AMEs (89% [17/19]) versus 11% [5/46]; $P < 0.0001$); they also had a greater number of AMEs (mean, 3.6 [range, 0 to 6] versus 0.2 [range, 0 to 5], respectively; $P < 0.0001$). Ninety-one percent (20/22) of AME+ isolates had KPC and/or ESBLs; 59% (13/22) had both KPC and ESBLs, and 32% (7/22) had either KPC or an ESBL. Only 2 AME+ isolates had neither KPC nor an ESBL.

We then determined the association between TEM, SHV, and AMEs among KPC+ and KPC− isolates (Table 4). Of note, only 4 of 65 isolates had CTX-M without TEM and/or SHV. Two of these were also KPC+, but none possessed any AME; all four were susceptible to all aminoglycosides tested (data not shown). Thus, we focused on isolates possessing either TEM or SHV. Of the 21 KPC+ isolates, all 11 that had both TEM and SHV had AMEs (Table 4). Of the 44 KPC− isolates, all 4 with both TEM and SHV had AMEs (Table 4). Isolates that had at least 2 of the 3 β-lactamases (KPC, TEM, or SHV) were significantly more likely than

TABLE 3 Percentage of isolates resistant to gentamicin, tobramycin, and amikacin, stratified by KPC/ESBL patterns

KPC/ESBL pattern	% (no.) of isolates with gentamicin MIC of ≥8 μg/ml	*P* value[a]	% (no.) of isolates with tobramycin MIC of ≥8 μg/ml	*P* value[a]	% (no.) of isolates with amikacin MIC of ≥32 μg/ml	*P* value[a]
KPC+/ESBL+ ($n = 13$)	100 (13)		100 (13)		8 (1)	
KPC+/ESBL− ($n = 8$)	25 (2)	0.0004	38 (3)	0.008	12.5 (1)	NS (0.78)
KPC−/ESBL+ ($n = 6$)	50 (3)	0.008	17 (1)	<0.0001	0 (0)	NS (0.57)
KPC−/ESBL− ($n = 38$)	0 (0)	<0.0001	3 (1)	<0.0001	0 (0)	NS (0.096)

[a] *P* values were calculated with KPC+/ESBL+ as a reference. NS, not significant.

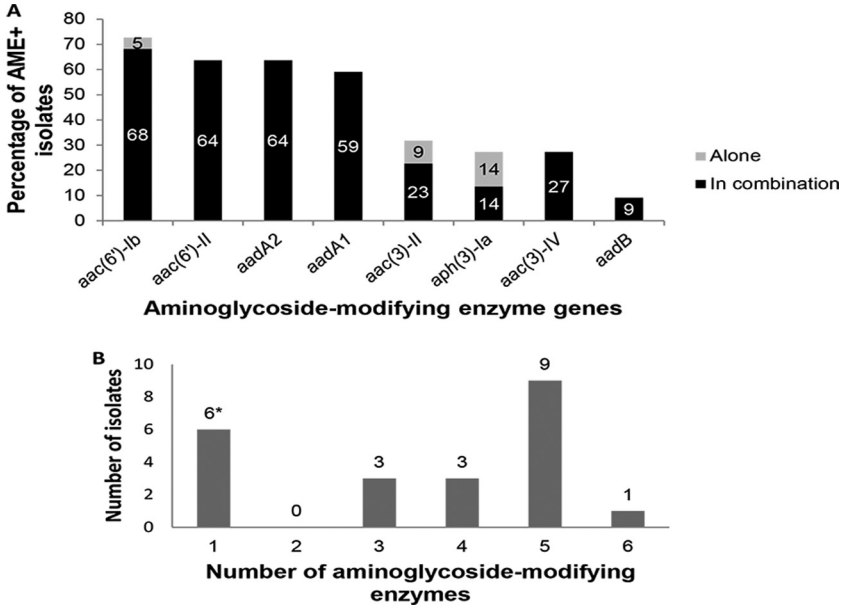

FIG 2 Description of AMEs in *Enterobacter* isolates. (A) Distribution of AMEs. AMEs that were present alone and those that are present with other AMEs are indicated by gray and black bars, respectively. (B) Number of AMEs possessed by the isolates. Seventy-three percent (16/22) of AME+ isolates possessed >1 AME; 68% (15/22) had AAC(6′)-Ib in combination with other AMEs. Twenty-seven percent (6/22) of isolates possessed only 1 AME (*): AAC(6′)-Ib ($n = 1$), APH(3′)-Ia ($n = 3$), or AAC(3)-II ($n = 2$).

other isolates to possess an AME (95% [19/20] versus 7% [3/45]; $P < 0.001$).

Aminoglycoside and PLZ MICs against *Enterobacter* spp. with different AME patterns. Median GEN, TOB, and AMK MICs were generally higher against AME+ than against AME− organisms (Fig. 4). There was a strong positive correlation between the number of AMEs and GEN and TOB MICs (Spearman $r = 0.79$ and 0.81, respectively; $P < 0.0001$) and a moderate correlation between the number of AMEs and AMK MICs ($r = 0.59$; $P < 0.0001$). There was no correlation between the number of AMEs and PLZ MICs ($r = 0.14$; $P = 0.19$).

The distribution of aminoglycoside MICs according to the number of AMEs is detailed in Fig. 4. Ninety-four percent (15/16) and 100% (16/16) of isolates with ≥3 AMEs were resistant to GEN and TOB, respectively.

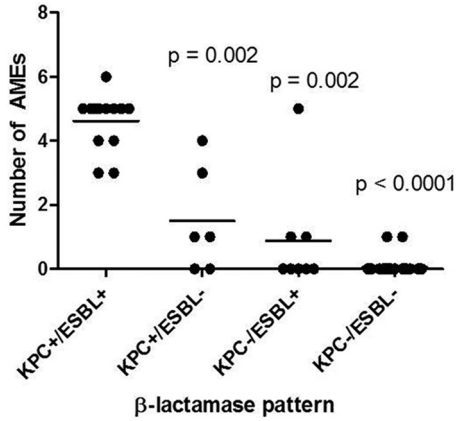

FIG 3 Association between the number of AMEs and presence or absence of KPC and ESBLs. Solid bars indicate means. *P* values were calculated with KPC+/ESBL+ as the reference.

DISCUSSION

There are several particularly noteworthy findings from this study. First, 62% (13/21) of our KPC+ *Enterobacter* isolates also possessed ESBLs, and 100% (13/13) of KPC+/ESBL+ isolates were resistant to the commonly used aminoglycosides GEN and TOB. In contrast, GEN and TOB resistance rates ranged from 0 to 33% among KPC+/ESBL−, KPC−/ESBL+, and KPC−/ESBL− isolates. Second, the diversity of AMEs was striking. Seventy-three percent (16/22) of AME+ isolates possessed multiple AMEs, and GEN and TOB MICs were significantly higher against such isolates than against isolates with ≤1 AME. Third, there was a strong association between the presence of KPC, ESBLs, and AMEs. KPC+/ESBL+ isolates were more likely than KPC+/ESBL−, KPC−/ESBL+, or KPC−/ESBL− isolates to carry one or more AMEs. Moreover, KPC+/ESBL+ isolates had a greater number of AMEs than isolates that were KPC+/ESBL−, KPC−/ESBL+, or KPC−/ESBL−. Finally, MICs of the novel aminoglycoside PLZ were low against all *Enterobacter* isolates, including those possessing AMEs. Taken together, the data establish that GEN and TOB

TABLE 4 Percentages of KPC+ isolates and KPC− isolates with AMEs, stratified by presence or absence of TEM and SHV

Isolate type	% (no./total) of isolates AME+			
	TEM+/SHV+ ($n = 15$)	TEM+/SHV− ($n = 7$)	TEM−/SHV+ ($n = 5$)	TEM−/SHV− ($n = 38$)
KPC+ ($n = 21$)	100 (11/11)	80 (4/5)	NA[a]	20 (1/5)
KPC− ($n = 44$)	100 (4/4)	0 (0/2)	0 (0/5)	6 (2/33)

[a] NA, not applicable.

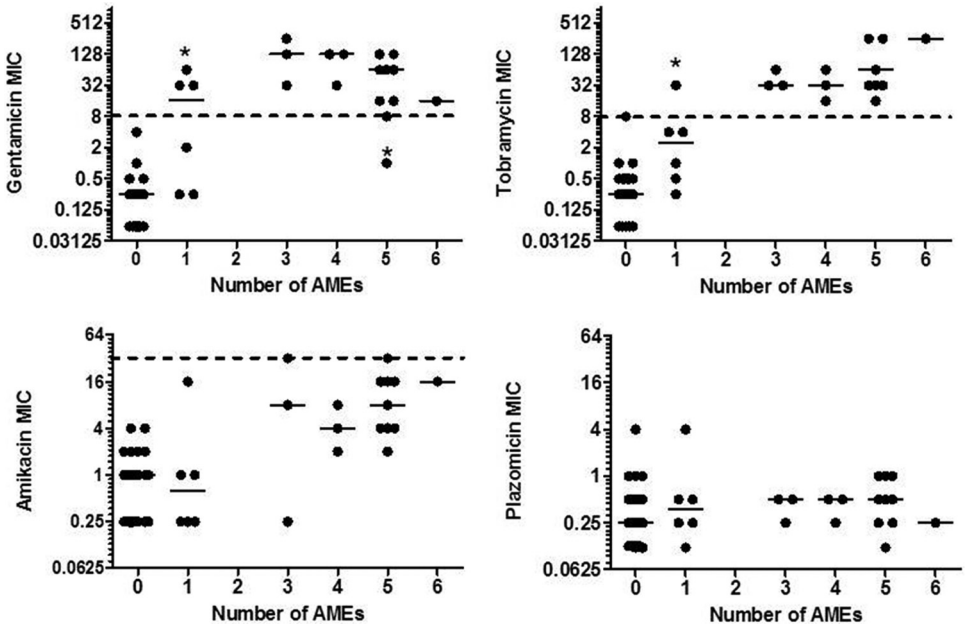

FIG 4 Distribution of aminoglycoside MICs based on the number of AMEs. Solid bars indicate median MICs. The dashed line passes through the CLSI breakpoint for intermediate status. As defined in this paper, MICs below the dashed line are sensitive, and those at or above the dashed line are resistant. Ninety-four percent (15/16) and 100% (16/16) of isolates with ≥3 AMEs were resistant to GEN and TOB, respectively. Three GEN-resistant isolates had a single AME: APH(3′)-Ia (MICs, 32, and 64 μg/ml) or AAC(3)-II (MIC, 32 μg/ml). One isolate with a GEN MIC of 1 μg/ml possessed the following AMEs: AAC(6′)-II, AAC(6′)-Ib, AAC(3)-IV, and ANT(3″)-1 (encoded by *aadA1* and *aadA2*). One isolate with a TOB MIC of 32 μg/ml possessed AAC(6′)-Ib alone. Asterisks highlight GEN- and TOB-resistant isolates with one AME and a GEN-susceptible isolate with 5 AMEs.

are limited as treatment options against KPC- and ESBL-producing *Enterobacter* infections at our center and that PLZ may represent a valuable addition to the antimicrobial armamentarium.

Previous studies have reported on the coexistence of carbapenemases, ESBLs, and AMEs in *Enterobacteriaceae* (29–34). Genes encoding AMEs have been found on the same plasmids and integrons as genes encoding KPC or ESBLs (35–39). Less commonly, KPC and ESBL genes may coexist on the same plasmids (37, 40). In addition, a single isolate may carry more than one plasmid, each with its own set of drug resistance genes (41). Our finding that the presence of at least two of the three β-lactamases (KPC, TEM, and SHV) predicted the presence of AMEs suggests that the gene determinants for these enzymes are carried together on the same plasmid; alternatively, these isolates may carry combinations of plasmids that harbor these resistance genes. In either scenario, the data indicate that the rapid detection of resistance determinants may be useful in informing antimicrobial treatment decisions (42).

Enterobacter isolates possessing APH(3′)-Ia or AAC(3)-II as the sole AME were resistant to GEN, in keeping with their ability to modify and inactivate GEN alone and both GEN and TOB, respectively (20). The individual contributions of other AMEs to GEN and TOB resistance were impossible to assess, since they were present almost exclusively in the company of multiple AMEs. The most common AMEs were AAC(6′)-Ib, which inactivates TOB, and AAC(6′)-II, which inactivates both GEN and TOB. Ninety-four percent (15/16) of isolates with AAC(6′)-Ib also had other AMEs that inactivate TOB (19). All 14 isolates with AAC(6′)-II had AAC(6′)-Ib; furthermore, 93% (13/14) of isolates with AAC(6′)-II had other AMEs that inactivate GEN. The predominance of AAC(6′)-Ib in our study and others is not surprising, as the gene encoding this enzyme is usually found within class I integrons (17, 43). Moreover, gene cassettes carrying other AME genes can be readily incorporated into class I integrons. As a result, AAC(6′)-Ib often coexists with other AMEs in isolates that are resistant to all currently available aminoglycosides (20).

AMK MICs in this study should be interpreted cautiously (8). Only 3% (2/65) and 11% (7/65) of the isolates were nonsusceptible to AMK using the CLSI and European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints, respectively (25, 44). These low rates were observed despite that fact that 73% (16/22) of AME+ isolates possessed AAC(6′)-Ib, which is reported to confer AMK resistance. In fact, AMK MICs below the susceptibility breakpoints are commonly observed among *Enterobacter* and other *Enterobacteriaceae* that possess AAC(6′)-Ib (8, 17, 45, 46). The clinical significance of susceptible MICs against bacteria carrying AMEs that are known to cause resistance is not established, and it is unclear if MICs or molecular markers are more relevant to treatment responses (47). We previously demonstrated that a majority of AAC(6′)-Ib+ carbapenem-resistant *K. pneumoniae* isolates showed regrowth during time-kill assays at achievable human serum concentrations of AMK despite being susceptible by CLSI criteria, suggesting that MICs may not be reliable predictors of activity (48). Indeed, the EUCAST states that should a member of the *Enterobacteriaceae* test as TOB intermediate or resistant and GEN and AMK susceptible, its AMK susceptibility status must be revised to "intermediate" (44). Until the clinical significance of these issues is resolved, we recommend that AMK be used with caution in the treatment of *Enterobacter* infections that are resistant to other aminoglycosides; at our center, such isolates are likely to harbor one or more AMEs, including AAC(6′)-Ib, that may result in suboptimal treatment responses.

Our results confirm previous findings of the excellent activity of PLZ against non-NDM-1-producing CRE (17, 23, 24). PLZ MICs were not affected by any KPC/ESBL pattern, nor were they affected by the number or type of AMEs. PLZ MICs were also consistently lower than those of the other aminoglycosides, even in the presence of AMEs. As PLZ was specifically designed to evade modifications conferred by most AMEs (23), these findings are not surprising. In a prior study, the only *Enterobacteriaceae* with PLZ MICs of >4 μg/ml produced 16S rRNA methyltransferases, enzymes known to be associated with NDM-1 and to confer high-level aminoglycoside resistance (23, 24). We demonstrated that both isolates with PLZ MICs of 4 μg/ml were negative for 16S rRNA methyltransferase genes *rmtB* and *armA*. Preliminary clinical data indicate that PLZ is infrequently associated with nephrotoxicity or ototoxicity (22), which suggests that this agent will have advantages over currently available aminoglycosides in both safety profile and spectrum of activity. A clinical trial of PLZ-based versus colistin-based combination regimens for CREs is ongoing (registration no. NCT01970371 at ClinicalTrials.gov).

Our data have several limitations. Only 8 and 6 of our clinical isolates carried KPC without ESBLs and ESBLs without KPC, respectively, and our results may have differed if we had had a greater number of such organisms. In addition, we described *Enterobacter* isolates from a single tertiary center in North America where NDM-1 (and therefore 16S rRNA methyltransferases) is uncommon, which potentially limits the applicability of our findings to other centers or regions.

In conclusion, we have shown high rates of aminoglycoside resistance among KPC- and ESBL-producing *Enterobacter*, directly linked to the higher frequency and number of AMEs. PLZ was consistently active against AME-producing isolates, suggesting that the agent may have an important role to play in treating multidrug-resistant *Enterobacter* infections in the future. It will be imperative to utilize this agent judiciously to limit the emergence of resistance. A full understanding of AMEs and other molecular mechanisms of aminoglycoside resistance will allow clinicians to rationally incorporate PLZ into treatment regimens. The development of molecular assays that accurately and rapidly predict antimicrobial responses among KPC- and ESBL-producing *Enterobacter* spp. should be a top research priority.

FUNDING INFORMATION

This work, including the efforts of Ryan K. Shields, was funded by HHS | National Institutes of Health (NIH) (K08AI114883). This work, including the efforts of Cornelius J. Clancy and M. Hong Nguyen, was funded by HHS | National Institutes of Health (NIH) (R21AI11103). This work, including the efforts of Yohei Doi, was funded by HHS | National Institutes of Health (NIH) (R21AI123747).

This project was supported by funding provided to the XDR Pathogen Laboratory by the University of Pittsburgh Medical Center.

REFERENCES

1. **Lazarovitch T, Amity K, Coyle JR, Ackerman B, Tal-Jasper R, Ofer-Friedman H, Hayakawa K, Bogan C, Lephart PR, Kaplansky T, Maskit M, Azouri T, Zaidenstein R, Perez F, Bonomo RA, Kaye KS, Marchaim D.** 2015. The complex epidemiology of carbapenem-resistant *enterobacter* infections: a multicenter descriptive analysis. Infect Control Hosp Epidemiol **36:**1283–1291. http://dx.doi.org/10.1017/ice.2015.186.
2. **Sievert DM, Ricks P, Edwards JR, Schneider A, Patel J, Srinivasan A, Kallen A, Limbago B, Fridkin S, National Healthcare Safety Network (NHSN) Team and Participating NHSN Facilities.** 2013. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009-2010. Infect Control Hosp Epidemiol **34:**1–14. http://dx.doi.org/10.1086/668770.
3. **Chow JW, Fine MJ, Shlaes DM, Quinn JP, Hooper DC, Johnson MP, Ramphal R, Wagener MM, Miyashiro DK, Yu VL.** 1991. *Enterobacter* bacteremia: clinical features and emergence of antibiotic resistance during therapy. Ann Intern Med **115:**585–590. http://dx.doi.org/10.7326/0003-4819-115-8-585.
4. **Harris PN, Ferguson JK.** 2012. Antibiotic therapy for inducible AmpC beta-lactamase-producing Gram-negative bacilli: what are the alternatives to carbapenems, quinolones and aminoglycosides? Int J Antimicrob Agents **40:**297–305. http://dx.doi.org/10.1016/j.ijantimicag.2012.06.004.
5. **Izdebski R, Baraniak A, Herda M, Fiett J, Bonten MJ, Carmeli Y, Goossens H, Hryniewicz W, Brun-Buisson C, Gniadkowski M, MOSAR WP2, WP3 and WP5 Study Groups.** 2015. MLST reveals potentially high-risk international clones of *Enterobacter cloacae*. J Antimicrob Chemother **70:**48–56. http://dx.doi.org/10.1093/jac/dku359.
6. **Ahn C, Syed A, Hu F, O'Hara JA, Rivera JI, Doi Y.** 2014. Microbiological features of KPC-producing *Enterobacter* isolates identified in a U.S. hospital system. Diagn Microbiol Infect Dis **80:**154–158. http://dx.doi.org/10.1016/j.diagmicrobio.2014.06.010.
7. **Pagès JM, Peslier S, Keating TA, Lavigne JP, Nichols WW.** 2016. Role of the outer membrane and porins in susceptibility of beta-lactamase-producing Enterobacteriaceae to ceftazidime-avibactam. Antimicrob Agents Chemother **60:**1349–1359.
8. **Kim SY, Park YJ, Yu JK, Kim YS.** 2011. Aminoglycoside susceptibility profiles of *Enterobacter cloacae* isolates harboring the *aac(6′)-Ib* gene. Korean J Lab Med **31:**279–281. http://dx.doi.org/10.3343/kjlm.2011.31.4.279.
9. **Wu W, Feng Y, Carattoli A, Zong Z.** 2015. Characterization of an *Enterobacter cloacae* strain producing both KPC and NDM carbapenemases by whole-genome sequencing. Antimicrob Agents Chemother **59:**6625–6628. http://dx.doi.org/10.1128/AAC.01275-15.
10. **Pereira PS, Borghi M, Albano RM, Lopes JC, Silveira MC, Marques EA, Oliveira JC, Asensi MD, Carvalho-Assef AP.** 2015. Coproduction of NDM-1 and KPC-2 in *Enterobacter hormaechei* from Brazil. Microb Drug Resist **21:**234–236. http://dx.doi.org/10.1089/mdr.2014.0171.
11. **Hossain A, Ferraro MJ, Pino RM, Dew RB, III, Moland ES, Lockhart TJ, Thomson KS, Goering RV, Hanson ND.** 2004. Plasmid-mediated carbapenem-hydrolyzing enzyme KPC-2 in an *Enterobacter* sp. Antimicrob Agents Chemother **48:**4438–4440. http://dx.doi.org/10.1128/AAC.48.11.4438-4440.2004.
12. **Wu Q, Liu Q, Han L, Sun J, Ni Y.** 2010. Plasmid-mediated carbapenem-hydrolyzing enzyme KPC-2 and ArmA 16S rRNA methylase conferring high-level aminoglycoside resistance in carbapenem-resistant *Enterobacter cloacae* in China. Diagn Microbiol Infect Dis **66:**326–328. http://dx.doi.org/10.1016/j.diagmicrobio.2009.10.003.
13. **Qin X, Yang Y, Hu F, Zhu D.** 2014. Hospital clonal dissemination of *Enterobacter aerogenes* producing carbapenemase KPC-2 in a Chinese teaching hospital. J Med Microbiol **63:**222–228. http://dx.doi.org/10.1099/jmm.0.064865-0.
14. **Zavascki AP, Machado AB, de Oliveira KR, Superti SV, Pilger DA, Cantarelli VV, Pereira PR, Lieberkmecht AC, Barth AL.** 2009. KPC-2-producing *Enterobacter cloacae* in two cities from Southern Brazil. Int J Antimicrob Agents **34:**286–288. http://dx.doi.org/10.1016/j.ijantimicag.2009.03.012.
15. **Bratu S, Landman D, Alam M, Tolentino E, Quale J.** 2005. Detection of KPC carbapenem-hydrolyzing enzymes in *Enterobacter* spp. from Brooklyn, New York. Antimicrob Agents Chemother **49:**776–778. http://dx.doi.org/10.1128/AAC.49.2.776-778.2005.
16. **Wendel AF, MacKenzie CR.** 2015. Characterization of a novel metallo-beta-lactamase variant, GIM-2, from a clinical isolate of Enterobacter cloacae in Germany. Antimicrob Agents Chemother **59:**1824–1825. http://dx.doi.org/10.1128/AAC.05062-14.
17. **Almaghrabi R, Clancy CJ, Doi Y, Hao B, Chen L, Shields RK, Press EG, Iovine NM, Townsend BM, Wagener MM, Kreiswirth B, Nguyen MH.** 2014. Carbapenem-resistant *Klebsiella pneumoniae* strains exhibit diversity in aminoglycoside-modifying enzymes, which exert differing effects on plazomicin and other agents. Antimicrob Agents Chemother **58:**4443–4451. http://dx.doi.org/10.1128/AAC.00099-14.
18. **Mingeot-Leclercq MP, Glupczynski Y, Tulkens PM.** 1999. Aminoglycosides: activity and resistance. Antimicrob Agents Chemother **43:**727–737.
19. **Shaw KJ, Rather PN, Hare RS, Miller GH.** 1993. Molecular genetics of

aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. Microbiol Rev **57:**138–163.

20. **Ramirez MS, Tolmasky ME.** 2010. Aminoglycoside modifying enzymes. Drug Resist Updat **13:**151–171. http://dx.doi.org/10.1016/j.drup.2010.08.003.
21. **Perez F, El Chakhtoura NG, Papp-Wallace KM, Wilson BM, Bonomo RA.** 2016. Treatment options for infections caused by carbapenem-resistant Enterobacteriaceae: can we apply "precision medicine" to antimicrobial chemotherapy? Expert Opin Pharmacother **17:**761–781. http://dx.doi.org/10.1517/14656566.2016.1145658.
22. **Karaiskos I, Souli M, Giamarellou H.** 2015. Plazomicin: an investigational therapy for the treatment of urinary tract infections. Expert Opin Invest Drugs **24:**1501–1511. http://dx.doi.org/10.1517/13543784.2015.1095180.
23. **Livermore DM, Mushtaq S, Warner M, Zhang JC, Maharjan S, Doumith M, Woodford N.** 2011. Activity of aminoglycosides, including ACHN-490, against carbapenem-resistant Enterobacteriaceae isolates. J Antimicrob Chemother **66:**48–53. http://dx.doi.org/10.1093/jac/dkq408.
24. **Galani I, Souli M, Daikos GL, Chrysouli Z, Poulakou G, Psichogiou M, Panagea T, Argyropoulou A, Stefanou I, Plakias G, Giamarellou H, Petrikkos G.** 2012. Activity of plazomicin (ACHN-490) against MDR clinical isolates of *Klebsiella pneumoniae*, *Escherichia coli*, and *Enterobacter* spp. from Athens, Greece. J Chemother **24:**191–194. http://dx.doi.org/10.1179/1973947812Y.0000000015.
25. **CLSI.** 2016. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 10th ed. Approved standard M07-A10. CLSI, Wayne, PA.
26. **Poulou A, Grivakou E, Vrioni G, Koumaki V, Pittaras T, Pournaras S, Tsakris A.** 2014. Modified CLSI extended-spectrum beta-lactamase (ESBL) confirmatory test for phenotypic detection of ESBLs among *Enterobacteriaceae* producing various beta-lactamases. J Clin Microbiol **52:**1483–1489. http://dx.doi.org/10.1128/JCM.03361-13.
27. **Dallenne C, Da Costa A, Decre D, Favier C, Arlet G.** 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. J Antimicrob Chemother **65:**490–495. http://dx.doi.org/10.1093/jac/dkp498.
28. **Noppe-Leclercq I, Wallet F, Haentjens S, Courcol R, Simonet M.** 1999. PCR detection of aminoglycoside resistance genes: a rapid molecular typing method for Acinetobacter baumannii. Res Microbiol **150:**317–322. http://dx.doi.org/10.1016/S0923-2508(99)80057-6.
29. **Fernandez-Rodriguez A, Canton R, Perez-Diaz JC, Martinez-Beltran J, Picazo JJ, Baquero F.** 1992. Aminoglycoside-modifying enzymes in clinical isolates harboring extended-spectrum beta-lactamases. Antimicrob Agents Chemother **36:**2536–2538. http://dx.doi.org/10.1128/AAC.36.11.2536.
30. **Ode T, Saito R, Kumita W, Sato K, Okugawa S, Moriya K, Koike K, Okamura N.** 2009. Analysis of plasmid-mediated multidrug resistance in *Escherichia coli* and *Klebsiella oxytoca* isolates from clinical specimens in Japan. Int J Antimicrob Agents **34:**347–350. http://dx.doi.org/10.1016/j.ijantimicag.2009.05.007.
31. **Wei DD, Wan LG, Yu Y, Xu QF, Deng Q, Cao XW, Liu Y.** 2015. Characterization of extended-spectrum beta-lactamase, carbapenemase, and plasmid quinolone determinants in *Klebsiella pneumoniae* isolates carrying distinct types of 16S rRNA methylase genes, and their association with mobile genetic elements. Microb Drug Resist **21:**186–193. http://dx.doi.org/10.1089/mdr.2014.0073.
32. **Partridge SR, Thomas LC, Ginn AN, Wiklendt AM, Kyme P, Iredell JR.** 2011. A novel gene cassette, aacA43, in a plasmid-borne class 1 integron. Antimicrob Agents Chemother **55:**2979–2982. http://dx.doi.org/10.1128/AAC.01582-10.
33. **Miró E, Grunbaum F, Gomez L, Rivera A, Mirelis B, Coll P, Navarro F.** 2013. Characterization of aminoglycoside-modifying enzymes in Enterobacteriaceae clinical strains and characterization of the plasmids implicated in their diffusion. Microb Drug Resist **19:**94–99. http://dx.doi.org/10.1089/mdr.2012.0125.
34. **Huang S, Dai W, Sun S, Zhang X, Zhang L.** 2012. Prevalence of plasmid-mediated quinolone resistance and aminoglycoside resistance determinants among carbapeneme non-susceptible *Enterobacter cloacae*. PLoS One **7:**e47636. http://dx.doi.org/10.1371/journal.pone.0047636.
35. **Gootz TD, Lescoe MK, Dib-Hajj F, Dougherty BA, He W, Della-Latta P, Huard RC.** 2009. Genetic organization of transposase regions surrounding blaKPC carbapenemase genes on plasmids from *Klebsiella* strains isolated in a New York City hospital. Antimicrob Agents Chemother **53:**1998–2004. http://dx.doi.org/10.1128/AAC.01355-08.
36. **Chmelnitsky I, Hermesh O, Navon-Venezia S, Strahilevitz J, Carmeli Y.** 2009. Detection of *aac(6′)-Ib-cr* in KPC-producing *Klebsiella pneumoniae* isolates from Tel Aviv, Israel. J Antimicrob Chemother **64:**718–722. http://dx.doi.org/10.1093/jac/dkp272.
37. **Chen L, Chavda KD, Melano RG, Hong T, Rojtman AD, Jacobs MR, Bonomo RA, Kreiswirth BN.** 2014. Molecular survey of the dissemination of two blaKPC-harboring IncFIA plasmids in New Jersey and New York hospitals. Antimicrob Agents Chemother **58:**2289–2294. http://dx.doi.org/10.1128/AAC.02749-13.
38. **Seni J, Falgenhauer L, Simeo N, Mirambo MM, Imirzalioglu C, Matee M, Rweyemamu M, Chakraborty T, Mshana SE.** 2016. Multiple ESBL-producing *Escherichia coli* sequence types carrying quinolone and aminoglycoside resistance genes circulating in companion and domestic farm animals in Mwanza, Tanzania, harbor commonly occurring plasmids. Front Microbiol **7:**142. http://dx.doi.org/10.3389/fmicb.2016.00142.
39. **Burke L, Humphreys H, Fitzgerald-Hughes D.** 22 March 2016. The molecular epidemiology of resistance in cefotaximase-producing *Escherichia coli* clinical isolates from Dublin, Ireland. Microb Drug Resist http://dx.doi.org/10.1089/mdr.2015.0154.
40. **Li JJ, Lee CS, Sheng JF, Doi Y.** 2014. Complete sequence of a conjugative incn plasmid harboring bla_{KPC-2}, bla_{SHV-12}, and qnrS1 from an *Escherichia coli* sequence type 648 strain. Antimicrob Agents Chemother **58:**6974–6977. http://dx.doi.org/10.1128/AAC.03632-14.
41. **Warburg G, Hidalgo-Grass C, Partridge SR, Tolmasky ME, Temper V, Moses AE, Block C, Strahilevitz J.** 2012. A carbapenem-resistant *Klebsiella pneumoniae* epidemic clone in Jerusalem: sequence type 512 carrying a plasmid encoding *aac(6′)-Ib*. J Antimicrob Chemother **67:**898–901. http://dx.doi.org/10.1093/jac/dkr552.
42. **Evans SR, Hujer AM, Jiang H, Hujer KM, Hall T, Marzan C, Jacobs MR, Sampath R, Ecker DJ, Manca C, Chavda K, Zhang P, Fernandez H, Chen L, Mediavilla JR, Hill CB, Perez F, Caliendo AM, Fowler VG, Jr, Chambers HF, Kreiswirth BN, Bonomo RA, Antibacterial Resistance Leadership Group.** 2016. Rapid molecular diagnostics, antibiotic treatment decisions, and developing approaches to inform empiric therapy: PRIMERS I and II. Clin Infect Dis **62:**181–189. http://dx.doi.org/10.1093/cid/civ837.
43. **Ramirez MS, Nikolaidis N, Tolmasky ME.** 2013. Rise and dissemination of aminoglycoside resistance: the *aac(6′)-Ib* paradigm. Front Microbiol **4:**121.
44. **Leclercq R, Canton R, Brown DF, Giske CG, Heisig P, MacGowan AP, Mouton JW, Nordmann P, Rodloff AC, Rossolini GM, Soussy CJ, Steinbakk M, Winstanley TG, Kahlmeter G.** 2013. EUCAST expert rules in antimicrobial susceptibility testing. Clin Microbiol Infect **19:**141–160. http://dx.doi.org/10.1111/j.1469-0691.2011.03703.x.
45. **Shen P, Jiang Y, Zhou Z, Zhang J, Yu Y, Li L.** 2008. Complete nucleotide sequence of pKP96, a 67 850 bp multiresistance plasmid encoding *qnrA1*, *aac(6′)-Ib-cr* and $bla_{CTX-M-24}$ from *Klebsiella pneumoniae*. J Antimicrob Chemother **62:**1252–1256. http://dx.doi.org/10.1093/jac/dkn397.
46. **Lindemann PC, Risberg K, Wiker HG, Mylvaganam H.** 2012. Aminoglycoside resistance in clinical *Escherichia coli* and *Klebsiella pneumoniae* isolates from Western Norway. APMIS **120:**495–502. http://dx.doi.org/10.1111/j.1600-0463.2011.02856.x.
47. **Jakobsen L, Sandvang D, Jensen VF, Seyfarth AM, Frimodt-Moller N, Hammerum AM.** 2007. Gentamicin susceptibility in *Escherichia coli* related to the genetic background: problems with breakpoints. Clin Microbiol Infect **13:**830–832. http://dx.doi.org/10.1111/j.1469-0691.2007.01751.x.
48. **Bremmer DN, Clancy CJ, Press EG, Almaghrabi R, Chen L, Doi Y, Nguyen MH, Shields RK.** 2014. KPC-producing *Klebsiella pneumoniae* strains that harbor AAC(6′)-Ib exhibit intermediate resistance to amikacin. Antimicrob Agents Chemother **58:**7597–7600. http://dx.doi.org/10.1128/AAC.03831-14.