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MECHANISMS OF RESISTANCE

# Activity of Ceftolozane-Tazobactam against Carbapenem-Resistant, Non-Carbapenemase-Producing *Pseudomonas aeruginosa* and Associated Resistance Mechanisms

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**ABSTRACT** Although carbapenems are effective for treating serious multidrug-resistant *Pseudomonas aeruginosa* infections, carbapenem-resistant *P. aeruginosa* (CRPA) is now being reported worldwide. Ceftolozane-tazobactam (C/T) demonstrates activity against many multidrug-resistant isolates. We evaluated the activity of C/T and compared its activity to that of ceftazidime-avibactam (C/A) using a well-characterized collection of non-carbapenemase-producing CRPA isolates. Forty-two non-carbapenemase-producing CRPA isolates from a previous study (J. Y. Lee and K. S. Ko, Int J Antimicrob Agents 40:168–172, 2012, https://doi.org/10.1016/j.ijantimicag.2012.04.004) were included. All had been previously shown to be negative for $bla_{IMP}$, $bla_{VIM}$, $bla_{SPM}$, $bla_{GIM}$, $bla_{SIM}$, and $bla_{KPC}$ by PCR. In the prior study, expression of *oprD*, *ampC*, and several efflux pump genes had been defined by quantitative reverse transcription-PCR. Here, antimicrobial susceptibility was determined by broth microdilution according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Time-kill curve assays were performed using three C/T- and C/A-susceptible CRPA isolates. Among 42 non-carbapenemase-producing CRPA isolates, overall susceptibility to C/T was 95.2%, compared to 71.4%, 42.9%, 23.8%, 21.4%, and 2.4% for C/A, ceftazidime, piperacillin-tazobactam, cefepime, and meropenem, respectively. The C/T resistance rate was significantly lower than that of C/A among isolates showing decreased *oprD* and increased *mexB* expression (5.1% versus 25.6%, $P = 0.025$, and 4.3% versus 34.8%, $P = 0.022$, respectively). In time-kill curve studies, C/T was less bactericidal than C/A against an isolate with decreased *oprD* and increased *ampC* expression. C/T was active against 95.2% of non-carbapenemase-producing CRPA clinical isolates. No apparent correlation of C/T MIC values with specific mutation-driven resistance mechanisms was noted.

**KEYWORDS** *Pseudomonas aeruginosa*, carbapenem resistant, ceftolozane-tazobactam

Although carbapenems remain effective in treating serious multidrug-resistant (MDR) *Pseudomonas aeruginosa* infections, carbapenem-resistant *P. aeruginosa* (CRPA) has emerged and is being reported as a nosocomial pathogen worldwide and particularly in debilitated or immunocompromised patients (1, 2). Infections caused by CRPA are of concern in many hospitals since they have been shown to reduce the

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**TABLE 1** Antimicrobial activities tested against non-carbapenemase-producing CRPA isolates[a]

| Antimicrobial agent | MIC (mg/liter) | | | No. (%) of isolates with result ($n$ = 42): | | |
|---|---|---|---|---|---|---|
| | $MIC_{50}$ | $MIC_{90}$ | Range | S | I | R |
| Imipenem | 16 | 32 | 8 to 32 | 0 (0) | 0 (0) | 42 (100) |
| Meropenem | 16 | 32 | 2 to >64 | 1 (2.4) | 9 (21.4) | 32 (76.2) |
| Piperacillin-tazobactam | 128/4 | >256/4 | 4/4 to >256/4 | 10 (23.8) | 3 (7.1) | 29 (69.0) |
| Ceftolozane-tazobactam | 2/4 | 4/4 | 1/4 to 32/4 | 40 (95.2) | 0 (0) | 2 (4.8) |
| Ceftazidime-avibactam | 8/4 | 16/4 | 2/4 to 32/4 | 30 (71.4) | NA | 12 (28.6) |
| Cefepime | 32 | >64 | 2 to >64 | 9 (21.4) | 8 (19.0) | 25 (59.5) |
| Ceftazidime | 16 | 64 | 2 to >64 | 18 (42.9) | 3 (7.1) | 21 (50.0) |
| Ciprofloxacin | 2 | 64 | ≤0.06 to >64 | 14 (33.3) | 8 (19.0) | 20 (47.6) |
| Amikacin | 16 | >128 | 2 to >128 | 25 (59.5) | 4 (9.5) | 13 (31.0) |
| Polymyxin B | 1 | 2 | 0.5 to 2 | 42 (100) | 0 (0) | 0 (0) |
| Colistin | 2 | 2 | 0.5 to 2 | 42 (100) | NA | 0 (0) |

[a]Abbreviations: CRPA, carbapenem-resistant *P. aeruginosa*; S, susceptible; I, intermediate; R, resistant; NA, not applicable.

likelihood of appropriate initial antimicrobial therapy and are associated with significant mortality (3).

While the production of carbapenemases, mainly Ambler class B metallo-β-lactamases (MBLs), is noteworthy in *P. aeruginosa* as a mechanism of carbapenem resistance (4), the presence of non-carbapenemase-mediated carbapenem resistance is far more common (5–7). Loss of outer membrane porin D (OprD) function in conjunction with another mechanism, such as overexpression of *ampC* or overexpression of efflux pumps, is the major determinant of resistance to carbapenems (5–7). Chromosomally encoded AmpC β-lactamase together with the efflux pump MexAB-OprM operon contributes to resistance of *P. aeruginosa* to many β-lactam antibiotics. Nevertheless, isolates with reduced susceptibility to carbapenems because of inactivation of *oprD* in conjunction with other mechanism, such as overexpression of *ampC* or overexpression of efflux pumps, sometimes show susceptibility to other β-lactams besides carbapenems (8–10).

Ceftolozane-tazobactam (C/T) is a novel antibiotic with broad-spectrum activity against Gram-negative bacteria, including MDR *P. aeruginosa*. Ceftolozane is an oxyimino-aminothiazolyl cephalosporin that has stability against chromosomal AmpC β-lactamases, overexpressed MexAB-OprM efflux pumps, and deleted OprD porins (11). Ceftolozane's affinity for the penicillin-binding proteins of *P. aeruginosa* accounts for its activity against this organism (12). Although tazobactam does not play a critical role in enhancing the activity of ceftolozane against *P. aeruginosa*, it extends the activity of ceftolozane alone against extended-spectrum-β-lactamase-producing *Enterobacteriaceae* (12). However, C/T is not active against *Klebsiella pneumoniae* carbapenemase (KPC) or metallo-β-lactamases (13, 14). Therefore, C/T demonstrates activity against many MDR isolates of *P. aeruginosa*, including carbapenem-resistant strains that do not produce a carbapenemase (14).

Here, we evaluated the activity of C/T and compared its activity to that of ceftazidime-avibactam (C/A) in non-carbapenemase-producing CRPA clinical isolates (6). In addition, we assessed strains for underlying C/T and C/A resistance mechanisms.

## RESULTS

**Antimicrobial susceptibility of C/T and C/A against *P. aeruginosa* and its correlation with resistance mechanisms.** Among 42 non-carbapenemase-producing CRPA isolates, overall susceptibility to C/T was 95.2%, compared to 71.4%, 42.9%, 23.8%, 21.4%, and 2.4% for C/A, ceftazidime, piperacillin-tazobactam, cefepime, and meropenem, respectively (Table 1). Only two isolates showed resistance to C/T.

Of 42 non-carbapenemase-producing CRPA isolates, 39 (92.9%) showed decreased *oprD* expression (≤30%) compared with that of PAO1 (Table 2). Strains with decreased *oprD* expression displayed median C/T and C/A MIC values of 2 and 8 mg/liter, respectively. The resistance rate for C/T was significantly lower than that for C/A among

**TABLE 2** MIC range and resistance rates for ceftolozane-tazobactam and ceftazidime-avibactam according to results for expression of *oprD*, efflux pumps, and chromosomal *ampC* among non-carbapenemase-producing CRPA clinical isolates

| Resistance mechanism | No. of isolates | MIC range (median) (mg/liter) | | % resistance | |
|---|---|---|---|---|---|
| | | Ceftolozane-tazobactam | Ceftazidime-avibactam | Ceftolozane-tazobactam | Ceftazidime-avibactam |
| Decreased *oprD* expression | | | | | |
| Positive (≤30% compared with PAO1)[a] | 39 | 1–32 (2) | 2–32 (8) | 5.1 | 25.6 |
| Negative (>30% compared with PAO1) | 3 | 2–4 (2) | 8–16 (16) | 0 | 66.7 |
| Overexpressed *mexB* | | | | | |
| Positive (≥3-fold compared with PAO1)[a] | 23 | 1–32 (2) | 2–32 (8) | 4.3 | 34.8 |
| Negative (<2-fold compared with PAO1) | 12 | 1–16 (2) | 2–16 (4) | 8.3 | 25.0 |
| Overexpressed *mexD* | | | | | |
| Positive (≥10-fold compared with PAO1) | 6 | 1–4 (2) | 2–8 (4) | 0 | 0 |
| Negative (<5-fold compared with PAO1) | 35 | 1–32 (2) | 2–32 (8) | 5.7 | 34.3 |
| Overexpressed *mexF* | | | | | |
| Positive (≥10-fold compared with PAO1) | 5 | 1–4 (2) | 2–16 (8) | 0 | 40 |
| Negative (<5-fold compared with PAO1) | 29 | 1–32 (2) | 2–32 (8) | 6.9 | 24.1 |
| Overexpressed *ampC* | | | | | |
| Positive (≥10-fold compared with PAO1) | 17 | 1–32 (4) | 2–16 (8) | 5.9 | 23.5 |
| Negative (<5-fold compared with PAO1) | 25 | 1–16 (2) | 2–32 (4) | 4.0 | 32.0 |

[a]$P < 0.05$ in comparison of percent resistance to ceftolozane-tazobactam with that to ceftazidime-avibactam.

isolates showing decreased *oprD* expression (5.1% versus 25.6%, $P = 0.025$). Among the isolates analyzed, 23 (54.8%) had elevated expression of *mexB*. Resistance to C/T was significantly lower than that to C/A among isolates showing elevated *mexB* expression (4.3% versus 34.8%, $P = 0.022$). Elevated expression of *mexD* was noted among 6 (14.3%) of the isolates. All isolates showing elevated expression of *mexD* were susceptible to both C/T and C/A. Overall, 17 (40.5%) isolates were considered to have a derepressed chromosomal *ampC*. The resistance rates for C/T and C/A among isolates showing derepressed chromosomal *ampC* were 5.9% and 23.5%, respectively, a statistically nonsignificant difference.

The resistance mechanisms described above were observed alone (15 isolates) or in combinations of two to four mechanisms in the isolates tested (Table 3). Overall, 10 resistance mechanisms or combinations thereof were observed, with decreased *oprD* expression alone being most prevalent (13 strains), followed by a combination of decreased expression of *oprD* and overexpression of *mexB* (9 isolates). Resistance to C/T was significantly lower than that to C/A among isolates showing decreased *oprD* and increased *mexB* expression (0% versus 55.6%, $P = 0.005$).

**TABLE 3** MIC range and resistance rates for ceftolozane-tazobactam and ceftazidime-avibactam according to results for resistance mechanisms among non-carbapenemase-producing CRPA clinical isolates

| Resistance mechanism | No. of isolates | MIC range (median) (mg/liter) | | % resistance | |
|---|---|---|---|---|---|
| | | Ceftolozane-tazobactam | Ceftazidime-avibactam | Ceftolozane-tazobactam | Ceftazidime-avibactam |
| Decreased *oprD* expression | 13 | 1–16 (2) | 2–16 (4) | 7.7 | 15.4 |
| Decreased *oprD* and increased *mexB* expression[a] | 9 | 1–4 (2) | 2–32 (16) | 0 | 55.6 |
| Decreased *oprD* and increased *mexY* expression | 1 | 1 | 2 | 0 | 0 |
| Decreased *oprD* and increased *ampC* expression | 3 | 1–4 (2) | 4–8 (4) | 0 | 0 |
| Decreased *oprD* and increased *mexB* and *ampC* expression | 4 | 2–32 (4) | 8–16 (16) | 25.0 | 50.0 |
| Decreased *oprD* and increased *mexY* and *ampC* expression | 1 | 2 | 16 | 0 | 100 |
| Decreased *oprD* and increased *mexB*, *mexD*, and *ampC* expression | 6 | 1–4 (2) | 2–8 (4) | 0 | 0 |
| Decreased *oprD* and increased *mexB*, *mexY*, and *ampC* expression | 2 | 2 | 8 | 0 | 0 |
| Increased *mexB* expression | 2 | 2 | 8–16 | 0 | 50 |
| Increased *mexY* and *ampC* expression | 1 | 4 | 16 | 0 | 100 |

[a]$P < 0.05$ in comparison of percent resistance to ceftolozane-tazobactam with that to ceftazidime-avibactam.

**TABLE 4** Antimicrobial resistance and genotype of three non-carbapenemase-producing CRPA isolates tested in a time-kill assay[b]

| Isolate no. | ST | MIC (mg/liter) | | | | | | | Expression of gene[a] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | IMP | MEM | C/T | C/A | FEP | CAZ | P/T | *oprD* | *mexB* | *ampC* |
| 42 | 277 | 32 | 8 | 1/4 | 4/4 | 8 | 8 | 8/4 | **0.0618** | 0.7769 | **61.3534** |
| 91 | 641 | 16 | 4 | 4/4 | 4/4 | 32 | 4 | 128/4 | **0.0001** | 1.2536 | 0.2649 |
| 186 | 233 | 16 | 16 | 2/4 | 8/4 | 8 | 8 | 16/4 | **0.0015** | **19.6462** | 0.4798 |

[a]Values in bold indicate a significant overexpression (or underexpression for *oprD*) of the corresponding gene according to the defined thresholds relative to PAO1 (see Table 2).
[b]Abbreviations: ST, sequence type; IMP, imipenem; MEM, meropenem; C/T, ceftolozane-tazobactam; C/A, ceftazidime-avibactam; FEP, cefepime; CAZ, ceftazidime; P/T, piperacillin-tazobactam.

**Killing effects of C/T and C/A on CRPA clinical isolates.** Three non-carbapenemase-producing CRPA clinical isolates susceptible to both C/T and C/A (genotypes ST277, ST641, and ST233) were selected for a time-kill assay using C/T and C/A (Table 4). Figure 1 shows the killing curves at C/T or C/A concentrations of 2 times, 4 times, and 8 times the MIC. Both C/T and C/A caused decreases in the number of CFU per milliliter over the 1- to 8-h time period for all strains at all multiples of MICs tested. The onset of bacterial killing showed a lag time of approximately 1 h in all strains. PAO1 had at least a 3-$\log_{10}$ decrease in the number of CFU per milliliter with both C/T and C/A at all MICs at 24 h (Fig. 1A and E). Both C/T and C/A were bactericidal after 8 h when tested at 2 times, 4 times, and 8 times the MIC against an isolate with downregulated *oprD* (Fig. 1C and G). C/T was less bactericidal against an isolate with downregulated *oprD* and overexpressed *ampC* (Fig. 1B and F). At the 8-h time point, all concentrations of C/T caused a $<$3-$\log_{10}$ decrease in CFU per milliliter. In addition, regrowth was observed at 12 h when tested at 2 times the MIC and at 24 h when tested at 4 times the MIC against this isolate (Fig. 1B and F). On the other hand, C/A was bactericidal against an isolate with downregulated *oprD* and overexpressed *mexB* at 8 h at all MICs tested (Fig. 1D and H). However, regrowth was observed at the 12-h time point when tested at 2 times and 4 times the MIC (Fig. 1D and H).

## DISCUSSION

Our C/T susceptibility data demonstrate that it is an active agent against non-carbapenemase-producing CRPA. In comparison to C/A MICs, C/T MICs were lower. Our study also demonstrates that decreased *oprD* transcription and increased transcription of efflux pump genes or *ampC* do not fully explain the correlation of C/T MIC values with specific mutation-driven resistance mechanisms. However, C/T was less bactericidal against an isolate having decreased *oprD* and increased *ampC* expression than against isolates having other resistance mechanisms, and C/A showed regrowth at the 12-h time point when tested at 2 times and 4 times the MIC against an isolate with decreased *oprD* and increased *mexB* expression in time-kill studies.

The increase in carbapenem resistance among *P. aeruginosa* clinical isolates is worrisome, because there has been little progress in the development of new antimicrobial agents targeting this organism (4, 15). Delaying the initiation of appropriate antimicrobial therapy is well established as being associated with increased morbidity and mortality in patients with severe *P. aeruginosa* infections (16, 17). In this setting, colistin has been recently deployed as a last-resort treatment option (18). However, concerns about nephrotoxicity, a well-known adverse effect of colistin, and challenging pharmacokinetics have led to limited use of this drug (19). C/T has shown potent *in vitro* activity against *Pseudomonas* species (20, 21). Previous studies showed C/T activity against 86% to 95% of clinical *P. aeruginosa* isolates, and when specifically evaluating more-resistant strains, 60 to 80% of ceftazidime-resistant and meropenem-resistant pseudomonal isolates displayed MICs to C/T of $\leq$8 mg/liter (22, 23). Consistent with this, our study showed 40 isolates (95.2%) to be susceptible to C/T among 42 non-carbapenemase-producing CRPA clinical isolates collected before the clinical availability of ceftolozane. All isolates were colistin susceptible,

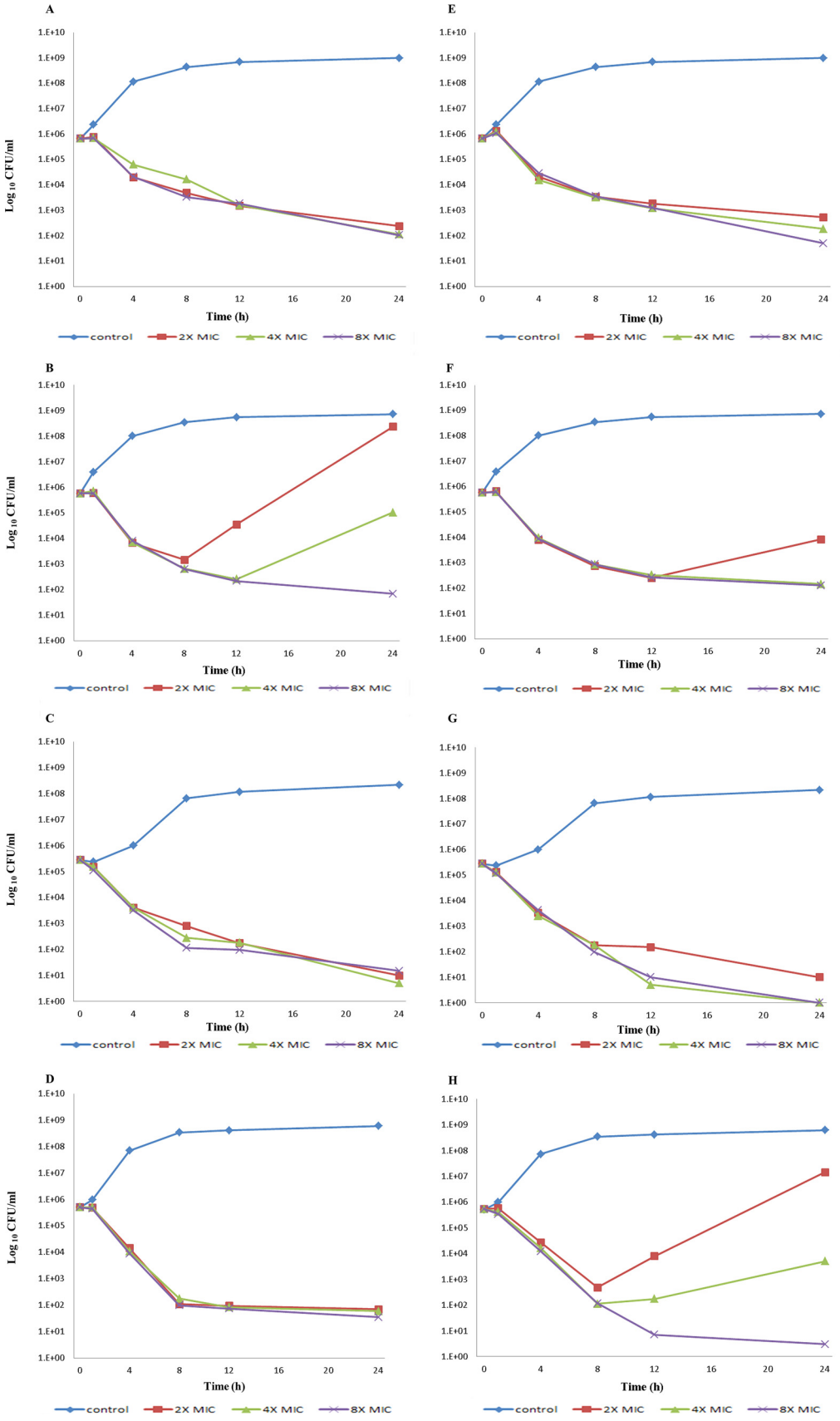


**FIG 1** Time-kill curves of carbapenem-resistant ceftolozane-tazobactam-susceptible and ceftazidime-avibactam-susceptible *P. aeruginosa* clinical isolates exposed to ceftolozane-tazobactam (A, B, C, and D) and ceftazidime-avibactam

(Continued on next page)

79.4% were C/A susceptible, 59.5% were amikacin susceptible, and <50% were susceptible to the remaining antibiotics tested.

Previous studies investigated the possible molecular mechanisms of *P. aeruginosa* resistance to C/T and C/A (12, 24–27). High-level resistance to C/T occurred only in a strain with multiple mutations leading to overexpression and structural modifications of AmpC (12). AmpC overexpression was suggested to contribute to resistance of *P. aeruginosa* to C/T, but underlying resistance mechanisms remain poorly defined. In the current study, two isolates showed resistance to C/T. The first showed decreased *oprD* expression, and the second showed decreased *oprD* expression combined with increased *ampC* and *mexB* expression. However, due to the apparent limited correlation of C/T MIC values with specific mutation-driven resistance mechanisms, we are unable to explain resistance mechanisms for C/T with decreased *oprD* transcription and increased transcription of efflux genes or of the *ampC* gene. Parenthetically, there was a correlation between MICs and resistance mechanism in 15 isolates with a metallo-β-lactamase (13 with $bla_{IMP-6}$ and 2 with $bla_{VIM-2}$), which showed MICs of ≥128/4 mg/liter for both C/T and C/A (data not shown). Intriguingly, nine isolates with decreased *oprD* expression combined with increased *mexB* expression did not display resistance to C/T, in contrast to the situation with C/A. Ceftolozane is known to not be affected by overexpressed MexAB-OprM because it is not a substrate of this pump, nor is it affected by deletion of OprD porins, because it does not enter bacterial cells through OprD (13). Further study will be needed to understand the drivers of resistance to C/T to support efforts for preserving the potency of this last-resort antibiotic.

Our study also showed antimicrobial effects of C/T and C/A against non-carbapenemase-producing CRPA clinical isolates using time-kill assays. C/T produced a decrease in the number of CFU per milliliter at 8 h for an isolate having decreased *oprD* and increased *ampC* expression; however, this combination was not bactericidal against this isolate, in contrast to C/A, which showed a bactericidal effect without regrowth. An important potential use of C/A is in the treatment of *P. aeruginosa* infections, as this drug has been shown to have potent inhibitory activity against the class C β-lactamase of *P. aeruginosa* (28). In addition, avibactam binds covalently and reversibly to β-lactamases (29). This reversibility is a unique feature that allows avibactam to undergo recyclization to inactivate another β-lactamase. We are uncertain of the clinical significance of regrowth after the 8-h time point, because both C/T and C/A clinical dosing regimens are every 8 h. Similar observations of regrowth in time-kill studies have been made with other commercially available β-lactam–β-lactamase-inhibitor combinations, such as piperacillin-tazobactam (30, 31). Despite this phenomenon, piperacillin-tazobactam has been in successful clinical use for many years, suggesting that regrowth in time-kill studies for β-lactam–β-lactamase-inhibitor agents might be an *in vitro* phenomenon that does not necessarily translate to clinical activity. With regard to lag time of bacterial killing, both C/T and C/A showed a lag in bacterial killing for all three isolates studied. β-Lactams bind to penicillin-binding proteins, stimulating an autolysin effect; turnover of the autolysin effect may result in a lag in killing (32, 33).

This study has some limitations. First, although we investigated the main resistance mechanisms of *P. aeruginosa* causing carbapenem resistance, we did not interrogate all resistance mechanisms. Although uncommon, class A extended-spectrum β-lactamases, such as TEM, SHV, CTX-M, PER, VEB, GES, and IBC families, have been detected in *P. aeruginosa*. Extended-spectrum β-lactamases from the class D OXA-type enzymes have also been encountered in *P. aeruginosa* (34). Second, even though these isolates were collected from eight South Korean hospitals, the sample size is too small to determine statistical significance. Therefore, further study will be needed to investigate

**FIG 1** Legend (Continued)
(E, F, G, and H). (A and E) PAO1. (B and F) An isolate with downregulated *oprD* and upregulated *ampC* (isolate 42). (C and G) An isolate with downregulated *oprD* (isolate 91). (D and H) An isolate with downregulated *oprD* and upregulated *mexB* (isolate 186). The lower limit of detection for this assay was 1 CFU/ml.

the correlation of C/T MIC values with specific resistance mechanisms using a large number of non-carbapenemase-producing CRPA isolates.

In conclusion, C/T showed excellent activity against non-carbapenemase-producing CRPA clinical isolates. We were unable to fully correlate C/T MIC values with specific mutation-driven resistance mechanisms.

## MATERIALS AND METHODS

**Bacterial isolates.** A total of 213 *P. aeruginosa* isolates (bacteremia, $n = 101$; urinary tract infection, $n = 112$) from eight South Korean hospitals assessed in a previous study (6) were considered for inclusion. Among 213 *P. aeruginosa* isolates, a total of 57 isolates (26.8%) resistant to imipenem and/or meropenem were determined to be resistant to carbapenems. Among the 57 CRPA isolates, 15 isolates were metallo-$\beta$-lactamase producers (13 $bla_{IMP-6}$ and 2 $bla_{VIM-2}$). Pathogens harboring carbapenemases, such as KPCs and metallo-$\beta$-lactamases, are known to be resistant to C/T (14), and these 15 isolates were not further studied in detail. Therefore, 42 non-carbapenemase-producing CRPA isolates, 14 of which were isolated from blood, 28 of which were isolated from urine, and all of which were negative for $bla_{IMP}$, $bla_{VIM}$, $bla_{SPM}$, $bla_{GIM}$, $bla_{SIM}$, and $bla_{KPC}$, were studied. These 42 isolates were evaluated for the presence of cryptic carbapenemases using the Carba NP test, as previously described (35, 36); they were all phenotypically negative for carbapenemase activity. Multilocus sequence typing (MLST) revealed 26 sequence types (STs). The expression of *oprD*, *ampC*, and several efflux pump genes had been previously defined by quantitative reverse transcription-PCR to define mechanisms conferring carbapenem resistance. Reduced *oprD* expression was considered relevant when it was ≤30% compared with that of *P. aeruginosa* PAO1. Strains were considered positive for *ampC*, *mexD*, *mexF*, or *mexY* overexpression when the corresponding mRNA level was at least 10-fold higher than that of PAO1, negative if it was lower than 5-fold, and borderline if it was between 5- and 10-fold. Strains were considered positive for *mexB* overexpression when the corresponding mRNA level was at least 3-fold higher than that of PAO1, negative if it was lower than 2-fold, and borderline if it was between 2- and 3-fold, according to previously defined criteria (37). All but three exhibited a relevant decrease in *oprD* expression compared to the reference strain. Twenty-nine overexpressed efflux pumps (primarily *mexB* but also *mexD*, *mexY*, and *mexF*) or *ampC*.

**Antimicrobial susceptibility testing.** In our previous work (6), susceptibility to 10 antimicrobial agents including imipenem, meropenem, piperacillin-tazobactam, cefepime, ceftazidime, tetracycline, ciprofloxacin, amikacin, polymyxin B, and colistin had been determined. In the current study, antimicrobial susceptibility to C/T and C/A was tested by broth microdilution according to the CLSI guidelines (38). Interpretation of susceptibility for all antimicrobial agents except C/A was done according to CLSI breakpoints (36). For C/A, the FDA susceptibility breakpoint (≤8/4 mg/liter) was applied. *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 served as quality control strains. All quality control results were within CLSI-specified ranges.

**Correlation of C/T or C/A activity with mechanisms of resistance.** The relationship between C/T or C/A activity and overexpression of efflux pumps or *ampC* and decreased *oprD* expression was also investigated.

***In vitro* time-kill studies.** Three CRPA isolates having different resistance mechanisms and PAO1 were selected for time-kill assays. Time-kill studies were performed according to a previously published study with some modifications (15). Briefly, freshly prepared colonies collected from the surface of an overnight agar culture were suspended in cation-adjusted Mueller-Hinton broth (CAMHB) and incubated for 1 to 2 h. Cultures were then diluted to a 0.5 McFarland standard (approximately $1.5 \times 10^8$ CFU/ml). An appropriate amount of bacteria was diluted in CAMHB to achieve a concentration of $5 \times 10^5$ CFU/ml in a final volume of 10 ml of CAMHB. Then, ceftolozane and ceftazidime were added to the prepared bacterial suspensions, so that the final drug concentration was 2 times, 4 times, or 8 times the MIC of ceftolozane and ceftazidime; tazobactam and avibactam were added to a final concentration of 4 mg/liter. A growth control with no antibiotic was also included. Tubes were incubated in a 37°C room air incubator with shaking (180 rpm); viability counts were performed at 0 h, 1 h, 4 h, 8 h, 12 h, and 24 h by removing 100 $\mu$l. A ≥3-$\log_{10}$ decrease in the number of CFU per milliliter was considered evidence of bactericidal activity.

**Statistical analysis.** Categorical variables were compared using Fisher's exact test. All tests were 2 sided, and $P$ values less than 0.05 were considered statistically significant. Statistical analysis was performed using PASW Statistics for Windows v.18.0 (SPSS Inc., Chicago, IL).

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