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BRIEF REPORT

In vitro Activity of Ceftolozane/Tazobactam Alone or with an Aminoglycoside Against Multi-Drug-Resistant *Pseudomonas aeruginosa* from Pediatric Cystic Fibrosis Patients

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ABSTRACT

Introduction: Gram-negative multi-drug resistance is an emerging threat among pediatric patients with cystic fibrosis (CF). Ceftolozane/tazobactam (C/T) is an extended-spectrum cephalosporin/beta-lactamase inhibitor combination that has been shown to maintain activity against MDR *P. aeruginosa* isolates. The understanding of C/T effectiveness in pediatric patients is extremely limited. Minimum inhibitory concentration (MIC) testing and time-kill analyses were performed to better understand the antimicrobial susceptibility and potential role of C/T.

Methods: Non-duplicate clinical respiratory MDR *P. aeruginosa* isolates ($n = 5$) from four pediatric CF patients were identified. MICs were determined for these isolates using CLSI broth microdilution methods. Time-kill analyses were performed using multiples of C/T alone, and combinations of C/T 2× and 8× the MIC with 30 mg/L tobramycin or 80 mg/L amikacin for all isolates. Cell counts were determined by serial dilution plating.

Results: Isolates had variable susceptibilities to C/T (range 0.5–8 mg/L), tobramycin (range 2 to >64 mg/L) and amikacin (range 8 to >256 mg/L). Time-kill analyses revealed an average of 0.71 (range −0.6 to 4.4), 1.50 (range 0.8–2.0) and 2.1 (range 1.2–3.4) log-kill at 4×, 8× and 16× the C/T MIC, respectively. At a tobramycin MIC of 32 mg/L, combination therapy showed synergistic benefit when the isolate was C/T susceptible. C/T and amikacin combination therapy showed synergistic activity at an amikacin MIC >256 mg/L when C/T MIC was 2 mg/L (4.7 log-kill at 2× C/T MIC and 4.0 log-kill at 8× C/T MIC).

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Conclusion: C/T appears to be a promising treatment option for treatment of MDR *P. aeruginosa* in pediatric CF patients, both alone and in combination with tobramycin or amikacin. Interestingly, the benefit of C/T combination therapy with amikacin may be more pronounced than with the addition of tobramycin. Further evaluation of such combination regimens in pediatric CF patients is warranted.

Keywords: Combination therapy; Cystic fibrosis; Pediatric; Pseudomonas; Time-kill

INTRODUCTION

The emergence of Gram-negative multi-drug resistance is an ongoing threat. Pediatric patients are not spared from these resistant organisms. Of particular concern is emerging Gram-negative resistance among pediatric patients with cystic fibrosis (CF), who are frequently exposed to broad-spectrum antibiotic therapy for the treatment of acute pulmonary exacerbations. Multi-drug-resistant *Pseudomonas aeruginosa* often leaves CF patients with few remaining treatment options and has a significant detrimental impact on patient care, including increased mortality [1]. New antimicrobial agents have been developed for the treatment of multi-drug-resistant organisms (MDRO), but data in pediatric patients are extremely limited.

Ceftolozane/tazobactam (Zerbaxa^{TM}) was approved by the FDA in December 2014 for the treatment of complicated intra-abdominal and urinary tract infections in adults [2]. Ceftolozane/tazobactam is an extended-spectrum cephalosporin/beta-lactamase inhibitor combination that has been shown to maintain activity against MDR *P. aeruginosa* strains, including respiratory strains from CF patients [3–6]. Further evaluation of ceftolozane/tazobactam drug activity against MDR *Pseudomonas* isolates from pediatric patients is warranted, given the potential utility of this antibiotic in the treatment of MDR *Pseudomonas* infection with limited remaining treatment options. To better understand the antimicrobial susceptibility and potential role of ceftolozane/tazobactam in children—used alone, and in combination with tobramycin or amikacin—we performed Minimum inhibitory concentrations (MICs) testing and time-kill analyses against *P. aeruginosa* isolates from pediatric CF patients.

METHODS

Microbiology

Five unique, non-duplicate clinical respiratory isolates of MDR *P. aeruginosa* (resistant to ≥3 classes as defined by MIC testing in the hospital microbiology laboratory) from four pediatric CF patients age 6–17 years were included. These isolates were collected at the discretion of the physician providing clinical care at Connecticut Children's Medical Center from 2014 to 2016. Isolates were identified through retrospective review of the monthly institution MDRO surveillance report and were obtained from the hospital microbiology laboratory. Once attained by the Center for Anti-Infective Research and Development (Hartford Hospital, Hartford, CT, USA), isolates were transferred onto Trypticase Soy Agar with 5% sheep blood (Becton, Dickinson, Sparks, MD, USA). The minimum inhibitory concentrations (MICs) were determined in triplicate using broth microdilution methodology, and are listed in Table 1. As recommended by Clinical

Table 1 Minimum inhibitory concentrations of five *P. aeruginosa* isolates against cefepime (*FEP*), ceftazidime (*CAZ*), ciprofloxacin (*CIP*), colistin (*CST*), aztreonam (*ATM*), piperacillin/tazobactam (*TZP*), meropenem (*MEM*), amikacin (*AMK*), fosfomycin (*FOF*), tobramycin (*TOB*) and ceftolozane/tazobactam (*C/T*)

Isolate	FEP	CAZ	CIP	CST	ATM	TZP	MEM	AMK	FOF	TOB	C/T
1554	64	>64	16	1	>64	256	16	32	64	>64	4
1555 (mucoid)	>64	>64	4	1	64	128	4	16	64	>64	4
1556	8	2	1	0.5	4	4	0.25	16	64	2	0.5
1557	8	4	2	1	1	2	0.25	256	>64	32	2
1558	16	32	4	2	32	>256	>64	8	64	32	8

Laboratory Standards Institute (CLSI), *P. aeruginosa* 27853 was utilized as a quality control strain and colony counts were performed on each isolate to verify the correct inoculum. All isolates were phenotypically assessed against ceftolozane/tazobactam, tobramycin, amikacin and other antibiotics according to CLSI guidelines [7, 8]. The modal MIC was used to characterize the isolate MICs. Merck (Kenilworth, NJ, USA) provided ceftolozane powder; tazobactam and all other antibiotics were purchased from Sigma Chemical (St. Louis, MO, USA).

Time-Kill Analyses

Time-kill analyses were performed on each of the 5 *P. aeruginosa* isolates. Each isolate was subcultured twice on Trypticase Soy Agar with 5% sheep blood, (Becton, Dickinson) and a bacterial suspension of 10^8 CFU/mL was prepared. Mueller–Hinton Broth (MHB) was inoculated with the bacterial suspension to a final suspension approximately 5×10^5 CFU/mL. Ceftolozane/tazobactam was added to achieve concentrations of 0.5×, 1×, 2×, 4×, 8×, 16× and 32× ceftolozane/tazobactam MICs alone, and combinations of ceftolozane/tazobactam 2× and 8× the MIC with 30 mg/L tobramycin and ceftolozane/tazobactam 2× and 8× the MIC with 80 mg/L amikacin for all isolates. Aminoglycoside concentrations were determined to mimic peak concentrations achieved with once-daily dosing in children. [9] Control experiments without active compound were conducted simultaneously with the time-kill studies. Final volumes for each bacterium-drug concentration were 10 mL and incubated at 37 °C. Samples were taken from each sample at 0, 3, 6, and 24 h from the time of adding the drug. Multiple 1:10 dilutions were made in saline and sub-cultured onto blood agar plates and incubated for 18–24 h, and mean bacterial densities were determined for each isolate. The minimal, accurately countable number of CFU/mL was determined to be 5×10^{-1} CFU/mL. [10] All studies were conducted in duplicate on different days, and the combined data are presented as mean bacterial density (CFU/mL) for all isolates. Bactericidal activity was defined as a decrease of $\geq$3-$\log_{10}$ from baseline bacterial density. Synergy was defined as a $\geq$2-$\log_{10}$ decrease in CFU/mL between the antibiotic combination and its most active constituent after 24 h when the number of surviving organisms in the presence of the combination was $\geq$2-$\log_{10}$ CFU/mL below the starting inoculum [10].

Compliance with Ethics Guidelines

This study was approved by the Connecticut Children's Medical Center Institutional Review Board, and does not contain any new studies with human or animal subjects performed by any of the authors.

RESULTS

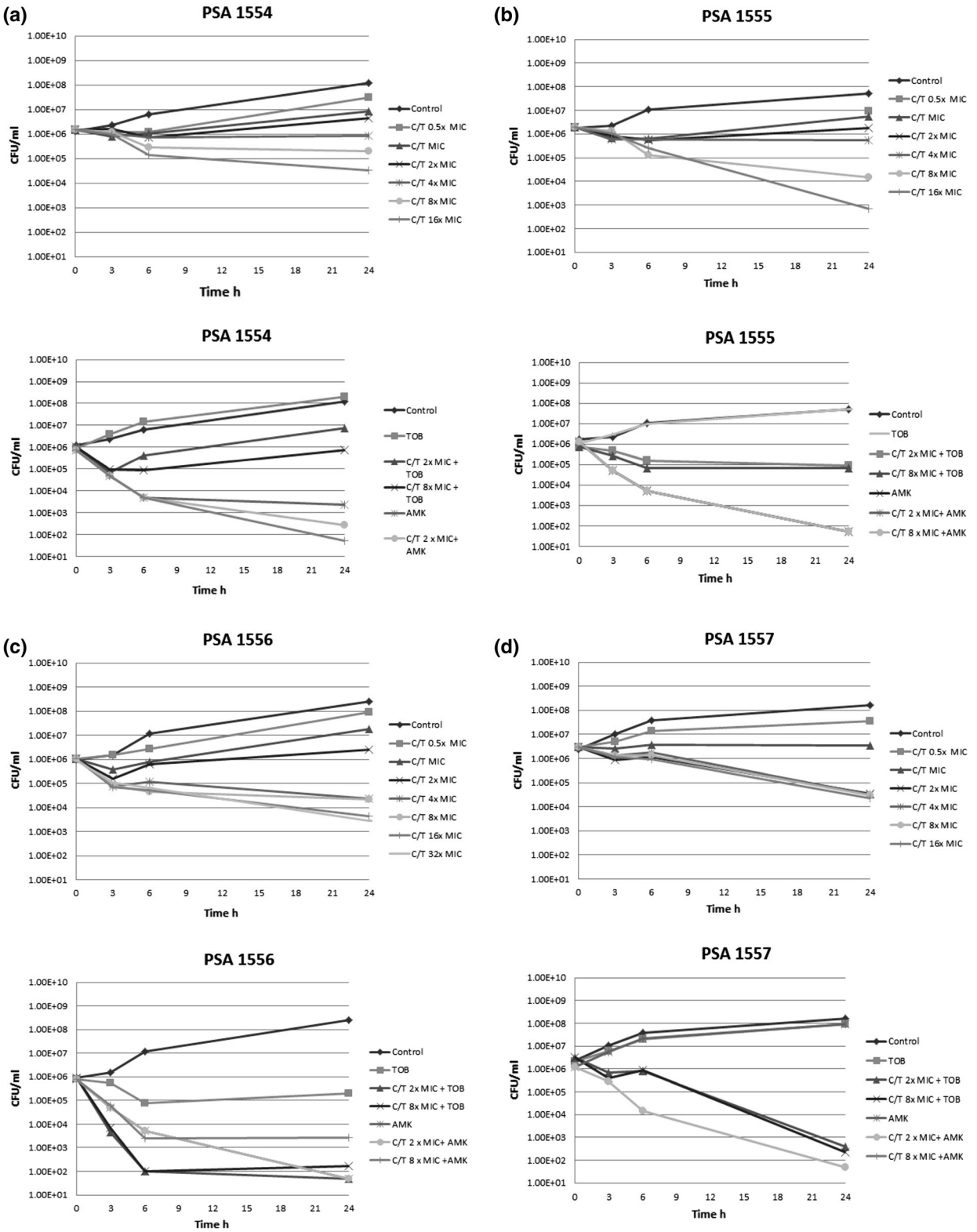
Isolates had variable susceptibilities to ceftolozane/tazobactam (range 0.5–8 mg/L), tobramycin (range 2 to >64 mg/L) and amikacin (range 8 to >256 mg/L). One isolate (1555) was identified as a mucoid strain. Mean bacterial densities over 24 h for all *P. aeruginosa* isolates tested against multiples of their ceftolozane/tazobactam MICs alone and in combination with either tobramycin or amikacin are shown in Fig. 1. Time-kill analyses revealed an average of 0.71 (range −0.6 to 4.4), 1.50 (range 0.8–2.0) and 2.1 (range 1.2–3.4) log-kill at 4×, 8× and 16× the ceftolozane/tazobactam MIC, respectively. Bactericidal killing was generally not seen until 16× ceftolozane/tazobactam MICs. At a tobramycin MIC of 2 mg/L, combination therapy with tobramycin and ceftolozane/tazobactam 2× and 8× the MIC showed a 4.3 and 3.7 log-kill, respectively. At a tobramycin MIC of 32 mg/L, combination therapy showed synergistic benefit when the isolate was ceftolozane/tazobactam susceptible (3.8 and 4.0 log-kill with 2× and 8× a ceftolozane/tazobactam MIC of 2 mg/L, respectively). No benefit of tobramycin combination therapy was seen among tobramycin non-susceptible isolates at a tobramycin MIC >64 mg/L.

When compared to the extent of killing with amikacin therapy alone, there was no additional benefit of amikacin and ceftolozane/tazobactam combination therapy at an amikacin MIC of 16 mg/L. Combination therapy with amikacin and ceftolozane/tazobactam 2× and 8× the MIC at an amikacin MIC of 8 mg/L resulted in a 2.2 and 4.6 log-kill, respectively, despite the isolate being ceftolozane/tazobactam non-susceptible (MIC 8 mg/L). Despite amikacin non-susceptibility, synergistic activity was seen with amikacin and ceftolozane/tazobactam combination therapy at an amikacin MIC >256 mg/L and ceftolozane/tazobactam MIC of 2 mg/L (4.7 log-kill at 2× C/T MIC and 4.0 log-kill at 8× ceftolozane/tazobactam MIC).

Fig. 1 Mean bacterial densities of five *P. aeruginosa* ► isolates over 24 h when exposed to multiple concentrations of C/T alone and in combination with TOB and AMK. **a** C/T MIC = 4 mg/L; TOB MIC >64 mg/L; AMK MIC = 32 mg/L, **b** C/T MIC = 4 mg/L; TOB MIC >64 mg/L; AMK = 16 mg/L; mucoid strain, **c** C/T MIC = 0.5 mg/L; TOB MIC = 2 mg/L; AMK MIC = 16 mg/L, **d** C/T MIC = 2 mg/L; TOB MIC = 32 mg/L; AMK MIC >256 mg/L, **e** C/T MIC = 8 mg/L; TOB MIC = 32 mg/L; AMK MIC = 8 mg/L. *MIC* minimum inhibitory concentration; for other abbreviatioms, see Table 1

DISCUSSION

To the best of our knowledge, this is the first study to perform time-kill analyses of ceftolozane/tazobactam alone and in combination with other agents against *P. aeruginosa* isolates from pediatric CF patients. These data suggest that killing of *P. aeruginosa* isolates appears to require concentrations of at least 8× the ceftolozane/tazobactam MIC, with the extent of ceftolozane/tazobactam activity decreasing as MICs increase. Given the multiples of ceftolozane/tazobactam MICs tested, these data are likely in agreement with


(a)
PSA 1554
CFU/ml
Time h
Control
C/T 0.5x MIC
C/T MIC
C/T 2x MIC
C/T 4x MIC
C/T 8x MIC
C/T 16x MIC
(b)
PSA 1555
CFU/ml
Time h
Control
C/T 0.5x MIC
C/T MIC
C/T 2x MIC
C/T 4x MIC
C/T 8x MIC
C/T 16x MIC
PSA 1554
CFU/ml
Time h
Control
TOB
C/T 2x MIC + TOB
C/T 8x MIC + TOB
AMK
C/T 2 x MIC+ AMK
PSA 1555
CFU/ml
Time h
Control
TOB
C/T 2x MIC + TOB
C/T 8x MIC + TOB
AMK
C/T 2 x MIC+ AMK
C/T 8 x MIC +AMK
(c)
PSA 1556
CFU/ml
Time h
Control
C/T 0.5x MIC
C/T MIC
C/T 2x MIC
C/T 4x MIC
C/T 8x MIC
C/T 16x MIC
C/T 32x MIC
(d)
PSA 1557
CFU/ml
Time h
Control
C/T 0.5x MIC
C/T MIC
C/T 2x MIC
C/T 4x MIC
C/T 8x MIC
C/T 16x MIC
PSA 1556
CFU/ml
Time h
Control
TOB
C/T 2x MIC + TOB
C/T 8x MIC + TOB
AMK
C/T 2 x MIC+ AMK
C/T 8 x MIC +AMK
PSA 1557
CFU/ml
Time h
Control
TOB
C/T 2x MIC + TOB
C/T 8x MIC + TOB
AMK
C/T 2 x MIC+ AMK
C/T 8 x MIC +AMK

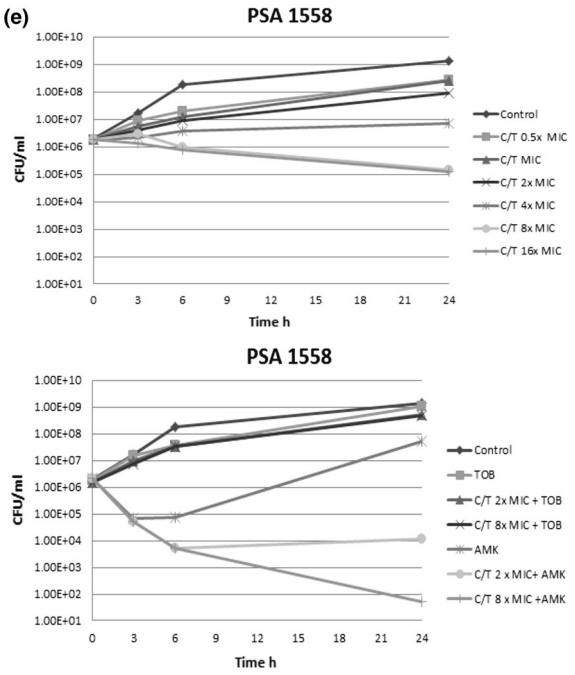


Fig. 1 continued

the concentration/MIC ratio of 6.6 suggested to optimize the in vivo antibiotic effect of ceftazidime against *P. aeruginosa* isolates from CF patients [11]. Further, the retained activity of ceftolozane/tazobactam against the tested mucoid *P. aeruginosa* strain supports previously published data, and suggests a potential role of this antimicrobial against mucoid strains from CF respiratory isolates [12]. The pharmacokinetics of ceftolozane/tazobactam in pediatric CF patients are yet to be explained, but a phase-one, open-label study evaluating the pharmacokinetics and safety of a single IV dose of ceftolozane/tazobactam in pediatric patients is currently enrolling (NCT02266706) [13]. Yet, the preserved ceftolozane/tazobactam MICs and susceptibility of *P. aeruginosa* isolates from pediatric CF patients reported here and in other studies is promising when considering the likelihood of attaining adequate ceftolozane/tazobactam pharmacodynamic targets [5].

The effect of tobramycin at concentrations equal to the MIC appears greater in isolates with a lower ceftolozane/tazobactam MIC. Overall, a synergistic effect of ceftolozane/tazobactam and tobramycin was evident among *P. aeruginosa* isolates that were largely ceftolozane/tazobactam and tobramycin susceptible and among isolates with low-level tobramycin resistance that remained ceftolozane/tazobactam susceptible. While the addition of tobramycin does not appear to enhance ceftolozane/tazobactam activity in isolates that are highly tobramycin resistant, the addition of amikacin to ceftolozane/tazobactam appears to provide synergistic activity against highly-amikacin resistant, ceftolozane/tazobactam-susceptible strains. Additionally, synergistic activity of amikacin and ceftolozane/tazobactam at 8× the MIC was seen when the isolate was ceftolozane/tazobactam resistant but amikacin susceptible.

The size of this study is a limitation, as only a small number of isolates ($n = 5$) from a small number of patients ($n = 4$) were evaluated.

CONCLUSION

Ceftolozane/tazobactam appears to be a promising treatment option for treatment of MDR *P. aeruginosa* in pediatric CF patients. Interestingly, the benefit of ceftolozane/tazobactam combination therapy with amikacin may be more pronounced than with the addition of tobramycin. Further evaluation of such combination regimens is warranted in pediatric CF patients, specifically when a better understanding of ceftolozane/tazobactam pharmacokinetics in

this population has been elucidated and dosing optimization strategies can be determined.

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Disclosures. Aimee M. Dassner, Christina Sutherland, Jennifer Girotto and David P. Nicolau declare that they have no conflict of interest.

Compliance with Ethics Guidelines. This study was approved by the Connecticut Children's Medical Center Institutional Review Board, and does not contain any new studies with human or animal subjects performed by any of the authors.

Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

REFERENCES

1. Lanes C, Pourcel C, Richardot C, et al. Diversity of β-lactam resistance mechanisms in cystic fibrosis isolates of *Pseudomonas aeruginosa*: a French multicenter study. J Antimicrob Chemother. 2013;68:1763–71.

2. ZERBAXA™ (ceftolozane/tazobactam) Current Prescribing Information. http://www.accessdata.fda.gov/drugsatfda_docs/label/2014/206829lbl.pdf. Accessed June 10, 2016.

3. Sutherland CA, Nicolau DP. Susceptibility profile of ceftolozane/tazobactam and other parenteral antimicrobials against *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* from US hospitals. Clin Therapeutics. 2015;37(7):1564–71.

4. Zamarano L, Juan C, Fernandez-Olmos A, et al. Activity of the new cephalosporin CXA-101 (FR264205) against *Pseudomonas aeruginosa* isolates from chronically-infected cystic fibrosis patients. Clin Microbiol Infect. 2010;16:1482–7.

5. Kuti JL, Pettit RS, Neu N, et al. Microbiological activity of ceftolozane/tazobactam, ceftazidime, meropenem and piperacillin/tazobactam against *Pseudomonas aeruginosa* isolated from children with cystic fibrosis. Diagn Microbiol Infect Dis. 2015;83:53–5.

6. Buehrle DJ, Shields RK, Liang C, et al. Evaluation of the in vitro activity of ceftazidime-avibactam and ceftolozane-tazobactam against meropenem-resistant *Pseudomonas aeruginosa* isolates. Antimicrob Agents Chemother. 2016;60(5):3227–31.

7. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-ninth edition, M07-A9. Wayne: CLSI; 2012.

8. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement (M100-S24). Wayne: CLSI; 2014.

9. Young DC, Zobell JT, Stockmann C, et al. Optimization of anti-pseudomonal antibiotics for

cystic fibrosis pulmonary exacerbations: V. Aminoglycosides. Pediatr Pulmonol. 2013;48(11):1047–61.

10. American Society for Microbiology. Instructions to authors. Antimicrob. Agents Chemother. 2016. http://jcm.asm.org/site/misc/2016AugustJCMITA.pdf. Accessed June 17, 2016.

11. Pharmacokinetic and safety study of ceftolozane/tazobactam in pediatric participants receiving antibiotic therapy for proven or suspected gram-negative infection or for peri-operative prophylaxis (MK-7625A-010). https://clinicaltrials.gov/ct2/show/NCT02266706. Accessed June 17, 2016.

12. Riera E, Macia MD, Mena A, et al. Anti-biofilm and resistance suppression activities of CXA-101 against chronic respiratory infection phenotypes of *Pseudomonas aeruginosa* strain PAO1. J Antimicrob Chemother. 2012;65:1299–404.

13. Manduru M, Mihm L, White RL, et al. In vitro pharmacodynamics of ceftazidime against *Pseudomonas aeruginosa* isolates from cystic fibrosis patients. Antimicrob Agents Chemother. 1997;41(9):2053–6.