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Antimicrobial Susceptibility Studies

Evaluation of *in vitro* activity of ceftolozane-tazobactam compared to other antimicrobial agents against *Pseudomonas aeruginosa* isolates from cystic fibrosis patients

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ABSTRACT

The *in vitro* activity of ceftolozane-tazobactam (C-T) was evaluated comparatively to other antibiotics against 188 *Pseudomonas aeruginosa* isolates collected from cystic fibrosis (CF) patients. Overall, the activity of C-T was comparable to colistin (susceptibility rate: 85.1% vs. 89.4%) but significantly higher than other antimicrobials. Particularly, C-T was active against 70% of meropenem nonsusceptible isolates and 64.1% of those nonsusceptible to beta-lactams. C-T was active against 70%, 58.1%, and 100% of multidrug-resistant, extensively drug-resistant (XDR), and pandrug-resistant isolates, respectively. No differences in C-T activity were found between isolates from children and adult patients, except for XDR ones significantly more susceptible in older patients. C-T and colistin exhibited comparable susceptibility rate (91.1% vs. 86.7%) also against 68 isolates collected during pulmonary exacerbations. Activity of C-T towards mucoid isolates was less than colistin (82.9% vs. 97.6%) but higher compared with other antibiotics. C-T represents a promising agent for treating CF lung infections.

1. Introduction

Carbapenems remain effective in treating serious multidrug-resistant (MDR) *P. aeruginosa* infections, although carbapenem-resistant *P. aeruginosa* has emerged worldwide as an important nosocomial pathogen, particularly in immunocompromised patients (Bodey et al., 1985; Poole, 2011). Infections caused by resistant *P. aeruginosa* are of concern in many hospitals since they are associated with a delayed start of appropriate antibiotic therapy and with significant mortality (Giamarellou, 2002). MDR *P. aeruginosa* infections are frequently resistant to carbapenems and other beta-lactams. *P. aeruginosa* resistance to beta-lactams is mediated through multiple mechanisms, including the acquisition of metallo-beta-lactamases, increased production of chromosomal AmpC, increased drug efflux, and changes in membrane permeability (Castanheira et al., 2014; Lister et al., 2009).

P. aeruginosa is the most common pathogen in cystic fibrosis (CF), colonizing the respiratory tract of approximately 65% of adult CF patients (Flume et al., 2009). Frequent acute pulmonary exacerbations during *P. aeruginosa* infection are responsible for progressive pulmonary decline, which contributes to early CF patient mortality (Flume et al., 2009). In these patients, the repeated exposure to intravenous antibiotics selects many *P. aeruginosa* MDR isolates (Chen et al., 2007; Smith et al., 2016); furthermore, the formation of intrinsically antibiotic-resistant biofilms during chronic infection renders treatment of exacerbations more challenging, highlighting the need for new antibiotics (Llanes et al., 2013).

Ceftolozane-tazobactam (C-T) is a novel fifth-generation cephalosporin-beta-lactamase inhibitor combination with broad-spectrum activity against Gram-negative bacteria, including MDR *P. aeruginosa*. C-T was approved by the U.S. Food and Drug Administration in 2014 and by the European Medicines Agency in 2015 for the treatment of complicated intra-abdominal infections, in combination with metronidazole, and complicated urinary tract infections (van Duin and Bonomo, 2016). Ceftolozane has stability against chromosomal AmpC-lactamases, overexpressed MexAB-OprM efflux pumps, and deleted OprD porins (Livermore et al., 2009); its activity against *P. aeruginosa* is due to its affinity for the penicillin-binding proteins (Zhanel et al., 2014). C-T demonstrates activity against many MDR *P. aeruginosa*, including carbapenemase-nonproducing carbapenem-resistant strains (Wright et al., 2017).

The goal of this study was to evaluate the *in vitro* activity of C-T against a collection of not duplicated, consecutive, *P. aeruginosa* isolates colonizing the respiratory tract of CF patients admitted to an Italian CF

Abbreviations: C-T, ceftolozane-tazobactam; CF, cystic fibrosis; MDR, multidrug-resistant; XDR, extensively drug-resistant; PDR, pandrug-resistant.

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center. This collection also included MDR, extensively drug-resistant (XDR), and pandrug-resistant (PDR) *P. aeruginosa* isolates.

2. Materials and methods

2.1. Bacterial isolates

During the period January 2010–June 2016, 188 not duplicated, consecutive, *P. aeruginosa* isolates from respiratory tract specimens of CF patients (age: <1 to 50 years old) were collected during routine clinic visits or hospitalization for pulmonary exacerbation at the CF center "Bambino Gesù" Hospital, Rome. Exacerbations were characterized by a cluster of symptoms and signs as previously described (Fuchs et al., 1994). Among 188 isolates tested, 40 were isolated as pure culture, whereas the remaining 144 were co-cultured with other bacterial or fungal species. Isolates were identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (Bruker Daltonics MALDI Biotyper) according to the manufacturer's instructions. Isolates were stored at −80 °C in Cryobank™ (Copan Diagnostics Inc; Murrieta, CA; USA) and subcultured twice onto sheep blood agar plates prior to testing.

2.2. Antimicrobial susceptibility testing

The following antibiotics were tested: ceftazidime (cephalosporin), meropenem (carbapenem), piperacillin-tazobactam (broad-spectrum penicillin combined with a beta-lactamase inhibitor), ciprofloxacin (fluoroquinolone), tobramycin (aminoglycoside), and colistin (polymyxin). MICs for ceftazidime, meropenem, piperacillin-tazobactam, ciprofloxacin, and tobramycin were determined using the MIC gradient method by MIC Test Strip (MTS, Liofilchem; Roseto degli Abruzzi, Italy), whereas MICs for both colistin and C-T were determined by the microdilution broth method using MICRONAUT-S MHK Pseudomonas 2 (Merlin Diagnostika GmbH; Germany), according to the manufacturers' recommendations. C-T was tested at concentrations of <1, 1, 2, 4, 8, and >8 μg/mL. Categorical interpretations for all antimicrobials were those found in EUCAST guidelines (http://www.eucast.org/clinical_breakpoints/). Particularly, susceptibility to C-T was considered for MIC ≤ 4/4 μg/mL, whereas resistance for MIC > 4/4 μg/mL. Quality control was performed using *P. aeruginosa* ATCC 27853, and results were interpreted according to EUCAST guidelines (http://www.eucast.org/ast_of_bacteria/qc_tables/). CLSI criteria were used to categorize an isolate as MDR (nonsusceptible to at least 1 agent in ≥3 antimicrobial categories tested), XDR (nonsusceptible to at least 1 agent in all but 2 or fewer antimicrobial categories tested), or PDR (nonsusceptible to all antimicrobial classes, including colistin) (Magiorakos et al., 2012), regardless of C-T susceptibility results.

This study received approval by the Ethical Committee of the "Bambino Gesù" Hospital.

2.3. Statistical analysis

Each susceptibility test was performed in triplicate and repeated on at least 2 different occasions. In the case of meropenem, isolates classified as "intermediate susceptible" were considered as "resistant". The significance between differences were statistically measured by chi-square test, considering as significant a *P* value less than 0.05.

3. Results

Overall, 188 not duplicated *P. aeruginosa* isolates were collected. The average patient age was 18.1 years, with 47.9% and 52.1% of male and female patients, respectively. A total of 98 and 90 *P. aeruginosa* isolates were respectively recovered from children (≤18 years old) and adult (>18 years old) patients.

The susceptibility profile of the strains considered as a whole is summarized in Table 1. The overall susceptibility to C-T was comparable to colistin (85.1% vs. 89.4%, respectively) but significantly higher than that observed for other antibiotics tested (71.8%, 71.8%, 66%, 63.8%, and 61.2%, respectively for ceftazidime, piperacillin-tazobactam, tobramycin, ciprofloxacin and meropenem; *P* at least <0.01 vs. C-T and colistin).

When comparing susceptibilities of meropenem-nonsusceptible isolates, C-T activity was comparable to that exhibited by colistin (susceptibility rate: 70.0% vs. 79.5%, respectively) but significantly higher than that observed for other antibiotics (susceptibility rate: 42.5%, 38.4%, 35.6% and 24.7%, respectively for piperacillin-tazobactam, ceftazidime, tobramycin, and ciprofloxacin; *P* at least <0.01).

A similar trend was observed for the isolates nonsusceptible to the 3 antipseudomonal beta-lactam agents tested in this study, namely, ceftazidime, meropenem, and piperacillin-tazobactam: C-T was significantly less active than colistin (susceptibility rate: 64.1% vs. 84.6%, respectively; *P*<0.05), although significantly more active compared with tobramycin and ciprofloxacin (susceptibility rate of 20.5% for both; *P*<0.001). Considering the isolates resistant to colistin, their susceptibility to C-T was comparable to that observed for ceftazidime, piperacillin-tazobactam, and tobramycin (75% vs. 65%, 65%, and 50%, respectively) but significantly higher than meropenem and ciprofloxacin (25% and 15%, respectively; *P* at least <0.01).

Overall, 20 (10.6%), 43 (22.8%), and 4 (2.1%) isolates were classified as MDR, XDR, and PDR, respectively. Considering MDR isolates, C-T showed comparable activity compared with piperacillin-tazobactam, colistin, ceftazidime, tobramycin, and meropenem (susceptibility rate: 70% vs. 70%, 65%, 60%, 45% and 30%, respectively) but significantly higher than that exerted by ciprofloxacin (30% susceptibility; *P*<0.05). Considering XDR isolates, C-T showed lower efficacy compared with colistin (susceptibility rate: 58.1% vs. 86% respectively; *P*<0.01) but significantly higher than that showed by other comparators (susceptibility rate: 16.3%, 16.3%, 16.3%, 11.6%, and 2.3%, respectively, for piperacillin-tazobactam, tobramycin, ciprofloxacin, ceftazidime, and meropenem; *P*<0.001).

Finally, it is worth noting that C-T is the only antibiotic active against all 4 PDR isolates.

The most effective antibiotic against isolates resistant to C-T was colistin (susceptibility rate: 82.2% vs. 39.3%, 28.6%, 25%, 21.3%, and 21.3%, respectively for piperacillin-tazobactam, ciprofloxacin, ceftazidime, tobramycin, and meropenem; *P* at least <0.01) (data not shown).

The antibiotic susceptibility was also stratified on the patients' age, and results are summarized in Table 2. The overall susceptibility rate to C-T was found comparable in both children and adult patients (87.6% and 82.2%, respectively). No statistically significant differences in C-T activity were found between age groups, except for XDR isolates whose susceptibility to C-T was higher in adult than younger patients (67.7% vs. 33.3%, respectively; *P*<0.05). The prevalence of both MDR and XDR isolates was higher in adult than in younger patients (MDR: 17.7% vs. 4.1%, respectively; *p*<0.01; XDR: 34.4% vs. 12.2%, respectively; *P*<0.001), whereas no differences were found regarding PDR isolates (susceptibility rate: 3.3% vs. 1%, respectively).

Considering the isolates from younger patients only, C-T showed comparable activity to other antibiotics tested, regardless of nonsusceptible/resistance patterns considered, except for XDR isolates against whom C-T was more active than ceftazidime and meropenem (susceptibility rate: 33.3% vs. 0% and 0%, respectively; *P*<0.05) but less active than colistin (susceptibility rate: 33.3% vs. 91.7%, respectively; *P*<0.01).

Different susceptibility trends were observed among isolates from adult patients only: C-T showed comparable activity to colistin but significantly higher than that exhibited by other antibiotics when tested against isolates considered as a whole, those nonsusceptible to meropenem, those simultaneously nonsusceptible to ceftazidime, meropenem, and piperacillin-tazobactam, and against XDR isolates (susceptibility rate to C-T: 82.2%, 75.9%, 71.4%, and 67.7%, respectively; *P* at least < 0.05). Against MDR isolates, C-T activity was comparable to piperacillin-tazobactam, ceftazidime, colistin, and tobramycin

Table 1
Activity of ceftolozane-tazobactam and comparators against 188 not duplicated *P. aeruginosa* CF isolates.

	MIC (μg/ml)[a]			Susceptibility[b] no. (%)		
Antimicrobial agent	50 %	90 %	Range	S	I	R
All isolates ($n = 188$)						
Ceftolozane-tazobactam	≤1	≥8	≤1–≥8	160 (85.1)	0	28 (14.9)
Ceftazidime	2	≥256	0.38–≥256	135 (71.8)	0	53 (28.2)
Ciprofloxacin	0.38	≥32	0.023–≥32	120 (63.8)	0	68 (36.2)
Colistin	0.5	4	0.5–≥8	168 (89.4)	0	20 (10.6)
Meropenem	0.5	≥32	0.032–≥32	115 (61.2)	24 (12.8)	49 (26.0)
Piperacillin-tazobactam	6	≥256	0.125–≥256	135 (71.8)	0	53 (28.2)
Tobramycin	1	384	0.064–≥1024	124 (66.0)	0	64 (34.0)
Meropenem-nonsusceptible isolates ($n = 73$)						
Ceftolozane-tazobactam	2	≥8	≤1–≥8	51 (70.0)	0	22 (30.0)
Ceftazidime	64	≥256	0.5–≥256	28 (38.4)	0	45 (61.6)
Ciprofloxacin	1.5	≥32	0.094–≥32	18 (24.7)	0	55 (75.3)
Colistin	0.5	≥8	0.5–≥8	58 (79.5)	0	15 (20.5)
Meropenem	≥32	≥32	3–≥32	0	49 (67.1)	24 (32.9)
Piperacillin-tazobactam	24	≥256	0.38–≥256	31 (42.5)	0	42 (57.5)
Tobramycin	12	≥1024	0.38–≥1024	26 (35.6)	0	47 (64.4)
Nonsusceptible to ceftazidime, meropenem, and piperacillin-tazobactam ($n = 39$)						
Ceftolozane-tazobactam	2	≥8	≤1–≥8	25 (64.1)	0	14 (35.9)
Ceftazidime	≥256	≥256	12–≥256	0	0	39 (100.0)
Ciprofloxacin	2	≥32	0.094–≥32	8 (20.5)	0	31 (79.5)
Colistin	0.5	≥8	0.5–≥8	33 (84.6)	0	6 (15.4)
Meropenem	≥32	≥32	4–≥32	0	6 (15.4)	33 (84.6)
Piperacillin-tazobactam	≥256	≥256	24–≥256	0	0	39 (100.0)
Tobramycin	16	≥1024	0.5–≥1024	8 (20.5)	0	31 (79.5)
Colistin-resistant isolates ($n = 20$)						
Ceftolozane-tazobactam	≤1	8	≤1–≥8	15 (75.0)	0	5 (25.0)
Ceftazidime	4	≥256	0.38–≥256	13 (65.0)	0	7 (35.0)
Ciprofloxacin	3	≥32	0.094–≥32	3 (15.0)	0	17 (85.0)
Colistin	≥8	≥8	4–≥8	0	0	20 (100.0)
Meropenem	≥32	≥32	0.094–≥32	5 (25.0)	4 (20.0)	11 (55.0)
Piperacillin-tazobactam	4	≥256	0.75–≥256	13 (65.0)	0	7 (35.0)
Tobramycin	4	≥1024	0.38–≥1024	10 (50.0)	0	10 (50.0)
MDR isolates ($n = 20$)						
Ceftolozane-tazobactam	≤1	≥8	≤1–≥8	14 (70.0)	0	6 (30.0)
Ceftazidime	8	≥256	0.38–≥256	12 (60.0)	0	8 (40.0)
Ciprofloxacin	1.5	≥32	0.094–≥32	6 (30.0)	0	14 (70.0)
Colistin	0.5	≥8	0.5–≥8	13 (65.0)	0	7 (35.0)
Meropenem	4	≥32	0.094–≥32	6 (30.0)	6 (30.0)	8 (40.0)
Piperacillin-tazobactam	8	≥256	0.75–≥256	14 (70.0)	0	6 (30.0)
Tobramycin	12	≥1024	0.5–≥1024	9 (45.0)	0	11 (55.0)
XDR isolates ($n = 43$)						
Ceftolozane-tazobactam	4	≥8	≤1–≥8	25 (58.1)	0	18 (41.9)
Ceftazidime	≥256	≥256	0.5–≥256	5 (11.6)	0	38 (88.4)
Ciprofloxacin	3	≥32	0.094–≥32	7 (16.3)	0	36 (83.7)
Colistin	0.5	≥8	0.5–≥8	37 (86.0)	0	6 (14.0)
Meropenem	≥32	≥32	1.5–≥32	1 (2.3)	7 (16.3)	35 (81.4)
Piperacillin-tazobactam	≥256	≥256	0.75–≥256	7 (16.3)	0	36 (83.7)
Tobramycin	48	≥1024	1–≥1024	7 (16.3)	0	36 (83.7)
PDR isolates ($n = 4$)						
Ceftolozane-tazobactam	n.a.	n.a.	≤1–4	4 (100.0)	0	0
Ceftazidime	n.a.	n.a.	≥256–≥256	0	0	4 (100.0)
Ciprofloxacin	n.a.	n.a.	3–≥32	0	0	4 (100.0)
Colistin	n.a.	n.a.	8–≥8	0	0	4 (100.0)
Meropenem	n.a.	n.a.	≥32–≥32	0	0	4 (100.0)
Piperacillin-tazobactam	n.a.	n.a.	≥256–≥256	0	0	4 (100.0)
Tobramycin	n.a.	n.a.	8–≥1024	0	0	4 (100.0)
XDR and PDR isolates ($n = 47$)						
Ceftolozane-tazobactam	4	≥8	≤1–≥8	29 (61.7)	0	18 (38.3)
Ceftazidime	≥256	≥256	0.5–≥256	5 (10.6)	0	42 (89.4)
Ciprofloxacin	3	≥32	0.094–≥32	7 (14.9)	0	40 (85.1)
Colistin	0.5	≥8	0.5–≥8	37 (78.7)	0	10 (21.3)
Meropenem	≥32	≥32	1.5–≥32	1 (2.1)	7 (14.9)	39 (83.0)
Piperacillin-tazobactam	≥256	≥256	0.75–≥256	7 (14.9)	0	40 (85.1)
Tobramycin	48	≥1024	1–≥1024	7 (14.9)	0	40 (85.1)

[a] n.a.= not applicable, due to the low number of strains.
[b] According to the EUCAST breakpoints: S = susceptible; I = intermediate; R = resistant.

(susceptibility rate: 75% vs. 75%, 68.8%, 68.8%, and 43.7%, respectively), whereas it exhibited higher activity than meropenem and ciprofloxacin (susceptibility rate: 25% and 18.9%, respectively; $p<0.01$).

Forty-one of the 188 isolates (21.8%) grew with a mucoid phenotype, mostly isolated from adult than younger patients (78% vs. 22%, $p<0.0001$). The susceptibility to C-T was less than colistin (82.9% vs. 97.6%, respectively; $P<0.001$) but higher compared with other antibiotics [51.2%, 48.8%, 48.8%, 43.9%, and 43.9%, respectively, for ceftazidime, tobramycin, piperacillin/tazobactam, meropenem, and ciprofloxacin (P at least <0.05) (data not shown)].

Table 2
Activity of ceftolozane-tazobactam and comparators against 98 *P. aeruginosa* CF isolates from children (≤18 years old) and against 90 isolates from adults (>18 years old).

	Susceptibility[a] no. (%) in children			Susceptibility[a] no. (%) in adults		
Antimicrobial agent	S	I	R	S	I	R
All isolates (children = 98; adults = 90)						
Ceftolozane-tazobactam	86 (87.6)	0	12 (12.4)	74 (82.2)	0	16 (17.8)
Ceftazidime	79 (80.6)	0	19 (19.4)	56 (62.2)	0	34 (37.8)
Ciprofloxacin	83 (84.7)	0	15 (15.3)	37 (41.1)	0	53 (58.9)
Colistin	93 (94.9)	0	5 (5.1)	75 (83.3)	0	15 (16.7)
Meropenem	79 (80.6)	8 (8.2)	11 (19.4)	36 (40)	16 (17.8)	38 (42.2)
Piperacillin-tazobactam	79 (80.6)	0	19 (19.4)	56 (62.2)	0	34 (37.8)
Tobramycin	80 (81.6)	0	18 (18.4)	43 (47.8)	0	47 (52.2)
Meropenem-nonsusceptible isolates (children = 19; adults = 54)						
Ceftolozane-tazobactam	10 (52.6)	0	9 (47.4)	41 (75.9)	0	13 (24.1)
Ceftazidime	5 (26.3)	0	14 (73.7)	23 (42.6)	0	21 (57.4)
Ciprofloxacin	8 (42.1)	0	11 (57.9)	10 (18.5)	0	44 (81.5)
Colistin	15 (78.9)	0	4 (21.1)	43 (79.6)	0	11 (20.4)
Meropenem	0	8 (42.1)	11 (57.9)	0	16 (29.6)	38 (70.4)
Piperacillin-tazobactam	7 (36.8)	0	12 (63.2)	24 (44.4)	0	20 (55.6)
Tobramycin	7 (36.8)	0	12 (63.2)	18 (33.3)	0	36 (66.7)
Nonsusceptible to ceftazidime, meropenem, and piperacillin-tazobactam (children = 11; adults = 28)						
Ceftolozane-tazobactam	5 (45.5)	0	6 (54.5)	20 (71.4)	0	8 (28.6)
Ceftazidime	0	0	11 (100)	0	0	28 (100)
Ciprofloxacin	3 (27.3)	0	8 (72.7)	5 (17.9)	0	23 (82.1)
Colistin	9 (81.8)	0	2 (18.2)	24 (85.7)	0	4 (14.3)
Meropenem	0	4 (36.4)	7 (63.6)	0	2 (7.1)	26 (92.9)
Piperacillin-tazobactam	0	0	11 (100)	0	0	28 (100)
Tobramycin	2 (18.2)	0	9 (81.8)	5 (17.9)	0	23 (82.1)
Colistin-resistant isolates (children = 5; adults = 15)						
Ceftolozane-tazobactam	4 (80)	0	1 (20)	11 (73.3)	0	4 (26.7)
Ceftazidime	2 (40)	0	3 (60)	11 (73.3)	0	4 (26.7)
Ciprofloxacin	2 (40)	0	3 (60)	1 (6.7)	0	14 (93.3)
Colistin	0	0	5 (100)	0	0	15 (100)
Meropenem	1 (20)	1 (20)	3 (60)	4 (26.7)	3 (20)	8 (53.3)
Piperacillin-tazobactam	2 (40)	0	3 (60)	11 (73.3)	0	4 (26.7)
Tobramycin	4 (80)	0	1 (20)	6 (40)	0	9 (60)
MDR isolates (children = 4; adults = 16)						
Ceftolozane-tazobactam	2 (50)	0	2 (50)	12 (75)	0	4 (25)
Ceftazidime	1 (25)	0	3 (75)	11 (68.8)	0	5 (31.2)
Ciprofloxacin	3 (75)	0	1 (25)	3 (18.9)	0	13 (81.1)
Colistin	2 (50)	0	2 (50)	11 (68.8)	0	5 (31.2)
Meropenem	2 (50)	0	2 (50)	4 (25)	6 (37.5)	6 (37.5)
Piperacillin-tazobactam	2 (50)	0	2 (50)	12 (75)	0	4 (25)
Tobramycin	2 (50)	0	2 (50)	7 (43.7)	0	9 (56.3)
XDR isolates (children = 12; adults = 31)						
Ceftolozane-tazobactam	4 (33.3)	0	8 (66.7)	21 (67.7)	0	10 (32.3)
Ceftazidime	0	0	12 (100)	5 (16.1)	0	26 (83.9)
Ciprofloxacin	3 (25)	0	9 (75)	4 (12.9)	0	27 (87.1)
Colistin	11 (91.7)	0	1 (8.3)	26 (83.9)	0	5 (16.1)
Meropenem	0	4 (33.3)	8 (66.7)	1 (3.2)	3 (9.7)	27 (87.1)
Piperacillin-tazobactam	2 (16.7)	0	10 (83.3)	5 (16.1)	0	26 (83.9)
Tobramycin	2 (16.7)	0	10 (83.3)	4 (12.9)	0	27 (87.1)
PDR isolates (children = 1; adults = 3)						
Ceftolozane-tazobactam	1 (100)	0	0	3 (100)	0	0
Ceftazidime	0	0	1 (100)	0	0	3 (100)
Ciprofloxacin	0	0	1 (100)	0	0	3 (100)
Colistin	0	0	1 (100)	0	0	3 (100)
Meropenem	0	0	1 (100)	0	0	3 (100)
Piperacillin-tazobactam	0	0	1 (100)	0	0	3 (100)
Tobramycin	0	0	1 (100)	0	0	3 (100)

[a] According to the EUCAST breakpoints: S = susceptible; I = intermediate; R = resistant.

Sixteen mucoid isolates (39%) were XDR, whereas 7 (17.1%) were MDR.

The variations in susceptibility rate to C-T were also monitored over the 6.5-year surveillance period, as shown in Fig. 1. Considering the strains as a whole, changes in the MIC distributions were minimal, resulting in a susceptibility rate that did not significantly change over the study period, ranging from 78.1% (year 2010) to 82.9% (year 2016). Similarly, no statistically significant differences were found stratifying the isolates on patient's age.

Sixty-eight out of 188 *P. aeruginosa* isolates were collected during pulmonary exacerbations, and their susceptibility profile is summarized in Table 3. Overall, C-T and colistin exhibited comparable activity (susceptibility rate: 91.1% and 86.7%), although higher compared to other antibiotics (susceptibility rate: 72%, 69.1%, 57.3%, 51.5%, and 47%, respectively, for ceftazidime, piperacillin-tazobactam, tobramycin, ciprofloxacin, and meropenem; P at least <0.01). The same trend was observed for the isolates collected from adult patients, whereas against the isolates from younger patients, all antibiotics tested showed comparable activity (susceptibility rates ranging from 72% of ciprofloxacin to 92% of C-T), with the exception for meropenem that resulted to be the less active (susceptibility rate: 68%; P<0.05 vs other antibiotics).

4. Discussion

In this study, the *in vitro* activity of C-T against a collection of *P. aeruginosa* CF isolates was evaluated and compared with commonly

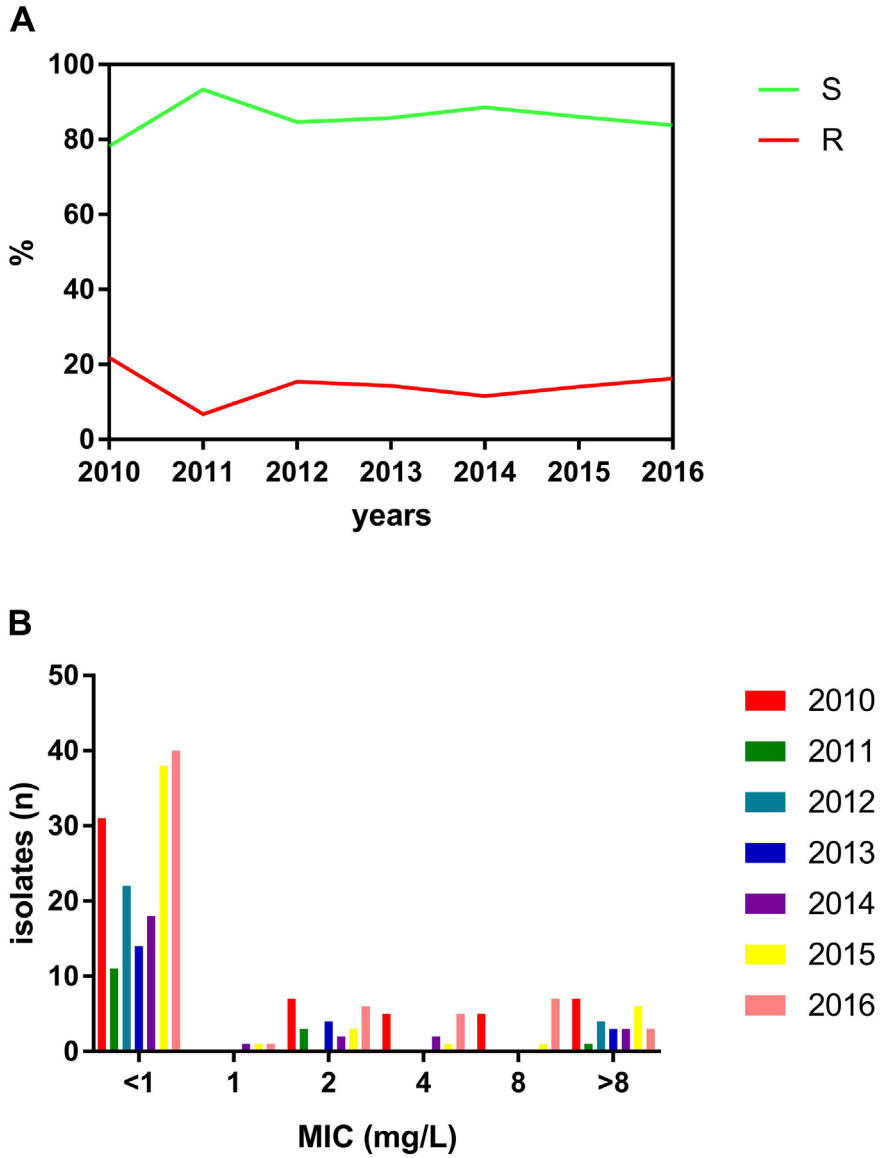

Fig. 1. Activity of ceftolozane-tazobactam (C-T) over the study period. MIC values were measured by the microdilution broth method using Micronaut-S MHK Pseudomonas 2 (Merlin Diagnostika GmbH; Germany). According to EUCAST guidelines (http://www.eucast.org/clinical_breakpoints/), a susceptibility breakpoint of ≤4/4 μg/mL was considered. **A**) Changes in the susceptibility rate over time, considering the isolates as a whole (overall) or stratified according to the patient's age (children: age ≤18 years old; adults: age >18 years old). **B**) MIC distribution over the study period, considering the isolates as a whole (n=188).

prescribed broad-spectrum antibiotics in the management of lung infections in CF patients.

Overall, our findings agree with previous studies confirming a relevant activity of C-T against *P. aeruginosa* CF isolates. Further, the activity of C-T was stable over time, with no significant changes in susceptibility rates during study period. The analysis of the susceptibility rates calculated by considering the 188 isolates as a whole indicated a comparable activity of C-T and colistin, but significantly higher compared with other

Table 3
Activity of ceftolozane-tazobactam and comparators against 68 *P. aeruginosa* isolates collected from CF patients during pulmonary exacerbation: 25 from children (≤18 years old) and 43 from adults (>18 years old).

	Susceptibility[a] no. (%) in children			Susceptibility[a] no. (%) in adults			Susceptibility[a] no. (%), overall		
Antimicrobial agent	S	I	R	S	I	R	S	I	R
All isolates (children = 25; adults = 43; overall = 68)									
Ceftolozane-tazobactam	23 (92.0)	0	2 (8.0)	39 (90.7)	0	4 (9.3)	62 (91.1)	0	6 (8.9)
Ceftazidime	19 (76.0)	0	6 (24.0)	30 (69.8)	0	13 (30.2)	49 (72.0)	0	19 (28.0)
Ciprofloxacin	18 (72)	0	7 (28)	17 (39.5)	0	26 (60.5)	35 (51.5)	0	33 (48.5)
Colistin	23 (92.0)	0	2 (8.0)	36 (83.7)	0	7 (16.3)	59 (86.7)	0	9 (13.3)
Meropenem	17 (68.0)	2 (8.0)	6 (24.0)	15 (34.9)	8 (18.6)	20 (46.5)	32 (47.0)	10 (14.8)	26 (38.2)
Piperacillin-tazobactam	19 (76.0)	0	6 (24.0)	28 (65.1)	0	15 (34.9)	47 (69.1)	0	21 (30.9)
Tobramycin	20 (80.0)	0	5 (20.0)	19 (44.2)	0	24 (55.8)	39 (57.3)	0	29 (42.7)

[a] According to the EUCAST breakpoints: S = susceptible; I = intermediate; R = resistant.

antibiotics tested. The same trend was observed considering only the isolates collected during pulmonary exacerbation, therefore confirming that C-T represents a good alternative candidate in the treatment of CF pulmonary exacerbations caused by *P. aeruginosa*.

An unanswered question regards the efficacy of C-T in adult CF patients with drug-resistant *P. aeruginosa* isolates. In fact, although the aggressive antimicrobial management of acute pulmonary exacerbations caused by *P. aeruginosa* has contributed to improvements in the life expectancy for CF adults, the repeated use of antibiotics caused the emergence of multiple resistances. Particularly, the persistence of MDR bacterial strains in the lungs has been associated with poorer clinical outcomes (Mustafa et al., 2016). In the present study, C-T demonstrated potent activity against highly resistant *P. aeruginosa* isolates. Overall, 70% of MDR isolates were susceptible to C-T whose activity was comparable to colistin, piperacillin-tazobactam, ceftazidime, and tobramycin. We can speculate that these findings are likely due to the occurrence of different types of mechanisms underlying the multidrug resistance associated with resistance patterns observed among MDR strains collected in this study. Significantly higher susceptibility rates to C-T were reported in a previous study where MDR isolates from CF children were 86% susceptible, 10% intermediate, and 4% resistant (Kuti et al., 2015).

In a previous study, C-T has been demonstrated to be effective against isolates from CF adult patients, including XDR and PDR strains (Zamorano et al., 2010). Besides colistin, we found C-T being largely the most effective antimicrobial against XDR isolates with an overall susceptibility rate of 58.1%. Although we did not find significant differences in the overall susceptibility rates of C-T between children and adults CF patients, the susceptibility of XDR isolates to C-T was higher in adults than in younger patients (67.7% vs. 33.3%, respectively). Our findings are in disagreement with Finklea et al. (2018) that found C-T to be active against only 38% of XDR *P. aeruginosa* isolates from adult CF patients. Susceptibility rates higher than 70% were reported in other studies, although in clinical settings other than CF (Castanheira et al., 2014; Shortridge et al., 2017). Strikingly, all 4 PDR *P. aeruginosa* isolates we found were susceptible to only C-T, contrarily to Finklea et al. (2018) that found no activity of C-T against 9 PDR isolates.

The discrepancies in susceptibility rates we observed with previous studies could be due both to the small sampling size — therefore warranting further work on a wider set of resistant *P. aeruginosa* strains — and the different breakpoints used for categorical classification. The EUCAST breakpoints we used for C-T in this study (susceptibility for MICs ≤4/4 μg/mL and resistance for MICs >4/4 μg/mL) are in fact different from those suggested by CLSI and adopted in previous studies (susceptibility for MICs ≤4/4 μg/mL, intermediate for MICs ≤8/4 μg/mL, and resistance for MICs ≤16/4 μg/mL). Moreover, it is noteworthy that the current susceptibility breakpoints for C-T (≤4/4 μg/mL) are based on the dosing protocol for the treatment of complicated intra-abdominal and urinary tract infections. For other types of infection, such as pulmonary infections, MICs clinical breakpoints for C-T could be different, and the use of different dosing strategies against *P. aeruginosa* isolates with higher MICs could have a positive impact on the clinical outcomes.

Carbapenem-resistant *P. aeruginosa* has emerged worldwide and is being reported as a nosocomial pathogen, particularly in debilitated or neutropenic individuals and CF patients (Britt et al., 2018; Mustafa et al., 2016). In agreement with previous findings (Shortridge et al., 2017), we observed that C-T activity was comparable to or lower than just colistin respectively against isolates nonsusceptible to meropenem or beta-lactam antibiotics, confirming this antibiotic to be very useful for the treatment of infections caused by *P. aeruginosa* with those resistance patterns. We also found that C-T resulted in the most effective antibiotic against isolates resistant to colistin.

One key factor contributing to the maintenance of chronic infection by *P. aeruginosa* in CF lung is the conversion to a mucoid phenotype, where bacteria overproduce exopolysaccharide alginate. Compared to nonmucoid isolates, mucoid *P. aeruginosa* exhibits enhanced resistance to multiple antibiotics (Goltermann and Tolker-Nielsen, 2017) and to host immune effectors (Limoli et al., 2014). In agreement with previous studies (Dassner et al., 2017; Rac et al., 2017), our findings showed that C-T retains a potent activity against 41 *P. aeruginosa* isolates that grew with mucoid phenotype (82.9% susceptibility), confirming a potential role of this antimicrobial in the treatment of chronic *P. aeruginosa* lung infections in CF patients.

Although maintenance antibiotic therapies remain a fundamental aspect for the treatment of pulmonary infections in CF patients and for reducing the frequency of exacerbations, there are differences in clinical practice with regard to the selection of appropriate antibiotics and the duration of therapy. A systematic review reported a paucity of evidence upon which to define best practice in the treatment of pulmonary exacerbations (Flume et al., 2009). It has been suggested that there is a discordance between the results of laboratory testing and clinical outcomes (Chmiel et al., 2014; Hurley et al., 2012), where antibiotic susceptibility test results for *P. aeruginosa* isolates do not predict outcomes when treating CF patients with pulmonary exacerbations (Smith et al., 2016). This represents an important clinical issue to define the best practices aimed at improving outcomes with antibiotic treatment of pulmonary exacerbations, especially in the light of the antimicrobial stewardship programs where the appropriate use of antibiotics is strongly recommended.

5. Conclusion

Overall, the results from this study confirm that *in vitro* activity of C-T against both susceptible and highly resistant *P. aeruginosa* CF isolates is generally comparable to colistin but higher than beta-lactams, aminoglycosides, and fluoroquinolones. Particularly, C-T appears to be a promising treatment option for treatment of MDR *P. aeruginosa* causing pulmonary exacerbations among CF patients. In this frame, the first successful use of C-T to treat an acute respiratory exacerbation of CF caused by MDR *P. aeruginosa* has been recently reported (Vickery et al., 2016). Further *in vitro* modeling and clinical evaluation in CF are, however, warranted to define the role of this antibiotic in the clinical practice.

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Authors' contribution

All authors have contributed to the study. GG and EF designed the study. GG and GDB prepared the initial manuscript. All authors contributed to the subsequent editorial revisions. EF and GL performed susceptibility tests. GG, AP, and GDB conducted the statistical analysis. All authors proofread the article.

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