Tedizolid is a promising antimicrobial option for the treatment of *Staphylococcus aureus* infections in cystic fibrosis patients

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Background: Tedizolid is a protein synthesis inhibitor in clinical use for the treatment of Gram-positive infections. Pulmonary MRSA infections are a growing problem in patients with cystic fibrosis (CF) and the efficacy of tedizolid-based therapy in CF pulmonary infections is unknown.

Objectives: To evaluate the *in vitro* and *in vivo* activity of tedizolid and predict the likelihood of tedizolid resistance selection in CF-background *Staphylococcus aureus* strains.

Methods: A collection of 330 *S. aureus* strains (from adult and paediatric patients), either of normal or small colony variant (SCV) phenotypes, gathered at three CF centres in the USA was used. Tedizolid activity was assessed by broth microdilution, Etest and time–kill analysis. *In vivo* tedizolid efficacy was tested in a murine pneumonia model. Tedizolid *in vitro* mutants were obtained by 40 days of exposure and progressive passages. Whole genome sequencing of clinical *S. aureus* strains with reduced susceptibility to tedizolid was performed.

Results: MRSA strain MIC₉₀s were tedizolid 0.12–0.25 mg/L and linezolid 1–2 mg/L; for MSSA strains, MIC₉₀s were tedizolid 0.12 mg/L and linezolid 1–2 mg/L. Two strains, WIS 441 and Seattle 106, with tedizolid MICs of 2 mg/L and 1 mg/L, respectively, had MICs above the FDA tedizolid breakpoint (0.5 mg/L). Tedizolid at free serum concentrations exhibited a bacteriostatic effect. Mean bacterial burdens in lungs (log₁₀ cfu/g) for WIS 423-infected mice were: control, 11.2±0.5; tedizolid-treated (10 mg/kg), 3.40±1.87; linezolid-treated (40 mg/kg), 4.51±2.1; and vancomycin-treated (30 mg/kg), 5.21±1.93. For WIS 441-infected mice the (log₁₀ cfu/g) values were: control, 9.66±0.8; tedizolid-treated, 3.18±1.35; linezolid-treated 5.94±2.19; and vancomycin-treated, 4.35±1.7.

Conclusions: These results suggest that tedizolid represents a promising therapeutic option for the treatment of CF-associated MRSA/MSSA infections, having potent *in vivo* activity and low resistance potential.

Introduction

MRSA is a major infectious human pathogen responsible for diseases ranging from skin and soft tissue infections to life-threatening pneumonia, both in hospital-acquired (HA) and community-acquired (CA) settings. β -Lactam resistance in MRSA involves the acquisition of PBP2a, a PBP with low affinity for β -lactams that can mediate cell wall assembly when the normal staphylococcal PBPs (PBP1 to 4) are inactivated by these agents.¹ *Staphylococcus aureus* is one of the earliest acquired and more prevalent pathogens infecting patients with cystic fibrosis (CF), a severe autosomal recessive disease that affects several organs, notably the lungs, predisposing CF patients to infections with potentially severe consequences. According to recent data from the CF registry in the USA, the prevalence of MSSA is around 70%, and that of MRSA around 26%, compared with 13% in Europe, 6%

in Canada and 3% in Australia.^{2,3} Emerging research has demonstrated that MRSA infections have a significant clinical impact on individuals with underlying chronic diseases, including CF, where antibiotic pressure and metabolic adaptations may favour the adaptability of *S. aureus* to establish long persistence and resistance. Numerous mechanisms associated with higher rates of antimicrobial failure have been reported in CF lung infections and other chronic MRSA infections, such as small colony variants (SCVs), biofilms and growth under anaerobic conditions.⁴ Moreover, chronic pulmonary infection with MRSA is thought to confer upon CF patients a worse overall clinical outcome and, in particular, results in an increased rate of decline in lung function. In this context, additional data regarding the antibiotic susceptibility of these specific strains are urgently needed to expand treatment options in cases of multiresistance.

It has recently been reported that linezolid (both IV and oral) is the most commonly utilized antimicrobial in CF patients, for both paediatric [IV 35/224 (16%); oral 41/224 (18%)] and adult [IV 44/235 (19%); oral 38/235 (16%)] inpatient treatment; IV vancomycin was the second most used antibiotic.⁵ Tedizolid phosphate (Sivextro®) is rapidly converted *in vivo* to tedizolid, the active moiety and an oxazolidinone antibiotic, by non-specific phosphatases.^{6,7} It was approved in 2014 for the treatment of acute bacterial skin and skin structure infections (ABSSSI) caused by Gram-positive bacteria, i.e. MRSA, *Streptococcus pyogenes*, *Streptococcus pneumoniae* and VRE, including some linezolid-resistant strains carrying the *cfr* gene, which causes methylation of 23S rRNA.⁸ Similar to linezolid, tedizolid works by binding to the 23S rRNA of the 50S subunit preventing the formation of the 70S initiation complex and inhibiting protein synthesis.^{8,9}

Tedizolid has shown substantial lung penetration;¹⁰ however, limited data are available regarding its efficacy in the treatment of CF MRSA-mediated infections. The *S. aureus* strains isolated from CF patients (CF-*S. aureus*) are well known to have altered metabolism and to possess MDR due to their chronic carriage and prolonged, repeated exposures to antibiotic treatments in the CF lung environment.¹¹ Thus, we hypothesized that tedizolid may constitute an alternative therapeutic option for MRSA infections detected in patients with underlying chronic diseases such as CF. In this work, we investigated the activity of tedizolid against 330 CF-*S. aureus* strains collected from geographically diverse CF centres in the USA. The goal of this study was to characterize, by *in vitro* and *in vivo* approaches, the antimicrobial activity of tedizolid in *S. aureus* collected from CF patient infections.

Materials and methods

Clinical CF strains

CF strains were isolated from patients' sputum cultures (Table 1). A collection of strains comprising either WT or SCV phenotypes was obtained from three academic medical institutions with large CF populations: the Center of Global Infectious Diseases (Seattle, WA, USA), UW Health (Madison, WI, USA) and Houston Methodist Hospital (Houston, TX, USA).

Culture conditions and antibiotics

Strains were grown in tryptic soy broth (TSB) (BD; Sparks, MD, USA) and Mueller–Hinton broth (MH) (BD) and on tryptic soy agar (TSA) with 5% sheep blood (BBL; Sparks, MD, USA) and MH agar (MHA) (BD). Tedizolid for *in vitro* use (TR-700) and tedizolid prodrug for *in vivo* use (TR-701) were provided by the study sponsor Merck & Co., Inc. (Rahway, NJ, USA); linezolid and vancomycin were obtained from Sigma. These antibiotics were reconstituted as recommended by the manufacturers. Antibiotic MICs were determined using Etest® (bioMérieux, Marcy l'Étoile, France) and the broth microdilution method using Mueller–Hinton II broth (MH) according to CLSI guidelines.¹² *S. aureus* ATCC 29213 was used as an internal control for MIC assays.

Susceptibility testing

Linezolid and vancomycin susceptibility testing were performed by both Etest and broth dilution. Tedizolid MICs were determined by the broth microdilution method in MH, following CLSI guidelines.¹² MBCs were determined by the broth microdilution technique, as per CLSI guidelines. Samples were plated from the wells at the MIC, and all the concentrations that did not show visible growth were observed for 3 log reduction compared with the inoculum.¹²

Table 1. Tedizolid (TZD) MICs for 330 CF-*S. aureus* strains determined by the microdilution method in MH and in comparison with linezolid (LZD) following CLSI guidelines

Strain characteristic	TZD status
MRSA (n=159; 48%)	
LZD-S (n=155; 46.8%; MIC ₉₀ =1–2 mg/L)	TZD-S (n=154; 46.5%; MIC ₉₀ =0.12–0.25 mg/L)
LZD-R (n=4; 1.2%)	TZD-R (n=0)
MSSA (n=171; 52%)	
LZD-S (n=170; 51.7%; MIC ₉₀ =1–2 mg/L)	TZD-S (n=170; 51.7%; MIC ₉₀ =0.12–0.25 mg/L)
LZD-R (n=1; 0.3%)	TZD-R (n=0)
	TZD-S (n=0)
	TZD-R (n=1; 0.3%)

S, susceptible; R, resistant.

Time–kill analyses

Analyses were performed on four representative CF strains following CLSI guidelines using human serum free-drug concentrations for linezolid (10.4 mg/L for a 600 mg dose), tedizolid (2.6 mg/L for a 200 mg dose)¹³ and vancomycin (10 mg/L for a 1000 mg dose) with an initial inoculum of 1×10^6 cfu/mL in 24-well microdilution plates.^{14,15} The numbers of cfu were counted by plating diluted samples on TSA at 0, 2, 4, 6, 8 and 24 h. Results were expressed as the number of cfu/mL versus time.

Spontaneous mutation frequency

Mid-log cultures (OD 600 nm; 0.8) were prepared from a representative number of CF and non-CF strains. Strains were collected and resuspended in PBS to a concentration of 3×10^9 cfu/mL and from each culture 5 mL aliquots were spread onto tedizolid MH plates. Initial cfu were enumerated by triplicate plating of serial dilutions of the starting inoculum in PBS. Plates were incubated for 5 days. Spontaneous mutation frequency was determined by dividing the number of colonies on plates containing tedizolid at 0.5–1 mg/L by actual plated cfu.

Tedizolid *in vitro* mutant selection

Serial passage selection was attempted in a representative number of CF strains, including the reference strain *S. aureus* ATCC 25213. Strain and control cultures (1.2×10^8 cfu) were serially passaged in the presence of subinhibitory concentrations of tedizolid, from 0.015 to 16 mg/L, in MH over 40 days to recover tedizolid-non-susceptible strains.

Galleria mellonella infection model

Groups of *G. mellonella* larvae (10 per group) were inoculated in the last left proleg with 10 µL of a bacterial suspension of strain WIS 441 (tedizolid MIC 2 mg/L) or WIS 423 (tedizolid MIC 0.25 mg/L) containing 1.5×10^6 cfu/mL, as previously described.^{15–17} Every trial included a group of 10 untreated larvae as an uninfected control group and 10 larvae injected with PBS as a method control. After 2 h, treatment was initiated with tedizolid (20 mg/kg),

linezolid (20 mg/kg) or vancomycin (10 mg/kg). Experiments were performed in at least three independent trials. Injected insects were monitored over 6 days at 37°C. By Day 6, pupa formation was recorded in surviving larvae.

In vivo efficacy of tedizolid in a CF-MRSA pneumonia model

Mice of the NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG) strain were used in this study (The Jackson Laboratory, Bar Harbor, ME, USA). Animals were fed a standard small laboratory animal diet and no restrictions were applied. Groups of three mice were infected with 50 µL of inoculum administered to the nares and inhaled into the lung. Treatment was commenced 2 h after challenge with IV injections of tedizolid 10 mg/kg, linezolid 40 mg/kg or vancomycin 30 mg/kg every 12 h; these regimens are comparable to human exposure. After 48 h of treatment, the mice were euthanized by carbon dioxide asphyxiation. Untreated control mice were assessed at the time of treatment initiation. Following euthanasia, the lungs were harvested using a sterile technique, homogenized, diluted, plated and incubated, and viable plate counts were recorded. Data were expressed as the mean change in log₁₀ cfu/lungs for three mice per treatment group compared with the organism burden at the start of therapy.

Animal ethics statement

All animal studies were approved by the Institutional Animal Care and Use Committee of the Houston Methodist Research Institute. To ensure protection and proper manipulation of animals, experiments were performed by trained personnel at the animal facility of the Houston Methodist Research Institute.

Statistical analysis and numbers of animals

Four therapy groups, with three animals per group, were considered for this study: control (infected, untreated); infected, tedizolid-treated; infected, linezolid-treated; and infected, vancomycin-treated. A total of ~15 mice were required for these experiments, including extras to cover for any problems. Statistical power was gained through the use of repeated measures analysis across points. The statistical analysis was performed using GraphPad Prism 7 software. The log-rank test was used to assess significant differences (*P*<0.05) for Kaplan–Meier survival curves. The unpaired Student’s *t*-test was used to determine significant differences (*P*<0.05) for log cfu/g. A minimum of three to five animals per group was considered to cover the statistical significance.

Genomic characterization and WGS

DNA of WIS 441 and Seattle 106 was prepared using the DNeasy Blood & Tissue Kit (QIAGEN). Libraries were prepared from purified DNA using the Nextera XT DNA Library Preparation Kit (Illumina) and sequenced with HiSeq 2000 instruments at the Epigenetics and Genomic Laboratory at Weill Cornell University, NY, USA. Genomes were assembled and SNPs were identified and compared with *S. aureus* N315 (GenBank BA000018) using the Lasergene 14 suite. Reads were aligned against N315 (PATRIC ID: 158879.11) and analysed using the PATRIC variation service 24, which uses BWA-MEM as the read aligner (<https://arxiv.org/abs/1303.3997>) and FreeBayes as the SNP caller (<https://arxiv.org/abs/1207.3907>).

Amplification and sequencing of ribosomal genes

Chromosomal DNA was isolated from *S. aureus* using the DNeasy Blood & Tissue Kit. PCR (Platinum PCR SuperMix High Fidelity; Invitrogen Corp., Carlsbad, CA, USA) was used to amplify each of the six *S. aureus* *rrn* operons (including the 5S, 16S and 23S rRNA genes) as previously described.¹⁸

Results

Tedizolid is active against MRSA/MSSA strains isolated from CF patients

The collection of CF-*S. aureus* strains used in the present study were either WT or SCV phenotypes identified at culture collection; the collection was obtained from three different CF centres. We screened a total of 330 strains, 159 MRSA and 171 MSSA. As shown in Table 1, tedizolid displayed activity against both MRSA (*n*=154 strains, 96.8%) and against MSSA (*n*=170 strains, 99.4%). Furthermore, for these tedizolid-susceptible strains, tedizolid MIC₉₀s were 0.12–0.25 mg/L and linezolid MIC₉₀s were 1–2 mg/L (Table 1). As shown in Table 2, two strains, WIS 441 and Seattle 106, had tedizolid MICs of 2 mg/L and 1 mg/L respectively, values that are above the FDA tedizolid breakpoint (0.5 mg/L). Overall, the activity of tedizolid was 8–16-fold lower than that of linezolid against CF MRSA and MSSA strains, independent of the phenotypic background of the strains.

Time–kill analysis

In vitro effectiveness of tedizolid was determined by time–kill experiments in subcultures at 0, 2, 4, 6, 8 and 24 h (initial inoculum 1×10⁶ cfu/mL) using a tedizolid human serum concentration of 2.6 mg/L (similar to human peak serum free-drug concentrations). Results are expressed by plotting mean colony counts (log₁₀ cfu) versus time as a measure of increase in killing at 24 h. Tedizolid (2.6 mg/L) was equally as active as linezolid (10.4 mg/L) in tedizolid-susceptible strains, resulting in a bacteriostatic effect, i.e. growth inhibition above 3 log₁₀ cfu reduction (Figure 1a and b). Interestingly, tedizolid (2.6 mg/L) was highly active in comparison with linezolid against strains resistant to both agents (e.g. tedizolid MICs 1–2 mg/L and linezolid MICs 32 mg/L); by contrast, no effects were observed with linezolid, which manifested as recurrent growth at 4–8 h (Figure 1c and d). These observations were confirmed by MIC/MBC analyses: tedizolid (MIC 0.12–0.25 mg/L; MBC 1–4 mg/L); tedizolid resistant (MIC 1–2 mg/L; MBC 4–16 mg/L).

In vivo efficacy of tedizolid in a waxworm model

The *in vivo* efficacy of tedizolid was tested using a *G. mellonella* (waxworm) *in vivo* model in two representative strains, WIS 423 (tedizolid susceptible) and WIS 441 (tedizolid resistant), mainly to compare the efficacy of tedizolid in strains displaying different tedizolid phenotypes. Groups of larvae (*n*=10) were inoculated with WIS 441 or WIS 423 strains; after the initial 2 h of incubation, worms were treated with tedizolid (20 mg/kg), linezolid (20 mg/kg) or vancomycin (10 mg/kg) and re-incubated for 24 h at 37°C.

Table 2. Phenotype and genotype characteristics of *S. aureus* clinical strains with reduced susceptibility to tedizolid

Strains	MIC (mg/L)		Mutations				
	linezolid	tedizolid	<i>cfr</i>	RNA 23S*	L3	L4	L22
WIS 441 (MRSA)	>32	2	NA	G2576T	no	no	no
Seattle 106 (MSSA)	>32	1	NA	G2576T	no	no	no

NA, no amplification.

Figure 1. *In vitro* effectiveness of tedizolid (TZD) determined by time-kill experiments using a concentration of 2.6 mg/L (similar to peak serum free-drug concentrations in humans). Subcultures were performed at 0, 2, 4, 6, 8 and 24 h (initial inoculum: 1×10^7 cfu/mL). Results are expressed by plotting mean log_{10} cfu versus time over 24 h. (a and b) Susceptible strains WIS 423 (MRSA) and Seattle 76 (MSSA), respectively: linezolid (LZD) MIC 2 mg/L; TZD MIC 0.25 mg/L. (c and d) Resistant strains WIS 441 (MRSA; LZD MIC >32 mg/L; TZD MIC 2 mg/L) and Seattle 106 (MSSA; LZD MIC >32 mg/L; TZD MIC 1 mg/L), respectively. * $P < 0.001$; # $P < 0.05$.

Treatment was repeated after 24 h. Worms were checked daily, and any deaths recorded, for a total of 6 days. As shown in Figure 2, groups of untreated WIS 423- or WIS 441-infected worms displayed very low survival rates ($\leq 30\%$). WIS 423-infected worms injected with tedizolid showed 90% survival at Day 2 and 80% at Day 6, similar to the percentages seen for those injected with linezolid and vancomycin (Figure 2a). Interestingly, tedizolid was more effective than linezolid against strain WIS 441, which exhibited a tedizolid MIC of 2 mg/L. Vancomycin was included as a control reference, displaying 80% survival of WIS 423-infected worms and 70%–80% of WIS 441-infected worms at Day 6 (Figure 2b). These data strongly suggest that tedizolid may represent an efficacious option for the treatment of CF-MRSA infections including those with MRSA strains such as WIS 441 with borderline tedizolid MIC of 2 mg/L.

Efficacy of tedizolid in a murine pneumonia model of CF infection

Four groups of three mice each were inoculated with 1.27×10^8 cfu of either WIS 423 or WIS 441 strains. Mice were infected with 50 μL of inoculum administered to the nares and inhaled into the lung. Treatments were commenced 2 h after challenge and given every 12 h for a total of 48 h. Mean lung densities $\pm$ SD (log_{10} cfu/g

of tissue) for WIS 423-infected mice were: control, 11.2 ± 0.5 ; tedizolid-treated (10 mg/kg), 3.40 ± 1.87 ; linezolid-treated (40 mg/kg), 4.51 ± 2.1 ; and vancomycin-treated (30 mg/kg), 5.21 ± 1.93 . For WIS 441-infected mice, the values were: control, 9.66 ± 0.8 ; tedizolid-treated (10 mg/kg), 3.18 ± 1.35 ; linezolid-treated (40 mg/kg), 5.94 ± 2.19 ; and vancomycin-treated (30 mg/kg), 4.35 ± 1.7 (Figure 3). These results suggest that tedizolid may offer a therapeutic option for the treatment of CF-MRSA, including cases with decreased susceptibility to both linezolid and tedizolid, as in the case of strains such as WIS 441.

Investigation of spontaneous mutations leading to reduced tedizolid susceptibility

We determined, in a representative number of strains, the frequency of spontaneous mutations conferring reduced susceptibility to tedizolid. As mentioned in the Materials and methods section, the frequency of mutations was assessed with two tedizolid concentrations, at $1 \times$ and $2 \times$ the strains' MICs and with an initial inoculum in the range of 1.21×10^9 to 2.4×10^9 cfu/mL. Under these conditions, no growth was observed on plates containing tedizolid for the majority of the tested strains and with values of mean frequency of mutations between 5.8×10^9 and 2.8×10^{10} (Table S1, available as [Supplementary data](#) at JAC Online). We found only one CF-MRSA strain, WIS 423, that had a reduced

Figure 2. *G. mellonella* infection of groups of larvae (10 per group) inoculated with PBS or bacterial suspensions containing 1.5×10^6 cfu/mL of WIS 423 or WIS 441 strains. A minimum of three independent experimental replicates were performed for each experiment. Survival data were plotted using the Kaplan–Meier method and expressed as percentage of survival versus time. Statistically significant differences were determined using the log-rank test (** $P < 0.01$).

Figure 3. Murine pneumonia model: mice ($n = 3$ per group) were inoculated with 1.27×10^8 cfu of strains WIS 423 and WIS 441. Mice were infected with 50 μ L of inoculum administered to the nares and inhaled into the lung. Treatments were commenced 2 h after challenge, then every 12 h. Mean lung bacterial densities $\pm$ SD (log₁₀ cfu/g of tissue) for WIS 423 were: control, 11.2 ± 0.5 ; tedizolid-treated (10 mg/kg), 3.40 ± 1.87 ; linezolid-treated (40 mg/kg), 4.51 ± 2.1 ; and vancomycin-treated (30 mg/kg), 5.21 ± 1.93 ; while for WIS 441 they were: control, 9.66 ± 0.8 ; tedizolid-treated (10 mg/kg), 3.18 ± 1.35 ; linezolid-treated (40 mg/kg), 5.94 ± 2.19 ; and vancomycin-treated (30 mg/kg), 4.35 ± 1.7 . The statistical analysis was performed using GraphPad Prism 7 software. The unpaired Student’s *t*-test was used to determine significant differences ($P < 0.05$) for log cfu/g.

susceptibility to tedizolid (MIC of 4 mg/L and mutation rate frequency of 4.8×10^8) (Table S1). Sequence analysis of 23S rRNA, *rplC*, *rplD* and *rplV* performed on the recovered WIS 423 tedizolid-mutant strain revealed that this strain possessed the G2576T mutation in one of the six copies of 23S rRNA. These results support the evidence that spontaneous mutation leading to reduced tedizolid susceptibility is a low frequency event, including for *Cf. aureus* strains.

Analysis of the ease of *in vitro* selection of tedizolid mutants

Although the probability of development of *in vitro*-selected tedizolid mutants may be extremely low, our rationale to test the ease

of *in vitro* mutant selection by tedizolid was based on the concept that MRSA from CF are mostly hypermutator strains,^{19,20} as manifested by the existence of a small proportion of the cell population that is able to resist high concentrations of antibiotics. We performed progressive tedizolid resistance selection by exposing tedizolid-susceptible strains in MH broth to stepwise 2-fold increasing concentrations of tedizolid alone for a total of 40 consecutive days. As shown in Table 3, there was a slight increase in tedizolid MICs from tedizolid MIC 0.06 mg/L up to 0.25 to 1 mg/L, which is above the susceptible breakpoint (0.5 mg/L), followed by an increase of tedizolid MICs to 1–4 mg/L at Day 40. In sharp contrast to tedizolid, we observed that for the ATCC 29213 strain selected by 40 parallel serial passages in linezolid, the MIC increased 8-fold to 4 mg/L whereas MICs of tedizolid remained

unchanged at 0.25 mg/L (data not shown). To further characterize the genetic changes associated with reduced tedizolid activity, individual colonies from the representative tedizolid-passaged strains recovered at Day 40 were analysed for changes in the sequence of the domain V region of 23S rRNA genes as well as the *rplC*, *rplD* and *rplV* genes. As shown in Table 4, multiple passages of and mutations of 23S rRNA (T2571/G2576) in the ATCC 33591 strain are required to achieve a tedizolid MIC increase to 2 mg/L. A similar observation was made for tedizolid mutants obtained from CF isolates, e.g. WIS 423 harbouring a G2576T mutation in three of six copies of the *rpl* gene, while for the WIS 526 strain G467A and G492A mutations were present in the *rplC* gene. Phenotypic analysis of the mutants recovered following 40 days of tedizolid exposure was determined by testing their susceptibility to other agents: chloramphenicol, linezolid, vancomycin, tetracycline and

gentamicin. The most notable change seen in WIS 526 and WIS 423 tedizolid mutants was their reduced susceptibility to chloramphenicol and linezolid while no changes were observed for vancomycin, tetracycline and gentamicin. The phenotypic changes observed in the tedizolid-recovered mutants were consistent with those seen in clinical *in vivo* tedizolid-resistant strains WIS 441 and Seattle 106 (Table 4). Altogether, these results suggest that the likelihood of *in vitro* tedizolid resistance selection is predictably low in CF-*S. aureus* strains, requiring multiple steps of tedizolid exposure.

Genes associated with *in vivo* tedizolid resistance

To investigate the genetic mutations associated with the *in vivo* CF-MRSA WIS 441 and Seattle 106 strains, the full genomes were sequenced and compared with the sequence of reference strain *S. aureus* N315 (PATRIC accession number 158879.11). Mutated genes were categorized by function to identify themes of bacterial physiology that may contribute to reduced susceptibility to tedizolid (Table 5). The most common non-synonymous SNPs were found by comparing the sequence of each strain with that of the *S. aureus* N315 reference strain. Common SNPs present in both WIS 441 and Seattle 106 were found in the sequence of the domain V region of the 23S rRNA gene, *rpl*, notably a G2576T mutation in three of the six copies of rRNA; additional mutations were found in ribosomal protein-associated genes, corresponding to *rplA* (encoding a 50S ribosomal protein L1) and *rpsQ* (30S ribosomal S17). Moreover, some SNPs were uniquely found in WIS 441 in *rplK* (50S ribosomal protein L11) and *rsmE* (16S rRNA methyltransferase). The mutations in *rpsQ* and *rplA* have not been previously characterized. We found additional non-synonymous SNPs in various cell wall-related genes [*tagG* (teichoic acid translocation permease), *tcaA* (TcaA protein) and *capB* (capsular genes)] as well

Table 3. MICs for parental strains and *in vitro* tedizolid serial-passage mutants obtained with subinhibitory concentrations of tedizolid over 40 days

Strain	Tedizolid MIC (mg/L)					
	Day 1	Day 5	Day 10	Day 15	Day 20	Day 40
ATCC 29213	0.5	0.5	0.25	0.25	0.5	0.5
ATCC 33591	0.5	0.5	0.25	0.5	1	1
WIS 423	0.5	0.25	0.25	0.25	1	4
WIS 526	0.5	0.5	0.25	0.12	1	4
Seattle 76	0.5	0.5	0.5	0.5	1	4
Seattle 118	0.5	0.5	0.5	0.5	1	4

Tedizolid MICs were stable until Day 15, followed by a slight decrease in susceptibility at Day 20 and afterwards, suggesting a low potential of resistance development in CF-MRSA.

Table 4. Phenotypic and genotypic characterization of tedizolid mutants obtained by progressive selection during 40 days of exposure (TZD/40 d) of CF strains (WIS 423 and WIS 526) in comparison with control strains ATCC 29213 and ATCC 33591

Strains	MIC (mg/L)						23S rRNA		Ribosomal proteins		
	CHL	GEN	LZD	VAN	TET	TZD	mutations	copies	<i>rplC</i>	<i>rplD</i>	<i>rplV</i>
ATCC 29213											
WT	8	0.25	2	1	0.25	≤0.25					
TZD passage	8	0.5	4	1	0.25	0.5					
ATCC 33591											
WT	32	0.5	1	1	0.5	≤0.25					
TZD passage	64	1	16	2	0.5	2	T2571C; G2576T	3 of 6			
WIS 423											
WT	2	1	2	2	1	≤0.25					
TZD passage	64	1	32	2	1	2	G2576T	3 of 6			
WIS 526											
WT	2	0.5	2	2	0.5	≤0.25					
TZD passage	16	0.5	4	1	0.5	2			G467A; G492A		
<i>In vivo</i> TZD mutants											
WIS 441	32	1	32	2	0.5	2	G2576T	3 of 6			
Seattle 106	32	1	32	2	1	2	G2576T	3 of 6			

Antimicrobial abbreviations: CHL, chloramphenicol; GEN, gentamicin; LZD, linezolid; VAN, vancomycin; TET, tetracycline; TZD, tedizolid.

Table 5. Most relevant mutations identified in tedizolid-resistant strains WIS 441 and Seattle 106

Gene	Mutations		Amino acid change	Function
	WIS 441	Seattle 106		
<i>prmA</i>	C>T		S210N	50S ribosomal protein L11 methyltransferase
<i>prmA</i>	T>C		I96V	50S ribosomal protein L11 methyltransferase
<i>rbgA</i>	A>G		S164G	ribosomal biogenesis GTPase
<i>rbgA</i>	T>A		D283E	ribosomal biogenesis GTPase
<i>rplA</i>	A>G	A>G	T92A	50S ribosomal protein L1
<i>rplC</i>			S145_H146delinsY	50S ribosomal protein L3
<i>rplK</i>	C>A		S111R	50S ribosomal protein L11
<i>rplO</i>		C>T	A135T	50S ribosomal protein L15
<i>rplS</i>	A>G		I48V	50S ribosomal protein L19
<i>rpsA</i>	G>C		H198D	30S ribosomal protein S1
<i>rpsA</i>		G>A	T2I	30S ribosomal protein S1
<i>rpsD</i>	G>A		V152I	30S ribosomal protein S4
<i>rpsD</i>	G>A		V177I	30S ribosomal protein S4
<i>rpsP</i>	G>T		D69Y	30S ribosomal protein S16
<i>rpsQ</i>	T>A	T>A	I77L	30S ribosomal protein S17
<i>SA1406</i>			E180K	16S rRNA methyltransferase RsmE
<i>SA1406</i>	A>G		V124A	16S rRNA methyltransferase RsmE
<i>adh1</i>	G>T	G>T	K325N	alcohol dehydrogenase
<i>capB</i>	T>C	T>C	C81R	capsular polysaccharide synthesis protein Cap5B
<i>tagG</i>	T>C	T>C	V227A	teichoic acid translocation permease
<i>sigA</i>	C>T	C>T	V253I	RNA polymerase sigma factor RpoD
<i>tcaA</i>	A>G	A>G	L218P	TcaA protein
<i>rrl</i>	G>T	G>T		23S rRNA methyltransferase

as stress-related genes [*sigA* (RNA polymerase sigma factor RpoD)] (Table 5). These results may suggest that a decrease in susceptibility to tedizolid is mainly associated with changes in genes associated with the previously described *rrl* gene (e.g. G2576T) and in the newly described ribosomal protein genes *rplA* and *rspQ*; however, the role of the latter in tedizolid resistance awaits functional experimental validation.

Discussion

The management of CF infections requires prompt and adequate antibiotic exposure and administration. The progressive and continued susceptibility of CF patients to respiratory infections due to *S. aureus*, including MRSA, remains an important concern. Chronic MRSA infection in CF patients is associated with worse outcomes and treatment eradication is a continual clinical challenge.

There are no guidelines or recommendations on the choice of antibiotics for CF pulmonary exacerbations with MRSA, resulting in the use of active antibiotics that varies between centres. The most frequently used therapies in current practice are trimethoprim/sulfamethoxazole (30%), linezolid (27%) and vancomycin (30%), with linezolid and vancomycin the most frequently used among inpatients.⁵

The pharmacokinetic data available from healthy subjects on tedizolid show that it is widely distributed throughout the body after administration of a 60 mg oral dose.²¹ In addition, tedizolid penetrates extensively into both extracellular (epithelial lining fluid) and intracellular (alveolar macrophages) compartments after once-daily oral dosing for 3 days.²¹ Tedizolid has demonstrated activity against MRSA and a safety profile in patients with CF, with good lung penetration and sputum concentrations that exceed the corresponding unbound plasma concentrations, indicating that tedizolid may be efficacious in the treatment of CF patients with pulmonary exacerbations.²² Consistent with this claim, we found in this study that tedizolid was active against MRSA isolated from CF infections from three different centres in the USA. The majority of the samples included in this study were collected from sputum from both adults and children during the period 2015–17. We found that tedizolid is equally as active as linezolid *in vitro* and *in vivo* against *S. aureus*, with the exception of two strains that showed decreased activity, with MICs above the tedizolid breakpoint [WIS 441 (MIC 2 mg/L) and Seattle 106 (MIC 1 mg/L)].

Resistance to tedizolid may be infrequent; modest increases in MICs may be seen in some isolates, as described here (tedizolid MIC 2 mg/L). Consistently, in the 2011–12 surveillance report that included 11 231 Gram-positive clinical isolates from the USA (84 centres) and Europe (115 centres), in 9 of 10 isolates that had a tedizolid MIC >1 mg/L (three *S. aureus*, five CoNS and one *Enterococcus faecium*) all had 23S rRNA or ribosomal protein mutations and had high linezolid MIC values.²³ We are unaware of the development of tedizolid resistance in CF clinical settings. Therefore, to gain an understanding of the ease of tedizolid resistance development in CF strains we investigated the spontaneous frequency of mutation. We found no differences in mutation frequency between CF and non-CF strains by a single exposure to tedizolid, at 1× or 2× MICs (Table S1). These findings are in agreement with previous work that has suggested very low frequency of resistance to TR-700.¹⁸

To determine whether serial passage may result in changes of tedizolid susceptibility in CF strains, we exposed a representative number of CF strains to subinhibitory concentrations of tedizolid by progressively escalating the exposure to tedizolid over the course of 40 days. We found that multiple passages (i.e. for 40 days) are required to achieve reduced tedizolid activity with MIC values of 2–4 mg/L; however, depending on the dosing, these antibiotic levels are still achievable. Moreover, this is in contrast with linezolid resistance, which emerges rapidly at day 3 of the 15 day therapeutic regimens. In CF patients, linezolid resistance is mainly associated with therapeutic regimens, a longer duration of treatment and the transmission of a resistant isolate from another patient.^{24,25}

Taking into account that the CF strains WIS 441 and Seattle 106 are clinical strains with reduced tedizolid susceptibility described for the first time, we were interested in determining the main genetic changes associated with decreased susceptibility to tedizolid

(i.e. in WIS 441 and Seattle 106). We found by whole-genome analyses that tedizolid-resistant strains harboured common mutations in genes associated with ribosomal 50S-related proteins (e.g. *rplA* and *rplC*), ribosomal 30S ribosomal protein S17 (e.g. *rpsQ*) and stress response genes (*sigA*), among others. Moreover, we found that resistance to linezolid in these two strains was due to chromosomal G2576T mutations in the 23S rRNA gene. Further epidemiological comparison between the genomes of strains WIS 441 and Seattle 106 showed that these isolates belonged to the ST72 lineage.²⁶ Interestingly, the ST72 lineage has been reported as associated with resistance to linezolid in *S. aureus* isolated from CF patients. This observation may suggest that there is a connection between the acquisition of resistance to the oxazolidinone class of antibiotics, including tedizolid, and specific *S. aureus* clonal types. In another study, phylogenetic analysis of CF strains suggests that CF patients are colonized by polyclonal populations of MRSA that represent an incredible reservoir for lateral gene transfer, and antibiotic-mediated phage induction may result in replication and high-frequency transfer, with the unintended consequence of promoting the spread of virulence and/or antibiotic resistance determinants, as recently demonstrated with ciprofloxacin and β -lactams in *S. aureus*.²⁷ Our study is the first providing evidence of tedizolid resistance and genome analyses from CF-*S. aureus* strains. The observed tedizolid *in vivo* efficacy against the WIS 441 strain may suggest that bioavailability and host factors may compensate and reduce mediated affinity effects. These data demonstrate the potential clinical utility of tedizolid as an alternative to linezolid for the treatment of *S. aureus* necrotizing pneumonia.

In conclusion, despite improvements in outcomes, the majority of CF patients still die from pulmonary complications and treatment of bacterial lung infection remains one of the primary goals of CF care. MRSA has emerged as a particularly vexing component of this challenge. We provide strong evidence that tedizolid may constitute an optimal alternative therapeutic option for the treatment of CF-*S. aureus* infections, extending its clinical utility in this vulnerable patient population.

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Transparency declarations

None to declare.

Supplementary data

Table S1 is available as [Supplementary data](#) at JAC Online.

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