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Antimicrobial Susceptibility Studies

Results of the Surveillance of Tedizolid Activity and Resistance Program: in vitro susceptibility of Gram-positive pathogens collected in 2011 and 2012 from the United States and Europe

Daniel F. Sahm [a,*], Jennifer Deane [a], Paul A. Bien [b], Jeffrey B. Locke [b], Douglas E. Zuill [b], Karen J. Shaw [b], Ken F. Bartizal [b]

[a] Eurofins Global Central Laboratories, Chantilly, VA, 20151, USA
[b] Trius Therapeutics, a Subsidiary of Cubist, San Diego, CA, 92121, USA

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ABSTRACT

The in vitro activity and spectrum of tedizolid and comparators were analyzed against 6884 Gram-positive clinical isolates collected from multiple US and European sites as part of the Surveillance of Tedizolid Activity and Resistance Program in 2011 and 2012. Organisms included 4499 *Staphylococcus aureus*, 537 coagulase-negative staphylococci (CoNS), 873 enterococci, and 975 β-hemolytic streptococci. The MIC values that inhibited 90% of the isolates within each group (MIC_{90}) were 0.25 μg/mL for *Staphylococcus epidermidis* and β-hemolytic streptococci and 0.5 μg/mL for *S. aureus*, other CoNS, and enterococci. Of 16 isolates with elevated tedizolid or linezolid MIC values (intermediate or resistant isolates), 10 had mutations in the genes encoding 23S rRNA (primarily G2576T), 5 had mutations in the genes encoding ribosomal proteins L3 or L4, and 5 carried the *cfr* multidrug resistance gene. Overall, tedizolid showed excellent activity against Gram-positive bacteria and was at least 4-fold more potent than linezolid against wild-type and linezolid-resistant isolates. Given the low overall frequency of isolates that would be resistant to tedizolid at the proposed break point of 0.5 μg/mL (0.19%) and potent activity against contemporary US and European isolates, tedizolid has the potential to serve as a valuable therapeutic option in the treatment of infections caused by Gram-positive pathogens.

1. Introduction

Tedizolid phosphate is a novel oxazolidinone prodrug that is rapidly converted by endogenous phosphatases to tedizolid, the microbiologically active moiety (Im et al., 2011; Shaw et al., 2008). Tedizolid exerts its antibacterial activity by binding to the peptidyl transferase center (PTC) of the 50S subunit of the bacterial ribosome, resulting in inhibition of protein synthesis. It has potent activity against a wide range of Gram-positive pathogens, including resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) (Im et al., 2011; Prokocimer et al., 2012; Schaadt et al., 2009; Shaw et al., 2008). Tedizolid demonstrates at least a 4-fold lower MIC value than linezolid, the only other currently marketed oxazolidinone antibiotic, against strains of staphylococci (including MRSA), streptococci, and enterococci (including VRE) (Brown and Traczewski, 2010; Schaadt et al., 2009). This increased potency is due to structural differences in the C- and D-rings, resulting in additional target site interactions with 23S rRNA residues that compose the PTC binding site (Shaw et al., 2008).

The pharmacokinetic and pharmacodynamic properties allow for once-daily administration of tedizolid, either orally or intravenously, at equivalent doses (Muñoz et al., 2013; Prokocimer et al., 2011, 2013). Two Phase 3, randomized, double-blind, noninferiority trials that compared 200 mg once-daily tedizolid for 6 days with 600 mg twice-daily linezolid for 10 days for the treatment of acute bacterial skin and skin structure infections (ABSSSI) demonstrated that treatment with tedizolid phosphate was non-inferior to linezolid and that tedizolid was generally well tolerated (Moran et al., 2014; Prokocimer et al., 2013).

The incidence of linezolid resistance development has been very low since its approval for clinical use in 2000 (Mendes et al., 2014b). However, some linezolid-resistant strains have emerged in patients without prior linezolid exposure and have been attributed to the clonal spread of strains from other hospitalized patients (Jones et al., 2006).

Oxazolidinone resistance is conferred by mutations or modifications that alter the conformation of the PTC binding site (Long and Vester, 2012). Mutations in chromosomal genes encoding 23S rRNA, most notably G2576T, have been found in the majority of linezolid-resistant isolates characterized to date (Long and Vester, 2012; Meka et al., 2004; Prystowsky et al., 2001; Tsiodras et al., 2001), with the degree of resistance correlating with the number of mutated gene copies

* Corresponding author. Tel.: +1-847-303-5003; fax: +1-847-303-5601.
E-mail addresses: dsahm@ihmainc.com (D.F. Sahm), jdeane@atcc.org (J. Deane), pbien74@gmail.com (P.A. Bien), jlocke@cidara.com (J.B. Locke), dzuill100@gmail.com (D.E. Zuill), kjshaw3@yahoo.com (K.J. Shaw), kbartizal@cidara.com (K.F. Bartizal).

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(Besier et al., 2008; Locke et al., 2009a; Marshall et al., 2002). Oxazolidinone resistance has also been associated with mutations in chromosomal genes encoding ribosomal proteins L3 and L4 (Locke et al., 2009a, 2009b; Wolter et al., 2005). Linezolid resistance can also arise from the presence of the plasmid-borne chloramphenicol-florfenicol resistance gene (*cfr*), which encodes a ribosomal methyltransferase (Cfr) (Mendes et al., 2008; Toh et al., 2007). Post-transcriptional Cfr methylation of 23S rRNA nucleotide A2503 sterically hinders the binding of multiple antimicrobial agents (Kaminska et al., 2010; Locke et al., 2010; Long et al., 2006; Smith and Mankin, 2008). Tedizolid retains activity against strains carrying *cfr* (that do not also possess chromosomally mediated linezolid resistance) due to its more compact A-ring hydroxymethyl side chain (Shaw et al., 2008).

The goal of the Surveillance of Tedizolid Activity and Resistance (STAR) Program is to compare the in vitro activity of tedizolid and other antimicrobials against a variety of clinically relevant Gram-positive pathogens and to monitor for the emergence of resistance. The Gram-positive pathogens chosen represent those relevant to the indication for ABSSSI, including those with significant resistance phenotypes such as MRSA and VRE.

2. Materials and methods

2.1. Bacterial isolate collection

Eurofins Global Central Laboratories (Chantilly, VA, USA) prospectively collected a total of 6884 non-duplicate, non-consecutive clinically significant isolates of Gram-positive bacteria from multiple locations in the United States and Europe. Of these, 3519 (51.1%) were collected in 2011, and 3365 (48.9%) were collected in 2012. The distribution of pathogen species in the US and Europe is shown in Table 1. Of the 6884 isolates, 5718 (83.1%) were collected from sites across the 9 US Census regions, and 1166 (16.9%) were collected from 6 countries in Europe (United Kingdom, France, Belgium, Spain, Germany, and Italy). In the US, isolates were collected from 40 sites in 2011 and 34 sites in 2012. In Europe, isolates were collected from 9 sites in 2011 and 6 sites in 2012.

2.2. Susceptibility testing

Upon receipt of the isolates, species identification was confirmed by MALDI Biotyper (Bruker, Fremont, CA, USA). Susceptibility testing was performed by broth microdilution in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI) and Eurofins Scientific standard operating procedures (CLSI, 2012a, 2012b). Quality control and interpretation of results were performed in accordance with CLSI M100. Susceptibility to all agents was classified according to CLSI break points (CLSI, 2012a). The tedizolid and linezolid MIC values of isolates with tedizolid MIC values >0.5 μg/mL and/or linezolid MIC values >4 μg/mL were confirmed by broth microdilution testing performed at Trius Therapeutics, a subsidiary of Cubist (San Diego, CA, USA), in accordance with CLSI guidelines.

Isolates were tested with MIC panels (Thermo Fisher Scientific, Cleveland, OH, USA) of antibiotics appropriate for their genera. Staphylococci and enterococci panels included the following agents: tedizolid, oxacillin (staphylococci only), ampicillin (enterococci only), erythromycin, clindamycin, daptomycin, linezolid, vancomycin, levofloxacin, tigecycline, gentamicin (staphylococci only), trimethoprim/sulfamethoxazole, gentamicin high-level aminoglycoside resistance (HLAR) (enterococci only), and streptomycin HLAR (enterococci only). Streptococci panels included 12 agents (tedizolid, ampicillin, penicillin, erythromycin, clindamycin, levofloxacin, trimethoprim/sulfamethoxazole, tetracycline, ceftriaxone, vancomycin, daptomycin, and linezolid).

2.3. Genetic analysis

Isolates with tedizolid MIC values >0.5 μg/mL and/or linezolid MIC values >4 μg/mL were analyzed for the presence of oxazolidinone resistance determinants. Screening for the presence of the *cfr* gene was performed by polymerase chain reaction (PCR) amplification and sequencing, as previously described (Locke et al., 2012). Sequence analysis of genes encoding 23S rRNA or ribosomal proteins L3 (*rplC*) and L4 (*rplD*) was performed through PCR amplification and sequencing, as previously described for *S. aureus* and *Staphylococcus epidermidis* (Locke et al., 2009a, 2009b; Meka et al., 2004; Pillai et al., 2002). The *Enterococcus faecalis* V583 genomic sequence (GenBank accession no. AE016830) was used to design *rplC* and *rplD* primers and was the source for the previously established set of 23S rRNA allele primers used in this study (Bourgeois-Nicolaos et al., 2007). Primers for analysis of *Enterococcus faecium* 23S rRNA, *rplC*, and *rplD* alleles were designed from the annotated *E. faecium* DO genome (GenBank accession no. CP003583.1). Mutations in genes encoding 23S rRNA were reported on the DNA level using *Escherichia coli* nucleotide numbering and mutations in the genes encoding ribosomal proteins L3 and L4 were reported on the protein level using amino acid residue numbering respective to the species analyzed.

3. Results

3.1. Overall activity of tedizolid

Over 2011 and 2012, tedizolid maintained a consistent and potent level of activity against key target pathogens. The activity profile of tedizolid and comparators is shown in Table 2, and the cumulative percentages of isolates inhibited at each tedizolid MIC value are shown in Table 3. Tedizolid MIC values ranged from ≤0.015 to 8 μg/mL, and 99.8% of tested isolates were inhibited at a tedizolid MIC value of ≤0.5 μg/mL. Only 13 of 6884 strains showed tedizolid MIC values ≥1 μg/mL (Table 4).

3.2. Activity of tedizolid against S. aureus

Tedizolid was highly active against staphylococci. Out of 4499 isolates of *S. aureus*, 99.9% had tedizolid MIC values of 0.5 μg/mL or less, and the modal MIC value was 0.25 μg/mL (Table 3 and Fig. 1A). MIC values for linezolid ranged from ≤0.25 to >4 μg/mL, with a modal value of 2 μg/mL and MIC_{90} of 2 μg/mL. Overall, 99.9% (4488 of 4492) of *S. aureus* isolates that were susceptible to linezolid had a tedizolid MIC value ≤0.5 μg/mL. These results were consistent over both geographic regions (data not shown). In addition, tedizolid maintained activity against *S. aureus* regardless of the methicillin susceptibility phenotype of the isolate (Table 4). The $MIC_{50/90}$ values against both MRSA and methicillin-susceptible *S. aureus* (MSSA) were 0.25/0.5 μg/mL; linezolid MIC_{50} and MIC_{90} values were 2 μg/mL for both MRSA and MSSA. However, the percentage of MRSA differed between continents. In the United States, 41.5% and 43.5% of *S. aureus* isolates were methicillin resistant in 2011 and 2012, respectively. In Europe, percentages of MRSA were lower, 24.3% in 2011 and 22.7% in 2012.

Seven *S. aureus* strains out of 4499 tested (0.2%) were resistant to linezolid, all with MIC values of 16 μg/mL (Table 4). Tedizolid MIC values for these strains ranged from 0.5 to 2 μg/mL. Analysis of resistance determinants showed that 4 of the 7 linezolid-resistant strains carried the *cfr* gene and no other chromosomal mutations. Of the 4 *cfr*-positive strains, tedizolid MIC values were 0.5 μg/mL for 3 strains and 1 μg/mL for 1 strain, which were 16- to 32-fold lower than the corresponding linezolid MIC values. For the 3 *cfr*-negative linezolid-resistant strains, sequence analysis of genes encoding 23S rRNA

Table 1
Distribution of organisms collected as part of the STAR Program in the United States and Europe in 2011 and 2012.

Location	Number of strains								
	S. aureus	*S. epidermidis*	Other CoNS	*E. faecalis*	*E. faecium*	Other enterococci	*S. agalactiae*	*S. pyogenes*	Other β-hemolytic streptococci
United States	3743	290	153	527	176	12	454	333	30
Europe	756	61	33	107	45	6	76	74	8
Total	4499	351	186	634	221	18	530	407	38