Comparative activity of tedizolid and glycopeptide combination therapies for the treatment of *Staphylococcus aureus* infections: an *in vitro* and *in vivo* evaluation against strains with reduced susceptibility to glycopeptides

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**Abstract**

**Purpose.** Glycopeptides are widely used for the treatment of meticillin-resistant *Staphylococcus aureus* (MRSA) infections. Although difficult to detect, isolates with reduced (GISA), hetero (hGISA) or complete (GRSA) resistance to glycopeptides are increasingly reported. Optimal therapy for such strains is unknown. We compared the *in vitro* and *in vivo* activity of tedizolid (TED), a recently licensed oxazolidonone, with vancomycin (VAN) and teicoplanin (TEIC) combined with fusidic acid (FD) or rifampicin (RIF) against *S. aureus* (SA) with reduced susceptibility to glycopeptides.

**Methods.** Susceptibility was determined for six (GISA, hGISA and GRSA) reference strains and 72 clinical MRSA isolates screened for hGISA/GISA-like phenotypes. Synergy and bactericidal activity were assessed using chequerboard and time-kill assays. The *G. mellonella* wax moth caterpillar model was used to measure the activity of TED and the combinations *in vivo*.

**Results.** Glycopeptide MICs (VAN/TEIC) ranged from 0.5–8/4 and 0.125–1 for TED. No significant synergy was noted when VAN/TEIC were combined with either RIF or FD. Time-kill assays confirmed that TED was bacteriostatic but superior to VAN and TEIC against GISA strains. In *G. mellonella* TED was more effective than TEIC monotherapy versus GISA strains. The combination of TEIC with RIF was the most effective combination overall, both *in vitro* and *in vivo*.

**Conclusions.** TED had good *in vitro* activity versus MRSA including those with reduced susceptibility to glycopeptides. Although bacteriostatic, it was effective in the *G. mellonella* model and superior to TEIC in the treatment of GISA. Although this supports the use of TED for MRSA and GISA, the TEIC/RIF combination also warrants further study.

**INTRODUCTION**

Glycopeptides have been used in the treatment of Gram-positive infections for more than 50 years, predominantly for meticillin-resistant *Staphylococcus aureus* (MRSA) infections. Although both VAN and TEIC usually retain excellent activity in routine laboratory susceptibility tests (MIC determinations), there is growing concern that their activity might soon be compromised. The earliest report of a *S. aureus* clinical isolate (Mu50) with reduced susceptibility to VAN (MIC 8 mg l⁻¹) came from Japan in 1997. Subsequently strains, frequently belonging to MRSA lineages, displaying either intermediate (GISA), hetero (hGISA), 'slow' (sVISA) or outright (GRSA) resistance to glycopeptides have been reported worldwide [1]. Although the mechanisms underlying resistance in GRSA isolates (horizontal acquisition of the *vanA* gene) have been identified, the reasons for reduced susceptibility are not fully understood. Emergence of the GISA and hGISA phenotype is likely an adaptive response promoted by mutations in multiple genes in two-component (*vraUTSR, walKR, grarRS*), quorum-sensing (*agr*) and transcriptional (*rpoB, agr*) regulatory systems involved in the control of peptidoglycan biosynthesis (*murZ, pbp2, sgtB, tarA, fmtA, lcpC*) and cell wall recycling [2]. The complexity underlying the generation and stability of the GISA and hGISA phenotype makes it difficult to detect amongst *S. aureus* strains recovered in clinical diagnostic laboratories.
The optimal antimicrobial treatment, whether empirical or definitive, for patients who may be infected with hGISA, GISA or GRSA strains is unclear. Glycopeptide monotherapy, with a dosing regimen tailored towards achieving individualized pharmacokinetic and pharmacodynamic (PK/PD) therapeutic targets, may be considered [3], although meta-analysis suggests poorer clinical outcomes in S. aureus infections in which the VAN MIC exceeds 1 mg l$^{-1}$ [4]. In the treatment of complicated MRSA infections, glycopeptides in combination with tetracyclines, co-trimoxazole, quinolones and β-lactams (piperacillin/tazobactam, cefazolin) have all been used with variable success [5]. In the United Kingdom, RIF and FD are often given as adjunctive agents for severe meticillin-susceptible (MSSA) and MRSA infections, including bloodstream infections and endocarditis [6, 7]. Newer drugs that specifically target resistant Gram-positive bacteria include lipoglycopeptides (daptomycin, dalbavancin, telavancin, oritavancin), glyyclcylines (tigecycline, eravaclycline), 5th-generation cephalosporins (ceftaroline, ceftobiprole) and the oxazolidonones (linezolid, tedizolid) [8].

Linezolid, although not bactericidal, is usually active against S. aureus with MRSA, hGISA, GISA and GRSA characteristics [9]. Other beneficial properties include some anaerobic activity, ease of administration (oral and injectable), favourable PK/PD profiles, no requirement for therapeutic drug monitoring (TDM) and secondary activities regarding staphylococcal toxin production (Panton Valentine Leukocidin Toxin) [10]. In clinical trials, linezolid has been found to be superior to glycopeptide therapy in the treatment of some MRSA infections, especially those involving the respiratory tract [11]. However, this has not been a consistent finding in meta-analysis of studies comparing the efficacy of oxazolidonones with glycopeptides [12, 13].

Tedizolid (TED) offers some advantages over linezolid in terms of duration, dosage and side effect profile when used for the treatment of acute bacterial skin and skin structure infections (ABSSIs) within the current licence. It is reported to be up to eightfold more potent than linezolid against S. aureus in standard MIC distribution comparisons [14]. Furthermore, TED retains activity against Staphylococci carrying the plasmid-encoded crfA gene, capable of conferring cross-resistance to linezolid, phenicolcs, lincomycins and streptogramins by methylation of the 23S rRNA target [15].

To explore whether TED could be recommended as a useful treatment for MRSA, hGISA and GISA infections, we compared the activity of TED, glycopeptides (VAN, TEC) and two commonly used glycopeptide combination therapies (RIF and FD) in vitro and also using a simple in vivo model of S. aureus infection.

**METHODS**

**Bacterial isolates, antibiotics and microbiological culture media**

S. aureus reference strains ATCC 25923 (MSSA), NCTC 12493 (MRSA), Mu50 (GISA), Mu3 (hGISA) and RN2240 were obtained from the National Collection of Type Cultures (NCTC) or the Network for Antimicrobial Resistance in S. aureus (NARSA) collection (Eurofins, VA). Seventy-two sequential non-duplicate clinical MRSA strains, isolated from clinical samples submitted as routine cultures to Barts and the London NHS Trust over a 3-month period in 2014, were also included.

Tedizolid (TR-700 Lot 33130169) and tedizolid phosphate (TR-701 Lot 04130103), were provided by Merck and Co, Inc. (Lexington, MA). TR-700 is the microbiologically active metabolite of tedizolid phosphate (TR-701) and was used in all *in vitro* susceptibility tests. TR-701 is the prodrug converted to TR-700 *in vivo* and was used for studies in *G. mellonella*. Teicoplanin, VAN, RIF and FD were sourced from Sigma-Aldrich (Poole, Dorset, UK). Dehydrated culture media were purchased from Oxoid (Basingstoke, Hampshire, UK), prepared and autoclaved according to the manufacturer’s instructions.

**Determination of glycopeptide susceptibility phenotypes**

All isolates were screened for growth and haemolysis on 5% sheep blood agar supplemented with 6 mg l$^{-1}$ VAN according to the assay described by Cafiso et al. [16]. Isolates lacking δ-haemolysis, when plated perpendicular to the β-haemolytic RN2240 strain, were assumed to have a dysfunctional agr system associated with the hGISA phenotype [17]. Glycopeptide MICs (VAN/TEIC) were determined using glycopeptide resistance detection (GRD) Etest strips (Biomerieux, Marcy-D’Etoile, France) with a 0.5 McFarland standard inoculum on MH2 agar [18]. Vancomycin and TEIC MICs were also determined individually using the macro-method on brain–heart infusion agar (BHI) with a heavier 2 McFarland inoculum [19]. Population analysis profiling (PAP) was used as the reference test for designation of any strain as GISA/hGISA. Modified PAP was performed according to the method of Wootton et al. [20], with serial dilution of a 0.5 McFarland suspension plated on BHI plates supplemented with VAN at 0, 0.5, 1, 2, 2.5, 4 and 8 mg l$^{-1}$. Colony counts were performed after 48 h incubation and plotted as log$_10$ c.f.u. ml$^{-1}$ against the VAN concentration. The area under the curve (AUC) was calculated and used to determine the PAP:AUC ratio compared to the AUC of hGISA Mu3. Criteria were a ratio of ≥0.90 for hGISA and ≥1.3 for a GISA isolate.

**Antimicrobial susceptibility testing and synergy assays**

Minimal inhibitory concentrations of TED (0.06–32 mg l$^{-1}$) were determined by broth microtitre dilution (BMD) in Mueller–Hinton 2 (MH2) cation-adjusted broth according to Clinical and Laboratory Standards institute (CLSI) methodology using a 50x stock solution of TR-700 prepared in dimethyl sulfoxide (DMSO). Plates were read after incubation for 48 h at 37°C. Synergy between glycopeptides (0–8 mg l$^{-1}$), RIF (0–64 mg l$^{-1}$) and FD (0–32 mg l$^{-1}$) was assessed in 96-well chequerboard assays with the fractional
inhibitory concentration index (FICI) used to determine either a synergistic (≤0.5), indifferent (0.5–4.0) or antagonistic (≥4.0) interaction. The bactericidal activity of compounds alone and in combination was assessed in time-kill assays against a 10⁶ c.f.u. ml⁻¹ inoculum of each S. aureus strain. Antimicrobials were added at final concentrations relative to EUCAST breakpoints for susceptibility: VAN/TEIC (2 mg l⁻¹), RIF (0.06 mg l⁻¹), FD (1 mg l⁻¹) and TED (0.5 mg l⁻¹). Viable counts were performed after 0, 2, 4, 6 and 24 h incubation and kill curves drawn by plotting log¹⁰ c.f.u. ml⁻¹ versus time.

**G. mellonella treatment assays**

The comparative efficacy of TED (TR-701), TEIC and a TEIC/RIF combination therapy for the treatment of hGISA/GISA was assessed in vivo using a G. mellonella/S. aureus therapeutic model [21]. G. mellonella larvae were obtained from Live Foods (Rooks Farm, Somerset, UK) and stored at 15 °C in wood shavings prior to use. Teicoplanin was used as the comparative glycopeptide due to the potential non-antimicrobial immunomodulatory effects of VAN on the G. mellonella response to infection [22].

Inoculum tests using 10⁴, 10⁵ and 10⁶ c.f.u./larvae were carried out to determine the infective dose required for 50% kill at 24 h (LD₅₀), and also the amount required for staggered killing of G. mellonella larvae over 96 h. Test inocula were prepared from stationary phase S. aureus cultures, washed, diluted and re-suspended in phosphate buffered saline (PBS). Infections were established by injection of 10 µl of the suspension directly into the larva haemocoel via a right proleg.

Treatment assays were conducted using 16 larvae per condition, infected as above. Larvae were chosen according to weight, and only those ranging from 225 to 275 mg were selected. Infected larvae were administered antimicrobials within 15–20 min via 10 µl injections through a left proleg at the following doses: TEIC 10 mg kg⁻¹, RIF 15 mg kg⁻¹, TED (TR-701)-15 mg kg⁻¹ [23]. Single and double injections of 10 µl of sterile PBS were used to control for both the trauma of injection and non-antimicrobial treatment. Non-infected larvae, incubated at 37 °C, were used to assess the health of each batch of caterpillars. Insect larvae were incubated in filter-lined Petri dishes at 37 °C and scored daily for survival (live/dead) for a total of 96 h. Experiments were performed in triplicate on separate occasions with different batches of insects for reproducibility. Pooled survival curves were plotted for each strain and treatment regimens compared using the log-rank test (Mantel–Cox) and the Chi-squared statistic (P<0.05).

**RESULTS AND DISCUSSION**

Using standard susceptibility testing methods (BMD) and EUCAST interpretative breakpoints (http://www.eucast.org/clinical_breakpoints), the majority of isolates were considered susceptible to glycopeptides (VAN/TEIC MIC≤1 mg l⁻¹). Reduced susceptibility to VAN and TEIC was seen in isolates Mu50 (GISA), Mu3 (hGISA) and SA 33 (Table 1). Amongst the 72 MRSA clinical isolates, 17 (24 %) failed to produce δ-haemolysis in the screening assay of which eight (11 %) also had raised MICs to VAN or TEIC in GRD or Macro Etests. Analysis of the PAP/AUC curves for these confirmed that only two in the collection (SA 33 and 75) could be assumed true hGISA (Table 1, Fig. S1, available in the online version of this article). The prevalence of hGISA and GISA amongst MRSA isolates associated with infections in our institution is therefore likely to be <3%.

Significant synergy (FICI≤0.5), when VAN or TEIC was combined with either RIF or FD could not be shown in the chequerboards. An additive effect was obtained when RIF was combined with VAN (FICI 0.74) or TEIC (FICI 0.6) versus MSSA ATCC 25923 and with VAN and FD versus MRSA NCTC 12493 (FICI 0.75). No antagonism was observed with any of the glycopeptide combinations using these assays.

All of the MSSA, MRSA, hGISA and the GISA type strains, the MRSA clinical isolates and 16/17 of the clinical isolates lacking δ-haemolysis, were susceptible to TED (MIC ≤0.5

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Phenotype</th>
<th>PAP/AUC ratio</th>
<th>MIC (µg ml⁻¹) Etest</th>
<th>MIC (µg ml⁻¹) BMD†</th>
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<td>GRD</td>
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<td>VAN</td>
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<tr>
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<td>1.5</td>
<td>&gt;32</td>
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<td>1.14</td>
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<tr>
<td><strong>S. aureus SA 75</strong></td>
<td>hGISA†</td>
<td>0.96</td>
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</table>

* hGISA=GRD MIC of VAN or TEIC≥8 mg l⁻¹.
† hGISA=MIC of VAN and TEIC≥8 mg l⁻¹, or TEIC≥12 mg l⁻¹ in Macro Etest.
‡ OXA, oxacillin; VAN, vancomycin; TEIC, teicoplanin; TED, tedizolid; RIF, rifampicin; FD, fusidic acid.
mg l⁻¹). Isolate SA 33 (TED MIC 1 mg l⁻¹) was classified as having intermediate susceptibility according to existing FDA breakpoints [24] (Table 1). In time-kill assays, TED (0.5 mg l⁻¹) had bacteriostatic effects (≤3 log reduction in c.f.u. ml⁻¹) but was superior to TEIC and VAN (2 mg l⁻¹) against those strains with GISA (Mu50) characteristics. The addition of RIF (0.06 mg l⁻¹) improved the killing activity of TEIC, except against the Mu50 (GISA) strain that also displayed high-level resistance to RIF (MIC>128 mg l⁻¹) (Fig. 1). Similar results were obtained in time-kill assays with hGISA SA 33 and 75 and using two MRSA isolates without hGISA properties. The combination of VAN and FD (1 mg l⁻¹) was as effective as TED against GISA/hGISA, but some antagonism was seen versus MSSA ATCC 25923 (Fig. 2; 2 log regrowth). The mechanism underlying this antagonism, and the prevalence, stability and implications for VAN/FD combination therapy in the treatment of MSSA, MRSA and non-(h)GISA infections, warrant further investigation.

Inoculum tests revealed that all S. aureus strains were pathogenic to G. mellonella. Although we did not specifically seek to identify differences in virulence or pathogenicity, there was variation in the kinetics of larval killing by different strains. This has been investigated by others in the same Galleria model, and it is possible that GISA strains may be attenuated or less fit than glycopeptide susceptible isolates [21, 25]. In our assays we observed 90–100% death within 48–96 h of infection with an inoculum of 10⁶ c.f.u./larva (Fig. S2). In treatment assays, staggered killing of non-treated larvae was also optimal with an inoculum of 10⁵ c.f.u./larva for infection with Mu3, Mu50, 33 and 75 but 10⁴ c.f.u./larva for those inoculated with ATCC 25923. Administration of TED 701 to uninfected larvae did not have any toxic effects on G. mellonella at up to 60 mg kg⁻¹.

Fig. 1. Time-kill curves of TED, VAN, TEIC and TEIC/RIF combination versus MSSA, hGISA and GISA Strains.
Comparison of different treatment regimens in *G. mellonella* infected with MSSA, hGISA and GISA isolates revealed differences in efficacy (Fig. 3). Treatment with either TEIC, TEIC/RIF or TED was effective against the ATCC 25923 MSSA type strain (*P*=0.004), although TED alone was inferior to either of the glycopeptide containing regimens (*P*<0.001). In the treatment of hGISA (Mu3, SA 33) and GISA (Mu50), TED compared to TEIC alone resulted in equal or improved survival (44–75 vs 0–75 %). However, the combination of TEIC and RIF was significantly better, with this regimen being superior to TED monotherapy in the treatment of MSSA, hGISA and GISA (*P*<0.01). Conversion of the TR-701 prodrug to the in vivo active TED compound must therefore proceed through the action of *Galleria* phosphatases in a similar way to the metabolism in humans and other mammalian infection models [26].

Overall, we found TED to have good activity against multidrug-resistant strains of *S. aureus*, including those with reduced susceptibility to glycopeptides. A larger surveillance study using 302 MRSA isolates found very potent activity of TED (MIC<sub>90</sub> 0.5 mg l<sup>-1</sup>), including against >200 with hVISA and VISA characteristics confirmed by PAP. Only two isolates with a TED MIC of 1 mg l<sup>-1</sup> were found in that study, both resistant to linezolid (MIC 8 mg l<sup>-1</sup>) but lacking cfrA [27]. Despite the promising activity illustrated by MIC distributions, it should be noted that the drug remains bacteriostatic. In time kill assays we noted this activity was preserved towards GISA and hGISA strains but superior to glycopeptides. Differences in the activity of TED and the glycopeptide combination therapies are interesting. Although synergy between VAN, TEIC, RIF and FD could not be demonstrated in chequerboard studies, the combination of TEIC and RIF was found to be bactericidal against...
RIF susceptible strains in time-kills. It was also the most effective therapy in the *G. mellonella* model, irrespective of TEIC or RIF susceptibility. Mutations in *rpoB*, encoding the β subunit of bacterial RNA polymerase, the target of RIF, have been linked with the development and evolution of GISA phenotypes [28]. Whether the addition of RIF would improve the outcome of glycopeptide therapy or select for resistance to both drugs therefore remains to be shown. A randomized controlled trial of the adjunctive use of RIF for *S. aureus* bacteraemia (ARREST, ISRCTN 37666216) is under way in the UK [29], and may provide useful data to answer this.

TED is currently licensed for short-term use in the treatment of complicated bacterial skin and soft tissue infections. The potent activity seen against *S. aureus* strains both in *vitro* and in *vivo*, including those with glycopeptide and linezolid resistance, suggests it may be increasingly useful in these infections, either as a monotherapy or an alternative to glycopeptide-containing combination regimens.

**Fig. 3.** Survival curves for *G. mellonella* infected with *S. aureus* ATCC 25923, Mu50, Mu3 and SA 33 treated with TED, TEIC, RIF and TEIC+RIF.

**Acknowledgements**
This work was presented in part at the 26th European Congress of Clinical Microbiology and Infectious Diseases, Amsterdam, 2016. We would like to thank Dr Stephanie Smith (Barts Health NHS Trust) for collection and preserving MRSA clinical isolates.

**Conflicts of interest**
The authors declare that there are no conflicts of interest.

**References**


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