

In vitro activity and in vivo efficacy of tigecycline alone and in combination with daptomycin and rifampin against Gram-positive cocci isolated from surgical wound infection

C. Silvestri · O. Cirioni · D. Arzeni · R. Ghiselli ·
O. Simonetti · F. Orlando · G. Ganzetti · S. Staffolani ·
L. Brescini · M. Provinciali · A. Offidani ·
M. Guerrieri · A. Giacometti

Received: 21 July 2011 / Accepted: 16 November 2011 / Published online: 11 December 2011
© Springer-Verlag 2011

Abstract The aim of this work was to determine the in vitro activity of tigecycline and its bactericidal effect for a large number of Gram-positive cocci, as well as to investigate its in vitro interaction with six clinically used antibiotics. In vivo, a wound model was established through the panniculus carnosus of BALB/c mice, and then inoculated with 5×10^7 colony-forming units (CFU) of *Staphylococcus aureus* or *Enterococcus faecalis*. For each bacterial strain, the study included an infected or non-infected group that did not receive any treatment, three groups singly treated with

tigecycline, rifampin, and daptomycin, and two groups that received tigecycline treatment plus rifampin or daptomycin. In the in vitro studies, tigecycline, daptomycin, and teicoplanin were active against all of the 48 Gram-positive isolates. The combination of tigecycline with rifampicin and daptomycin was synergistic against *S. aureus* and *Enterococcus* spp. In the in vivo studies, all groups treated with single drugs showed statistically significant results compared to the control group. The two groups treated with a combination of drugs showed the highest antimicrobial efficacy. In conclusion, our results suggested a strong activity of tigecycline alone and in combination with other antimicrobial agents against multi-resistant Gram-positive organisms isolated from wound infections.

C. Silvestri · O. Cirioni · D. Arzeni · S. Staffolani · L. Brescini ·
A. Giacometti
Institute of Infectious Diseases and Public Health,
Università Politecnica delle Marche—Ospedali Riuniti,
Ancona, Italy

R. Ghiselli · M. Guerrieri
General Surgery and Surgery Methodology Clinic,
Università Politecnica delle Marche—Ospedali Riuniti,
Ancona, Italy

O. Simonetti · G. Ganzetti · A. Offidani
Dermatological Clinic,
Università Politecnica delle Marche—Ospedali Riuniti,
Ancona, Italy

F. Orlando · M. Provinciali
Experimental Animal Models for Aging Units,
Research Department, I.N.R.C.A.—I.R.R.C.S.,
Ancona, Italy

O. Cirioni (✉)
Clinica delle Malattie Infettive, c/o Ospedali Riuniti di Ancona,
Via Conca, 71,
60126 Ancona, AN, Italy
e-mail: o.cirioni@univpm.it

Introduction

Gram-positive infections have become a serious problem, especially in the nosocomial setting, and their treatment is often complicated by the emergence of multidrug-resistant pathogens [1, 2]. Surgical site infections are the second most common cause of these infections; the US Centers for Disease Control and Prevention (CDC) estimates that about 500,000 surgical site infections occur annually in the United States [3, 4]. They are often the biological summation of several factors, such as the inoculum of bacteria introduced into the wound during the procedure, the unique virulence of contaminants, the microenvironment of each wound, and the integrity of the patient's host defense mechanisms [3–5]. *Staphylococcus aureus* and *S. epidermidis* are among the

most frequent causes of these infections [6]. Roughly, 40% of the general population are colonized with *S. aureus* and, therefore, carry an increased risk for infection associated with surgery, dialysis, or intravascular devices. At the same time, enterococci, which have traditionally been regarded as low-grade pathogens, have emerged as an increasingly important cause of nosocomial infection. The rise in hospital-acquired enterococcal infection has been, in part, due to the increased use of broad-spectrum antibiotics and the rising number of severely ill patients [7]. The increasing multidrug resistance of these bacteria due to overuse and failure to apply basic infection control policies and procedures has created a need for the development of new antimicrobial agents to treat these infections [1–4, 8, 9]. In particular, in the last decade, enterococci have demonstrated an increasing frequency of multidrug resistance, including high-level resistance to aminoglycosides, penicillins, chloramphenicol, tetracycline, and glycopeptides [10, 11]. Most enterococcal infections are caused by *Enterococcus faecalis*, which causes 80–90% of human enterococcal infections and is more likely to be resistant even to antibiotics of last resort [7, 10].

A way to overcome the problems of this emergence is in an increased effort to search for antimicrobial compounds with new mechanisms of action and in the use of synergistic antimicrobials [11–13].

Tigecycline is a member of the glycylcyclines. It has a broader range of activity, covering infections caused not only by resistant Gram-positive bacteria, but also by many multiply resistant Gram-negative organisms, including those producing extended-spectrum β -lactamases, but little is currently known about the activity of tigecycline-based combinations [14, 15].

The aim of the present study was to evaluate the in vitro activity of tigecycline against a large number of Gram-positive cocci, as well as to investigate its efficacy, both alone and in combination with other conventional drugs, in an animal model of wound staphylococcal and enterococcal infection.

Materials and methods

Isolates

A total of 48 nonduplicate clinical isolates were studied. They included 12 strains each of *E. durans*, *E. faecalis*, *E. faecium*, and methicillin-resistant *S. aureus* (MRSA), respectively. MRSA ATCC 43300 and *E. faecalis* ATCC 29212 were included as reference strains. The clinical isolates were cultured from specimens obtained from patients who underwent surgical treatment from July 2005 to December 2010.

Strains were identified by the API 20 STAPH and API 20 STREP System (bioMérieux, Marcy, L'Etoile, France).

Antimicrobial agents

The following antibiotics were evaluated: amikacin (Sigma-Aldrich, St. Louis, MO), daptomycin (Novartis Pharma Scherz AG, Bern, Switzerland), imipenem (Merck, Sharp & Dohme, Milan, Italy), levofloxacin (Aventis Pharma AG, Zurich, Switzerland), rifampin (Sigma-Aldrich, St. Louis, MO), teicoplanin (Sanofi-Aventis, Milan, Italy), and tigecycline (Wyeth Pharmaceuticals, Inc., Philadelphia, PA). Stock solutions were prepared in physiological solution and stored at -80°C until they were used. The concentration range assayed for amikacin and teicoplanin was 0.25–64 $\mu\text{g/ml}$, and for daptomycin, imipenem, levofloxacin, rifampin, and tigecycline, the concentration range was 0.015–8.0 $\mu\text{g/ml}$.

MIC determination

Minimum inhibitory concentrations (MICs) were determined by a broth microdilution method with cation-adjusted Mueller–Hinton broth (Becton Dickinson Italia, Milan, Italy) and an inoculum of 5×10^5 according to the procedures outlined by the Clinical and Laboratory Standards Institute (CLSI), formerly the National Committee for Clinical Laboratory Standards (NCCLS) [16]. The MIC was taken as the lowest drug concentration at which observable growth was inhibited. For daptomycin, the growth media was supplemented with Ca^{2+} to a final concentration of 50 $\mu\text{g/mL}$. For tigecycline, fresh broth was used (less than 12 h old). Experiments were performed in triplicate. *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 were included in each set of experiments.

Synergy studies

In interaction studies, all the strains were used to test the antibiotic combination by a checkerboard titration method using 96-well polypropylene microtiter plates. The wells were inoculated with 10^5 colony-forming units (CFU)/ml and the plates were incubated for 24 h at 35°C [17].

Fractionary inhibitory concentrations (FICs) were calculated as the MIC of drug A or B in combination/the MIC of drug A or B alone, and the FIC index (FICI) was obtained by adding the FIC values. FICIs were interpreted as follows: ≤ 0.5 , synergy; >0.5 and < 4 , indifferent; and ≥ 4 , antagonistic [17, 18].

Time–kill assay

Synergism for daptomycin and tigecycline against *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 were also tested

in time–kill experiments. Tubes containing brain heart infusion (BHI) were inoculated with 1×10^5 to 5×10^6 CFU/ml of the test organism. Each antibiotic was tested alone (1/2 MIC) and in combination. For synergism, antibiotics were added to the tubes at concentrations equivalent to 1/2 MIC and incubated in a shaking water bath at 35°C. Aliquots were removed at time 0, 3, 6, and 24 h of incubation and serially diluted in sodium chloride solution for the determination of viable counts. Diluted samples were plated on trypticase soy agar and incubated at 35°C for 18 h [17].

Synergy was defined as $\geq 2 \log_{10}$ decrease in the CFU/ml between the combination and its most active component after 3, 6, and 24 h and the number of surviving organisms in the presence of the combination being $\geq 2 \log_{10}$ below the starting inoculum at 0 h.

Animals

Adult male BALB/c mice weighting 40 to 50 g were used for all the experiments ($n=12$ per group). All animals were housed in individual cages under constant temperature (22°C) and humidity with a 12-h light/dark cycle, and had access to chow and water as much as desired throughout the study. The environment was temperature- and humidity-controlled, with lights on and off at 06.30 AM and 06.30 PM, respectively. The study was approved by the animal research ethics committee of the I.N.R.C.A.–I.R.R.C.S., Ancona, Italy.

Preparation of inoculum

The quality control strains methicillin-susceptible *S. aureus* (MSSA) ATCC 29213 and vancomycin-susceptible *E. faecalis* ATCC 29212 were used in the in vivo setting. Bacteria were grown in BHI broth. When bacteria were in the log phase of growth, the suspension was centrifuged at 1,000g for 15 min, the supernatant discarded, and the bacteria were re-suspended and diluted into sterile saline to achieve a concentration of approximately 5×10^7 CFU/ml.

Mouse wound infection model

Rifampicin and daptomycin were chosen for the in vivo studies since they were shown to be synergic with tigecycline in the in vitro studies. For each strain, the study included an infected or not infected group that did not receive any treatment, three groups that received singly intraperitoneal treatment with tigecycline (1.5 mg/kg), rifampin (10 mg/kg), and daptomycin (7 mg/kg), a group that received intraperitoneal tigecycline plus rifampin, and, finally, a group where intraperitoneal tigecycline plus daptomycin at the same dosages as the singly treated groups was given. The mice were anesthetized by an intramuscular injection of ketamine (50 mg/kg body weight) and xylazine

(8 mg/kg body weight), and hair on the back was shaved and the skin cleansed with 10% povidone–iodine solution. Using a 1.0 × 2.0-cm template, one full-thickness wound was established through the panniculus carnosus on the back subcutaneous tissue of each animal. A small gauze was placed over each wound and then inoculated with 1 ml of 5×10^7 CFU of control strains [19]. The pocket was closed by means of skin clips [20]. This procedure results in a local abscess at 24 h. One wound was created per animal. The animals were returned to individual cages and thoroughly examined daily. After 24 h, in the control animals, the wound was opened, the gauze removed for quantitative bacterial culture, and treatment was initiated. Intraperitoneal treatments were administered daily for 7 days.

Animals were euthanized and a 1 × 2-cm area of skin, including the wound, was excised aseptically. Skin samples were homogenized in 1 ml of phosphate-buffered saline (PBS) using a stomacher. The quantization of viable bacteria was performed by culturing serial dilutions (0.1 ml) of the bacterial suspension on blood agar plates. All plates were incubated at 37°C for 48 h and evaluated for the presence of bacteria. The organisms were quantized by counting the number of CFU per plate. The limit of detection for this method was approximately 10 CFU/g.

Statistical analysis

All results are presented as group means with standard deviation. Statistical analysis was performed using analysis of variance (ANOVA). Significance was accepted when the p -value was <0.05 .

Results

All isolates were susceptible to tigecycline, daptomycin, and teicoplanin (Table 1).

Interestingly, tigecycline, for each species considered, showed lower values of susceptibility than the other drugs. All isolates of *S. aureus* and *E. faecium* showed high rates of resistance to levofloxacin (range 8.0–16 µg/ml). *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 showed MIC values as established by the CLSI.

High rates of synergism were observed for *E. durans* when tigecycline was combined with imipenem (75%). The combination of tigecycline with rifampicin and daptomycin was synergistic against all isolates of *Enterococcus* spp., including the control strain, while the better combination for *S. aureus* was obtained when tigecycline was combined with daptomycin and rifampin (75%), including the control strain. Interestingly, no antagonism was observed (Table 2).

Table 1 Minimum inhibitory concentration (MIC) range, MIC₅₀, and MIC₉₀ values for each species versus amikacin (Ak), daptomycin (Dap), imipenem (Ipm), levofloxacin (Lev), rifampicin (Ra), teicoplanin (Tec), and tigecycline (Tig)

Clinical isolates	No. of isolates		Ak	Dap	Ipm	Lev	Ra	Tec	Tig
<i>E. durans</i>	12	MIC range	8–128	0.5–4.0	1.0–16	0.5–16	0.25–8.0	0.5–8.0	0.015–0.125
		MIC ₅₀	16	1.0	4.0	1.0	1.0	0.5	0.03
		MIC ₉₀	128	2.0	8.0	8.0	8.0	8.0	0.125
<i>E. faecalis</i>	12	MIC range	8.0–128	0.25–4.0	0.5–16	0.5–16	0.06–4.0	0.25–8.0	0.015–0.25
		MIC ₅₀	64	2.0	2.0	4.0	1.0	0.5	0.06
		MIC ₉₀	128	4.0	4.0	16	4.0	4.0	0.25
<i>E. faecium</i>	12	MIC range	1.0–64	0.25–4.0	0.125–8.0	8.0–16	0.25–4.0	0.5–8.0	0.015–0.25
		MIC ₅₀	16	1.0	1.0	16	2.0	1.0	0.06
		MIC ₉₀	64	4.0	8.0	16	4.0	4.0	0.25
<i>S. aureus</i>	12	MIC range	1.0–16	0.125–1.0	0.125–32	8–32	0.06–4.0	0.5–8.0	0.015–0.5
		MIC ₅₀	4.0	0.25	1.0	16	0.06	2.0	0.125
		MIC ₉₀	16	1.0	32	32	4.0	4.0	0.125

For ATCC staphylococcal and enterococcal control strains, the time–kill experiments (Figs. 1 and 2) confirmed that tigecycline plus daptomycin was more active than either drug alone ($p < 0.05$).

In the in vivo setting, for staphylococcal infection, the mean bacterial numbers in challenged but untreated controls ($5.8 \times 10^7 \pm 0.7 \times 10^7$ CFU/ml) were significantly higher than those recovered from all treatment groups (Table 3). Specifically, tigecycline alone reduced the bacterial numbers to $4.6 \times 10^3 \pm 0.4 \times 10^3$ CFU/ml. A comparable reduction in bacterial load was also obtained following the administration of intraperitoneal daptomycin or rifampin ($3.6 \times 10^3 \pm 0.3 \times 10^3$ and $7.0 \times 10^3 \pm 1.3 \times 10^3$, respectively). Finally, the greatest bacterial inhibition was obtained in the group that received tigecycline and intraperitoneal daptomycin or rifampin ($1.2 \times 10^1 \pm 0.1 \times 10^1$ and $2.8 \times 10^1 \pm 0.4 \times 10^1$, respectively) ($p < 0.01$).

For enterococcal infection, we observed the same pattern of results. All groups treated with single drugs showed statistically significant results compared to the control group. Similarly to the staphylococcal group infection, the two groups treated with a combination of drugs showed the highest efficacy in the inhibition of bacterial load. The results are summarized in Table 4.

Discussion

The increasing prevalence of resistant Gram-positive bacteria that are commonly responsible for wound infections restricts the choice of drugs for their treatment [1, 3]. Multidrug-resistant strains further complicate the problem, as empiric antibiotic therapy usually remains inadequate or inappropriate in such patients.

Since the 1960s, at least five major clones of MRSA have spread worldwide. Initially, they were found only among patients with hospital contact, but, already by the 1990s, distinct community-associated strains emerged. In contrast to methicillin resistance, significant resistance to glycopeptides among enterococci was not detected until these agents were in use for almost three decades. This resistance was firstly described among enterococcal species in Europe in 1988. Antimicrobial combinations have been used clinically with the aim of increasing treatment efficacy and decreasing the emergence of drug-resistant mutants [21]. Tigecycline has been shown to be one of the most promising therapeutic options for treating multidrug-resistant infections. It is bacteriostatic against a broad spectrum of aerobic and anaerobic Gram-positive (including MRSA and vancomycin-resistant enterococci) and Gram-negative organisms. It acts by

Table 2 Percentage of synergy (S) and indifference (I) between tigecycline (Tig) in combination with a second agent: amikacin (Ak), daptomycin (Dap), imipenem (Ipm), levofloxacin (Lev), rifampicin (Ra), and teicoplanin (Tec)

Clinical isolates	No. of isolates	Tig/Ak		Tig/Dap		Tig/Ipm		Tig/Lev		Tig/Ra		Tig/Tec	
		S	I	S	I	S	I	S	I	S	I	S	I
<i>E. durans</i>	12	17%	83%	42%	58%	75%	25%	8%	92%	42%	58%	0%	100%
<i>E. faecalis</i>	12	17%	83%	100%	0%	42%	58%	25%	75%	67%	33%	0%	100%
<i>E. faecium</i>	12	17%	83%	58%	42%	58%	42%	17%	83%	100%	0%	17%	83%
<i>S. aureus</i>	12	0%	100%	75%	25%	58%	42%	17%	83%	75%	25%	33%	67%

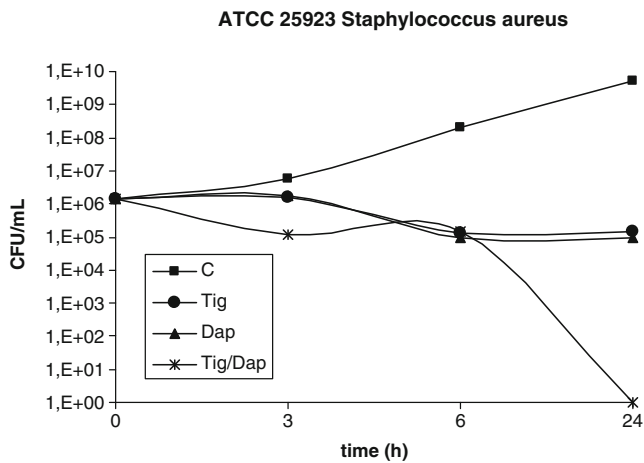


Fig. 1 Killing curve for *Staphylococcus aureus* of tigecycline (Tig) (1/2 MIC: 0.125 µg/ml), daptomycin (Dap) (1/2 MIC: 0.25 µg/ml), and the combination of tigecycline/daptomycin (Tig/Dap) (0.125/0.25 µg/ml)

preventing translation through a reversible binding interaction that blocks the association of charged tRNA with the ribosome [22–25].

According to the literature, our results showed a good in vitro activity of tigecycline against enterococci and MRSA isolates [26, 27]. In fact, all isolates were susceptible to tigecycline. These strains were also highly susceptible to teicoplanin and daptomycin, while they showed high rates of resistance to levofloxacin.

For each species, we observed a good synergic activity of tigecycline in combination with all the drugs tested, with the exception of teicoplanin. In particular, the better combination was obtained for tigecycline/daptomycin and tigecycline/rifampin for all strains of *Enterococcus* spp. To better corroborate the in vitro data, we performed an animal model

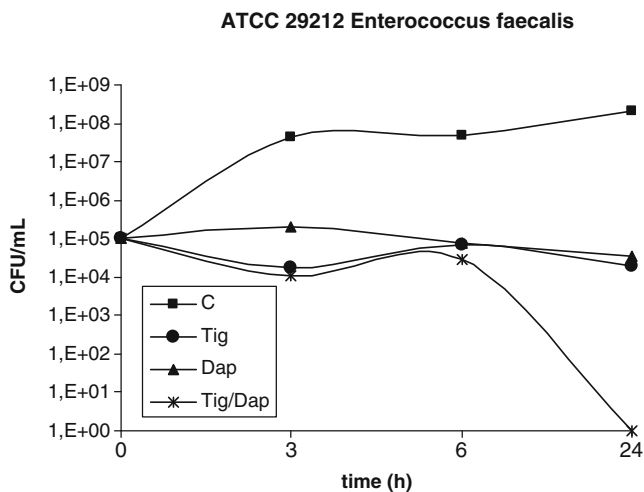


Fig. 2 Killing curve for *Enterococcus faecalis* of tigecycline (Tig) (1/2 MIC: 0.125 µg/ml), daptomycin (Dap) (1/2 MIC: 1.0 µg/ml), and the combination of tigecycline/daptomycin (Tig/Dap) (0.125 /1.0 µg/ml)

Table 3 Quantitative culture of excised tissues after drug administration in staphylococcal wound infection

Treatment	CFU/ml
Uninfected	<10
Infected untreated	$5.8 \times 10^7 \pm 0.7 \times 10^7$
Tig (1.5 mg/kg) ^a	$4.6 \times 10^3 \pm 0.4 \times 10^{3b}$
Ra (10 mg/kg) ^a	$7.0 \times 10^3 \pm 1.3 \times 10^{3b}$
Dap (7 mg/kg) ^a	$3.6 \times 10^3 \pm 0.3 \times 10^{3b}$
Tig (1.5 mg/kg) plus Ra (10 mg/kg)	$2.8 \times 10^1 \pm 0.4 \times 10^{b,c}$
Tig (1.5 mg/kg) plus Dap (7 mg/kg)	$1.2 \times 10^1 \pm 0.1 \times 10^{1b,c}$

^a Tig, tigecycline; Ra, rifampin; Dap, daptomycin

^b Groups treated with intraperitoneal antibiotics showed significant improvement compared to the group without treatment. ANOVA, * $p < 0.001$

^c Groups treated with combined antibiotics showed significant improvement compared to the singly treated groups. ANOVA, * $p < 0.001$

of surgical wound infection. This infection was determined by both a staphylococcal and an enterococcal strain. The drugs that we chose to use in the in vivo model were rifampin and daptomycin, since they were shown in the in vitro studies to be synergic with tigecycline.

For both bacterial strains, all groups treated with single drugs showed a statistically significant result compared to the control group. As expected, tigecycline showed good activity both against staphylococcal and enterococcal isolates. Interestingly, our data indicates that both the combination therapy groups showed the highest antibacterial efficacy. This study emphasizes the importance of combination therapy in infections due to nosocomial isolates of Gram-positive cocci. The lack of antagonism is an encouraging outcome, suggesting that tigecycline may prove to be effective not only in monotherapy, but also in combination

Table 4 Quantitative culture of excised tissues after drug administration in enterococcal wound infection

Treatment	CFU/ml
Uninfected	<10
Infected untreated	$6.48 \times 10^7 \pm 1.2 \times 10^7$
Tig (1.5 mg/kg) ^a	$5.9 \times 10^3 \pm 1.1 \times 10^{3b}$
Ra (10 mg/kg) ^a	$8.4 \times 10^3 \pm 1.6 \times 10^{3b}$
Dap (7 mg/kg) ^a	$4.2 \times 10^3 \pm 0.8 \times 10^{3b}$
Tig (1.5 mg/kg) plus Ra (10 mg/kg)	$3.7 \times 10^1 \pm 0.5 \times 10^{b,c}$
Tig (1.5 mg/kg) plus Dap (7 mg/kg)	$1.4 \times 10^1 \pm 0.3 \times 10^{1b,c}$

^a Tig, tigecycline; Ra, rifampin; Dap, daptomycin

^b Groups treated with intraperitoneal antibiotics showed significant improvement compared to the group without treatment. ANOVA, * $p < 0.001$

^c Groups treated with combined antibiotics showed significant improvement compared to the singly treated groups. ANOVA, * $p < 0.001$

therapy. Combination therapy has already been described in several in vitro and in vivo studies [12, 17, 28, 29]. The main conclusion of the in vitro studies was that the interaction of tigecycline with other antimicrobials produced primarily an indifferent response and very rarely showed antagonism. However, they also revealed a number of synergisms which were potentially interesting from both microbiological and clinical points of view. In particular, synergism was observed when tigecycline was combined with rifampin against *Enterococcus* spp., *S. pneumoniae*, *Brucella melitensis*, and *Enterobacter* spp. These combinations have a clinical relevance, as the organisms they target belonged to potentially problematic multi-resistant species and to bacteria inadequately inhibited by tigecycline. In the in vivo reports, synergy was also described when tigecycline was combined with daptomycin for *E. faecium* bacterial endocarditis [29]. Our study confirmed the data from these previous studies. It displayed consistent beneficial activity of tigecycline alone and in combination with other antimicrobial agents against Gram-positive organisms, emphasizing that it may be a useful option to treat staphylococcal and enterococcal wound infections.

References

- Cormican MG, Jones RN (1996) Emerging resistance to antimicrobial agents in Gram-positive bacteria. Enterococci, staphylococci and nonpneumococcal streptococci. *Drugs* 51:S6–S12
- Linden PK (1998) Clinical implications of nosocomial gram-positive bacteremia and superimposed antimicrobial resistance. *Am J Med* 104:24S–33S
- Bratzler DW, Houck PM; Surgical Infection Prevention Guidelines Writers Workgroup et al (2004) Antimicrobial prophylaxis for surgery: an advisory statement from the National Surgical Infection Prevention Project. *Clin Infect Dis* 38:1706–1715
- Burke JP (2003) Infection control—a problem for patient safety. *N Engl J Med* 348:651–656
- Hirsch T, Koerber A, Jacobsen F et al (2010) Evaluation of toxic side effects of clinically used skin antiseptics in vitro. *J Surg Res* 164:344–350
- El-Azizi M, Rao S, Kanchanapoom T et al (2005) In vitro activity of vancomycin, quinupristin/dalfopristin, and linezolid against intact and disrupted biofilms of staphylococci. *Ann Clin Microbiol Antimicrob* 4:2
- Lode HM (2009) Clinical impact of antibiotic-resistant Gram-positive pathogens. *Clin Microbiol Infect* 15:212–217
- Bouza E (2009) New therapeutic choices for infections caused by methicillin-resistant *Staphylococcus aureus*. *Clin Microbiol Infect* 15(Suppl 7):44–52
- Moellering RC Jr (1998) Problems with antimicrobial resistance in gram-positive cocci. *Clin Infect Dis* 26:1177–1178
- Arias CA, Contreras GA, Murray BE (2010) Management of multidrug-resistant enterococcal infections. *Clin Microbiol Infect* 16:555–562
- Diekema DJ, BootsMiller BJ, Vaughn TE et al (2004) Antimicrobial resistance trends and outbreak frequency in United States hospitals. *Clin Infect Dis* 38:78–85
- Giacometti A, Cirioni O, Kamysz W et al (2005) In vitro activity and killing effect of temporin A on nosocomial isolates of *Enterococcus faecalis* and interactions with clinically used antibiotics. *J Antimicrob Chemother* 55:272–274
- Spellberg B, Guidos R, Gilbert D et al (2008) The epidemic of antibiotic-resistant infections: a call to action for the medical community from the Infectious Diseases Society of America. *Clin Infect Dis* 46:155–164
- Montefour K, Frieden J, Hurst S et al (2008) *Acinetobacter baumannii*: an emerging multidrug-resistant pathogen in critical care. *Crit Care Nurse* 28:15–25
- Labthavikul P, Petersen PJ, Bradford PA (2003) In vitro activity of tigecycline against *Staphylococcus epidermidis* growing in an adherent-cell biofilm model. *Antimicrob Agents Chemother* 47:3967–3969
- Clinical and Laboratory Standards Institute (CLSI) (2003) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A6. National Committee for Clinical Laboratory Standards (NCCLS), Villanova, PA
- Petersen PJ, Labthavikul P, Jones CH (2006) In vitro antibacterial activities of tigecycline in combination with other antimicrobial agents determined by checkerboard and time-kill kinetic analysis. *J Antimicrob Chemother* 57:573–576
- Rand KH, Houck HJ, Brown P et al (1993) Reproducibility of the microdilution checkerboard method for antibiotic synergy. *Antimicrob Agents Chemother* 37:613–615
- Simonetti O, Cirioni O, Ghiselli R et al (2008) RNAIII-inhibiting peptide enhances healing of wounds infected with methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 52:2205–2211
- Kugelberg E, Norström T, Petersen TK et al (2005) Establishment of a superficial skin infection model in mice by using *Staphylococcus aureus* and *Streptococcus pyogenes*. *Antimicrob Agents Chemother* 49:3435–3441
- Lin MY, Hayden MK (2010) Methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*: recognition and prevention in intensive care units. *Crit Care Med* 38:S335–S344
- Peterson LR (2008) A review of tigecycline—the first glycylcycline. *Int J Antimicrob Agents* 32:S215–S222
- Hoban DJ, Bouchillon SK, Johnson BM et al; Tigecycline Evaluation and Surveillance trial (TEST Program) Group (2005) In vitro activity of tigecycline against 6792 Gram-negative and Gram-positive clinical isolates from the global Tigecycline Evaluation and Surveillance Trial (TEST Program, 2004). *Diagn Microbiol Infect Dis* 52:215–227
- Moland ES, Craft DW, Hong SG et al (2008) In vitro activity of tigecycline against multidrug-resistant *Acinetobacter baumannii* and selection of tigecycline–amikacin synergy. *Antimicrob Agents Chemother* 52:2940–2942
- Rathe M, Kristensen L, Ellermann-Eriksen S et al (2010) Vancomycin-resistant *Enterococcus* spp.: validation of susceptibility testing and in vitro activity of vancomycin, linezolid, tigecycline and daptomycin. *APMIS* 118:66–73
- Farrell DJ, Turnidge JD, Bell J et al (2010) The in vitro evaluation of tigecycline tested against pathogens isolated in eight countries in the Asia-Western Pacific region (2008). *J Infect* 60:440–451
- Ippolito G, Leone S, Lauria FN et al (2010) Methicillin-resistant *Staphylococcus aureus*: the superbug. *Int J Infect Dis* 14(Suppl 4): S7–S11
- Entenza JM, Moreillon P (2009) Tigecycline in combination with other antimicrobials: a review of in vitro, animal and case report studies. *Int J Antimicrob Agents* 34:8.e1–8.e9
- Rybak MJ, McGrath BJ (1996) Combination antimicrobial therapy for bacterial infections. Guidelines for the clinician. *Drugs* 52:390–405