Coagulase-negative staphylococci as a cause of infections related to intravascular prosthetic devices: limitations of present therapy

T. Schulin and A. Voss

University Medical Center St Radboud, Department of Medical Microbiology, Nijmegen, The Netherlands

Coagulase-negative staphylococci (CNS) are an important cause of catheter-related bloodstream infections. This review will shed light on the pathogenesis related to biofilm formation, and will discuss antimicrobial susceptibility of CNS to older and newer antibiotics, as well as therapeutic options.

Clin Microbiol Infect 2001: 7 (Supplement 4): 1–7

INTRODUCTION

Over recent decades, coagulase-negative staphylococci (CNS) have become a frequent cause of nosocomial catheter-related bloodstream infections [1–3]. CNS, due to their ability to produce biofilm, are especially likely to colonize and infect indwelling vascular catheters and port systems. This colonization can lead to bacteremia, which is an important complication in patients cared for in hematology, hemodialysis, intensive care and neonatal units where intravascular (IV) catheters are a crucial part of their management. The increasing resistance of CNS to antimicrobial agents [4,5] makes the treatment of catheter-related infections increasingly difficult. An understanding of the pathogenesis of CNS is an important consideration in selecting the appropriate therapy. New drugs are essential to circumvent existing resistance mechanisms, including biofilm formation, if treatment of such infections is to be successful.

PATHOGENICITY

The pathogenic potential of CNS is generally thought to be low [6–8]. Clinical observations have lead to the assumption that most CNS isolated from the bloodstream are contaminants. There are still no defined criteria that differentiate between colonization and true bacteremia. In most instances, the clinical picture and isolation of CNS from more than one set of blood cultures may indicate true infection [9]. The pathogenicity of CNS has been the subject of much investigation, in order to improve our understanding of CNS bloodstream infections associated with IV catheter material.

Corresponding author and reprint requests: T. Schulin, University Medical Center St Radboud, Department of Medical Microbiology (440 MMB), PO Box 9101, 6500 HB Nijmegen, The Netherlands
Tel: +31 24 3619560
Fax: +31 24 3540216
E-mail: t.schulin@mmb.azn.nl

BIOFILM FORMATION

Genes that influence biofilm formation and pathogenicity

The adherence to catheter material and the formation of biofilm plays an important role in the pathogenesis of catheter-related infections with CNS [10–12]. Two phases are involved in biofilm formation. Initially, the bacteria rapidly adhere to the foreign material, due to various factors such as the nature of the polymer material and bacterial surface proteins [13–15]. Secondly, the bacteria produce over time an extracellular matrix that consists mainly of teichoic acid and sugars. The bacteria thereby become imbedded in the biofilm in the form of multilayered cell clusters. Polysaccharide intercellular adhesin (PIA), which is the gene product of the icaADBC operon, has recently been described to play a crucial role in biofilm formation [16,17]. This has furthermore been shown in an in vivo IV catheter-associated infection model in rats [14]. Within the biofilm the bacteria form tight microcolonies and appear to be tolerant of or resistant to a variety of antimicrobial agents, despite in vitro susceptibility by conventional testing [18]. The molecular basis of polymer-associated infections due to staphylococci has been reviewed recently [19,20].

Ziehbuhr *et al* found that the ica gene locus was more often present in CNS isolated from blood cultures than in mucosal isolates and that these strains more often formed a multilayered biofilm [21]. Genetic investigations revealed that the ica as well as the mecA gene, conferring β-lactam resistance, were detected significantly more often in infecting strains than in contaminating strains [22].

Influence of antibiotics on biofilm formation

Antibiotics can influence the mechanisms of adherence and of slime production in CNS in different ways; various studies have been undertaken to elucidate these effects. Rachid *et al.* [23] investigated whether subinhibitory concentrations of antibiotics influenced the expression of the icaADBC operon in CNS. They found that sub-MIC levels of quinupristin/dalfopristin or tetracycline enhanced expression, leading to higher polysaccharide intracellular adhesin production; whereas penicillin, oxacillin, clindamycin, gentamicin, ofloxacin, vancomycin and teicoplanin had no influence and erythromycin led to only a moderate increase in expression. In contrast, Wilcox *et al.* [24] described that vancomycin and teicoplanin in sub-MIC concentration enhanced adherence to polystyrene and silicon rubber. Similar effects have been described by Dunne [25], who showed biofilm enhancement by subinhibitory concentrations of cefamandole and vancomycin in some but not all strains of CNS. No enhancement was observed in concentrations at or above the MIC. Interstrain variations were noted in all studies.

Subinhibitory levels of ciprofloxacin increased the expression of fibronectin-binding proteins in ciprofloxacin-resistant *Staphylococcus aureus* [26], resulting in enhanced adherence to catheter material. Although *Staphylococcus epidermidis* also adheres to fibronectin [27], no such effect has been described in this species so far.

ANTIBIOTIC SUSCEPTIBILITY

Influence of biofilms on antibacterial activity

Although most clinical isolates of CNS are highly susceptible to vancomycin when tested in vitro as dispersed planktonic bacteria, these organisms are resistant or tolerant to this agent when embedded in a biofilm [28]. The physical barrier of the biofilm matrix to penetration by the relatively large vancomycin molecule had originally been proposed as a possible mechanism, but subsequent studies have shown that high vancomycin concentrations can be achieved in biofilms. The combination of teicoplanin or vancomycin with rifampicin or amikacin can increase activity on sessile bacteria. With these combinations, sterilization of Vialon and polyvinylchloride catheters was achieved in one study [29]. Despite rapid diffusion and high levels of vancomycin and rifampin in the CNS biofilm, bacteria were still viable after 72 h, with an increased MIC and MBC to rifampin, but not to vancomycin [30]. Hamilton-Miller *et al.* investigated the activity of ciprofloxacin and quinupristin/dalfopristin against CNS in biofilms and found that both agents were able to kill sessile bacteria slowly [31]. Some studies propose that the slime of the biofilm itself diminished the activity of glycopeptide antibiotics [32,33] and pefloxacin [34], thus leading to treatment failure in some instances. Rifampicin activity was not decreased, and for other antibiotics, only a moderate effect was seen [34]. Biofilm eradication in vivo and in vitro was studied in two isogenic CNS strains, which differed in their ability to form biofilm. Using amikacin, levofloxacin, rifampicin and teicoplanin, the slime-negative strain was better eradicated than its slime-positive parent strain [18]. Routine sensitivity tests often fail to predict therapeutic success. In an in vivo model of device-related infection, Widmer *et al.* [35] showed that the clinical outcome could reliably be predicted by testing drug efficacy on stationary and adherent micro-organisms, but not by minimal inhibitory concentrations.

Influence of clarithromycin on biofilm

Clarithromycin is a macrolide antibiotic; most strains of methicillin-resistant CNS (MR-CNS) are also resistant to macrolides. Therefore, treatment of infections due to MR-CNS with macrolides is not considered an option. However, there is evidence that macrolides can influence biofilm production of CNS. In a study from Japan [36] it was shown that treatment of catheters colonized with CNS with noninhibitory doses of clarithromycin resulted in the eradication of slime-like material. As a result, other antibiotics could more easily reach the bacteria on the catheter surface. This phenomenon has also been described for mucoid *Pseudomonas aeruginosa* [37–40] and has led to new treatment options for infections by this organism.

Methicillin-resistant coagulase-negative staphylococci

Staphylococcus epidermidis strains that are resistant to methicillin are cross-resistant to all other β-lactam antibiotics, although sometimes they appear to be susceptible in vitro. More than 80% of the strains isolated from nosocomial infection are methicillin-resistant, in contrast to more than 80% being susceptible to methicillin in community-acquired infections [41,42]. It has been found that the mecA gene conferring methicillin resistance is more often found in infecting than in colonizing strains of CNS [22]. In another study mecA-positive phase variants of *S. epidermidis* lacked mecA expression and had a strongly reduced adherence capacity, thus offering further evidence that the expression of methicillin resistance in CNS has a possible influence on virulence factors [43].

Epidemiological studies of CNS isolated from bloodstream infections in North America and Europe have shown methicillin resistance to be detected in 60%–80% of strains, respectively [44,45]. From 1996 through 1999, 70% of the *S. epidermidis* strains and 90% of the *Staphylococcus haemolyticus* strains isolated from bloodstream infections in Germany were

also resistant to methicillin [46]. The correlation of methicillin resistance in CNS and the influence on virulence is flawed by the use of different breakpoints (NCCLS, 0.5 mg/L; BSCA, 2.0 mg/L) leading to reports of different methicillin resistance percentages within the same strain collection, namely 70% vs. 40%, respectively [47].

Quinolone resistance

Resistance to fluoroquinolones, especially in MRSE, has emerged over the last two decades. In part, this increase in resistance is attributed to an increased use of ciprofloxacin in treating critically ill patients [48]. Excretion of ciprofloxacin into sweat may be an additional factor that promotes the selection of drug-resistant skin bacteria including CNS [49,50]. In 461 bloodstream isolates of MR-CNS studied in Germany, 72.8% of *S. epidermidis* and all *S. haemolyticus* strains were resistant to ciprofloxacin [46].

Vancomycin and teicoplanin resistance in CNS

The glycopeptide antibiotics vancomycin and teicoplanin are often last-resort antibiotics used in the treatment of infections caused by MR-CNS. However, resistance to these agents can be produced in a step-wise manner in vitro [51–53], although only in a small percentage of strains, and not to a high level of resistance. Clinical strains of glycopeptide-resistant *S. haemolyticus* were not reported until 1986 [54–58]. In one study [59], 362 clinical isolates of CNS were investigated; 23.2% were intermediate and 1.7% resistant to teicoplanin, in contrast to less than 0.3% for vancomycin (74% of teicoplanin-resistant strains belonged to the species *S. epidermidis*, and 20% to *S. haemolyticus*). In the UK, 6.5% of 769 isolates of CNS were teicoplanin resistant, in contrast to only 0.5% of vancomycin-resistant strains [47]. However, local prevalence varied, with 26% teicoplanin-resistant CNS in one of the centres. In a large European study, 1594 CNS from bloodstream infections were tested for their susceptibility to glycopeptides. None of the isolates was resistant to vancomycin and 0.7% were resistant to teicoplanin [60]. Selection of resistant strains whilst on treatment [61–64] has been described for *S. haemolyticus* [65] and also for *S. epidermidis* [66]. In one study, teicoplanin-intermediate or -resistant strains were found in 49.2% of patients with CNS infection after receiving glycopeptide treatment [67]. Increased use of teicoplanin was correlated to an increase in MICs for teicoplanin in CNS [68]. In contrast, Cercenado *et al.* [69] noticed that teicoplanin-intermediate strains were exclusively isolated from a patient with no prior teicoplanin treatment. Recently, the emergence of glycopeptide resistance in CNS has been extensively reviewed by Biavasco *et al.* [70].

SUSCEPTIBILITY TO NEWER AGENTS IN VITRO AND IN VIVO AND IN BIOFILM

Quinupristin/dalfopristin

Quinupristin/dalfopristin is a new semisynthetic injectable streptogramin antibiotic with a high in vitro bactericidal activity against Gram-positive bacteria, including multidrug-resistant staphylococci [71,72]. In two German in vitro studies of 735 strains (141 isolates from clinical material and 594 CNS from bloodstream infections) all isolates were susceptible to quinupristin/dalfopristin showing an $MIC_{90} < 1$ mg/L [46,73]. Similar results were reported from The Netherlands, where 36 CNS from endocarditis were inhibited by quinupristin/dalfopristin concentrations <1 mg/L [74]. In 675 bacteremia isolates from the SENTRY program [3], the MIC_{90} for CNS to quinupristin/dalfopristin was 0.5 mg/L. The activity of quinupristin/dalfopristin was also studied in biofilms; over a course of 48 h, sessile bacteria were killed by 2/3 $\log_{10}$ CFU/mL, which is comparable with the activity of ciprofloxacin in biofilm [31]. In another study, quinupristin/dalfopristin had a greater bactericidal effect on CNS in biofilm than did flucloxacillin, glycopeptides, erythromycin and ciprofloxacin [75].

Newer quinolones

The newer quinolones demonstrate higher activity against Gram-positive bacteria, including ciprofloxacin-resistant strains [76]. However, they play only a secondary role in the treatment of bloodstream infections caused by CNS.

Linezolid

Linezolid is the first member of a new class of antibiotics, the oxazolidinones. These have an unique mode of action and are highly active against a number of Gram-positive isolates, including methicillin-and methicillin/teicoplanin-resistant CNS with MIC < 4 mg/L [47,72,73,77,78]. Measurements of linezolid concentrations in staphylococcal biofilms produced endoluminally on dialysis catheters have been carried out. Although linezolid concentrations in the biofilm were lower than those for vancomycin, there was a 91% reduction in biofilm-associated bacterial counts [79]. Linezolid has nearly complete oral bioavailability, as well as favourable pharmacokinetic and toxicity profiles, making this antibiotic an attractive alternative in the treatment of multidrug-resistant staphylococcal infections [80,81]. Also, linezolid has been anecdotally effective in treating a patient suffering from a foreign body infection with MR-CNS, where vancomycin therapy had failed, further information is awaited with interest before its role in such infections can be recommended [82].

Agents under investigation

LY 333328 is a novel glycopeptide with a higher bactericidal activity against organisms resistant to older glycopeptides. It is active in vitro against CNS (MIC < 4 mg/L) [83–88], including oxacillin/teicoplanin-resistant strains [89].

Evernimicin is an oligopeptide antibiotic with bacteriostatic activity against a number of Gram-positive organisms [90–94]. MICs are generally lower than those for vancomycin. The MIC of evernimicin was < 1 mg/L among 1427 CNS tested [93].

Daptomycin (LY 146032) is a cyclic polypeptide belonging to the peptolide class of antibiotics. It has a bactericidal effect on Gram-positive pathogens similar to that of the glycopeptides. Among CNS, including methicillin-resistant strains, it is more active than vancomycin, quinupristin/dalfopristin and linezolid [72].

Glycylcyclin (GAR936) is a novel tetracycline analog that demonstrates bacteriostatic activity against Gram-positive organisms resistant to older compounds of this class [95].

No studies on the influence of biofilm-associated bacteria have been undertaken to date. More studies are needed to assess any potential role in the treatment of CNS infections.

THERAPY

The optimal management of intravenous catheter related infections has not been established [96,97]. Furthermore, the duration of therapy has not been well defined. In general, a 5-7 day course of antibiotics should be sufficient in most cases of infection caused by CNS. Patients with catheter-related coagulase-negative staphylococcal bacteremia have been treated successfully without catheter removal. However, catheter retention can lead to a recurrence of bacteremia in up to 20% of the cases [98].

In most cases of catheter-associated infection due to staphylococci, the therapy of choice is currently a glycopeptide, on account of the high percentage of β-lactam-resistant strains of CNS. The antistaphylococcal activity of rifampin is higher that that of other compounds, and the combination of glycopeptide/rifampin has been shown to improve the activity of antibiotics on staphylococci embedded in biofilms [29]. However, in the light of increasing resistance to glycopeptides among staphylococci as well as in enterococci, use of those agents should be restricted. Souvenir *et al*. concluded that nearly 50% of patients with positive blood-cultures for CNS were treated unnecessarily [99]. The relatively poor action of glycopeptides on CNS embedded in biofilms endorses the search for more potent agents [100].

Linezolid, which is active against multidrug-resistant CNS and can be given orally in a twice-daily dose, has been shown to be effective in eliminating bacteria in catheter-related biofilms. These advantages over the glycopeptides raise its potential as a candidate for treating catheter-related staphylococcal infections. However, further experience is necessary which should also include defining the optimum duration of therapy.

REFERENCES

1. Rupp ME, Archer GL. Coagulase-negative staphylococci: pathogens associated with medical progress. *Clin Infect Dis* 1994; **19:** 231–43.
2. Edmond MB, Wallace SE, McClish DK, Pfaller MA, Jones RN, Wenzel RP. Nosocomial bloodstream infections in United States hospitals: a three-year analysis. *Clin Infect Dis* 1999; **29:** 239–44.
3. Pfaller MA, Jones RN, Doern GV, Kugler K. Bacterial pathogens isolated from patients with bloodstream infection. frequencies of occurrence and antimicrobial susceptibility patterns from the SENTRY antimicrobial surveillance program (United States and Canada, 1997). *Antimicrob Agents Chemother* 1998; **42:** 1762–70.
4. Livermore DM. Antibiotic resistance in staphylococci. *Int J Antimicrob Agents* 2000; **16 (Suppl. 1):** 3–10.
5. Witte W. Antibiotic resistance in Gram-positive bacteria: epidemiological aspects. *J Antimicrob Chemother* 1999; **44 (Suppl. A):** 1–9.
6. Huebner J, Goldmann DA. Coagulase-negative staphylococci: role as pathogens. *Annu Rev Med* 1999; **50:** 223–36.
7. Goldmann DA, Pier GB. Pathogenesis of infections related to intravascular catheterization. *Clin Microbiol Rev* 1993; **6:** 176–92.
8. Kloos WE, Bannerman TL. Update on clinical significance of coagulase-negative staphylococci. *Clin Microbiol Rev* 1994; **7:** 117–40.
9. Crump JA, Collignon PJ. Intravascular catheter-associated infections. *Eur J Clin Microbiol Infect Dis* 2000; **19:** 1–8.
10. Muller E, Takeda S, Shiro H, Goldmann D, Pier GB. Occurrence of capsular polysaccharide/adhesin among clinical isolates of coagulase-negative staphylococci. *J Infect Dis* 1993; **168:** 1211–8.
11. Ishak MA, Groschel DH, Mandell GL, Wenzel RP. Association of slime with pathogenicity of coagulase-negative staphylococci causing nosocomial septicemia. *J Clin Microbiol* 1985; **22:** 1025–9.
12. Baddour LM, Smalley DL, Kraus AP, Lamoreaux WJ, Christensen GD. Comparison of microbiologic characteristics of pathogenic and saprophytic coagulase-negative staphylococci from patients on continuous ambulatory peritoneal dialysis. *Diagn Microbiol Infect Dis* 1986; **5:** 197–205.
13. Baldassarri L, Donnelli G, Gelosia A, Voglino MC, Simpson AW, Christensen GD. Purification and characterization of the staphylococcal slime-associated antigen and its occurrence among Staphylococcus epidermis clinical isolates. *Infect Immun* 1996; **64:** 3410–5.
14. Rupp ME, Ulphani JS, Fey PD, Mack D. Characterization of Staphylococcus epidermidis polysaccharide intercellular adhesin/hemagglutinin in the pathogenesis of intravascular catheter-associated infection in a rat model. *Infect Immun* 1999; **67:** 2656–9.
15. Ziebuhr W, Lossner I, Rachid S, Dietrich K, Gotz F, Hacker J. Modulation of the polysaccharide intercellular adhesin (PIA) expression in biofilm forming Staphylococcus epidermidis. Analysis of genetic mechanisms. *Adv Exp Med Biol* 2000; **485:** 151–7.
16. Mack D, Nedelmann M, Krokotsch A, Schwarzkopf A, Heesemann J, Laufs R. Characterization of transposon mutants of biofilm-producing Staphylococcus epidermidis impaired in the accumulative phase of biofilm production: genetic identification of a hexosamine-containing polysaccharide intercellular adhesin. *Infect Immun* 1994; **62:** 3244–53.

17. McKenney D, Hubner J, Muller E, Wang Y, Goldmann DA, Pier GB. The ica locus of *Staphylococcus epidermidis* encodes production of the capsular polysaccharide/adhesin. *Infect Immun* 1998; **66:** 4711–20.
18. Schwank S, Rajacic Z, Zimmerli W, Blaser J. Impact of bacterial biofilm formation on in vitro and in vivo activities of antibiotics. *Antimicrob Agents Chemother* 1998; **42:** 895–8.
19. von Eiff C, Heilmann C, Peters G. New aspects in the molecular basis of polymer-associated infections due to staphylococci. *Eur J Clin Microbiol Infect Dis* 1999; **18:** 843–6.
20. Mack D. Molecular mechanisms of *Staphylococcus epidermidis* biofilm formation. *J Hosp Infect* 1999; **43 (Suppl.):** S113–S125.
21. Ziebuhr W, Heilmann C, Gotz F *et al* Detection of the intercellular adhesion gene cluster (ica) and phase variation in *Staphylococcus epidermidis* blood culture strains and mucosal isolates. *Infect Immun* 1997; **65:** 890–6.
22. Frebourg NB, Lefebvre S, Baert S, Lemeland JF. PCR-Based assay for discrimination between invasive and contaminating *Staphylococcus epidermidis* strains. *J Clin Microbiol* 2000; **38:** 877–80.
23. Rachid S, Ohlsen K, Witte W, Hacker J, Ziebuhr W. Effect of subinhibitory antibiotic concentrations on polysaccharide intercellular adhesin expression in biofilm-forming *Staphylococcus epidermidis*. *Antimicrob Agents Chemother* 2000; **44:** 3357–63.
24. Wilcox MH, Finch RG, Smith DG, Williams P, Denyer SP. Effects of carbon dioxide and sub-lethal levels of antibiotics on adherence of coagulase-negative staphylococci to polystyrene and silicone rubber. *J Antimicrob Chemother* 1991; **27:** 577–87.
25. Dunne WM Jr. Effects of subinhibitory concentrations of vancomycin or cefamandole on biofilm production by coagulase-negative staphylococci. *Antimicrob Agents Chemother* 1990; **34:** 390–3.
26. Bisognano C, Vaudaux PE, Lew DP, Ng EY, Hooper DC. Increased expression of fibronectin-binding proteins by fluoroquinolone-resistant *Staphylococcus aureus* exposed to subinhibitory levels of ciprofloxacin. *Antimicrob Agents Chemother* 1997; **41:** 906–13.
27. Raad I. Intravascular-catheter-related infections. *Lancet* 1998; **351:** 893–8.
28. König C, Schwank S, Blaser J. Factors compromising antibiotic activity against biofilms of *Staphylococcus epidermidis*. *Eur J Clin Microbiol Infect Dis* 2001; **20:** 20–6.
29. Pascual A, Ramirez DA, Perea EJ. Activity of glycopeptides in combination with amikacin or rifampin against *Staphylococcus epidermidis* bio-films on plastic catheters. *Eur J Clin Microbiol Infect Dis* 1994; **13:** 515–7.
30. Dunne WM Jr, Mason EO Jr, Kaplan SL. Diffusion of rifampin and vancomycin through a *Staphylococcus epidermidis* biofilm. *Antimicrob Agents Chemother* 1993; **37:** 2522–6.
31. Hamilton-Miller JM, Shah S. Activity of quinupristin/dalfopristin against *Staphylococcus epidermidis* in biofilms: a comparison with ciprofloxacin. *J Antimicrob Chemother* 1997; **39 (Suppl. A):** 103–8.
32. Darouiche RO, Dhir A, Miller AJ, Landon GC, Raad II, Musher DM. Vancomycin penetration into biofilm covering infected prostheses and effect on bacteria. *J Infect Dis* 1994; **170:** 720–3.
33. Farber BF, Kaplan MH, Clogston AG. *Staphylococcus epidermidis* extracted slime inhibits the antimicrobial action of glycopeptide antibiotics. *J Infect Dis* 1990; **161:** 37–40.
34. Souli M, Giamarellou H. Effects of slime produced by clinical isolates of coagulase-negative staphylococci on activities of various antimicrobial agents. *Antimicrob Agents Chemother* 1998; **42:** 939–41.
35. Widmer AF, Frei R, Rajacic Z, Zimmerli W. Correlation between in vivo and in vitro efficacy of antimicrobial agents against foreign body infections. *J Infect Dis* 1990; **162:** 96–102.
36. Yasuda H, Ajiki Y, Koga T, Yokota T. Interaction between clarithromycin and biofilms formed by *Staphylococcus epidermidis*. *Antimicrob Agents Chemother* 1994; **38:** 138–41.
37. Bui KQ, Banevicius MA, Nightingale CH, Quintiliani R, Nicolau DP. In vitro and in vivo influence of adjunct clarithromycin on the treatment of mucoid *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 2000; **45:** 57–62.
38. Kondoh K, Hashiba M, Baba S. Inhibitory activity of clarithromycin on biofilm synthesis with *Pseudomonas aeruginosa*. *Acta Otolaryngol Suppl* 1996; **525:** 56–60.
39. Yasuda H, Ajiki Y, Koga T, Kawada H, Yokota T. Interaction between biofilms formed by *Pseudomonas aeruginosa* and clarithromycin. *Antimicrob Agents Chemother* 1993; **37:** 1749–55.
40. Ichimiya T, Yamasaki T, Nasu M. In-vitro effects of antimicrobial agents on *Pseudomonas aeruginosa* biofilm formation. *J Antimicrob Chemother* 1994; **34:** 331–41.
41. Archer GL. Molecular epidemiology of multiresistant *Staphylococcus epidermidis*. *J Antimicrob Chemother* 1988; **21 (Suppl. C):** 133–8.
42. Caputo GM, Archer GL, Calderwood SB, DiNubile MJ, Karchmer AW. Native valve endocarditis due to coagulase-negative staphylococci. Clinical and microbiologic features. *Am J Med* 1987; **83:** 619–25.
43. Mempel M, Feucht H, Ziebuhr W, Endres M, Laufs R, Gruter L. Lack of mecA transcription in slime-negative phase variants of methicillin-resistant *Staphylococcus epidermidis*. *Antimicrob Agents Chemother* 1994; **38:** 1251–5.
44. Diekema DJ, Pfaller MA, Jones RN *et al* Trends in antimicrobial susceptibility of bacterial pathogens isolated from patients with bloodstream infections in the USA, Canada and Latin America. SENTRY Participants Group. *Int J Antimicrob Agents* 2000; **13:** 257–71.
45. Fluit AC, Jones ME, Schmitz FJ, Acar J, Gupta R, Verhoef J. Antimicrobial susceptibility and frequency of occurrence of clinical blood isolates in Europe from the SENTRY antimicrobial surveillance program, 1997 and 1998. *Clin Infect Dis* 2000; **30:** 454–60.
46. von Eiff C, Reinert RR, Kresken M, Brauers J, Hafner D, Peters G. Nationwide German multicenter study on prevalence of antibiotic resistance in staphylococcal bloodstream isolates and comparative in vitro activities of quinupristin-dalfopristin. *J Clin Microbiol* 2000; **38:** 2819–23.
47. Henwood CJ, Livermore DM, Johnson AP *et al* Susceptibility of Gram-positive cocci from 25 UK hospitals to antimicrobial agents including linezolid. *J Antimicrob Chemother* 2000; **46:** 931–40.
48. Mulder JG, Kosterink JG, Degener JE. The relationship between the use of flucloxacillin, vancomycin, aminoglycosides and ciprofloxacin and the susceptibility patterns of coagulase-negative staphylococci recovered from blood cultures. *J Antimicrob Chemother* 1997; **40:** 701–6.
49. Hoiby N, Jarlov JO, Kemp M *et al* Excretion of ciprofloxacin in sweat and multiresistant *Staphylococcus epidermidis*. *Lancet* 1997; **349:** 167–9.
50. Hoiby N, Johansen HK. Ciprofloxacin in sweat and antibiotic resistance. The Copenhagen Study Group on Antibiotics in Sweat. *Lancet* 1995; **346:** 1235.
51. Watanakunakorn C. In-vitro induction of resistance in coagulase-negative staphylococci to vancomycin and teicoplanin. *J Antimicrob Chemother* 1988; **22:** 321–4.
52. Mouton RP, Mulders SL. LY146032: activity and resistance development in vitro. *J Antimicrob Chemother* 1987; **20:** 513–7.
53. Herwaldt L, Boyken L, Pfaller M. In vitro selection of resistance to vancomycin in bloodstream isolates of *Staphylococcus haemolyticus* and *Staphylococcus epidermidis*. *Eur J Clin Microbiol Infect Dis* 1991; **10:** 1007–12.
54. Del Bene VE, John JF Jr, Twitty JA, Lewis JW. Anti-staphylococcal activity of teicoplanin, vancomycin, and other antimicrobial agents: the significance of methicillin resistance. *J Infect Dis* 1986; **154:** 349–52.
55. Wilson AP, O'Hare MD, Felmingham D, Gruneberg RN. Teicoplanin-resistant coagulase-negative staphylococcus. *Lancet* 1986; **2:** 973.
56. Franson TR, Kammer RB, Cooper GL, Preston DA, Brown RA. Vancomycin-resistant staphylococcus. *N Engl J Med* 1987; **317:** 766–7.
57. Schwalbe RS, Stapleton JT, Gilligan PH. Emergence of vancomycin

resistance in coagulase-negative staphylococci. *N Engl J Med* 1987; **316:** 927–31.

58. Garrett DO, Jochimsen E, Murfitt K *et al* The emergence of decreased susceptibility to vancomycin in *Staphylococcus epidermidis*. *Infect Control Hosp Epidemiol* 1999; **20:** 167–70.
59. Goldstein FW, Coutrot A, Sieffer A, Acar JF. Percentages and distributions of teicoplanin-and vancomycin-resistant strains among coagulase-negative staphylococci. *Antimicrob Agents Chemother* 1990; **34:** 899–900.
60. Gruneberg RN, Hryniewicz W. Clinical relevance of a European collaborative study on comparative susceptibility of Gram-positive clinical isolates to teicoplanin and vancomycin. *Int J Antimicrob Agents* 1998; **10:** 271–7.
61. Sieradzki K, Roberts RB, Serur D, Hargrave J, Tomasz A. Heterogeneously vancomycin-resistant *Staphylococcus epidermidis* strain causing recurrent peritonitis in a dialysis patient during vancomycin therapy. *J Clin Microbiol* 1999; **37:** 39–44.
62. Aubert G, Passot S, Lucht F, Dorche G. Selection of vancomycin- and teicoplanin-resistant *Staphylococcus haemolyticus* during teicoplanin treatment of *Staphylococcus epidermidis* treatment. *J Antimicrob Chemother* 1990; **25:** 491–3.
63. Veach LA, Pfaller MA, Barrett M, Koontz FP, Wenzel RP. Vancomycin resistance in *Staphylococcus haemolyticus* causing colonization and bloodstream infection. *J Clin Microbiol* 1990; **28:** 2064–8.
64. Schwalbe RS, Ritz WJ, Verma PR, Barranco EA, Gilligan PH. Selection for vancomycin resistance in clinical isolates of *Staphylococcus haemolyticus*. *J Infect Dis* 1990; **161:** 45–51.
65. Spanik S, Trupl J, Studena M, Krcmery V. Breakthrough nosocomial bacteraemia due to teicoplanin-resistant *Staphylococcus haemolyticus* in five patients with acute leukaemia. *J Hosp Infect* 1997; **35:** 155–9.
66. Krcmery V Jr, Trupl J, Drgona L, Lacka J, Kukuckova E, Oravcova E. Nosocomial bacteremia due to vancomycin-resistant *Staphylococcus epidermidis* in four patients with cancer, neutropenia, and previous treatment with vancomycin. *Eur J Clin Microbiol Infect Dis* 1996; **15:** 259–61.
67. Maugein J, Pellegrin JL, Brossard G, Fourche J, Leng B, Reiffers J. In vitro activities of vancomycin and teicoplanin against coagulase-negative staphylococci isolated from neutropenic patients. *Antimicrob Agents Chemother* 1990; **34:** 901–3.
68. Sloos JH, van de Klundert JA, Dijkshoorn L, van Boven CP. Changing susceptibilities of coagulase-negative staphylococci to teicoplanin in a teaching hospital. *J Antimicrob Chemother* 1998; **42:** 787–91.
69. Cercenado E, Garcia-Leoni ME, Diaz MD *et al* Emergence of teicoplanin-resistant coagulase-negative staphylococci. *J Clin Microbiol* 1996; **34:** 1765–8.
70. Biavasco F, Vignaroli C, Varaldo PE. Glycopeptide resistance in coagulase-negative staphylococci. *Eur J Clin Microbiol Infect Dis* 2000; **19:** 403–17.
71. Garcia R, Raad I. In vitro study of the potential role of quinupristin/dalfopristin in the treatment of catheter-related staphylococcal infections. *Eur J Clin Microbiol Infect Dis* 1996; **15:** 933–6.
72. Rybak MJ, Hershberger E, Moldovan T, Grucz RG. In vitro activities of daptomycin, vancomycin, linezolid, and quinupristin-dalfopristin against Staphylococci and Enterococci, including vancom. *Antimicrob Agents Chemother* 2000; **44:** 1062–6.
73. von Eiff C, Peters G. Comparative in-vitro activities of moxifloxacin, trovafloxacin, quinupristin/dalfopristin and linezolid against staphylococci. *J Antimicrob Chemother* 1999; **43:** 569–73.
74. Mouton JW, Endtz HP, den Hollander JGBN, Verbrugh HA. In-vitro activity of quinupristin/dalfopristin compared with other widely used antibiotics against strains isolated from patients with endocarditis. *J Antimicrob Chemother* 1997; **39 (Suppl. A):** 75–80.
75. Gander S, Finch R. The effects of exposure at constant (1 h) or exponentially decreasing concentrations of quinupristin/dalfopristin on biofilms of Gram-positive bacteria. *J Antimicrob Chemother* 2000; **46:** 61–7.
76. Hardy D, Amsterdam D, Mandell LA, Rotstein C. Comparative in vitro activities of ciprofloxacin, gemifloxacin, grepafloxacin, moxifloxacin, ofloxacin, sparfloxacin, trovafloxacin, and other antimicrobial agents against bloodstream isolates of Gram-positive cocci. *Antimicrob Agents Chemother* 2000; **44:** 802–5.
77. Jorgensen JH, McElmeel ML, Trippy CW. In vitro activities of the oxazolidinone antibiotics U-100592 and U-100766 against *Staphylococcus aureus* and coagulase-negative Staphylococcus species. *Antimicrob Agents Chemother* 1997; **41:** 465–7.
78. Rybak MJ, Cappelletty DM, Moldovan T, Aeschlimann JR, Kaatz GW. Comparative in vitro activities and postantibiotic effects of the oxazolidinone compounds eperezolid (PNU-100592) and linezolid (PNU-100766) versus vancomycin against *Staphylococcus aureus*, coagulase-negative staphylococci, *Enterococcus faecalis*, and *Enterococcus faecium*. *Antimicrob Agents Chemother* 1998; **42:** 721–4.
79. Wilcox MH, Kite P, Mills K, Sugden S. In situ measurement of linezolid and vancomycin concentrations in intravascular catheter-associated biofilm. *J Antimicrob Chemother* 2001; **47:** 171–5.
80. Clemett D, Markham A. Linezolid. *Drugs* 2000; **59:** 815–27.
81. Diekema DI, Jones RN. Oxazolidinones: a review. *Drugs* 2000; **59:** 7–16.
82. Chien JW, Kucia ML, Salata RA. Use of linezolid, an oxazolidinone, in the treatment of multidrug-resistant Gram-positive bacterial infections. *Clin Infect Dis* 2000; **30:** 146–51.
83. Garcia-Garrote F, Cercenado E, Alcala L, Bouza E. In vitro activity of the new glycopeptide LY333328 against multiply resistant Gram-positive clinical isolates. *Antimicrob Agents Chemother* 1998; **42:** 2452–5.
84. Harland S, Tebbs SE, Elliott TS. Evaluation of the in-vitro activity of the glycopeptide antibiotic LY333328 in comparison with vancomycin and teicoplanin. *J Antimicrob Chemother* 1998; **41:** 273–6.
85. Fraise AP, Andrews J, Wise R. Activity of a new glycopeptide antibiotic (LY333328) against enterococci and other resistant Gram-positive organisms. *J Antimicrob Chemother* 1997; **40:** 423–5.
86. Biavasco F, Vignaroli C, Lupidi R, Manso E, Facinelli B, Varaldo PE. In vitro antibacterial activity of LY333328, a new semisynthetic glycopeptide. *Antimicrob Agents Chemother* 1997; **41:** 2165–72.
87. Jones RN, Barrett MS, Erwin ME. In vitro activity and spectrum of LY333328, a novel glycopeptide derivative. *Antimicrob Agents Chemother* 1997; **41:** 488–93.
88. Schwalbe RS, McIntosh AC, Qaiyumi S *et al* In vitro activity of LY333328, an investigational glycopeptide antibiotic, against enterococci and staphylococci. *Antimicrob Agents Chemother* 1996; **40:** 2416–9.
89. Zeckel ML, Preston DA, Allen BS. In vitro activities of LY333328 and comparative agents against nosocomial Gram-positive pathogens collected in a 1997 global surveillance study. *Antimicrob Agents Chemother* 2000; **44:** 1370–4.
90. Jones RN, Marshall SA, Erwin ME. Antimicrobial activity and spectrum of SCH27899 (Ziracin) tested against Gram-positive species including recommendations for routine susceptibility testing methods and quality control. Quality Control Study Group. *Diagn Microbiol Infect Dis* 1999; **34:** 103–10.
91. Urban C, Mariano N, Mosinka-Snipas K, Wadee C, Chahrour T, Rahal JJ. Comparative in-vitro activity of SCH 27899, a novel everninomicin, and vancomycin. *J Antimicrob Chemother* 1996; **37:** 361–4.
92. Nakashio S, Iwasawa H, Dun FY, Kanemitsu K, Shimada J. Everninomicin, a new oligosaccharide antibiotic. its antimicrobial activity, post-antibiotic effect and synergistic bactericidal activity. *Drugs Exp Clin Res* 1995; **21:** 7–16.
93. Jones RN, Hare RS, Sabatelli FJ. Ziracin susceptibility testing, G.T. in vitro Gram-positive antimicrobial activity of evernimicin (SCH 27899), a novel oligosaccharide, compared with other antimicrobials: a multicentre international trial. *J Antimicrob Chemother* 2001; **47:** 15–25.

94. Fuchs PC, Barry AL, Brown SD. In vitro activities of SCH27899 alone and in combination with 17 other antimicrobial agents. *Antimicrob Agents Chemother* 1999; **43:** 2996–7.
95. Boucher HW, Wennersten CB, Eliopoulos GM. In vitro activities of the glycylcycline GAR-936 against Gram-positive bacteria. *Antimicrob Agents Chemother* 2000; **44:** 2225–9.
96. Oppenheim BA. Optimal management of central venous catheter-related infections – what is the evidence? *J Infect* 2000; **40:** 26–30.
97. Raad I. Management of intravascular catheter-related infections. *J Antimicrob Chemother* 2000; **45:** 267–70.
98. Raad I, Davis S, Khan A, Tarrand J, Elting L, Bodey GP. Impact of central venous catheter removal on the recurrence of catheter-related coagulase-negative staphylococcal bacteremia. *Infect Control Hosp Epidemiol* 1992; **13:** 215–21.
99. Souvenir D, Anderson DE Jr, Palpant S *et al* Blood cultures positive for coagulase-negative staphylococci: antisepsis, pseudobacteremia, and therapy of patients. *J Clin Microbiol* 1998; **36:** 1923–6.
100. Raad I, Alrahwan A, Rolston K. *Staphylococcus epidermidis*. emerging resistance and need for alternative agents. *Clin Infect Dis* 1998; **26:** 1182–7.