History and evolution of antibiotic resistance in coagulase-negative staphylococci: Susceptibility profiles of new anti-staphylococcal agents

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Abstract: Coagulase-negative staphylococci (CNS) are a heterogenous group of Gram-positive cocci that are widespread commensals among mammalia. Unlike their coagulase-positive counterpart, *Staphylococcus aureus*, CNS produce few virulence patterns and normally refrain from invading tissue. Yet, not only can CNS cause infections in normal host tissue, but modern medicine has also seen their rise as opportunists that display adherence to medical device materials to produce a protective biofilm. CNS have historically been more resistant to antimicrobials, including the β-lactam antibiotics, than *S. aureus* and some hospitals reveal rates of oxacillin resistance in CNS approaching 90%. Cross resistance to non-β-lactam agents has been a recurrent theme over the past 40 years in the CNS. Thus, there has been a pressing need for newer antimicrobial agents with good antistaphylococcal activity. Those new agents tend to have excellent antistaphylococcal activity include daptomycin, linezolid, oritavancin, telavancin, tigecycline, dalbavancin, new quinolones, and ceftibiprole, several of which have unique mechanisms of action. The MIC_{90} for these new compounds typically ranges from 0.5–4 μg/mL. Staphylococcal biofilm formation is quite common in CNS infections and markedly increases the MIC for most older antimicrobials. Several of the newer agents offer some promise of penetration of biofilm to inhibit or kill adherent staphylococci. CNS will likely remain a major cause of infections in the modern age, evolve further antimicrobial resistance mechanisms, and require development of newer antimicrobials for curative therapy.

Keywords: coagulase-negative staphylococcus, *Staphylococcus epidermidis, Staphylococcus haemolyticus*, *Staphylococcus lugdenesis*, biofilm, new antibiotics, antibiotic resistance

Introduction

The group of Gram-positive bacteria identified as coagulase-negative staphylococci (CNS), usually harmless commensals, have become important, commonly isolated pathogens in clinical microbiology laboratories around the world (Kloos and Bannerman 1994; Cerca et al 2005; Arciola et al 2006; Bayram and Balci 2006; Caierão et al 2006; Widerström et al 2006). Over the last several decades, infection with CNS has been characterized as related to "medical progress" (Rupp and Archer 1994). Accordingly, CNS, as human pathogens, are usually associated with healthcare settings and occur in patients who are immunocompromised or harboring indwelling polymer or metallic devices (Bisno 1995).

By 1985, there were 19 recognized species of CNS, 8 of which exhibited a possible association with human infection (Kloos and Jorgensen 1985). At that time, the most commonly isolated of these pathogenic species were *Staphylococcus epidermidis* and *S. haemolyticus*. Typing of CNS in the late 1980s was primarily based on biochemical testing profiles such as the one proposed by Kloos and Schleifer in 1975 (Kloos and Schleifer 1975). This scheme used certain biochemical markers including novobiocin resistance; fermentation of: sucrose, fructose, galactose, ribose, lactose,

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Table 1 Coagulase-negative staphylococci reported to cause infections in humans[1]

Species	Common site/type of infection
S. capitis	Urinary tract, skin, blood, CSF shunt
S. caprae	Urinary tract, blood, access sites, prosthetic joints
S. cohnii	Wounds, joints, meninges
S. epidermidis	Widespread sites; wounds, access devices
S. haemolyticus	Native valve, wound, bone, and joint
S. hominis	Blood, lung, breast
S. lugdunensis	Endothelium, heart valves
S. pasteurii	Orthopedic implants
S. saprophiticus	Urinary tract
S. scheiferi	Nosocomial infections, CSF shunts, pacemakers
S. warneri	Widespread sites; neonatal intensive care units
S. xylosus	Blood

[1]Derived from Tristan et al (2006) and Koksal et al (2007).

turanose, melezitose, xylose, mannose, trehalose, mannitol, xylitol, and maltose; and phosphatase nitrate reduction and use of arginine. With character types defined through major and minor biochemical routes, a simplified scheme emerged for speciating staphylococci of human origin (Kloos and Schleifer 1975).

Other early speciation methods included antimicrobial susceptibility profile typing, multilocus enzyme electrophoresis, and cellular fatty acid analysis (Birnbaum et al 1991). Due to the intensive labor involved in all of these early typing schemes and because of the assumption of the low virulence of this group of organisms, most isolates with morphology of Gram-positive cocci in clusters and positive for catalase production without coagulase production were described simply as CNS. Clinical debate continued about the clinical or therapeutic utility of speciating CNS; nevertheless, evidence emerged suggesting that particular species of CNS may be associated with specific diseases, readdressing the question of a need for speciation (Kloos and Bannerman 1994).

In the US, bacteremia due to unspeciated CNS have increased from 9% to 27% from 1980 to 1989 (Schaberg et al 1991). Over the last decade, the numbers of CNS species have grown to total 32, with half of these species isolated from human tissue and blood samples (von Eiff et al 2002). Consequently, development of commercial automated systems such as the Microscan Pos ID, Rapid Pos ID (Baxter Diagnostic Inc., MicroScan Division, West Sacramento, CA, USA), and VITEK (bioMérieux Vitek Inc., Hazelwood, MO, USA) have facilitated more rapid testing of staphylococci by using computer software to analyze combined biochemical and antibiotic susceptibility profiles (Kloos and George 1991; Hussain et al 1998; Caierão et al 2006). These robotic systems scan incubated trays using fluorimetric and photometric readings to record flouresence, turbidity, and colormetric signals which indicate relative growth in each well (Ligozzi et al 2002).

Each automated system uses a unique set of biochemical tests and species parameters. Small differences in identification methods, however, have the potential to cause inconsistencies in results when comparing one system's speciation of a microbial isolate to another (Perl et al 1994). Moreover, automated systems can cause outright misidentification even at the genus level. In one report, results from an automated typing system were compared to those of a 16s rRNA speciation for 19 clinical bacterial isolates (Ben-Ami et al 2005). The automated system identified all of the isolates as Kocuria, while the 16s rRNA sequence subsequently identified all 19 isolates as CNS, 18 *S. epidermidis* and 1 *S. hamolyticus*; nevertheless, the automated identification of CNS at the levels of species and subspecies presents potential value for clinical correlation and therapeutic intervention (Rupp and Archer 1994). Other work recently presented suggests *S. epidermidis* isolates may be misidentified even as *S. auricularis*, so it is clear that erroneous typing could delay appropriate and necessary therapeutic intervention (John et al 2006).

Regardless of species specificity, treatment of CNS infection has become increasingly difficult due to the high prevalence of antibiotic-resistant strains. Widely used antibiotics including penicillins, particularly semi-synthetic penicillins, cephalosporins, macrolides, aminoglycosides, and tetracyclines, have proven to be ineffective in inhibiting several prevalent species of CNS, thus, augmenting the need for new and effective antimicrobials (Cerca et al 2005; Gaudioso de Allori et al 2006; Arciola et al 2006). To add to the resistance problem, multi resistance in CNS, as in *S. aureus*, is carried out on a staphylococcal chromosome cassette (SCC) which almost always includes the *mec*A gene for resistance to semi-synthetic penicillins (SCC*mec*) (Hanssen et al 2004). SCC*mec* resides in the chromosome as several cassette variants I-V.

CNS are ubiquitous in nature, residing on skin and mucous membranes (Costa et al 2004). When exposed to medical devices, the CNS anchor themselves to a polymer surface via van der Waal's forces, hydrophobic interactions, and polarity, ultimately forming a thick biofilm (Vuong et al 2003; Mack et al 2006). Production of biofilm reduces the organism's susceptibility to specific antimicrobials which

Table 2 List of antimicrobial classes potentially active against coagulase-negative staphylococci

Class
Aminoglycosides
Carbapenems
Cephalosporins
Choramphemicols
Diaminopyrimidines
Fluoroquinolones
Fusidic acid
Glycopeptides
Lincomycins
Lipopeptides
Macrolides
Oxazolidinones
Polymyxins
Rifampicins
Semi-synthetic penicillins
Sulfonamides
Tetracyclines
Dihydrofolate reductase inhibitors (trimethoprim)

are highly active against planktonic cultures. The problem of antibiotic biofilm interaction has recently become a stimulus to develop antibiotics that can inhibit initial microbial adhesion to polymer surfaces as well as growth on planktonic cultures (Cerca et al 2005).

In this paper we will review the general problem of antibiotic resistance in CNS and discuss the susceptibility and resistance, where data are available, for specific species. Additionally, in view of a recent expansion in new anti staphylococcal agents, we will address the susceptibility profiles of some of these newer agents.

Pre-1994 susceptibility literature

Antimicrobial susceptibility in CNS became an issue in the 1970s when these species were isolated as pathogens in prosthetic valve endocarditis infections of cerebrospinal fluid shunt infections (Keys and Hewitt 1973; Shoenbaum et al 1975). There were earlier harbingers that new resistance genes were emerging, specifically those whose products would inhibit the binding of semisynthetic penicillins, agents that had been developed to resist the effects of extracellular staphylococcal β-lactamases. Methicillin was the predominant semisynthetic penicillin used in early therapy so the term "methicillin resistance" became a generic term for resistance to β-lactam antibiotics based on the inability of these agents to bind to the new penicillin-binding protein product of *mec*A, PBP-2a (Sutherland and Rollison 1964; Bentley et al 1967). During the 1970s it became clear that methicillin resistance was more prevalent in CNS (MRCNS) than in methicillin-resistant *S. aureus* (MRSA), an observation that continues to be true today.

During the 1970s and early 1980s, the problem of multiresistance in CNS was reported primarily in cases of device related infections particularly prosthetic valve endocarditis (Archer 1978). In a group of *S. epidermidis* associated with device infections, 56% were resistant to methicillin and 70% were resistant to cefoxitin and cephardine (Archer 1978). For the subsequent period through 1994, Archer and Climo have reviewed the literature (Archer and Climo 1994) in which they report an escalation of resistance for almost all antimicrobial classes excluding glycopeptides: β-lactams aminoglycosides, trimethoprim, rifampin, fluoroquinolones, macrolides, and tetracyclines. Therefore, in this current paper we will review the post-1994 literature on the subject.

Post-1994 susceptibility literature

The trends in resistance in CNS over the last 13 years have continued to show escalation in the frequency and expression of resistance determinants. Resistance to β-lactam agents (methicillin resistance) has remained the foremost strain determinant. Strains of MRCNS are often linked to multiresistance, like their MRSA counterparts, thus presenting an ongoing therapeutic challenge. The discovery of specific chromosomal cassettes carrying the *mec*A gene as well as multiple other genes has provided a new backdrop against which evolution of CNS species and their respective SCC*mec* can be measured (Mongkolrattanothai et al 2004; Noto and Archer 2006). A dichotomy between methicillin-susceptible CNS (MSCNS) and MRCNS permeates the literature on susceptibility testing. In fact, these groups are usually tested and reported separately. For example the analysis of US key bloodstream bacterial isolates from 1995, 1996, and 1997 showed that among 43,789 CNS, 42% were MSCNS and 58% were MRCNS (Sahm et al 1999). Moreover, in this group of MRCNS the rate of vancomycin minimum inhibitory concentration (MIC) of 4 μg/mL increased from 1.8% to 4.8% from 1995 to 1997.

That database, SENTRY, was expanded to other countries during 1997–1999 (Diekema 2001). Interestingly, while rates of methicillin resistance among CNS varied widely among 13 European countries, the rate was around 70% for 5 global locations; Canada, USA, Latin America, Europe, and the West Pacific. Co-resistance patterns were also reported for the five regions based on oxacillin resistance. For example in the US trimethoprim-sulfamethoxazole (SXT) resistance was about 17% in MSCNS compared with nearly 57% in MRCNS. Similar trends were seen for gentamicin,

Table 3 New agents with good antibacterial activity against coagulase-negative staphylococci

Agent	Year introduced	Mechanism of action	MIC_{90} CNS MSCNS/MRCNS	MIC_{90} *S. aureus* MSSA/MRSA	Reference
Ceftibiprole	N.A.	Binds to abberant penicillin binding protein,PBP-2A	1.0/2.0	0.5/2.0	Bogdanovich et al 2005a
Daptomycin	2003	Cell membrane lysis	0.5/0.5	0.5/0.5	Critchley et al 2003 Petersen et al 2002
Dalbavancin	N.A.	Blocks cell-wall synthesis like teichoplanin	0.06/0.06	0.06/0.06	Lin et al 2005
Linezolid	2000	Binds to tRNA	4.0/4.0	4.0/4.0	Noskin et al 1999
Moxifloxacin	2002	Interferes with topoisomerase II and IV	0.12/4.0	0.12/4.0	Hardy et al 2000
Televancin	N.A.	Blocks the synthesis of phospholipids in cell membranes	0.5/1.0	0.5/0.5	King et al 2004
Tigecycline	2003	Blocks 30s ribosome	0.5/0.5	0.5/0.5	Frische et al 2004 Gales et al 2005 Sader et al 2005

Abbreviations: CNS, coagulase-negative staphylococci; MSCNS/MRCNS, methicillin-susceptible/methicillin-resistant CNS; MSSA/MRSA, methicillin-susceptible *S. aureus*/ methicillin-resistant *S. aureus*.

ciprofloxacin, clindamycin, and erythromycin both in US and global sites (Diekema et al 2001). For most antimicrobials, intensive care units (ICUs) tend to show the highest rates of antimicrobial resistance (Fridkin et al 1999). In a massive study to determine the focus of antibiotic resistance in different areas for over 80 hospitals, the rates of MRCNS were significantly higher in ICU – than in non-ICU patients and were higher in non-ICU patients than in outpatients. These data suggest that the major risk for MRCNS is associated with more complex hospital environments.

A similar large study was conducted in which staphylococci were collected from 20 regional health centers in several countries across Europe, Asia, and Latin America (Sanches et al 2000). The CNS were divided into methicillin-resistant *S. epidermidis* (MRSE), methicillin-resistant *S. haemolyticus* (MRSH), and other MRCNS and their susceptible counterparts. Cross resistance was most common in the methicillin-resistant groups. For example, rates of SXT, ciprofloxacin, and gentamicin were 64%, 50.5%, and 72.3% respectively. MRSH showed even higher frequencies of cross resistance. These trends in cross resistance among CNS were different than those for MRSA, suggesting that different selection pressures are at work for the two groups.

Another study tested susceptibility of only skin and soft tissue isolates in 283 US hospitals and 301 hospitals in Europe for 9 antimicrobials, including amoxicillin-clavulanate, cefotaxime, cetriaxone, ciprofloxacin, erythromycin, gentamicin, levofloxacin, trimethoprim (TMP)/sulfamethoxazole (SMX), and vancomycin. For MSCNS almost all isolates were susceptible to amoxicillin-clavulanate, suggesting that clavulanate remains very active against staphylococcal β-lactamase (Jones et al 2003). For the fluoroquinolones, ciprofloxacin averaged about 90% susceptibility and, where tested, levofloxacin was active against slightly more strains. For example, in Italy 83.3% of isolates were susceptible to ciprofloxacin versus 85.7% for levofloxacin. Ranges of activity for gentamicin (86.1%–96.4%) and TMP/sulfamethizole (SMZ) (88.1%–92.7%) were active in a similar range. Over 25% of isolates were resistant to erythromycin whereas all isolates were susceptible to vancomycin. For MRCNS the resistance trends were, in general, similar in European and US isolates with some exception. For example, ciprofloxacin resistance was 65.0%–66.7% across four European countries (France, Germany, Italy, and Spain) versus a range of 38.1%–47.0% in the US. Gentamicin resistance was higher in Europe (range of 50.2%–61.3%) in the US (32.4%). No vancomycin resistance was detected.

Cuevas et al studied the trends of resistance in clinical isolates of CNS in Spain for five periods from 1986 to 2002 (Cuevas et al 2004). For all years together, 28% of strains were community in origin and 72% were nososcomial. Oxacillin resistance rose steadily from 32.5% in 1986 to 61.3% in 2002. Gentamicin resistance rates peaked in 1994 at 41.4% but dropped to 27.85% in 2002. Most interestingly, ciprofloxacin resistance rose from 1.1% in 1986 to 44.9% in 2002. Although there was no vancomycin resistance, there was one isolate in the 1996 study resistant to teichoplanin, an observation made earlier in one isolate of *S. epidermidis* from a corneal infection (Pinna et al 1999). Nosocomial strains were more likely to demonstrate resistance. In another study done in Uppsala, Sweden to determine more specifically how an ICU stay would influence CNS resistance, high rates of oxacillin

Table 4 Ideal characteristics of anti-coagulase-negative staphylococcal antimicrobials

- Ease of synthesis, isolation
- Evidence that bactericidal concentration (MBC) closely approaches the inhibitory concentrations (MIC)
- Excellent extracellular and intracellular activity
- Pharmacokinetics/pharmacodynamics favorable to long intermittent dosing
- Prolonged post-antibiotic effect
- Good biofilm penetration
- Potential biofilm inhibition
- Lack of antagonism with other anti-staphylococcal agents
- Slow evolution of in vitro and in vivo resistance
- Low indices of systemic and organ toxicity

and ciprofloxacin resistance (92% and 83% respectively) in 20 ICU patients were correlated with longer lengths of stay. Genotyping of the ICU CNS isolates found 32 pulse field types involved, but 16 genotypes colonized more than 1 individual suggesting some clonality among these CNS isolates.

In a study from Greece of intravenous catheter infections associated with bacteremia, over a 2-year period CNS were responsible for nearly 60% of infections (Paragioudaki et al 2004). For these pathogens, in 1999, the resistance rate was 72% for ampicillin, oxacillin, ceftazidime, ceftriaxone, cefaclor, amoxicillin/clavulanate, and impenem, with slightly lower rates for SXT, ciprofloxacin, and amikacin. There was no vancomycin resistance. Rates dropped slightly in the year 2000 for the same antimicrobials. The redundancy of resistance rates may be due to spread of virulent CNS clones as was observed earlier at the University of Patras (Spiliopoulou et al 2003).

Several studies combined automated species identification with antimicrobial susceptibility testing. From Italy, Ariciola et al reported resistance levels in 15 species of 193 isolates found to colonize orthopedic implants (Arciola et al 2006). In the 5 most prevalent species – *S. hominis*, *S. haemolyticus*, *S. capitis*, *S. warnerii*, *S. cohenii* – resistance to penicillin was similar (51%–66%). Most oxacillin resistance was seen in *S. haemolyticus* whereas no oxacillin resistance was present in *S. warneri*. Multiple antibiotic resistance genes were most likely to be found in *S. haemolyticus*, averaging 2.8 for that species. Though the data are small in number, this study suggests that both careful susceptibility testing along with species identification is helpful in certain device-related infections.

CNS remain the major cause of postcataract endophthalmitis. Recchia et al found that nearly 60% of pathogens from such cases were CNS and 91% of these were *S. epidermidis* (Recchia et al 2006). In analyzing 2 groups, for the 5 years before and after 1994, a significant increase in resistance was seen for ciprofloxacin (20 to 38%) and cefazolin (19 to 40%). TMP/SMZ resistance stated relative stable, around 27%.

The study of device-related antimicrobial resistance has been hampered by lack of good models. Work with newer models has determined activity of various antibacterial solutions activity against different bacteria after adherence to silicone catheter segments (Sherertz et al 2006). Results showed that *S. epidermidis* was the most susceptible in terms of log kill over 24 hours to most antimicrobials tested and, in particular, to a complex solution of minocycline, EDTA, and ethanol.

New individual agents

Several new antimicrobials with good anti-staphylococcal activity have been marketed recently or will likely soon be marketed: ceftobiprole, dalbavancin, daptomycin, linezolide, tigecycline and telavancin. We will discuss the reports that have included CNS in the susceptibility profile.

Linezolid

This oxazolidinone was introduced into clinical practice (in the US) in 2000. Its mechanism of action involves the binding of N-formylmethionyl-tRNA to the ribosome with the 50s subunit, but not the 30s subunit of the ribosome (Shinabarer 1999). There are no comparative trials of linezolid therapy for CNS infections. With an MIC_{90} = 1–4 μg/mL, it has become a major option for therapy in resistant and recalcitrant staphylococcal infections including those due to CNS (Davidson and Low 2004). In the largest in vitro experience as of its 1999 publication, Noskin et al studied 365 oxacillin-susceptible and 441 oxcillin-resistant CNS (Noskin et al 1999). Both groups had the same MIC_{50} and MIC_{90}, 2 and 4 μg/mL respectively. At that time, only vancomycin of the agents studied was more active. Data submitted for US registration of linezolid showed MIC_{90} values of both methicillin-resistant and methicillin-resistant *S. epidermidis* of 1–4 μg/mL (Pfizer data on file). Postmarketing surveillance programs reported for 870 CNS isolates an MIC_{90} of 1 μg/mL (Ross et al 2005). Only one isolate had an MIC $>$ 8 μg/mL.

In vitro development of resistance occurs very slowly with serial passage but when it does occur, it is related to two independently isolated point mutations at G2447U and G2576U of the 23S rRNA (Shinabarger 1999). Resistance in the clinical setting, interestingly, has involved a report of linezolid-resistant CNS (Potoski et al 2006). The University of Pittsburgh Medical Center experienced this cluster of 25 linezolid-resistant CNS isolates with a single *S. epidermidis* clone, shown by pulse-field gel

electrophoresis similarity, that infected 21 patients. The linezolid MIC was >256 μg/mL in 24 of the 25 isolates. In this group of patients, previous linezolid use was a risk factor for development of resistance.

Tigecycline

Tigecycline is a minocycline derivitative with excellent activity against resistant Gram-positive bacteria. A South American study analyzed 47 MSCNS and 180 MRCNS for tigecycline susceptibility (Gales et al 2005). Both groups displayed an MIC_{90} of 0.5 μg/mL (identical for *S. aureus*) and 100% were susceptible to 2 μg/mL. In a 2004 publication 321 MSCNS and 1111 MRCNS mirrored Gales study with MIC_{90} of 0.5 μg/mL for both groups (Frische et al 2004). In a larger study of 3574 CNS, Sader et al found tigecycline exhibited excellent activity against blood stream pathogens (Sader et al 2005). Again the demonstrated MIC_{90} was 0.5 μg/mL for CNS and for *S. aureus*. Data supporting this level of activity were also derived in another study comparing the activity of a new quinolone, DX-619, with other Gram-positive agents (Bogdanovich et al 2005b). The MIC_{90} of tigecycline for 61 MRCNS and 67 MSCNS was 0.5 μg/mL. DX-619 comparatively had an MIC_{90} 0.06 μg/mL. In another study, limited number of glycopeptide-intermediate susceptible isolated of CNS (22) have been studied for tigecycline and daptomycin susceptibility. In one small set of 8 *S. epidermidis* isolates and 4 *S. haemolyticus* isolates, the MIC was again 0.5 μg/mL for tigecycline, 2.0 μg/mL for arbekacin and 1.0 μg/mL for daptomycin (LaPlante and Rybak 2004). The minimum bactericidal concentration (MBC):MIC ratio was 4 (no specifics given). Time-kill experiments for tetracycline at 4 times the MIC against glycopeptide-intermediate *S. epidermidis* revealed similar killing to daptomycin but significantly more activity than vancomycin at 24 hours. Thus, as a tetracycline derivative, tigecycline is surprisingly active against susceptible and resistant CNS and has some bactericidal effect.

Daptomycin

Daptomycin, an older cyclic glycopeptide that had been studied for several decades, was recently brought back to clinical life. It made its clinical debut in 2003 for approved use in skin and soft tissue infection and has been shown recently to be clinically non-inferior to vancomycin in clinical trials of staphylococcal bacteremia (Fowler et al 2006). Daptomycin probably has several mechanisms of action that involve perturbation of calcium-dependent channels to disrupt the cytoplasmic membrane, perhaps first by altering the membrane potential (Silverman et al 2001; Friedman et al 2006). One of the largest studies of daptomycin-CNS interactions consisted of 1126 CNS (as verified by Vitek coagulase testing) taken from 6,973 gram-positive isolates collected from patient specimens at 50 hospitals distributed throughout the nine US Bureau of the Census regions (Critchley et al 2003). Daptomycin was consistently active with an MIC range of 0.015–2.0 μg/mL and an MIC_{90} of 0.5 μg/mL. Daptomycin and quinupristin/dalfopristin were the most active agents seen in this analysis. As an anti-staphylococcal agent, daptomycin may have other advantages like a very low spontaneous resistance rates seen during serial passage, and the fact that resistant mutants had lower virulence in an animal model (Silverman et al 2001). When daptomycin was tested for in vitro activity in Mueller-Hinton broth supplemented with 50 mg of calcium per liter, MICs were about 4-fold lower (Petersen et al 2002). In a comparative study against tigecycline, and other Gram-positive agents, the MIC_{90} of daptomycin and tigecycline for MSCNS was 0.25 μg/mL and 0.5 μg/mL respectively but was 8- and 2-fold higher for MRCNS, respectively. In comparison, the MIC rose multifold for other agents like erythromycin, levofloxacin, and teicoplanin. Resistance to daptomycin has been shown in the laboratory with serial passage. Elegant experiments of genome comparison revealed that the non-susceptible mutants were altered in *Mpr*F, a lysylphosphatidylglycerol synthetase (Silverman et al 2006).

New fluoroquinolones

Several new quinolones have excellent activity against CNS. As stated above (see daptomycin), a des-F(6)-quinolone, DX-619 was compared to 6 other quinolones. For strains that had an MIC_{90} of 16.0 μg/mL, the DX-619 MIC_{90} was 0.125 μg/mL, one tube lower than another new quinolone agent, sitafloxacin (Bogdanovich et al 2005b). Interestingly, killing for DX-619 of a vancomycin-nonsusceptible CNS was complete at 4 × MIC and >log4 at 12 hours for 2 × MIC. Of the currently used fluoroquinolones, moxifloxacin is probably the most frequently used. Its MIC_{90} for MSCNS was 1.0 μg/mL and for MRCNS was MSCNS was 4.0 μg/mL.

New cephalosporins

Historically, β-lactam antibiotics have produced excellent results against methicillin-susceptibile staphylococci. Production of aberrant penicillin-binding proteins like PBP-2A bypass the effect of β-lactams. Thus, there has been a search to find β-lactams that themselves bind to PBP-2A and thus render strains susceptible. Ceftobiprole is one of a new group

of cephalosporins that are active against methicillin-resistant staphylococci. MRSA are susceptible to ceftobiprole at an MIC_{50}/MIC_{90} of 2/2 and MRCNS are similarly susceptible with a range of 0.125–4 μg/mL (Bogdanovich et al 2005a). Ceftobiprole also demonstrates excellent time-kill kinetics and in *S. aureus* a good post-antibiotic effect (PAE) at MIC and sub-MIC concentrations (Pankuch and Applebaum 2006). In a study of isolates from diabetic foot infections, staphylococci were speciated and had excellent ceftibiprole MIC_{90} values (μg/mL): methicillin-susceptible *S. aureus* (MSSA), 0.5; MRSA 1.0; *S. epidermidis* 1.0; *S. haemolyticus* 2.0, and *S. ludgenensis* 0.5.

New glycopeptides

Several derivatives of vancomycin have been developed and brought to clinical trials. Telavancin is a rapidly bactericidal lipoglycopeptide with excellent staphylococcal activity. In a study of telavancin versus standard therapy for treatment of skin and soft tissue infection, CNS caused 13 of 122 staphylococcal infections (Stryjewski et al 2006). The MIC_{90} for telavancin was 0.25 μg/mL (0.06–0.25) among CNS isolates that had a MIC_{90} of 2.0 for oxacillin. Televancin also is one of the few newer agents to exhibit activity in biofilms (Gander et al 2005). A 3-log decrease in 2 CNS isolates was demonstrated in a so-called Sorbarod biofilm model.

Dalbavancin is another lipoglycopeptide that inhibits cell-wall synthesis. In a British study of 92 isolates from 60 hospital laboratories, 100% of the isolates were inhibited by 1 μg/mL of drug (Mushtaq et al 2004). The E-test MIC correlation was excellent with broth dilution but less correlation with agar dilution methodology (Fritsche et al 2006). In another study of dalbavancin compared to other agents including oritavancin, an experimental glycopeptide, dalbavancin had exceptional activity at the MIC_{90} level (0.06 μg/mL) for both MSCNS and MRCNS (King et al 2004). In that study, ortivancin had an MIC_{90} of 2.0 μg/mL and 4.0 μg/mL for MSCNS and MRCNS. Interestingly, dalbavancin has a very long half-life (7.5 days) that, when marketed, will challenge prescribers who are used to much shorter dosing intervals.

Other agents

Other agents are on the Gram-positive therapy horizon, but very few have shown enough promise to come to clinical trials. One agent, API-1252, from a group called enoyl-acyl carrier protein reductases, has shown excellent antistaphylococcal activity. The MICs for *S. epidermidis* isolates from Canada to API-1252 were excellent, with MIC_{90} for methicillin-suscetible *S. epidermidis* (MSSE) and MRSE of 0.06 and 0.03 μg/mL respectively, only slightly higher than the MIC_{90}s for *S. aureus* (Karlowsky et al 2007). A second group worth mentioning are the acyldepsipeptides (ADEPs) which have a unique mechanism of action and are quite active against Gram-positive bacteria. ADEPs activate the protease function of ClpP proteins to cause a dysregulation of proteolysis (Brötz-Osterhelt et al 2005). The MIC_{50} for *S. aureus* of one ADEP, is as low as 0.05 μg/mL, but data for CNS have yet to be published.

The effect of staphylococcal biofilm on antimicrobial susceptibility

Biofilms consists of a complex extracellular matrix that can have profound effects on the ability of antimicrobials to inhibit and kill bacteria and fungi. Biofilms are extremely common in infections due to CNS, bacteria that have special sets of genes that regulate and determine cell-wall associated adhesion and biofilm production (Mack et al 2006). The regulation of biofilm production for most staphylococci involves complex schemes (*agr*, *sar*, *sig*B) that result in sets of genes being turned on and off depending on the current cellular environment. Biofilm regulation involves up and down regulation of genes that mediate information storage, cellular processes and signaling, metabolism and other functions (Yao et al 2006).

It has been known several years that biofilms interact with antimicrobials (Khardori et al 1991). Early work suggested that subtherapeutic levels of certain agents like fluoroquinolones and glycopeptides would not prevent attachment of bacteria but that β-lactam agents and vancomycin could inhibit aggregative activity (Rupp and Hamer 1998). The mechanism of biofilm-associated antimicrobial resistance is likely multifactorial. Unlike other methods to test antimicrobial susceptibility, methods that address biofilm susceptibility to antimicrobial agents have been needed to define fully the influence of biofilm on susceptibility (Patel 2005). To that end, very recent work comparing planktonic bacteria to biofilm-grown ones has shown large differences in susceptibility. Almost all single agents studied (linezolid, cefazolin, oxacillin, vancomycin, gentamicin, azithromycin, ciprofloxacin and fusidic acid) had a marked increase in the MBC versus the MIC using what is called a modification of the Calgary Biofilm Device (Saginur et al 2006). Rifampin alone had some predictable equivalence between the MIC and the MBC. For *S. epidermidis* isolates, certain combinations, almost always containing rifampin, were bactericidal against ≥90% of biofilms (examples rifampin/vancomycin/gentamicin; rifampicin/ciprofloxacin/fusidic acid), but

a smaller number of antibiotic combinations were active against biofilm-grown bacteria than against planktonic bacteria. Strains of *S. lugdenensis* in biofilm have been shown to be particularly susceptible to moxifloxacin, suggesting that some fluoroquinolones do have good biofilm activity (Frank et al 2007). In the same model, vancomycin was shown to be non-bactericidal, thus suggesting that biofilm contributes to the phenomenon of tolerance or lack of killing in certain glycopeptides.

The effects of antimicrobials seem to be different at the time of adherence versus the later stage of mature biofilm formation. In a study to test interaction of specific agents with biofilm, dicloxacillin was more active at both stages than cefazolin and vancomycin. Finally, biofilm susceptibility may be staphylococcal species specific (Cerca et al 2005). An Argentine study looked at 5 CNS species and found that of 88 biofilm producing strains, resistance to 2 or more agents was seen in 29% of *S. haemolyticus*, 24% of *S. saprophyticus*, and 20% of *S. epidermidis* (Gaudioso de Allori et al 2006). Such work implies that rapid evaluation of biofilm-associated resistance should be integrated into clinical consideration. Furthermore, their in vitro findings can be translated into clinically applicable devices.

Tigecycline and N-acetylcysteine have been embedded into vascular catheters. Such combinations are not only inhibitory of biofilm growth in vitro but also act synergistically against MRSA and MRSE (Aslam et al 2007).

Concluding remarks

Coagulase negative staphylococci comprise many species which have become increasing pathogenic to man by virtue of a changing host of demographics. CNS are relatively more resistant than their *S. aureus* counterparts but have fewer virulence factors. They do have a penchant for forming biofilms on device materials and devitalized tissue, making biofilm-associated CNS more resistant to penetration by antimicrobial agents. There are now good methods to show that biofilms markedly increase the inhibitory concentration of standard antistaphylococcal agents. Hospital CNS have broad spectrum resistance and usually carry SCC*mec* cassettes that make their host resistant not only to β-lactam but a wide array of currently available antimicrobials. There are several newly marketed agents including linezolid, tigecycline, and daptomycin that have excellent activity against CNS including one species that is almost always pathogenic, *S. lugdenesis*. Other new, non-marketed agents including variations on vancomycin and new peptide agents show promise in vitro. Like their forebearers, however, there is always the spectre of evolving resistance, and only time will determine how rapidly resistance does emerge in the clinical arena.

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