Update on Clinical Significance of Coagulase-Negative Staphylococci

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INTRODUCTION

As a group, the coagulase-negative *Staphylococcus* species (CNS) are among the most frequently isolated bacteria in the clinical microbiology laboratory (236, 246). One of the major problems facing the laboratory is distinguishing clinically significant, pathogenic strains of CNS from contaminant strains (164). The vast majority of infections (or diseases) assumed to be caused by CNS are a significant consequence of hospitalization. Recent reports on surveillance data taken from the National Nosocomial Infections Surveillance System during the late 1980s and early 1990s have indicated that CNS are among the five most commonly reported pathogens (in fifth place at 9 to 9.7%, compared

with 10 to 11.2% for *Staphylococcus aureus*) in hospitals conducting hospital-wide surveillance (145, 271). CNS were the most frequently reported pathogens in nosocomial bloodstream infections (27 to 27.9%, with *S. aureus* next at 16 to 16.5%). The ranking and infection rates of CNS were quite similar among hospitals conducting surveillance in intensive care units (ICUs) and in those conducting surveillance hospital-wide. In contrast to the situation in the 1970s, major shifts have occurred in the decade of the 1980s and in the early 1990s in the etiology of nosocomial infection. Most noticeably, the shifts have been toward the more antibiotic-resistant pathogens, of which the CNS are a major group (12, 113, 271). Current antibiotic-prescribing practices, including preoperative antibiotic prophylaxis, have led to the selection of antibiotic-resistant organisms (271).

The increasing importance of CNS also may be due in part to the growing appreciation of this group of organisms as opportunistic pathogens and to the increase in the use of transient or permanent medical devices, such as intravascu-

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lar catheters and prosthetic devices, in seriously ill and immunocompromised patients (i.e., intensive care patients, premature newborns, and cancer and transplant patients). CNS infections often can be life-threatening in these patients. CNS are a major component of the normal flora of the cutaneous ecosystem, including the skin and mucous membranes (165, 167, 175). In the cutaneous ecosystem, CNS generally have a benign relationship with their host and function as commensal or saprophytic organisms. However, if the cutaneous organ system has been damaged by trauma, inoculation by needles, or direct implantation of foreign bodies, these organisms can gain entry to the host. Depending upon their ability to adhere to host or foreign body surfaces, breach or avoid the host immune system, multiply, and produce products that damage the host, they may develop the lifestyle of a pathogen.

The primary aim of this review is to update information concerning the identification and clinical significance of CNS that has accumulated since the last comprehensive review by Pfaller and Herwaldt (246). Research on the CNS has proceeded on several fronts, including the identification of new species and subspecies, development of more accurate and rapid methods for identifying species and subspecies and for epidemiological typing of strains, the use of new antibiotics for therapy, and greater understanding of antibiotic resistance mechanisms, genetic transfer systems, and pathogenic mechanisms.

HISTORICAL PERSPECTIVE

In 1958, Smith and coworkers (283) noted the potential pathogenicity of CNS by collecting data from patients with septicemia. Several years later, Pulverer and Halswick (255) reported on 128 cases of endocarditis believed to be caused by CNS. Prior to the 1970s, clinicians and microbiologists generally regarded CNS as contaminants in clinical specimens and S. aureus as the only pathogenic Staphylococcus species. In his Theodor Billroth Memorial Lecture at the Fifth International Symposium on Staphylococci and Staphylococcal Infections, Pulverer (254) shared his frustration with the medical community when he said, "In 1965 we sent a paper (255) entitled 'Coagulase-negative staphylococci as pathogenic agents' to one of the leading medical journals in Germany. We had great problems in convincing the editors that we had no joke in mind but wished to report seriously about a lethal case of CNS endocarditis. . . . In 1964, we observed this rather malignant disease in a 57-year-old man who died several months later despite a long-lasting and high-dosage penicillin treatment." Brandt and Swahn (37) reported that more than 1% of all cases of endocarditis may be due to CNS.

In 1965, Wilson and Stuart (318) reported that CNS were found in pure culture in 53 of 1,200 (4.4%) cases of wound infections. In 1971, Pulverer and Pillich (256) investigated the incidence of CNS pyogenic infections in Cologne, Germany, presenting data for the years 1960, 1969, and 1970. CNS were found in about 10% of all pyogenic lesions observed in hospital patients, and in about 50% of these cases, CNS were believed to be present in pure culture. In 1962, Pereira (240) reported that a certain group of CNS (now known as S. saprophyticus) caused urinary tract infections (UTIs). A few years later, Gallagher and coworkers (96) and Mabeck (201) also presented evidence that CNS cause UTIs. In 1971, Holt (131) reported the colonization of ventriculoatrial shunts by CNS. Colonization was usually followed by septicemia. Looking through the literature,

Pulverer (254) collected data from 2,276 ventriculoatrial or peritoneal shunt operations and estimated that 8% of the patients acquired shunt infections, with 58% of the cases probably caused by CNS.

In light of recent advances in staphylococcal systematics and epidemiological typing methods, conclusions concerning the etiology of CNS infections reported prior to the 1980s should be made with some caution. For many of the early studies reporting CNS in infections, sound methodologies were not available for the determination of repeated or pure cultures of organisms. However, during the last decade, considerable progress in the classification of staphylococci and in the development of methods for identifying them at the genus, species, subspecies, and strain levels has been made (167, 171, 175, 245, 247). These newer systematics have not only made clinicians more aware of the variety of CNS present in clinical specimens, but also enhanced the credibility of CNS as etiologic agents.

By the 1980s, the range of infections believed to be caused by CNS, and especially by S. epidermidis, was quite wide and included bacteremia (24, 207); native valve endocarditis (NVE) and prosthetic valve endocarditis (7, 11, 260); osteomyelitis (204, 233); pyoarthritis (204); peritonitis during continuous ambulatory dialysis (182, 267); mediastinitis (34); prostatitis (45, 310); infections of permanent pacemakers (48), vascular grafts and intravascular catheters (242, 243), cerebrospinal fluid shunts (100), and prosthetic joints and a variety of orthopedic devices (38, 54, 218); and UTIs (148, 156, 192). The CNS species S. saprophyticus was often regarded as a more important opportunistic pathogen than S. epidermidis in human UTIs, especially in young, sexually active females (3, 206, 307). It was considered to be the second most common cause of acute cystitis or pyelonephritis in these patients. Several other CNS species have been implicated at low incidence in a variety of infections. S. haemolyticus is the second most frequently encountered CNS species in the clinical laboratory. This species has been implicated in NVE (43), septicemia (102), peritonitis (114), wound, bone, and joint infections (85, 229), and UTIs (148). S. warneri is believed to be the cause of some cases of vertebral osteomyelitis (159), NVE (60), and UTIs (192). S. hominis has been associated with endocarditis (85), peritonitis (85), septicemia (36), and arthritis (85). Some of the earlier reports indicating an association of S. hominis with infections may have been in error due to the confusion of this species with phosphatase-negative strains of S. epidermidis (171). S. simulans has been associated with some cases of chronic osteomyelitis and pyarthrosis (204).

IDENTIFYING THE ETIOLOGIC AGENT

Understanding Staphylococcus Communities

Currently, there are 31 species recognized in the genus Staphylococcus (Table 1). About one-half of these are indigenous to humans and include S. aureus (a coagulase-positive species), S. epidermidis, S. haemolyticus, S. saprophyticus, S. cohnii, S. xylosus (276), S. capitis, S. warneri, S. hominis, S. simulans (173), S. saccharolyticus (162), S. auricularis (174), S. caprae (67), S. lugdunensis, and S. schleiferi (276). Eight subspecies have also been described, four of which have been given names. S. capitis subsp. ureolyticus (19) and S. cohnii subsp. urealyticum (177) are indigenous to humans and other primates. Species that are indigenous to other animals and birds may be found occasionally on humans, especially when recent contact has been made.

TABLE 1. Currently recognized *Staphylococcus* species and subspecies

Species	Subspecies	Natural host(s)[a]	Reference(s)
S. aureus	aureus	Humans, mammals, birds	265
S. aureus	anaerobius	Sheep	64
S. epidermidis		Humans (domestic mammals)	276
S. capitis	capitis	Humans	173
S. capitis	ureolyticus	Humans, some primates	19
S. caprae		Humans, goats	67
S. saccharolyticus		Humans	162
S. warneri		Humans, primates, domestic mammals	173
S. haemolyticus		Humans, primates, (domestic mammals)	276
S. hominis		Humans	173
S. lugdunensis		Humans	93
S. auricularis		Humans, primates	174
S. cohnii	cohnii	Humans	177, 276
S. cohnii	urealyticum	Humans, primates	177
S. saprophyticus		Humans, mammals	276
S. xylosus		Humans, mammals, birds	276
S. arlettae		Mammals, birds	274
S. equorum		Horses, cattle	274
S. kloosii		Mammals	274
S. gallinarum		Poultry, birds	67
S. muscae		Domestic mammals, (flies)	117
S. felis		Cats	139
S. simulans		Humans, mammals	173
S. carnosus		Meat and fish products, unknown	272
S. piscifermentans		Fermented fish	294
S. intermedius		Mammals, birds	115
S. delphini		Dolphins	303
S. schleiferi	schleiferi	Human infections, unknown	93
S. schleiferi	coagulans	Dogs	140
S. hyicus		Pigs, cattle, goats	66
S. chromogenes		Cattle, horses, goats	66, 116
S. caseolyticus		Cattle, whales	275
S. lentus		Domestic mammals, dolphins	273
S. vitulus		Meat products, domestic mammals, whales	309
S. sciuri		Mammals, birds	176

[a] Parentheses indicate that the species is probably transient on the host.

The largest populations of human staphylococci are usually found in regions of the skin and mucous membranes surrounding openings to the body surface (165, 172). The population of staphylococci living in moist habitats, such as the anterior nares, axillae, and inguinal and perineal areas, may reach densities of 10^3 to 10^6 CFU/cm^2 of surface, and that in relatively dry habitats or the extremities may reach 10 to 10^3 CFU/cm^2. Some *Staphylococcus* species and subspecies demonstrate a marked preference for certain habitats. For example, *S. capitis* subsp. *capitis* prefers the human head and produces very large populations on the scalp following puberty (165). It is also found on other regions of the adult head such as the forehead, face, eyebrows, and external auditory meatus in moderate-sized to large populations. *S. capitis* subsp. *ureolyticus* is found on regions of the head in rather small populations but also can be found on a variety of other body sites, being more widely distributed than *S. capitis* subsp. *capitis* (19). *S. auricularis* is one of the major species living in the adult external auditory meatus (165, 174). The coagulase-positive species *S. aureus* demonstrates a habitat preference for the anterior nares in adults (165). It is somewhat more widely distributed over the body in preadolescent children. This species is especially adapted to damaged or traumatized tissue or skin. *S. saprophyticus* is usually found in small, transient populations on a variety of body sites, but this species possesses surface properties that allow it to adhere readily to urogenital cells (57). The

predominant *Staphylococcus* species of humans, *S. epidermidis*, is widely distributed over the body surface (165, 172). It usually produces very large populations in the anterior nares, axillae, inguinal and perineal areas, and toe webs. *S. hominis* and *S. haemolyticus* are most numerous on skin sites where apocrine glands are found, such as in the axillae and inguinal and perineal areas (165, 172). Generally, they can also colonize the drier regions of skin (e.g., on the extremities) more successfully than other species. *S. warneri* and *S. lugdunensis* are widely distributed over the body, though their population size is usually quite small (165, 172, 276). *S. caprae*, although originally isolated from goats (hence the name), has been found on human skin in very small numbers and in human clinical specimens (17, 157). Human isolates of this species could be distinguished from some goat isolates on the basis of *Sma*I restriction enzyme digests of chromosomal DNA and to some extent on the basis of their cellular fatty acids (CFAs) (17). *S. schleiferi* subsp. *schleiferi* has been isolated from human clinical specimens but has not yet been reported on the skin of healthy people (276). The natural host or host preference for this subspecies has not been determined. *S. xylosus* is very widespread in nature and occasionally may be isolated from humans. This species is most often found on people handling animals (165, 172).

The widespread distribution of staphylococci over the body surface and their relatively large total population size

make specimen collection a real challenge. Unless careful and thoughtful procedures are used to isolate organisms from the focus of infection, it is a difficult task to distinguish the etiologic agent(s) from contaminating normal flora. Specimen quality is largely determined by how the clinical specimen is collected and how well it reflects the infectious disease problem.

Specimen Collection and Processing

The isolation and enumeration of staphylococci from clinical specimens are routine operations in the clinical laboratory. Some recommended procedures for collecting and processing specimens are described in the American Society for Microbiology's *Manual of Clinical Microbiology*, fourth and fifth editions (14, 194), and the American Public Health Association's *Diagnostic Procedures for Bacterial Infections*, seventh edition (312). Ideally, specimens should be taken from the focus or foci of infection without collecting surrounding normal flora.

Blood cultures for detecting bacteremia. CNS are a major cause of hospital-acquired bacteremia, and in most cases the focus of infection is an intravascular catheter (78). Many of the patients are in the ICU or in neonatal ICUs (NICUs). Accurate diagnosis depends on the clinical presentation and the isolation of identical organisms (same strain or clonal population) from repeated cultures. Accuracy is imperative when diagnosing bacteremia associated with infections of indwelling medical devices, because device removal may be necessary to eradicate the infection. Quantitative blood cultures are helpful for the diagnosis of central venous catheter (CVC) infections in patients in ICUs. The finding of a 5- to 10-fold increase in the concentration of bacteria drawn via the CVC, in comparison with the concentration of bacteria drawn via a peripheral catheter, has been suggested to indicate a CVC-related bacteremia (87). Colony counts of CNS from blood drawn from vascular access devices frequently exceed 100 CFU/ml. One semiquantitative count method by which CVC-related bacteremia can be confirmed is to remove the distal 5- to 7-cm segment of the catheter and roll the catheter onto a culture plate. A count of >15 CFU has been suggested to indicate CVC-related sepsis (10, 203). However, in one study, only 4% of semiquantitative count results had clinical impact (315). An alternative method would be to rinse the catheter surface with broth (55, 196). The numbers of bacteria detected with broth methods should be higher than those detected with imprint methods (314). It has been suggested that new laboratory techniques that do not require the removal of a catheter are needed to guide therapeutic decisions so as to reduce a potential risk to the patient and lower the cost of the laboratory test (315).

NVE is often associated with CNS bacteremia. The most convincing laboratory findings include the rapid isolation of CNS from more than one blood culture, a high intensity of bacteremia (CFU per milliliter), and the presence of the same strain(s) in sequential isolations (4, 158). Generally, in patients with suspected bacterial endocarditis, three blood cultures are sufficient to isolate the etiologic agent (141). However, in patients who have received antimicrobial agents before blood collection, a total of four to six separate blood cultures may be necessary to isolate the etiologic agent. With NVE, the timing of collection is usually not critical because the bacteremia is continuous, a hallmark feature of NVE and a rather common feature of prosthetic valve endocarditis. Blood cultures are usually collected separately within a 24-h period at no less than hourly

intervals. It is essential that blood for culture be collected aseptically (e.g., using commercial prep kits or by cleaning the skin with 80 to 95% alcohol and then applying 2% iodine or iodophor at the venipuncture site and waiting for at least 1 min before making the venipuncture). Furthermore, it is preferable to collect blood from more than one venipuncture site in the event that a contaminant is accidentally introduced at one of the sites. Strain identification may be complicated by the phenotypic variation observed in CNS isolated from blood or infected tissue in patients with NVE or prosthetic valve endocarditis (8, 63, 76, 217). Phenotypic variation may involve changes in colony morphology, phage type, biotype, serotype, adherence properties, and/or antibiotic susceptibility pattern. Some of this variation may be reflected in changes of plasmid profile and/or restriction endonuclease fragment patterns. In most reported situations, variation involved the gain or loss of only one feature or, less frequently, a small number of features.

A variety of different CNS species have been implicated in NVE. In one case believed to be caused by *S. warneri*, five of six blood culture sets drawn over a 2-day period were positive for this species (321). Gram-positive cocci were observed in the damaged aortic and mitral valves even though cultures were negative. In another case of *S. warneri* NVE, four of six blood cultures were positive for this species (155). It would have been even more convincing that *S. warneri* was the etiologic agent in the above cases had the strains of this species been identified.

S. lugdunensis was found in a series of seven blood cultures in a recent case of NVE in a patient with a long history of vascular disease (280). In addition, culture of an intravenous catheter tip and mitral valve tissue of the patient demonstrated the presence of *S. lugdunensis* in large and moderate numbers, respectively. Etienne et al. (75) have reported three cases of *S. lugdunensis* NVE. They found that four to eleven blood cultures yielded only this species. In one of these cases, the aortic, mitral, and tricuspid valves were culture positive for *S. lugdunensis*. In a recent case of NVE in a patient with congenital heart disease, *S. simulans* was isolated from four blood cultures collected consecutively in 1 day at 4-h intervals (144). Analyses of plasmid profiles, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profiles of the extracellular proteins, and immunoblotting of proteins suggested that all isolates were members of the same strain, thereby implicating it as the etiologic agent. In a second reported case of *S. simulans* NVE, this species was isolated from all seven blood culture sets taken from the patient (213). Cultures of the damaged aortic valve leaflet and vegetation were negative. Multiple positive blood cultures have also been observed in cases of NVE caused by the CNS species *S. capitis* (15, 195), *S. epidermidis* (43, 158), *S. saprophyticus* (282), and *S. saccharolyticus* (313).

CNS are regarded as major opportunistic pathogens isolated from blood cultures in NICUs (82, 90, 281). These staphylococci pose a substantial risk of nosocomial bacteremia among infants with very low birth weights (44, 89). Diagnosis of bacteremia has been made on the basis of one or more positive blood cultures growing a single morphologic type (strain) or species of CNS as the sole isolate (281). Cultures growing multiple strains or species of CNS and/or other normal skin flora are regarded as probably contaminated. Since the blood volume of the very smallest babies is so low, only a single blood culture is usually obtained. For these babies, the neonatologist usually places a greater reliance on clinical criteria than on the microbiologic data.

Freeman et al. (89) identified CNS from the NICU on the basis of their colonial morphology, antibiotic susceptibility, and species identity in an attempt to identify the etiologic agent. Carlos et al. (44) used a combination of typing systems, including biotyping, antimicrobial susceptibility, and plasmid pattern, to identify an endemic strain of *S. epidermidis* producing bacteremia in the NICU. Bialkowska-Hobrzanska and coworkers (30) have employed both restriction endonuclease fingerprinting of the chromosomal DNA and plasmid profile analysis to identify strains of CNS isolated from bacteremic neonates. Using restriction endonuclease fingerprinting and DNA-DNA hybridization with different antibiotic resistance gene probes, Low et al. (198) demonstrated the presence of an endemic strain of *S. haemolyticus* causing bacteremia in NICU patients.

The interpretation of blood cultures positive with CNS is sometimes met with uncertainties, subtleties, and inherent pitfalls (42). The mere presence of microorganisms in blood denotes neither active multiplication nor harmful consequences. Furthermore, contamination can occur at any point between the manufacture of the blood culture system and the final subculture. Traditionally, clinicians have regarded growth in more than one bottle or culture set as evidence of a true-positive culture. True bacteremias are classified as transient, intermittent, or continuous on the basis of whether a very few, some, or all of a series of blood cultures are positive. Transient bacteremias are frequently the result of the traumas and activities of everyday life as well as medical and dental procedures. Most are probably harmless. However, prior to removal by normal clearance mechanisms, some CNS may find a haven in a damaged tissue or organ and cause infection. Transient bacteremias may be a prelude to endocarditis, hematogenous osteomyelitis, and many infections related to foreign implants (42). Intermittent bacteremia usually reflects an established infection extrinsic to the bloodstream. In general, localized infections that give rise to bacteremia are more serious and are associated with a higher mortality rate than localized infections without positive blood cultures (212). Continuous or sustained CNS bacteremia is characteristic of intravascular infections (e.g., endocarditis). Not only are all or nearly all blood cultures positive, but also the intensity of bacteremia (CFU per milliliter) tends to be remarkably even. In general, isolation of the same strain of CNS from multiple blood cultures implies that the organism is continuously present in the bloodstream and, therefore, together with clinical indications may be regarded as a potential etiologic agent.

Nevertheless, the evidence provided by blood cultures is largely indirect, and in the case of localized infections it would be better to identify the organism in situ at the focus of infection, although this is not always possible. Perhaps this may be accomplished in the future by either biopsy, specific staining, or demonstration of specific antigens or DNA sequences. Future studies may show that in some cases more than one strain or species of CNS may be present and active in a focus of infection. Furthermore, due to different growth rates of some CNS in conventional broth blood culture media, slower growing strains or species may be overlooked. This situation represents a pitfall that can be largely circumvented by the direct culture of blood (e.g., lysis-centrifugation system). The use of a second or backup blood culture system is recommended, not only when clinical findings are consistent with NVE due to a fastidious microorganism but also when CNS are suspected (212). Another pitfall is created when microbiologists select for identification CNS colonies that are only 24 to 48 h old. The

young colonies have not yet developed strain- and species-specific morphologic features and often look alike. Older colonies that have been incubated for 72 to 96 h and then stored for 2 days at room temperature develop distinctive features that can aid in strain and species identification (167, 171).

Tissue, exudate, and prosthesis cultures. Examination of tissues and fluids in association with or surrounding a site of infection may reveal the etiologic agent. For example, in a postoperative infection of a hip prosthesis in a compromised patient, *S. schleiferi* subsp. *schleiferi* was found in all samples taken from subcutaneous tissues, synovial fluid, the femur, and the hip, representing areas associated with the infection site (146). Aspiration of the joint space and washing of orthopedic prostheses with broth commonly yield the infecting bacteria. In a compromised patient with a postoperative infection caused by introduction of an umbrella device, *S. schleiferi* subsp. *schleiferi* was isolated in a series of nine blood cultures taken over a period of 15 days (146). To minimize the risk of false-negative cultures in a CNS infection of a prosthetic device, ultrasonic oscillation may be used to shake off from prosthetic surfaces adherent organisms embedded in a biofilm matrix (25, 301). Methods that used ultrasonic oscillation of explanted vascular graft material demonstrated a significant increase in the incidence of cultures positive for *S. epidermidis* compared with standard blood agar plate and broth culture techniques. Recent studies by Bandyk and coworkers (16) have indicated that ultrasonically oscillated explanted graft material (biofilm culture) yielded bacteria, typically a CNS, in more than 80% of vascular prosthesis infections. None of the 15 patients included in their study exhibited bacteremia or bacteria in Gram-stained smears of perigraft exudates, suggesting no or little release of bacterial cells from the adherent biofilm matrix while the prosthesis was present in the patient. In a leukemic patient with hepatosplenic abscesses, *S. epidermidis* was recovered from cultures of biopsied hepatic and splenic tissue and cultures of perisplenic and perihepatic exudates. A Gram-stained smear revealed staphylococci in the splenic and hepatic tissues (232). The patient responded characteristically to vancomycin therapy, with complete resolution of the hepatic lesions. In a case of cervical adenitis believed to be caused by *S. epidermidis*, this species was obtained in pure culture from the drainage of an abscess located in the right posterior cervical triangle (268). Clinical improvement occurred only with drainage and treatment with vancomycin.

Urine cultures for detecting bacteriuria. UTIs are among the most common bacterial infections. They can be categorized as being complicated or uncomplicated. In general, complicated UTIs occur in patients with a history of recurrent infections, signs or symptoms of upper tract disease, or coexisting conditions such as pregnancy, immunosuppression, or structural anomalies of the urinary tract. By contrast, uncomplicated UTIs occur in patients who are otherwise healthy and who have a history of lower tract symptoms of short duration; they are not accompanied by fever or flank pain. Approximately 80% of all UTIs are caused by *Escherichia coli*. The CNS species *S. saprophyticus* accounts for as much as 10 to 11%, especially in young adult women (190, 307). A urine culture is usually indicated when there are (i) complicated or uncertain clinical features; (ii) a history of UTI in the past 3 weeks, indicating possible relapse; (iii) symptoms for more than 7 days; (iv) recent hospitalization or catheterization, indicating the possibility of a nosocomial infection; (v) pregnancy; or (vi) diabetes.

Traditionally, colony counts of ≥100,000 CFU/ml in two or more cultures of midstream urine indicate a significant bacteriuria or UTI (160, 285). However, since CNS grow relatively slowly in urine, it has been suggested that lower colony counts of 100 to 100,000 CFU/ml should be considered an appropriate range for significant bacteriuria due to these organisms (132, 184, 251, 287). Some investigators suggest that CNS should be considered a urinary tract pathogen only when they are present in pure culture (107, 108) or are associated with no more than one other species (123). Of course, repeated isolation of a particular strain in pure culture is most convincing. A freshly voided, midstream, clean-catch sample is usually satisfactory for making a determination of infection. Suprapubic aspiration may be indicated in patients who have a low bacterial count in clean-catch specimens, in neonates, and in young infants. Contamination rates should be very low with this procedure. Sometimes UTI due to *S. saprophyticus* may be accompanied by a bacteremia with the same organism (103, 110, 191), attesting to the invasive ability of *S. saprophyticus*. Invasive *S. saprophyticus* infections, such as acute pyelonephritis, can elicit an antibody response that might be useful for diagnostic purposes in patients with UTI (127).

The designation of CNS as pathogens in the etiology of chronic bacterial prostatitis is controversial (215, 248, 286). Nickel and Costerton (227), as well as several other earlier investigators (26, 183, 310), have presented evidence to suggest that *S. epidermidis* and *S. saprophyticus* can be associated with the clinical syndrome of chronic prostatitis and most likely are implicated in the pathogenesis of the prostatic inflammation. These CNS fulfilled the criteria of bacterial localization as set forth by Meares and Stamey (216), who compared CFU in expressed prostatic secretions and bladder urine. In one study of three men with chronic prostatitis, bacterial localization within the tissue was also demonstrated by ultrastructural examination of prostate biopsies (227). Scanning electron microscopy demonstrated rigid coccal cells and microcolonies of coccal bacteria adherent to the ductal wall epithelium which were enveloped in a dehydrated slime matrix. Transmission electron microscopy demonstrated sparse bacterial biofilms containing gram-positive cocci within the intraductal space of the prostate. Furthermore, cultures of the prostate biopsies confirmed the presence of *S. epidermidis*. It is of interest that none of the patients responded to appropriate antibiotic therapy (trimethoprim-sulfamethoxazole, doxycycline, and norfloxacin) based on the culture and susceptibility results. These results might be explained by the fact that the CNS were sequestered within intraprostatic biofilms and in this "hibernating" state are relatively refractory to antibiotic therapy and host defenses.

Practical Approaches to the Problems

Until it becomes feasible to identify CNS species, subspecies, and strains in situ, at the focus of infection, we must rely on the laboratory culture and isolation of CNS from clinical specimens. The first step in attempting to identify the etiologic agent usually involves culturing a fresh specimen on nonselective agar medium directly or following an enrichment in broth. Of these choices, direct plating on nonselective agar provides the most accurate assessment of the proportion and total CFU of each type of CNS organism present in the clinical specimen. Plating on selective agar or broth enrichment may be necessary to select CNS from certain kinds of clinical specimens (e.g., sputum or feces, in

which other bacteria might predominate) or to select a specific CNS species (e.g., with novobiocin to select *S. saprophyticus* from the urinary tract). One of the pitfalls of enrichment is that selective agents commonly used may favor the growth of certain CNS species, subspecies, or strains. This would distort the original population structure. It is generally regarded that repeated isolation of a particular strain in pure culture from a series of specimens provides good evidence of causality. Nevertheless, in the process of obtaining a specimen from a normally sterile site and in specimens taken from unsterile regions of the body, culture purity may not be absolute or even practical. On primary isolation plates, a relatively small number of colonies are examined to assess purity, and these usually represent only a small percentage of the total bacteria actually present in the specimen. It would be more practical to consider that repeated isolation of a particular predominant strain from a series of specimens provides good evidence of causality. Of course, the best evidence would be obtained from specimens taken from normally sterile sites. It is more difficult to assess the clinical significance of mixed cultures in which two or more species, subspecies, and/or strains are present in significant proportions in a series of specimens. Nevertheless, a mixed infection may be suspected if the combination involves only two or three different organisms and they are found in a series of specimens collected within a short time interval. Some infections may even require the synergistic relationship of different species. For example, in a rabbit osteomyelitis model (210), *S. epidermidis* alone caused a low percentage of osteomyelitis but this species in combination with *Bacteroides thetaiotaomicron* caused osteomyelitis in 95% of the rabbits.

In the examination of the primary isolation plate, usually a blood agar plate, a decision is made to select a certain colony or group of colonies for identification. As mentioned in the preceding section, this decision is usually made too early, at a time when colonies of CNS have not yet developed strain- and species-specific features. Failure to hold plates for several days can result in the following errors: (i) selection of more than one species, subspecies, or strain if two or more colonies are sampled to produce an inoculum, which could lead to errors in identification; (ii) selection of an organism that is not the etiologic agent if the specimen contains two or more different species, subspecies, or strains; and (iii) incorrect labeling of a mixed culture as a pure culture. The CNS subspecies *S. capitis* subsp. *capitis* and *S. cohnii* subsp. *cohnii* pose a problem in that different strains can often not be distinguished on the basis of colony morphology. Colonies of these subspecies are unpigmented and opaque (i.e., appear white) and demonstrate little variation in colony morphology. CNS strain identity based on colony morphology is discussed further below (see Strain Identification, Conventional Techniques). In the future, it may be possible to identify colonies of species, subspecies, and strains not only by their morphology but also on the basis of colony hybridization with probes to detect specific nucleic acid sequences. Genetic approaches to screening colonies should greatly facilitate the quantitation of individual clonal populations.

IDENTIFICATION OF NEW *STAPHYLOCOCCUS* SPECIES AND SUBSPECIES

Since the review by Pfaller and Herwaldt (246) in 1988, several new *Staphylococcus* species and subspecies have been recognized; these include *S. lugdunensis* (93), *S. schlei-*

TABLE 2. Differentiation of the new human *Staphylococcus* species and subspecies from closely related species and subspecies[a]

Character	Determination[b] in given species									
	S. epidermidis	*S. capitis* subsp. *capitis*	*S. capitis* subsp. *ureolyticus*	*S. caprae*	*S. lugdunensis*	*S. saprophyticus*	*S. cohnii* subsp. *cohnii*	*S. cohnii* subsp. *urealyticum*	*S. schleiferi* subsp. *schleiferi*	*S. schleiferi* subsp. *coagulans*
Colony size >6 mm	−	−	−	d	d	+	d	+	−	d
Colony pigment	−	−	(d)	−	d	d	−	d	−	−
Coagulase	−	−	−	−	−	−	−	−	−	+
Clumping factor	−	−	−	−	(+)	−	−	−	+	−
Thermonuclease	−	−	−	−	−	−	−	−	+	+
Alkaline phosphatase	+[c]	−	−	(+)	−	−	−	+	+	+
Pyrrolidonyl arylamidase	−	−	(d)	d	+	−	−	d	+	ND
Ornithine decarboxylase	(d)	−	−	−	+	−	−	−	−	−
Urease	+	−	+	+	d	+	−	+	−	+
β-Glucosidase	(d)	−	−	−	+	d	−	−	−	ND
β-Glucuronidase	−	−	−	−	−	−	−	+	−	ND
β-Galactosidase	−	−	−	−	−	+	−	+	(d)	ND
Novobiocin resistance	−	−	−	−	−	+	+	+	−	−
Acid (aerobically) from[d]:										
D-Trehalose	−	−	−	(+)	+	+	+	+	d	−
D-Mannitol	−	+	+	+	−	d	d	+	−	d
D-Mannose	(+)	+	+	+	+	−	(d)	+	+	+
D-Turanose	(d)	−	−	(+)	(d)	+	−	−	−	ND
Maltose	+	−	+	(+)	+	+	(d)	(+)	−	−
Sucrose	+	(+)	+	−	+	+	−	−	−	d

[a] The new human species and subspecies include *S. capitis* subsp. *ureolyticus*, *S. lugdunensis*, *S. cohnii* subsp. *urealyticum*, and *S. schleiferi* subsp. *schleiferi*.

[b] +, 90% or more strains positive; −, 90% or more strains negative; d, 11 to 89% of strains positive. ND, not determined. Parentheses indicate a delayed reaction.

[c] Alkaline phosphatase activity is negative for approximately 6 to 15% of strains of *S. epidermidis*, depending on the population sample. A significant number of clinical isolates have been phosphatase negative.

[d] All species listed do not produce acid (aerobically) from D-xylose, D-cellobiose, L-arabinose, and raffinose.

feri subsp. *schleiferi* (93), *S. schleiferi* subsp. *coagulans* (140), *S. capitis* subsp. *ureolyticus* (19), *S. cohnii* subsp. *urealyticum* (177), *S. muscae* (117), *S. piscifermentans* (294), and *S. vitulus* (309). Those found on humans and/or in human clinical specimens include *S. lugdunensis*, *S. schleiferi* subsp. *schleiferi*, *S. capitis* subsp. *ureolyticus*, and *S. cohnii* subsp. *urealyticum*. In addition, it is now recognized that *S. caprae* (67) can be found occasionally on humans and in human clinical specimens and, like the others, should be regarded as an opportunistic pathogen in humans (17, 157).

Conventional Methods

Conventional methods for determining phenotypic characters that differentiate the new human *Staphylococcus* species and subspecies are shown in Table 2 (140, 167, 171, 175). *S. lugdunensis* can be distinguished from all other CNS species by its rapid positive ornithine decarboxylase activity (93). *S. schleiferi* subsp. *schleiferi* can be distinguished from all other CNS species by its positive thermonuclease and clumping factor activities (93). This subspecies can be separated from *S. schleiferi* subsp. *coagulans* by its negative tube coagulase test, positive clumping factor activity, and negative urease activity (140). Hébert (121) has described several additional tests that might be useful in distinguishing *S. schleiferi* subsp. *schleiferi* from *S. lugdunensis*. *S. lugdunensis* is resistant to polymyxin B and bacitracin (10 U),

but *S. schleiferi* is susceptible to these antibiotics. *S. capitis* subsp. *ureolyticus* can be differentiated from *S. capitis* subsp. *capitis* on the basis of its positive urease activity and acid production from maltose (19). This subspecies can be distinguished from the close relative *S. caprae* by its negative alkaline phosphatase activity, acid production from sucrose, and lack of acid production from trehalose and turanose (67). *S. cohnii* subsp. *urealyticum* can be differentiated from *S. cohnii* subsp. *cohnii* on the basis of its positive alkaline phosphatase, urease, β-glucuronidase, and β-galactosidase activities (177). Although character analysis by many of the conventional methods requires 1 to 3 days before a final identification can be made, conventional methods are quite accurate and have served as a source of reference for studying the accuracy of rapid identification systems. A numerical code system for the reference identification of *Staphylococcus* species and subspecies based on the results of 18 primary conventional biochemical tests has been recently proposed by Rhoden et al. (259). The system, referred to as the Centers for Disease Control *Micrococcaceae* profile system, identified more than 95% of the 824 strains tested. Of the new species and subspecies, *S. lugdunensis* was well represented in this system (45 strains), but *S. schleiferi* (1 strain), *S. capitis* subsp. *ureolyticus* (9 strains), and *S. cohnii* subsp. *urealyticum* (10 strains) were underrepresented. *S. caprae* was represented by only two

strains. Despite the small number of strains analyzed for certain species, the *Micrococcaceae* profile system approach appears to be a reasonable alternative for laboratories that require reference identification for members of the *Micrococcaceae*, including CNS.

Commercial Rapid Identification Systems

To expedite the identification process for use in the clinical laboratory, several manufacturers have developed rapid species and subspecies identification kits or automated systems requiring only a few hours to 1 day for completing tests. The major companies marketing products for the identification of CNS species and subspecies include the following: (i) bioMérieux Vitek, Inc., Hazelwood, Mo. (API STAPH IDENT, STAPH Trac System, ID 32 STAPH, RAPiDEC STAPH System, and Gram Positive Identification Card for use with the automated Vitek and Vitek Jr.); (ii) Baxter Diagnostics Inc., MicroScan Division, West Sacramento, Calif. (MicroScan Pos ID panel, Pos Combo Type 6 panel, MicroScan Rapid Pos ID panel, and Rapid Pos Combo Type 1 panel for use with the automated auto SCAN-W/A system); (iii) Becton Dickinson Microbiology Systems, Cockeysville, Md. (Minitek Gram-Positive Set); (iv) Becton Dickinson Diagnostic Instrument Systems, Towson, Md. (Sceptor *Staphylococcus* MIC/ID Panel and Sceptor Gram Positive Breakpoint/ID Panel); (v) Biolog, Haywood, Calif. (GP MicroPlate test panel); and (vi) MIDI, Newark, Del. (Microbial Identification System).

Identification of most human *Staphylococcus* species with the commercial systems can be made with an accuracy of 70 to >90%. Identification of *Staphylococcus* species and subspecies has improved somewhat with the Baxter Diagnostics MicroScan Pos ID and Rapid Pos ID panel systems (169) and the bioMérieux Vitek Gram Positive Identification Card (18) by increasing the data bases. It is expected that the reliability of these and other commercial systems will continue to increase as the result of growing data bases and the addition of more discriminating tests. The new *Staphylococcus* species and subspecies have been incorporated recently into several data bases. Accuracy in the identification of *S. lugdunensis* and *S. schleiferi* can be increased significantly by the addition of the ornithine decarboxylase test (now employed in the bioMérieux Vitek ID 32 STAPH) and the thermonuclease test (Remel Laboratories, Inc., Lenexa, Kans.), respectively.

STRAIN IDENTIFICATION

The identification of strains of CNS has become important since the recognition of the clinical significance of CNS. Several reviews have emphasized the need for strain identification and suggest requirements for an epidemiological typing scheme for CNS (33, 49, 50, 167, 171, 234, 246). The identification of strains is important in monitoring the reservoir and distribution of CNS involved in nosocomial infections and in determining the etiologic agent. The rationale is that the repeated isolation of a particular strain is more clinically significant than solely the repeated isolation of a species. This review divides the strain typing methods into two categories, conventional and molecular. For a typing scheme to be useful, it must be sensitive, specific, reproducible, affordable, and timely. The information gained from strain delineation of CNS will improve methods in prevention, diagnosis, and therapy.

Conventional Methods

Colony morphology. Strain identity should at least start with good characterization of colony morphology. Presently, it is the only practical way of discerning prospective strains or clones on primary isolation plates. The clinical microbiologist's first encounter with a CNS isolate is on the primary isolation plate, where colonies are screened and selected as inoculum for identification. In most laboratories, colonies are screened within 18 to 24 h, when most species or strains appear the same. As shown in Fig. 1, three strains of *S. epidermidis* are indistinguishable after 24 h at 35°C, resulting in the false assessment that the plate contains a pure culture. Thus, the inoculum taken from this plate for further studies could be a mixed culture, or if only one colony is used, the predominant strain or etiologic agent may be missed. The best method allows well-isolated colonies to develop over a period of several days at incubation temperatures of 30 to 35°C on a suitable medium and then 2 days longer at room temperature (167, 171). For most species, more than 90% of the isolates can be differentiated after incubation for 72 h, and an even higher percentage can be differentiated if colonies are then allowed to stand at room temperature. Colonies of the same strain exhibit similar features of size, consistency, edge, profile, luster, and color. This technique works best on those CNS species that demonstrate a translucent colony type and/or pigment variation, e.g., *S. epidermidis*, *S. warneri*, *S. lugdunensis*, *S. hominis*, *S. haemolyticus*, *S. simulans*, *S. saprophyticus*, and *S. xylosus*. One word of caution: a variant morphotype(s) may be produced by certain strains that could then be misclassified as different strains. In this situation, additional studies, such as plasmid and/or chromosomal analysis, should clarify the relationship of each morphotype.

Antibiograms and biotyping. The use of antibiograms and biotyping has been extensively reviewed previously (33, 49, 171, 234, 246). More recently, groups have been working on a typing scheme that uses a combination of several techniques (120, 122, 124). Hébert et al. (122) use a combination of the API Staph-Ident biochemical profile along with adherence and synergistic hemolysis to define biotypes of CNS. This scheme, with the addition of five antibiotic disks (novobiocin, polymyxin B, bacitracin, furazolidone, and Taxo A) and pyroglutamyl-β-naphthylamide hydrolysis, allowed strains within the species *S. lugdunensis* and *S. schleiferi* to be separated into several biotypes (121). Herwaldt et al. (124) incorporated the API Staph Trac, antibiotic profile, slime production, and synergistic hemolysis for successful strain discrimination. Upon incorporation of plasmid analysis, they were able to further differentiate between strains. Ludlam et al. (199) studied the antibiograms of 50 isolates of CNS. The isolates showed 25 distinct patterns, giving a discrimination of 50%. The antibiogram alone gave 66% of the discriminatory power of their scheme, which also included biotyping, phage typing, and plasmid analysis. They achieved 95% of the discriminatory power of the scheme when they used plasmid analysis along with antibiograms or biotyping and phage typing along with antibiograms.

With the ease in obtaining an antibiogram and a biotype, these techniques in combination would provide a moderate degree of strain delineation for those laboratories that are not able to incorporate more advanced techniques. However, several precautions should be noted: (i) a strain's susceptibility pattern may vary because of plasmid instability and mutation (167, 171); and (ii) certain biochemical
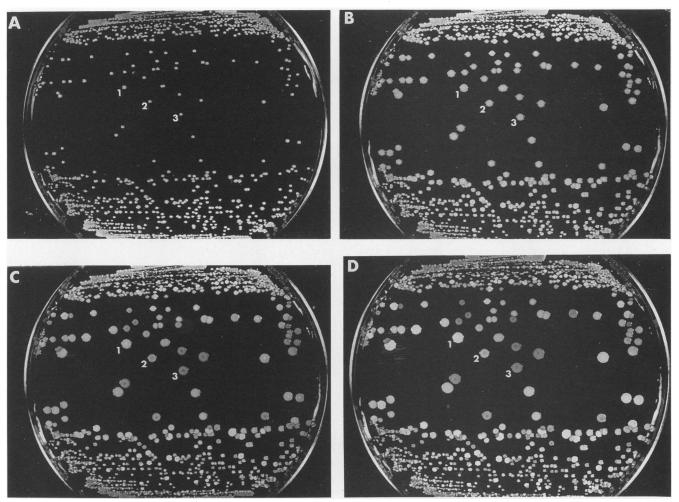
FIG. 1. Colonies of three strains of *S. epidermidis* grown on tryptic soy agar with 5% sheep blood. Plates were photographed over a 5-day period. (A) 24-h incubation at 35°C; (B) 48-h incubation at 35°C; (C) 72-h incubation at 35°C; (D) 72-h incubation at 35°C followed by 2 days at room temperature. The numbers 1, 2, and 3 represent the three different strains. Strains were further identified on the bases of biotype, antibiogram, plasmid profile, and restriction enzyme analysis of genomic DNA.

characters demonstrate clonal variation, and upon successive subculturing, variants may accumulate. Because of these problems, it is recommended that more than one typing system be incorporated to achieve a higher degree of strain delineation.

Phage typing. Bacteriophage typing has become an established system for typing *S. aureus*. Phage sets have been available for *S. epidermidis*, but they are not internationally standardized. A recent report of using phages for *S. epidermidis* typing again demonstrated the inability to standardize the technique and type the strains of this species (81). The phages of the *S. epidermidis* isolates exhibited a wide host range, and tests displayed low reproducibility. These problems decreased the discriminatory value of the technique. The typeability of *S. saprophyticus* also has been investigated. Pereira and Melo Cristino (241) were able to type 134 of 297 strains with high reproducibility. However, the phages were not absolutely specific for *S. saprophyticus*, since they also typed certain strains of *S. cohnii* and *S. xylosus*. These investigators were able to increase discrimination when they added plasmid profiling. Rosdahl et al. (264) found that strains of *S. haemolyticus* were seldom

phage typeable, and multiply resistant strains of *S. haemolyticus*, *S. epidermidis*, and *S. hominis* were rarely typeable. Perhaps these observations may be explained by the spread of multiply resistant clones that lack phage typeability or by the acquisition of resistance plasmids and prophages that may prevent lysis by the typing phages. Due to the frequent isolation of multiply resistant CNS, problems would arise for clinical laboratories that would use phage typing. For phage typing to be more useful, new phages that type *S. epidermidis* and multiply resistant isolates of CNS must be selected. Once again, this technique may be more valuable if it is used in combination with other typing techniques or for epidemic rather than endemic situations (49).

Molecular Approaches

CFA analysis. Cellular fatty acid (CFA) analysis has received little attention as a method for strain delineation, perhaps because of the need to standardize substrates and growth conditions to obtain reproducible results. Kotilainen et al. (180) found CFA analysis comparable to standard

techniques (antibiogram, biotype, and plasmid profiles) for distinguishing between multiple CNS blood isolates. Their results showed that numerous morphologically identical isolates of a strain from a patient gave a correlation value of >95, while numerous nonidentical isolates of the same species from a patient gave a correlation of <95. The study showed some strain discrimination; however, more studies are necessary to show the epidemiological usefulness of this method. CFA analysis is relatively inexpensive, simple, and quick, and a large number of isolates can be tested at one time. As indicated in the review by Welch (311), CFA analysis combined with numerical correlation analysis for subgrouping isolates may prove to be of some use in strain identification.

Pyrolysis-mass spectrometry. The recent application of pyrolysis-mass spectrometry for strain discrimination of CNS has given encouraging results (91, 92). In this technique, the organisms are pyrolyzed, the pyrolysates are examined by mass spectrometry, and the results are analyzed and compared mathematically to produce a dendrogram (9). Investigators have found pyrolysis-mass spectrometry to be comparable to typing schemes that incorporate antibiogram, biotype, and plasmid analysis (91, 92). This technique is relatively inexpensive (after the initial cost of equipment has been made), rapid, and reproducible. It is necessary to include epidemiologically unrelated control strains so that the significance of the different spectrograms of the strains can be estimated.

Multilocus enzyme electrophoresis. Multilocus enzyme electrophoresis is based on analysis of the electrophoretic profile of genetically controlled variants of metabolite enzymes (isoenzymes). These isoenzymes are distinguished on the basis of their movement in a starch or polyacrylamide gel and reaction with specific stains. The advantages of this technique are the ease in performance, the availability of reagents, a high degree of reproducibility, and the stability of the profiles (245). The disadvantage is the expense of the enzymes and staining reagents. Musser et al. (222) incorporated the technique successfully to study the genetic structure among strains of *S. aureus* that cause toxic shock syndrome. In early studies of CNS isoenzymes for species identification, it was apparent that this method could distinguish strains of certain species (323). Further analysis of the technique is necessary to determine its usefulness in strain identification of CNS and the numbers and types of enzymes necessary to differentiate between strains.

Plasmid analysis. Plasmid profiling and the restriction endonuclease analysis of specific plasmids can serve as a valuable typing system, especially for those strains that carry multiple plasmids. Recent reviews have given the advantages and disadvantages of plasmid profiling (171, 246, 247). The CNS that often carry multiple plasmids are *S. epidermidis, S. haemolyticus, S. hominis, S. capitis, S. warneri, S. saprophyticus, S. cohnii* and *S. xylosus*, while *S. auricularis* and *S. lugdunensis* seldom have plasmids or have only one or two (167). Restriction enzyme analysis of the plasmids may further extend the sensitivity of this technique and is particularly useful for differentiating plasmids of the same size. However, some common plasmids are highly conserved and often have identical fragment patterns irrespective of the strain, for example, the small tetracycline plasmids and the small macrolide-lincosamide-streptogramin B (MLS) resistance plasmids. One major disadvantage of this technique is that plasmids are somewhat unstable elements, and the lack or addition of one plasmid may not truly designate a different strain. The technique is a relatively

simple and inexpensive way to discriminate between strains and thereby, in combination with other strain typing methods, will be useful in epidemiology studies (247).

Whole-cell polypeptide analysis. Whole-cell protein profiling employs gel electrophoresis of cellular proteins. The detection methods include using either Coomassie blue staining, ^{35}S-methionine (radio-PAGE), or immunoblotting. SDS-PAGE with staining by Coomassie blue and radio-PAGE examine all major bacterial proteins, while immunoblotting examines surface-exposed antigens that are immunoreactive to antibodies. SDS-PAGE with Coomassie blue staining has produced distinct banding patterns for species of CNS (56). Maggs and Pennington (202) took the technique further to show that it could discriminate between clones of *S. capitis* inhabiting different regions of the skin of human subjects. Several studies have compared the detection methods (39, 71, 298). Thomson-Carter and Pennington (298) compared SDS-PAGE and immunoblotting. Both were reproducible; immunoblotting was more sensitive, and SDS-PAGE was easier to perform. Dryden et al. (71) found that SDS-PAGE had a higher discriminatory power (69%) than immunoblotting (57%). They thought that both techniques were technically demanding, and standardization of each step was necessary for reproducibility. Brown et al. (39) enhanced immunoblotting by using multiple antisera (from three strains of CNS); however, when the technique was compared with radio-PAGE, it was still inferior.

Chromosomal analysis. Chromosomal DNA analysis by restriction endonuclease fingerprinting has been used to type various pathogens, but little research has been done on CNS. Bialkowaka-Hobrzanska et al. (30) studied a total of 48 isolates of *S. epidermidis* and 19 isolates of *S. haemolyticus* for optimal conditions and reliable restriction endonuclease fingerprinting analysis. They investigated 12 restriction endonucleases and found *Cla*I, *Pst*I, *Bgl*II, and *Sac*I to be the most discriminatory. They concluded that restriction by *Cla*I was more discriminatory than plasmid profiling, and the results were stable. Wilton et al. (319) screened 13 enzymes including *Cla*I; however, they found that *Bcl*I gave the most distinct banding patterns, with excellent reproducibility. This technique lacks standardization, and the banding patterns are often difficult to analyze because of the large number of fragments generated by the restriction enzymes and the single electrofield used to separate fragments. Recently, two new approaches to chromosomal analysis, ribotyping and field inversion gel electrophoresis or pulsed-field gel electrophoresis (PFGE), have shown promise in the identification of different strains.

Ribotyping incorporates the use of nucleic acid probes to highlight specific rDNA-containing bands upon restriction of the chromosomal genome. Several groups have begun to investigate the usefulness of this technique to further resolve strain identification (29, 62, 142, 297). DeBuyser and co-workers (62) used radiolabelled 16S rDNA from *Bacillus subt*·· and noted different rRNA gene restriction patterns between various species and strains of CNS following *Hind*III and *Eco*RI cleavage of total DNA. Thomson-Carter et al. (297) found similar results with their study of 22 strains of seven different species; however, they used 16S plus 23S rRNA from *E. coli* as a probe. Bialkowska-Hobrzanska et al. (29) studied 78 strains and 15 species by comparing ribotyping, using the above probe, with *Cla*I cleavage and no probe. They found that the patterns given by ribotyping were easier to read, yet slightly less discriminatory. Izard et al. (142) examined the intraspecific typing ability of ribotyping on 86 strains of *S. epidermidis*. Upon digestion with *Eco*RI and

*Hind*III, they found 11 and 10 ribotypes, respectively. Discriminatory power varied from 14.3 to 15.1% with the use of one enzyme. When both enzymes were used, the discriminatory power was 31.6%.

Field inversion gel electrophoresis and PFGE allow the use of restriction enzymes that infrequently cut chromosomal DNA and therefore separate large DNA fragments. This in turn allows a better interpretation of the banding patterns. Goering and Duensing (104) used field inversion gel electrophoresis to examine strains of methicillin-resistant *S. epidermidis*. They found that strain interrelationships could be established on the basis of *Sma*I-generated chromosomal restriction fragment length polymorphisms. Goering and Winters (105) made their technique more rapid by preparing and analyzing the DNA in a total time of 2 days without a decrease in sensitivity or reproducibility. Preliminary studies on the use of PFGE for strain delineation of CNS have shown promise (17, 99). George and Kloos (99) have incorporated PFGE in the study of *S. epidermidis*, *S. capitis* subsp. *capitis*, *S. capitis* subsp. *ureolyticus*, and *S. caprae* for strain identification and genome sizing. Upon restriction of the DNA with *Sma*I, they found considerable conservation in fragment patterns for different strains of *S. capitis* subsp. *capitis*, *S. capitis* subsp. *ureolyticus*, and *S. caprae*. On the other hand, *S. epidermidis* displayed a variety of different patterns (Fig. 2). A small percentage of *S. epidermidis* strains demonstrated clonal variation in their fragment pattern, involving a size change in one or two of the bands. Bannerman et al. (17) found that strains of *S. caprae* isolated from goats produced fragment patterns different from those produced by strains of *S. caprae* isolated from humans. Further studies of different CNS species and the use of different enzymes are needed to determine how well field inversion gel electrophoresis and PFGE will discriminate among strains of CNS.

It must be noted that the methodology and interpretation of the molecular typing techniques discussed in this review are in need of standardization before they can be used routinely in the clinical laboratory.

ANTIBIOTIC SUSCEPTIBILITIES

In Vitro Susceptibility Testing and Resistance

Nosocomial infections caused by methicillin-resistant CNS still pose a serious problem for health care institutions. The detection of resistance in these strains, as reviewed by Pfaller and Herwaldt (246), has been hampered due to the variability in standard techniques used in determining methicillin resistance. Woods et al. (322) have described methods that detect a high percentage of methicillin-resistant strains of *S. epidermidis*. They recommend the use of a direct inoculum and either (i) a 24-h oxacillin disk diffusion test at 35°C, followed by continued incubation for a total of 48 h for isolates demonstrating intermediate resistance, or (ii) an oxacillin agar screen conducted at 35°C for up to 48 h. Several new molecular techniques that examine the genotype directly have been under investigation. These include the use of a *mecA* gene probe (5, 291, 302) and the PCR (220, 253). Archer and Pennell (5) found the *mecA* probe to be more sensitive than broth microdilution and more specific than agar dilution in identifying methicillin-resistant strains of CNS and obtained results within 24 h. They concluded that this method, which detects the gene for an altered penicillin-binding protein (PBP2a), could be used as a standard for the detection of methicillin resistance. The PCR
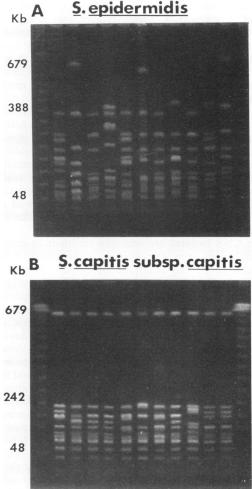

FIG. 2. PFGE of *Sma*I-generated restriction patterns of the chromosome of different isolates of *S. epidermidis* and *S. capitis* subsp. *capitis*.

technique was found to be more sensitive than DNA hybridization, and it provided data in less than 5 h (253). One control or regulation in the expression of PBP2a has been shown to be a regulatory function of an inducible *blaZ* gene from a penicillinase plasmid (302). In penicillinase-negative strains, a region designated *mecR* on the methicillin resistance determinant reduces the overall expression of resistance by negatively regulating the synthesis of PBP2a (269, 295). The presence of the *mecR* region may account for the delay in the detection of methicillin resistance by standard clinical procedures. Others have found methicillin resistance in strains that did not contain *mecA* (291). They hypothesize that a protein may be involved in protecting the cell through a high degree of cross-linking of peptidoglycan. PBP2a or low-affinity penicillin-binding proteins presumably similar to PBP2a have been shown in a variety of CNS, including strains of *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. simulans*, *S. saprophyticus*, *S. sciuri*, *S. capitis*, *S. warneri*, and *S. caprae* (5, 220, 250, 289, 291). Such a widespread distribution of methicillin resistance within the CNS may be due to or at least initiated by the transfer of the *mecA* gene among the CNS and *S. aureus*. Archer and Scott (6) have found a conjugative transfer gene (*tra* gene) in the CNS

species *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. simulans*, *S. warneri*, *S. saprophyticus*, and *S. capitis*. The *tra* gene was located in 22 of 176 methicillin-resistant CNS. They concluded that transfer genes are usually found on plasmids that also encode gentamicin resistance in multiresistant isolates. More studies are warranted to determine other mechanisms involved in the spread of methicillin resistance among the CNS.

With the increased isolation of clinically significant CNS, interest in their susceptibility to various antimicrobial agents and the establishment of resistance to various agents has also increased. As reviewed by Pfaller and Herwaldt (246), *S. epidermidis* has become resistant to many commonly used antibiotics and may be a reservoir for antibiotic resistance genes in hospitals. Because of the widespread resistance to antibiotics, the usefulness of the newest families of antimicrobial agents targeted for CNS, the glycopeptides and the quinolones, has received much attention.

When CNS isolates are multiply resistant to commonly used antibiotics, the glycopeptide vancomycin has been considered to be the antibiotic of choice. However, the isolation of clinical strains of *S. haemolyticus* with a decreased susceptibility to vancomycin has been reported (94, 278, 304). Although rare, these isolates may signal the beginning of resistance to an important antibiotic against CNS infections. Several groups have tried to induce resistance in strains of *S. epidermidis* and *S. haemolyticus* by using a broth or agar selection method to determine whether resistance to vancomycin can be easily obtained and thereby may become important (125, 277, 304, 308). Watanakunakorn (308) reported that of 18 strains of *S. haemolyticus* studied by passage in broth media containing vancomycin, only 4 strains developed a three- to fourfold increase in the vancomycin MIC, with a range of 1 to 8 μg/ml. Herwaldt et al. (125) found that increases in the MICs for 21 *S. haemolyticus* strains ranged from 4 to 32 μg/ml by the broth selection method and 8 to 32 μg/ml by the agar selection method. They found that seven *S. epidermidis* strains analyzed by the agar method demonstrated an increase in the range of 8 to 16 μg/ml. With the increased likelihood that clinical laboratories will encounter vancomycin-resistant strains of CNS, it becomes important to rapidly identify those strains that are likely to develop resistance to vancomycin. One possible approach that has received little attention and needs more research is the use of imipenem disks to determine vancomycin-resistant subpopulations. Schwalbe et al. (277) found that *S. haemolyticus* strains selected by their growth on brain heart infusion agar containing 12 μg of vancomycin per ml produced a double zone of growth around imipenem disks. The inner zone contained vancomycin-resistant subpopulations of *S. haemolyticus*. In contrast, Herwaldt et al. (125) selected vancomycin-resistant *S. haemolyticus* strains by sequential exposure of strains to subinhibitory concentrations of vancomycin. Only some strains that became resistant to vancomycin produced a double zone of growth around imipenem disks.

Decreased susceptibility of strains of *S. haemolyticus* and *S. epidermidis* to another glycopeptide, teicoplanin, has been more prevalent than that found with vancomycin (20, 109, 161, 197). When determining the breakpoints of resistance and susceptibility, several groups have expressed concern over their inability to correlate results (47, 119, 197). Variations in the media, inoculum size, and incubation times and reliance on the interpretative breakpoints for vancomycin may account for some discrepancies; therefore, it is necessary to specifically standardize the susceptibility test-

ing of teicoplanin. However, Kenny et al. (161) incorporated and recommended a different interpretive criteria for MIC testing of teicoplanin (8 μg/ml, susceptible; 16 μg/ml, moderately susceptible; ≥32 μg/ml, resistant). Upon the use of these breakpoints, they found that errors occurred in <1.0% of the total numbers of isolates. Teicoplanin has been used with some success as an alternative to vancomycin in the treatment of moderate and severe infections by CNS (35). However, Brunet et al. (41) reported a patient who recovered from an *S. haemolyticus* infection after treatment with vancomycin following treatment failure with teicoplanin. Even though teicoplanin may have fewer side effects than vancomycin, it must be given in higher doses than first thought necessary, and therefore it may prove to be less useful than vancomycin (249). Teicoplanin may be reserved only for those patients who cannot tolerate vancomycin.

With the continued emergence of multiply resistant CNS, newer glycopeptides continue to be tested for their activity against CNS. Decaplanin has been found to be less active than either teicoplanin or vancomycin (225), and resistance has been discovered in strains of CNS, particularly *S. haemolyticus* (270). Another set of new glycopeptides includes derivatives of teicoplanin (MDL 62208, MDL 62211, and MDL 62873). These antibiotics were found to be more active than the parent, teicoplanin, and vancomycin (31, 153). However, clinical trials have not been completed to establish the toxicity of the new derivatives. LY264826, a novel glycopeptide, showed promising activity that was at least comparable to those of teicoplanin and vancomycin (46, 263). The genetic and biochemical mechanisms responsible for resistance to glycopeptides in CNS are not clear. Resistance has not been shown to be transmissible among the CNS; however, selection pressures may influence the spread of a resistant strain from an infected patient throughout the hospital environment. Therefore, precautions should be taken to prevent the dissemination of resistant strains (149) and to limit the use of glycopeptides only to the most severe cases of CNS infection.

The quinolones are a promising group of antibiotics that have a broad spectrum of activity. However, with the increased use of this group of antibiotics, particularly ciprofloxacin, there have been reports of resistant CNS (21, 23, 72, 101, 181). Studies have indicated that resistance to one quinolone may predispose the isolate to become resistant to other quinolones (21, 257, 296, 319). Some investigators have tried to induce resistance to the quinolones by serial passages in the presence of the antibiotics (23, 224, 284). Exposure to subinhibitory concentrations can raise the MICs for CNS to resistant levels. This may be a problem in vivo if the antibiotic concentration at the site of infection is near the MIC for the test strain (23). Studies on newer quinolones continue (1, 32, 88, 95, 221, 224, 257, 262, 284). Aldridge (1) found that the investigative fluoroquinolones, CI-960 and WIN 57273, exhibited more activity than ciprofloxacin against CNS. Exposure of CNS to WIN 57273 caused the appearance of only a low number of single-step spontaneous mutants (88). Levofloxacin appears to be more active than ciprofloxacin (95). Temofloxacin (32), T-3761 (221), and sparfloxacin (257) appear to be comparable to ciprofloxacin. Importantly, resistance to sparfloxacin appeared to emerge less rapidly following exposure to increasing concentrations than that to ciprofloxacin (284). Although the mechanism of resistance to quinolones has not been clearly established in CNS, resistance mechanisms in other bacteria have been through alterations in the DNA gyrase (224, 319). These alterations have been associated with

chromosomal gene changes (59, 319). Because of continued selection pressures, it may be only a matter of time before plasmid-mediated resistance arises in CNS.

The glycopeptides and the quinolones gained more attention in the past few years, but studies of the macrolide and cephalosporin groups of antibiotics are also expanding. Several new macrolides have been investigated for activity against the CNS and have been compared with erythromycin. In summary, the newer macrolides are comparable to erythromycin, yet resistance is already being found (86, 118, 128, 208, 258). Similar results are found with the new cephalosporins (74, 152, 154, 163, 193, 279). MLS resistance was investigated to find its prevalence in the CNS. MLS resistance was found in isolates of *S. epidermidis*, *S. haemolyticus*, *S. saprophyticus*, *S. hominis*, *S. simulans*, *S. cohnii*, *S. warneri*, *S. capitis*, *S. xylosus*, and *S. sciuri* (147, 170, 266). Jenssen et al. (147) found that of 151 CNS, 53 were constitutively resistant, 25 were inducibly resistant, and 11 displayed a novel phenotype of erythromycin-inducible resistance to erythromycin and a streptogramin but not to a lincosamide (MS resistance). The molecular mechanism was unclear. Ross et al. (266) briefly characterized the MS resistance phenotype and concluded that the MS phenotype was not due to an altered expression of MLS resistance determinants (*erm* genes). More studies are needed to help elucidate the mechanism for MS resistance.

Many clinical laboratories use standard agar dilution or broth microdilution procedures for antimicrobial susceptibility testing, including the determination of MICs. However, these techniques are time-consuming and require careful quality control to produce reliable results. The E test is currently under investigation as a new technique for determining MICs. The E test, which is based on diffusion of an antibiotic gradient from a plastic strip on inoculated agar medium, provides a simple agar diffusion method and a MIC result (13). The new method was found to be as reliable as the standard methods for the determination of MICs for CNS (13, 40, 133, 226). However, in tests of oxacillin, it was necessary to supplement Mueller-Hinton agar with sodium chloride (133, 226). The E test appears to be an accurate, simple, and reproducible alternative method for antimicrobial susceptibility testing of CNS.

Species and Subspecies Patterns

Some *Staphylococcus* species and subspecies behave differently under antibiotic pressure. This variation has led to the recognition of certain interesting species and subspecies patterns. *S. epidermidis*, *S. hominis*, *S. haemolyticus*, and *S. warneri* tend to develop resistance more readily than other CNS species (165). Resistance to penicillin, tetracycline, and/or erythromycin is frequently encountered in CNS with the exceptions of *S. auricularis* and *S. capitis* subsp. *capitis* (165). Although cutaneous populations of *S. capitis* subsp. *capitis* develop resistance slowly when put under continued antibiotic pressure, they will eventually show resistance and replace susceptible populations. *S. capitis* subsp. *ureolyticus* develops resistance slightly more rapidly than *S. capitis* subsp. *capitis* and upon doing so increases its habitat range. The increase in habitat range has been observed with certain other *Staphylococcus* species (166). *S. cohnii* and *S. xylosus* have been shown to be intrinsically resistant to lincomycin (167). The human isolates exhibiting intrinsic novobiocin resistance (*S. saprophyticus*, *S. cohnii*, and *S. xylosus*) show a slight intrinsic penicillin resistance (167) and decreased susceptibility to quinolone antibiotics (21). Fortunately, *S.*

lugdunensis and *S. schleiferi* rarely appear to develop resistance to most antibiotics (84); however, antibiotic resistance in *S. lugdunensis* has begun to emerge (187). *S. lugdunensis* (121) and *S. epidermidis* (120) have been found to be more resistant to polymyxin B than other CNS species. Recently, *S. lugdunensis* (121) and *S. haemolyticus* (120) were shown to exhibit resistance to 10 U of bacitracin. As mentioned, clinical isolates of *S. haemolyticus* with resistance to vancomycin and teicoplanin are being isolated with more frequency. The knowledge gained from these findings may provide better management of infections caused by specific species of CNS.

PATHOGENESIS

Microbial Properties Associated with Virulence

CNS are a major cause of foreign body infections, e.g., of intravascular catheters, catheters for continuous ambulatory peritoneal dialysis (CAPD), fluid shunt systems, prosthetic heart valves, joint prostheses, and pacemaker electrodes, and they may also be involved in the pathogenesis of fibrous capsular contracture after mammoplasty with silicon prostheses and the toxic lens syndrome after implantation of artificial eye lenses (48, 58, 70, 106, 156, 209, 230, 267, 290). Early-onset infections occur within several days or weeks after surgery or catheterization, and in most of these cases, introduction of the etiologic agent(s) takes place during surgery or insertion of the catheter (178). On the other hand, late-onset infections start after a much longer interval of several weeks, months, or years, with the etiologic agent(s) being introduced at the time of surgery, insertion of the catheter, or during bacteremia of another origin (48). *S. epidermidis* and, to a lesser extent, other species of the *S. epidermidis* species group (e.g., *S. haemolyticus*, *S. capitis*, *S. hominis*, and *S. warneri*) are the principal pathogens of foreign body infections (49, 143).

The process of foreign body infections proceeds by several important steps. The first step involves the adhesion of bacteria to biomaterials (mainly synthetic polymers). Nonspecific adhesion of cells to solid surfaces under in vitro conditions mainly involves electrostatic and hydrophobic interactions (83, 143), besides the hydrodynamic forces of the liquid medium that influence the transport of the cells to the surface. In general, strains of *S. epidermidis* that demonstrate high hydrophobicity adhere more strongly to polymer surfaces (200). Specific adhesion of *S. epidermidis* RP-62A to silastic catheter surfaces can be mediated by a capsular polysaccharide-adhesin (PS/A) (300). PS/A is a large (>500,000 molecular weight) polymer of galactose and arabinose in a 1:1 molar ratio (219). Purified PS/A inhibits adherence of *S. epidermidis* to catheters and elicits antibodies that block adherence and stabilize the extracellular structure surrounding cells, presumed to be a capsule. PS/A appears to enhance the very early stages of colonization of biomaterials; i.e., the initial adherence can be measured after 15-min contact of materials with broth cultures. Recent studies have indicated that *S. capitis* subsp. *ureolyticus*, in addition to *S. epidermidis*, is capable of producing PS/A (168). A high percentage of clinical isolates of both species produces PS/A and biofilm. There is also a suggestion that long-term colonizing strains on the skin of healthy individuals produce significant amounts of PS/A and biofilm. PS/A is highly immunogenic in its purified form and may play a role in protective immunity. In animal models of catheter-related bacteremia (179) and intra-aortic catheter-related endocardi-

tis (293), it has been shown that the major defense mechanism achieved through immunization with PS/A is opsonophagocytic killing by peripheral blood leukocytes. However, during experimental infection, there is no immune response to PS/A, perhaps as a result of the immunosuppressive effects of teichoic acid. In addition to PS/A, there is some evidence that, at least in some strains of *S. epidermidis*, a proteinaceous adhesin mediates in vitro attachment to polymer surfaces (129, 130, 235). In recent studies, Timmerman et al. (299) found that a 220-kDa proteinaceous surface antigen of *S. epidermidis* 354 mediates attachment to polystyrene. Immunogold electron microscopic studies showed the presence of this antigen on the bacterial surface and also on what may be interpreted as fimbria-like surface projections.

In vivo adhesion is probably a very complex situation, for both polymer and bacteria undergo changes in the dynamic environment of the host (61, 316). Polymers and bacterial cells become coated with a variety of serum and tissue fluid components, e.g., fibronectin, fibrinogen, collagen, and vitronectin, that may influence adhesion. Specific interactions between bacterial binding proteins or structures and the serum and tissue components on the polymer surface may overrule the nonspecific forces and perhaps even some of the specific in vitro adhesins mentioned above. Different species of CNS have different capacities to bind and agglutinate serum and tissue proteins (238, 239, 292). Paulsson et al. (237) have developed a rapid particle agglutination assay to detect the interaction of different species of CNS with collagen, laminen, fibronectin, and vitronectin immobilized on latex beads. The results of their study showed that cells of strains of *S. haemolyticus* reacted more strongly than cells of strains of *S. epidermidis*, although no significant difference in cell surface hydrophobicity or charge could be demonstrated. The cell surface receptors of *S. haemolyticus* were more heat and protease resistant than *S. aureus* receptors. Strains of *S. saprophyticus* isolated from UTIs showed a high capacity to adhere to laminen, a connective-tissue protein. *S. haemolyticus* and *S. epidermidis* cells bound to both N-terminal (29-kDa) and C-terminal (120-kDa) fragments of fibronectin. In addition to mediating adhesion to polymer surfaces, the serum and tissue proteins may facilitate binding and colonization in open wounds and damaged tissues (305). In wound infections, binding to fibronectin and collagen is most likely the first step in tissue colonization (306).

It is now generally understood that following adherence of CNS to foreign bodies, the second step in infection involves the production of slime (extracellular slime substance [ESS]) (83, 143, 317). Scanning electron microscopic studies have shown the extensive production of ESS by staphylococci, especially *S. epidermidis*, ultimately resulting in encased multiple layers of bacteria (242). One of the roles of ESS is the formation of a biofilm on the surface of biomaterials which may function as a penetration barrier to antibiotics (77, 242). Once established, the bacterial biofilm is very difficult to remove. Information on the chemical composition of ESS and the regulation of its production is still incomplete, although recent studies by Hussain and coworkers (135, 136, 138) have indicated that ESS produced by *S. epidermidis* in a chemically defined medium contains glycerol phosphate, D-alanine, glucose (most strains), and N-acetylglucosamine. Ester linking of D-alanine, the absence of lipids, and the presence of constituent monomers in simple molar proportions strongly suggest that the isolated polymer or glycoconjugate is glycerol teichoic acid. The

inhibition of ESS production by tunicamycin further supports this view, because this antibiotic is known to interfere with the synthesis of teichoic acids by being a glycosylation blocker (137, 244). The inhibition by 5-fluorouracil, a compound believed to act chiefly in nucleic acid biosynthesis, might be due to the production of fluorinated derivatives of the UDP-sugars that are precursors of teichoic acids (261). ESS is a water-soluble substance and is loosely bound to the staphylococcal cell. It might remain close to the organism that produces it only when the cells grow on a surface. It may not be surprising that ESS is composed chiefly of teichoic acid considering that glycerol teichoic acid is a component of *S. epidermidis* and certain other CNS cell walls (73). Earlier studies suggesting that mannose and galactose are major components of ESS may have been misleading in that these sugars may have been derived from the complex culture media supporting growth (69, 136). When grown in air versus air enriched with carbon dioxide, CNS adhere differently and have altered cell surface protein profiles and carbohydrate content (65). When CNS previously identified as negative for ESS production by the standard tube test of Christensen et al. (53) were reexamined by the tissue culture plate test (51), two classes were recognized with respect to the effect of oxygenation on ESS production (22). The class I phenotype was positive for slime production under aerobic conditions but not under anaerobic conditions, whereas the class II phenotype produced little or no slime under either aerobic or anaerobic conditions. The authors proposed that since oxygen is subject to concentration fluctuations in human hosts, these findings could have important implications regarding the pathogenicity of individual strains of CNS. Some strains of *S. epidermidis* and other CNS species that are classified as ESS negative by the above methods can still cause infection. Perhaps, in these strains, ESS is produced in very small quantities in vitro (187) but in sufficient quantities in vivo to maintain infection. The production of biofilm, including both the properties of adherence and ESS, can be assayed radiochemically when CNS are grown in tubes (plastic scintillation vials) in a chemically defined medium containing [^{14}C]glucose (134). The ability to produce biofilm can also be determined for individual colonies of CNS by using a Congo red agar test (27).

Some strains of *S. saprophyticus* have the ability to produce ESS, though it may be somewhat different from that produced by *S. epidermidis* (126). Urea is essential for ESS production by *S. saprophyticus*, but not for that by *S. epidermidis*. ESS of *S. saprophyticus* may be a risk factor for the development of urinary stones, especially in urine in which the urea concentration is high. Infection-induced urinary stones most often contain mucoproteins and carbohydrates in a slime-like matrix associated with crystals of stuvite and apatite. This amorphous substance is believed to be of bacterial origin (214, 228). ESS may play a role in UTIs associated with catheterization, though this has not yet been confirmed. The urease of *S. saprophyticus* has been shown to be a major factor required for invasiveness in bladder tissue (97). The production of urease is probably also one of the reasons why this species is sometimes associated with urinary calculi. *S. saprophyticus* also displays a tissue specificity, i.e., for uroepithelial cells of the urogenital tract (57, 205). The receptor-mediated adherence is believed to be the first major step in the development of UTI. A major surface protein (Ssp) of *S. saprophyticus* that may be involved in interactions of this species with eukaryotic cells has been identified recently (98).

Host-Bacterium Interactions

There is growing evidence that ESS interferes with host defense mechanisms in addition to its role in the formation of biofilm. *S. epidermidis* ESS can inhibit the proliferation of human peripheral mononuclear cells (mainly T lymphocytes) after they are stimulated with polyclonal immunomodulators (111). More recently, it has been shown that ESS (or glycocalyx) preparations from *S. epidermidis* and *S. lugdunensis* do not have a direct inhibitory effect on T-cell proliferation, but rather directly stimulate monocyte production of prostaglandin E_2, and that it is this activity that in turn contributes to the inhibition of T-lymphocyte proliferation (288). This activation of monocytes results not only in prostaglandin E_2 production but also in human interleukin-1 and tumor necrosis factor alpha production and secretion, factors that promote the acute inflammatory responses (28, 68). ESS could also be shown to interfere with blastogenesis of B cells and subsequent immunoglobulin production (112).

In addition to its effect on the immune system, ESS of *S. epidermidis* can have a significant effect on opsonophagocytosis mechanisms. This includes (i) inhibition of the ability of polymorphonuclear leukocytes to migrate directly toward a known chemotactic stimulus (79, 151); (ii) degranulation of specific granules (lactoferrin) (150), possibly leading to a decreased intracellular killing ability; (iii) inhibition of polymorphonuclear leukocyte chemiluminescence, a response that results from oxygen-dependent metabolic activity that normally occurs during phagocytosis and intracellular oxygen-dependent killing (2, 79); and (iv) inhibition of interactions of the bacterial surface with opsonins, such as complement and/or immunoglobulin G, which promote phagocytosis and killing (143). Whether surface-exposed proteins serve as targets for opsonization is unknown. However, studies by Plaunt and Patrick (252) have demonstrated the presence of four immunodominant proteins (18, 41, 48, and 51 kDa) produced by strains of *S. epidermidis*. Their identification is perhaps a first step toward understanding the host defense mechanisms responsible for maintaining a commensal relationship with this organism. ESS or capsular material of *S. simulans* can have an antiphagocytic effect (231). ESS can impair the oxidative burst or responses of rabbit alveolar macrophages, thereby compromising their effectiveness in host defense (223). Macrophages most likely play a major role in host interactions with foreign bodies present in the host for long periods.

Animal Models

Mouse models for foreign body infections involving subcutaneous abscess formation have provided information concerning the pathogenicity of CNS species and the efficiency of antibiotic treatment on infections of catheters. The most recent investigations have been performed by Lambe et al. (185–187, 189), who used a modified mouse model of Christensen et al. (52) to assess the formation of abscesses. The modified model involved preadhering the bacteria to a catheter before subcutaneous implantation and monitoring the infection for 7 days. Studies on the pathogenicity of several different species of CNS, using the 7-day model, indicated that *S. schleiferi* was the most virulent CNS species (187); furthermore, this species usually did not require a foreign body to produce an abscess (80). *S. epidermidis* (168, 186) and *S. lugdunensis* (186, 187) have been found to cause moderate to severe abscess formation. *S. epidermidis* sometimes required and *S. lugdunensis* gen-

erally required the presence of a foreign body for abscess formation (80). *S. hominis* (186), *S. warneri* (186), and *S. capitis* (168, 186) produced fewer abscesses than any of the other CNS investigated, yet they were found to be pathogens when a foreign body was present. With the less virulent species, it was necessary to use a larger mouse population to obtain statistically significant results (186). Strains of *S. epidermidis*, *S. capitis*, and *S. haemolyticus* differing in their duration of colonization on normal human skin have also been tested for abscess formation with the mouse model (168). It was found that strains of *S. epidermidis* and *S. capitis* that persisted for more than 2 months to several years on skin were more virulent than those that persisted for less than 2 months. *S. haemolyticus* strains usually persisted for less than 2 months, and although some strains produced low-grade infections, they could not be recovered from the implanted catheter or surrounding tissue. The presence of an abscess correlated well with biofilm production and the presence of PS/A.

In addition to determining pathogenicity on the basis of abscess formation, the mouse model has been used to determine the effect of antibiotics on catheter infections and duration of colonization by *S. epidermidis* (189, 211). Lambe et al. (189) found clindamycin and, to a somewhat lesser extent, cefazolin to be effective in limiting abscess formation. Mayberry-Carson et al. (211) concluded from their studies with ciprofloxacin that prophylaxis before implantation of the catheter significantly reduced the rate of abscess formation. Continued treatment subsequent to implantation was necessary to prevent infection.

The rabbit tibia model has been employed to examine the role of *S. epidermidis* and *B. fragilis*, alone and in combination, in experimentally induced foreign-body-associated osteomyelitis (188). In this model, a catheter was implanted into the medullary cavity of the tibia. Lambe et al. (188) found that only two of the five animals infected with *S. epidermidis* developed culture-positive osteomyelitis. All six animals infected with both microorganisms developed culture-positive osteomyelitis. Transmission and scanning electron microscopy showed that when the microorganisms are involved in a mixed infection *S. epidermidis* predominates on the foreign body and *B. fragilis* predominates in the infected bone and marrow. Mayberry-Carson et al. (210) used a different rabbit tibia model to investigate the in vivo efficacy of ciprofloxacin therapy on polymicrobic osteomyelitis. They concluded that, although relatively high tissue levels of ciprofloxacin were attained, little therapeutic effect was observed. Furthermore, these studies demonstrated that an infection may require the synergistic relationship of two species and that antibiotic treatment against one of them may not eradicate the other.

CONCLUSIONS

The clinical significance of CNS continues to increase as strategies in medical practice lead to more invasive procedures such as the replacement of damaged or missing body parts with synthetic materials and the widespread use of catheters. The most vulnerable to infection by CNS are hospitalized patients, especially those who are premature, very young, or old and those who are immunocompromised and/or suffering from chronic diseases. The proportion of people who are immunocompromised continues to increase. Young, sexually active females who are particularly prone to UTIs by the CNS species *S. saprophyticus* are an exception to the preceding groups.

Since CNS as a group are widespread on the human body and can produce very large populations, distinguishing the etiologic agent(s) from contaminating normal flora is a serious challenge to the clinical laboratory. To some extent, the solution to the problem will be facilitated by the quality of the specimen obtained from the patient and how accurately the specimen represents the infectious problem. Culture identification should proceed to the species and strain levels. A much stronger case can be made for a specific etiologic agent if the same strain is repeatedly isolated from a series of specimens than if different strains of one or more CNS species are isolated. Strain identity can be based initially on colony morphology on the primary isolation plate. Identification can then proceed by the use of one or more molecular approaches to gain information on the genotype. Over the past 5 years, several new *Staphylococcus* species and subspecies have been discovered. It is expected that, as more laboratories continue to search for unusual staphylococci and the number of isolates increases over time, some of the rarer species and subspecies will be recognized.

Many of the CNS species are commonly resistant to antibiotics that are in current use for staphylococcal infections, with the exception of vancomycin. The use of antibiotics in hospitals has provided a reservoir of antibiotic resistance genes and has promoted the accumulation of multiply resistant CNS strains. Most attempts at modifying existing antibiotics or synthesizing new ones have met with limited success, for ultimately CNS, and especially members of the *S. epidermidis* species group, develop resistant populations. Of considerable concern is the widespread distribution of methicillin resistance among CNS species and genetic exchange between CNS and *S. aureus*.

At present, the main focus on mechanisms of pathogenesis has been with foreign body infections and the role of specific adhesins and slime produced by *S. epidermidis*. There is now some understanding of the sequence of events leading to the establishment of biofilm on polymers, though the story is not complete and may be somewhat different for the establishment of infection in native tissue. It is now clear that biofilm can act as a barrier to antibiotics and limit the effectiveness of antibiotic therapy. Furthermore, slime can reduce the immune response and opsonophagocytosis, thereby interfering with host defense mechanisms. Animal model studies have indicated that biofilm production and the ability to produce infections are properties of not only certain strains of *S. epidermidis* but also certain strains of *S. capitis*, *S. lugdunensis*, and *S. schleiferi*. In these models, the severity and extent of infection were less for strains of *S. hominis*, *S. haemolyticus*, and *S. warneri*. Studies are warranted to compare the mechanisms of pathogenesis used by each of the CNS species, with the assumption that some differences will be found among them. Mechanisms of pathogenesis have begun to be understood for the urinary tract pathogen *S. saprophyticus*, and they appear to involve receptor-mediated adherence to uroepithelial cells and the production of urease. The role of slime produced by this species is uncertain. Future studies should ultimately bring together the population, cellular, and molecular aspects of adaptive strategies used by CNS species. As we become more aware of the various strategies used by these organisms, we will be in a better position to compromise their defense mechanisms and improve treatment, and perhaps even to prevent their colonization of biomaterials.

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