Species Identification of Coagulase-Negative Staphylococci from Urinary Tract Isolates

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A new scheme for identification of coagulase-negative staphylococci was applied to 138 consecutive urinary isolates of coagulase-negative staphylococci. The most common species were *Staphylococcus epidermidis* (53%), *S. hominis* (12%), and *S. haemolyticus* (10%). *S. saprophyticus* comprised only 5%. The disk method for antibiotic susceptibility for all species grouped together disclosed resistance most commonly to penicillin (35%), tetracycline (33%), methicillin (27%), and sulfonamide (24%). This pattern was also seen specifically with *S. epidermidis*. Further studies are needed to determine the incidence of species-specific antibiotic resistance and species-specific infection by site. This may be of particular interest in those patients with nosocomial infections due to coagulase-negative staphylococci.

Coagulase-negative staphylococci commonly cause urinary tract infections, particularly in young females (1, 8, 10, 11, 13, 19). Several studies employing suprapubic aspiration of urine leave no doubt about the pathogenicity of these organisms in the urinary tract (1, 10). Among urinary coagulase-negative staphylococci, novobiocin-resistant species in particular are most prevalent (13). In 1975 Kloos and Schleifer (9) introduced a simplified scheme for routine identification of newly characterized coagulase-negative staphylococci based on morphology, biochemical reactions, antibiotic susceptibility, and cell wall composition. Very few studies have applied this new scheme to the classification of urinary coagulase-negative staphylococci (15, 16). No studies have attempted to relate patterns of antibiotic resistance to these newly named species from clinical sources. This report relates our experience with classifying a large number of urinary isolates of coagulase-negative staphylococci.

MATERIALS AND METHODS

The 138 consecutive strains of staphylococci used in this study were isolated from urine cultures during 1976 in the Clinical Bacteriology Laboratory, Medical University Hospital. Isolates were classified by the method of Kloos and Schleifer (9) if they were the sole isolate present in greater than 10,000 organisms per ml or the predominant organism when isolated with 1 other organism with a combined colony count of 10,000/ml. The organisms were identified as coagulase-negative staphylococci by Gram stain, catalase production, glucose fermentation, and the absence of coagulase production by the tube method (7). The organisms were further confirmed as staphylococci by

resistance to lysostaphin (9) and susceptibility to Furoxone (5). Hemolysis and colony morphology were observed on bovine and sheep blood agar plates (5% in Trypticase soy agar, Baltimore Biological Laboratory) after 1, 3, and 5 days of incubation at 37°C. Nitrate production was determined by inoculating 5 ml of indole-nitrate broth (Baltimore Biological Laboratory), incubating for 48 h, and observing color development after addition of sulfanilic acid and alpha-naphthylamine. Negative results were confirmed by addition of zinc dust. Acid production from carbohydrates was recorded at 24 h, 48 h, 5 days and 2 weeks in tubes containing 5 ml of purple broth base (Baltimore Biological Laboratory) and 1% of the filter-sterilized carbohydrate. Novobiocin susceptibility was determined by streaking a loopful of saline suspension containing 10^7 to 10^8 colony-forming units per ml to P agar containing 1.6 µg of novobiocin per ml (9). Any growth was considered evidence of novobiocin resistance. Additionally, three strains of *Staphylococcus saprophyticus* provided by W. E. Kloos produced abundant growth on the novobiocin-containing P agar and overgrew novobiocin disks containing 2.5 µg of novobiocin. Known non-*S. saprophyticus* strains did not grow on novobiocin containing P agar and were markedly inhibited with disk testing. Controls were plates of P agar without novobiocin. Other antibiotic susceptibilities by the method of Bauer and Kirby (2) were determined for the following antimicrobial agents: chloramphenicol, erythromycin, penicillin, methicillin, tetracycline, sulfasoxazole, cephalothin, and clindamycin.

RESULTS

Of the 141 isolates reported from the Clinical Microbiology Laboratory as coagulase-negative staphylococci, two were corynebacteria, and one was a micrococcus. This micrococcus was the only Furoxone-resistant strain. The total num-

ber and percentage of each species are shown in Table 1. *S. epidermidis*, *S. hominis*, and *S. haemolyticus* comprised 79% of the isolates. All of the isolates were placed in one of the designated species, except for six strains which could not be classified. Because hemolysis is an important trait in the classification scheme, we attempted to compare hemolysis on sheep versus bovine blood agar. The percentage of isolates which produced either alpha- or beta-hemolysis on sheep blood agar (22 and 58%, respectively) was similar to that produced on bovine blood agar (35 and 59%, respectively).

The patterns of antibiotic resistance are shown in Table 2. A total of 24% of all strains were sensitive to all antibiotics tested. The incidence of resistance was greatest for penicillin (35%), tetracycline (33%), methicillin (27%), and sulfonamide (24%) and lower for erythromycin (15%) and clindamycin (13%). Only five isolates (4%) were resistant to cephalothin.

DISCUSSION

This study substantiates the feasibility of classifying coagulase-negative staphylococci isolated from the urinary tract from patients with a variety of disorders. Our findings regarding the frequency of occurrence of various species vary from other reports. We did not find a preponderance of novobiocin-resistant organisms, that is, *S. saprophyticus* or *S. cohnii* (1, 6, 14). In

other surveys (14, 15), *S. saprophyticus* was by far the most commonly isolated coagulase-negative staphylococcus from the urine. Nord et al. (15) studied 248 inpatients and outpatients and classified their coagulase-negative staphylococci isolated from urine, using the scheme of Kloos and Schleifer. *S. saprophyticus* (44%), *S. epidermidis* (23%), and *S. haemolyticus* (14%) were the predominating species. In Nord's series, 7% of isolates from urine were *S. hominis* versus 14% in our series (15). In our series, *S. haemolyticus* accounted for 11% of isolates versus 14% in Nord's series. *S. cohnii* has been described as a cause of urinary tract infection (14) and also has been isolated from high vaginal and periurethral sites (8). The 7% of *S. cohnii* in our study suggests that *S. cohnii* may be another novobiocin-resistant, coagulase-negative staphylococcus of importance especially in the hospitalized patient (16).

Patterns of antibiotic resistance in coagulase-negative staphylococci are important because coagulase-negative staphylococci tend to be more resistant than their coagulase-positive counterparts (12, 17). One crucial aspect of this problem in coagulase-negative staphylococci is resistance to the semisynthetic penicillins, so-called methicillin resistance. Methicillin resistance in *S. aureus* is very unusual in this country, whereas about 22% of coagulase-negative staphylococci compiled from various American hospitals are resistant to methicillin (Bac Data, Medical Information Systems, Inc., Clifton, N. J.). In our hospital, about 20% of all coagulase-negative staphylococci, including urinary isolates, are methicillin resistant. This percentage probably increases with hospital stay and with exposure to antibiotics, particularly the semisynthetic penicillins (3). In our present study, 27% of all coagulase-negative staphylococci were methicillin resistant, with higher percentages of resistance seen only for penicillin and tetracycline. This indeed may reflect the large numbers of inpatients in our study and their exposure to the semisynthetic penicillins. Except for *S. epidermidis*, the number of isolates for each species

TABLE 1. Distribution of coagulase-negative staphylococci from urine

Species	No.	Percent
<i>S. epidermidis</i>	73	53
<i>S. hominis</i>	16	12
<i>S. haemolyticus</i>	14	10
<i>S. cohnii</i>	9	7
<i>S. saprophyticus</i>	7	5
<i>S. simulans</i>	7	5
<i>S. capitis</i>	5	4
<i>S. xylosus</i>	1	1
<i>S. warneri</i>	0	0
Unidentifiable	6	4

TABLE 2. Antibiotic susceptibility patterns in urinary isolates of coagulase-negative staphylococci

Species (no.)	Chloramphenicol	Erythromycin	Penicillin	Methicillin	Tetracycline	Sulfonamide	Cephalothin	Clindamycin
<i>S. epidermidis</i> (73)	19	25	48	30	53	37	1	19
<i>S. hominis</i> (16)	19	0	31	13	32	25	6	0
<i>S. haemolyticus</i> (12)	0	21	50	14	21	29	14	14
<i>S. cohnii</i> (9)	11	22	33	33	33	44	0	22
<i>S. saprophyticus</i> (7)	14	14	0	71	43	29	0	0
<i>S. simulans</i> (7)	0	29	29	29	57	14	0	14
<i>S. capitis</i> (5)	0	0	0	0	0	20	0	0
<i>S. xylosus</i> (1)	0	100	100	100	100	100	0	0

is too small for generalizations about species-specific antibiotic resistance. Noteworthy trends are the increased methicillin resistance in *S. epidermidis* and *S. saprophyticus* and the low overall antibiotic resistance in *S. capitis*. Of the 37 methicillin-resistant strains, only 5 were cephalothin-resistant. All cephalothin-resistant strains were methicillin resistant. Some caution, however, must be exercised in interpreting the high degree of cephalosporin sensitivity, because other factors such as inoculum size, temperature of incubation, and duration of incubation may greatly affect assessment of cephalosporin susceptibility of staphylococci (18).

Our study raises several other points for further investigation. More work using this new scheme for classification needs to be done to detect the true incidence of various species and the peculiarities in pathogenesis related to various species both in outpatients and inpatients. Oeding and Digraanes (16) have already shown that novobiocin-sensitive, coagulase-negative staphylococci are more commonly isolated from inpatients than outpatients. Additional work could include antibody coating of urinary isolates to relate species to upper- and lower-urinary tract infection. Determination of the effect of the hospital environment of species-specific infections by site may be helpful in evaluating the pathogenic potential of a given species. Finally, future analysis of species-specific antibiotic resistance patterns could resolve the question of whether certain species by their very nature are more antibiotic resistant. In this age of the comprised host and wide-spread use of indwelling plastic devices, the pathological importance of coagulase-negative staphylococci seems destined to increase.

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LITERATURE CITED

1. Bailey, R. R. 1973. Significance of coagulase-negative staphylococcus in urine. *J. Infect. Dis.* **127**:179-182.
2. Bauer, A. W., W. M. Kirby, J. C. Sherris, and M. Turk. 1966. Antibiotic susceptibility testing by a standardized disk method. *Am. J. Clin. Pathol.* **45**:493-496.
3. Bentley, D. W. 1973. Methicillin resistance in *Staphylococcus epidermidis* epidemiologic studies. *Contrib. Microbiol. Immunol.* **1**:650-658.
4. Bentley, D. W., H. Riaz-Ul, R. A. Murphy, and M. H. Lepper. 1968. Biotyping, an epidemiological tool for coagulase-negative staphylococci. *Antimicrob. Agents Chemother.* **1967**, p. 54-59.
5. Curry, J. C., and E. Borovian. 1976. Selective medium for distinguishing micrococci from staphylococci in the clinical laboratory. *J. Clin. Microbiol.* **4**:455-457.
6. Digraanes, A., and P. Oeding. 1975. Characterization of *Micrococcaceae* from the urinary tract. *Acta Pathol. Microbiol. Scand. Sect. B.* **83**:373-381.
7. Facklam, R., and P. B. Smith. 1976. The gram-positive cocci. *Hum. Pathol.* **17**:187-194.
8. Gill, P., and M. Sellin. 1977. Micrococci and urinary infection. *Lancet* **ii**:986.
9. Kloos, W. E., and K. H. Schleifer. 1975. Simplified scheme for routine identification of human *Staphylococcus* species. *J. Clin. Microbiol.* **1**:82-88.
10. Mabeck, C. E. 1969. Studies in urinary tract infection. II. Urinary tract infection due to coagulase-negative staphylococci. *Acta Med. Scand.* **186**:39-45.
11. Mabeck, C. E. 1969. Significance of coagulase-negative staphylococcal bacteriuria. *Lancet* **ii**:1150-1152.
12. Marsik, F. J., and J. T. Parisi. 1973. Significance of *Staphylococcus epidermidis* in the clinical laboratory. *Appl. Microbiol.* **25**:11-14.
13. Maskell, R. 1974. Importance of coagulase-negative staphylococci as pathogens in the urinary tract. *Lancet* **i**:1155-1158.
14. Namayar, F., J. de Graaf, and D. M. MacLaren. 1977. Micrococci and urinary tract infection. *Lancet* **ii**:1223-1224.
15. Nord, C.-E., S. Holta-Oie, A. Ljungah, and T. Wadstrom. 1976. Characterization of coagulase-negative staphylococcal species from human infections. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Suppl.* **5**:105-111.
16. Oeding, P., and A. Digraanes. 1977. Classification of coagulase-negative staphylococci in the diagnostic laboratory. *Acta Pathol. Microbiol. Scand. Sect. B.* **85**:136-142.
17. Sabath, L. D., F. F. Barrett, C. Wilcox, D. A. Gerstein, and M. Finland. 1969. Methicillin resistance of *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Antimicrob. Agents Chemother.* **1968**, p. 302-306.
18. Sabath, L. D., and S. J. Wallace. 1971. Factors influencing methicillin resistance in staphylococci. *Ann. N.Y. Acad. Sci.* **182**:258-266.
19. Williams, D. N., M. E. Lund, and D. J. Blazevec. 1976. Significance of urinary isolates of coagulase-negative *Micrococcaceae*. *J. Clin. Microbiol.* **3**:556-559.