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Characterization of *Micrococcaceae* Strains Isolated from the Human Urogenital Tract by the Conventional Scheme and a Micromethod

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Two hundred and twelve *Micrococcaceae* isolates were obtained from 82 men with nongonococcal urethritis, 24 women with vaginitis, and 54 girls with vulvovaginitis. Identification and biotyping of these strains were carried out by using the simplified scheme of Kloos and Schleifer (W. E. Kloos and K. H. Schleifer, J. Clin. Microbiol. 1:82–88, 1975) and the commercially available API Staph test (DMS Staph Trac). *Staphylococcus epidermidis* occurred in about half of these isolates. There was no statistical difference between the urethral and vaginal specimens, except for *S. haemolyticus* found primarily in males and for *S. simulans* and *S. aureus* found primarily in girls between the ages of 1 and 12 years. *S. saprophyticus*, a major cause of urinary tract infections in young women, was never isolated from the vagina, suggesting the probability of another reservoir.

Coagulase-negative staphylococci (CNS) are no longer considered only contaminants but disease-causing agents (8, 13, 19, 27, 30). This is especially true for CNS isolated from the urinary tract. One of these CNS, *Staphylococcus saprophyticus*, is now recognized as a common cause of urinary tract infection (17), and this observation raises the question of the possible reservoirs of this microorganism.

We observed in preliminary studies a heterogenicity of staphylococcal strains in urogenital specimens which prompted us to perform species identification with the conventional simplified scheme of Kloos and Schleifer (20) in comparison to the commercially available API Staph (DMS Staph Trac; API System, La Balme les Grottes, France).

Bacterial strains. A total of 212 strains isolated on tryptic soy agar with 5% sheep blood (BioMerieux, Marcy l'Etoile, France) incubated 48 h in a candle jar at 37°C were identified as *Micrococcaceae* according to classical characteristics, clustering gram-positive cocci by Gram stain and catalase positive (21). All the strains were isolated from urogenital specimens submitted to the Bacteriology Laboratory, Children's Hospital, Bordeaux, France. A total of 114 strains were from 82 men with nongonococcal urethretis, 29 strains were from 24 women with vaginitis, and 69 strains were from 54 girls with vulvovaginitis.

The following 23 reference strains, grouped according to the method of Skerman et al. (29), were included in the study: *S. aureus* ATTC 12600, *S. intermedius* H 11/68, *S. hyicus* subsp. *hyicus* NCTC 10350, *S. hyicus* subsp. *chromogenes* NCTC 10530, *S. simulans* ATCC 27848, *S. capitis* ATCC 27840, *S. epidermidis* ATCC 14990, *S. warneri* ATCC 27836, *S. haemolyticus* DSM 20263, *S. hominis* ATCC 27844, *S. saprophyticus* CCM 883, *S. xylosus* DSM 20266, *S. cohnii* DMS 20260, *S. sciuri* ATCC 29062, *S. lentus* ATCC 29070, *Stomatococcus mucilaginosus* CCM 2417, *Micrococcus kristinae* ATCC 25570, *M. luteus* ATCC 4698, *M. varians* ATCC 15306, *M. roseus* ATCC 186, *M. nishinomiyaensis* CCM 2140, *M. sedentarius* ATCC 14392, *M. lylae* ATCC 27566.

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All the strains were maintained in a semisolid medium (Institut Pasteur Production [IPP], Marnes La Coquette, France) at room temperature until used.

Conventional identification. Identification to the genus level was studied by testing the susceptibility to the following substances: lysostaphin, 200 μg/ml (Sigma Chemical Co., St. Louis, Mo.) on P agar (20); vibriostatic compound O/129 (IPP) on P agar (5); nitrofurantoin, 300 μg per disk (IPP) (16); and bacitracin, 10 U per disk (IPP), on Mueller-Hinton agar (12).

The strains were identified to the species level by using the following protocol of Kloos and Schleifer (20): coagulase with oxalated rabbit plasma (IPP) in a 4-h test at 37°C, followed by 20 h at room temperature; hemolysis on human blood agar; nitrate reduction; and acid production from 10 carbohydrates (D-ribose, D-xylose, α-lactose, sucrose, maltose, D-mannitol, D-fructose, L(+)arabinose, D-trehalose, and xylitol) observed under aerobic conditions with Purple Agar Base Medium (Difco Laboratories, Detroit, Mich.). The protocol was completed with the alkaline phosphatase test (Pathostaphkit, BioMérieux), and by measuring urease (Christensen medium) and arginine dihydrolase (Moeller medium) activity, fibrinogen affinity (Staphyslide, BioMérieux) (7), and susceptibility to lysostaphin (50 μg/ml; Sigma) on P agar and to novobiocin (5 μg per disk; A.S. Rosco, Taatstrup, Denmark) on Mueller-Hinton agar (20). For *Micrococcus* speciation we also used penicillin disks, oxacillin disks (IPP), and dimethylphenylene diamine disks for oxidase reaction. We used the simplified scheme of Kloos and Schleifer (20) with complementary tests (Table 1) to identify *Staphylococcus* species, the scheme of Kloos et al. (22) for *Micrococcus* species, and the scheme of Bergan and Kocur (2) for *Stomatococcus* species.

API Staph. The API Staph strip contains 20 microtubes, one control, and nineteen tests, including utilization of 14 carbohydrates (glucose, fructose, mannose, maltose, lactose, trehalose, mannitol, xylitol, melibiose, raffinose, xylose, sucrose, α-methyl-glucoside, and *N*-acetyl-glucosamine), nitrate reduction, alkaline phosphatase activity, Voges-Proskauer reaction, and arginine and urea hydrolysis.

The strip was inoculated with a bacterial suspension (McFarland 2 opacity standard) and incubated at 37°C for 24

TABLE 1. Complement to the simplified scheme of Kloos and Schleifer for identification of staphylococci[a]

Position[b]	Identification	ARF	Nov	Arg	Ure	Phs
1	*S. aureus*	+	−	+	(+)	+
2	*S. intermedius*	−	−	(+)	+	+
3	*S. xylosus*	−	+	−	+	+
	S. sciuri	−	+	−	−	+
4	*S. cohnii*	−	+	−	−	(−)
5	*S. capitis*	−	−	+	−	−
6	*S. epidermidis*	−	−	(+)	+	(+)
7, 7b	*S. saprophyticus*	−	+	−	+	−
8, 8b	*S. warneri*	−	−	+	+	−
9, 9b	*S. haemolyticus*	−	−	+	−	−
10,10b	*S. hominis*	−	−	−	+	−
11,11b	*S. simulans*	−	−	+	+	−
	S. hyicus	−	−	+	+	+

[a] Abbreviations: ARF, affinity reaction for fibrinogen; Nov, resistance to novobiocin (5 μg per disk); Arg, arginine hydrolysis; Ure, urea hydrolysis; Phs, alkaline phosphatase activity. Symbols: −, >90% negative; (−), 70 to 90% negative; (+), 70 to 90% positive; +, >90% positive.

[b] Based on the simplified scheme of Kloos and Schleifer.

and 48 h. The reading was done according to the recommendations of the manufacturer, with the exception of the inoculum, which was prepared from a blood agar instead of P agar.

The results were recorded on report sheets. A seven digit number was derived for each isolate by tabulating the positive reactions of tests in groups of three. This number was compared with the profile register provided by the manufacturer (October 1982 edition).

Lysostaphin results were considered to differentiate *Staphylococcus* spp. from *Micrococcus* spp. according to the recommendations of the manufacturer.

Eight field strains were identified as *Micrococcus*. In addition to the seven reference strains, they also had the following characteristics: lysostaphin resistance, nitrofurantoin resistance, vibriostatic compound O/129 susceptibility, and an inhibition diameter bacitracin/nitrofurantoin ratio of >1.5.

A total of 204 strains were identified as *Staphylococcus* spp., along with the 15 reference strains, by the following characteristics: lysostaphin and nitrofurantoin susceptibility, vibriostatic compound O/129 resistance, and an inhibition diameter bacitracin/nitrofurantoin ratio of <1.2. Two reference strains, *S. capitis*, *S. hominis*, and 47 field isolates were partially inhibited by lysostaphin.

None of these strains were from the genus *Stomatococcus* which is lysostaphin resistant and vibriostatic compound O/129 and nitrofurantoin susceptible.

The conventional method permitted the identification of 94.3% of the *Micrococcaceae*. Among 12 unidentified *Staphylococcus* strains (5.7%), 1 was sucrose-negative *S. hominis* and 11 corresponded with position 6 in the simplified scheme of Kloos and Schleifer, with the exception of mannitol utilization. The results are summarized on Table 2. With the API Staph, 89.1% of the strains could be identified to the species level. The identification of only one *Micrococcus* species, *M. varians*, could be achieved with this technique. Among the reference strains, only two, *S. aureus* and *S. intermedius*, could not be differentiated. Seven strains yielded profiles which fell between two species. The numerical profile for 11 strains were not found in the analytical data. A total of 184 (86.4%) strains were correctly identified. Identification of all *S. aureus*, *S. simulans*, *S. saprophyticus*, *S. capitis*, and *M. varians* were correct. Discrepancies were found with 10% of *S. epidermidis*, identified as *S. haemolyticus* (2 strains), *S. aureus* (1 strain), *S. warneri* (1 strain), *S. capitis* (2 strains), and *Staphylococcus* sp. (4 strains). Also one *S. hominis* strain was identified as *S. warneri*, and one *S. cohnii*, four *S. haemolyticus*, two *S. warneri*, and five *M. luteus* isolates were not identified. On the other hand, four *Staphylococcus* sp. were identified as *S. epidermidis* (2 isolates) and *S. haemolyticus* (2 isolates) by the scheme of Kloos and Schleifer.

There was a wide variety of *Micrococcaceae* organisms found colonizing the human urogenital tract (Table 3). In addition, *S. epidermidis* occurred in about half of these isolates. Among the other CNS, only *S. xylosus* was not encountered. *Micrococcus* sp. was rarely found (3.7%). There was statistically significant difference for detection of *S. haemolyticus* between urethral and vulvovaginal specimens ($P < 0.001$) and urethral and vaginal specimens ($P < 0.01$) and for *S. simulans* and *S. aureus* between vulvovaginal and urethral specimens ($P < 0.001$).

The conventional method is accurate but of little value in routine diagnosis because it is more time-consuming to prepare and slower in producing results (up to 3 days) (23). The practical aspect of a micromethod is evident. The API

TABLE 2. Accuracy of the API Staph for identification of *Micrococcaceae*[a]

Organism	No. identified		No. (%) correctly identified by API Staph
	Conventional scheme	API Staph	
S. epidermidis	98	90	88 (90%)
S. haemolyticus	42	42	38 (90%)
S. aureus	19	20	19 (100%)
S. simulans	12	12	12 (100%)
S. hominis	7	6	6 (86%)
S. cohnii	5	4	4 (80%)
S. warneri	5	5	3 (60%)
S. saprophyticus	2	2	2 (100%)
S. capitis	2	5	2 (100%)
Staphylococcus sp.	12	18	7
M. luteus	5	0	
M. varians	3	3	3 (100%)
Micrococcus sp.	0	5	

[a] Of 212 isolates, 200 (94.3%) were identified by the conventional scheme, and 189 (89.1%) were identified by API Staph. API Staph correctly identified 184 of 212 (86.8%) isolates.

TABLE 3. Distribution of the Urogenital isolates of *Micrococcaceae*

Species	No. of strains (%)	Urethral isolates (%)	Vaginal isolates (%)	Vulvovaginal isolates (%)
S. epidermidis	98 (46.2)	45 (39.4)	16 (55.1)	37 (53.6)
S. haemolyticus	42 (19.8)	38 (33.3)	1 (3.4)	3 (4.3)
S. aureus	19 (8.9)	6 (5.2)	2 (6.9)	11 (15.9)
S. simulans	12 (5.7)	0 (0.0)	2 (6.9)	10 (14.5)
S. hominis	7 (3.3)	4 (3.5)	1 (3.4)	2 (2.9)
S. cohnii	5 (2.3)	4 (3.5)	1 (3.4)	0 (0.0)
S. warneri	5 (2.3)	1 (0.8)	2 (6.9)	2 (2.9)
S. saprophyticus	2 (0.9)	2 (1.7)	0 (0.0)	0 (0.0)
S. capitis	2 (0.9)	0 (0.0)	2 (6.9)	0 (0.0)
Staphylococcus sp.	12 (5.7)	6 (5.2)	2 (6.9)	4 (5.8)
M. luteus	5 (2.3)	5 (4.3)	0 (0.0)	0 (0.0)
M. varians	3 (1.4)	3 (2.6)	0 (0.0)	0 (0.0)
All isolates	212	114 (53.7)	29 (13.6)	69 (32.5)

Staph system has been tested by different researchers (4, 14, 25) after the pioneer work of Brun et al. (6), with results comparable to those found in this study.

Sometimes species identification is not sufficient (8, 10). Sewell et al., in a study of CNS of clinical significance, found that 93% of the strains were *S. epidermidis* (28). It is important to have a strain fingerprint of epidemiological value to differentiate among these isolates and among the nonspeciated staphylococci. The API Staph is interesting in this respect.

If one assumes that identification obtained by conventional biochemical tests according to the method of Kloos and Schleifer were correct (there is approximately a 2% chance of error according to DNA hybridization [23]), discrepancies occurred in 12 cases with API Staph (6.3%) among the bacteria identified by this method. We can postulate that the species identification of unidentified strains by Kloos and Schleifer would be correct, but the verification would involve DNA hybridization with prototype strains.

Several studies found a predominance of *S. saprophyticus* and, to a lesser extent, *S. simulans* in urine samples under pathogenic conditions and mainly in young women, with 23% *S. saprophyticus* and 7% *S. simulans* (1), 9% *S. saprophyticus* and 12% *S. simulans* (11), 11% *S. saprophyticus* and 6% *S. simulans* (15), and 17% *S. saprophyticus* and 2% *S. simulans* (26). Hovelius et al. in a study of men with urethritis, found *S. saprophyticus* in 20% of the patients versus 7.1% in the control group (18), which is contradictory to our results. They also described recently an association between *S. saprophyticus* and urinary tract infection of elderly men, the majority with indwelling catheters (17).

These observations raise the question of the possible reservoir for *S. saprophyticus*. In 1966, Cox found staphylococci in 22 normal female urethras among 52 studied, but he did not differentiate the strains, named *S. albus*, except by hemolysis (9). In a study of the periurethral flora of 160 women, aged 18 to 38, Bollgren et al. found results similar to ours, in which a variety of species, in particular 12.5% *S. aureus* and 1.9% *S. simulans*, were observed with a predominance of *S. epidermidis* at a frequency lower than usually found in general studies of CNS and in the absence of *S. saprophyticus* (3). Our results and those of Bollgren et al. are not in agreement with the hypothesis of Hovelius and Mårdh that the periurethral and urethral flora of women could be the reservoir for *S. saprophyticus* (17). The possibility of an alimentary origin for this bacterium and of a gastrointestinal reservoir must be considered. Coagulase-negative, novobiocin-resistant staphylococci have been found more frequently in the skin flora of animals than of humans and in meat and milk (24). Additional work is needed in this direction.

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