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Identification of *Micrococcaceae* in Clinical Bacteriology

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The cellular morphology, identifying physiological characteristics, and a key to the human genera of *Micrococcaceae* are presented with flow charts for identification of aerobic and anaerobic isolates. These flow charts can be amended as desired, depending upon the degree of accuracy desired. *Micrococcaceae* isolates in a 350-bed private general hospital during a 15-week period are tabulated to show relative numbers of the different genera and species, with their probable relationship to infection or contamination. Only 11 of the 220 *Micrococcaceae* isolates were not *Staphylococcus*; no *Sarcina* or *Peptococcus* were isolated. Of the *Staphylococcus* isolates, 61% were *S. epidermidis*. Almost 18% of the *S. aureus* isolates were coagulase-negative. Of the *S. aureus* isolates, 80% of the coagulase-positive isolates were infecting agents, as were 67% of the coagulase-negative *S. aureus* isolates, compared to only 48% of *S. epidermidis* isolates. Two of four *Gaffkya* isolates but only one of seven *Micrococcus* isolates were infecting agents. If coagulase production is used as the sole criterion for speciation of staphylococci, and *Micrococcus* is not differentiated from *Staphylococcus*, the term "coagulase-negative staphylococci" does not differentiate three distinct levels of pathogenicity. Coagulase-negative *S. aureus* is more virulent than *S. epidermidis* or *Gaffkya*, which are more virulent than *Micrococcus* or *Sarcina*.

The family *Micrococcaceae* consists of the genera *Micrococcus*, *Staphylococcus*, *Gaffkya*, *Sarcina*, and the obligately anaerobic *Methanococcus* and *Peptococcus*. *Methanococcus* consists of methane-producing saprophytes and is the only genus not reported from clinical material. *Micrococcaceae* are heterotrophic catalase-positive gram-positive cocci which usually divide in two or three planes to produce packets or masses, as opposed to streptococci, which are catalase-negative and usually divide in one plane to produce chains. Members of the *Micrococcaceae*, however, may occur singly or in pairs or even in short chains, and the Gram reaction varies from strong to weak (i.e., readily decolorized). No visible gas is produced by the aerobic species, although the anaerobic species may produce methane, carbon dioxide, or hydrogen.

The aerobic members of the family may be divided into two groups according to their nucleic acid base content (3, 19; A. E. Auletta and E. R. Kennedy, Bacteriol. Proc., p. 23, 1966). Group I consists of *Gaffkya* and *Staphylococcus*, which ferment glucose anaerobically and whose nucleic acid bases contain approximately 32% guanine plus cytosine. Members of Group II, consisting of *Micrococcus* and the aerobic sarcinae, are unable to ferment glucose anaerobically, and their guanine plus cytosine content is approximately 66%. Differentiation by nucleic acid base content is impractical in the clinical laboratory, but determination of glucose fermentation is easy and useful.

Baird-Parker (1, 2) reviewed and evaluated suggested classifications of *Micrococcus* and *Staphylococcus*, and from his own observations has proposed a classification of these genera based on physiological characteristics. Other criteria have been suggested (14, 15). Until some other official classification is accepted, however, that in *Bergey's Manual* is the one which should be used, and the information in this paper is based on that classification.

Staphylococcus. This is the best known of the genera clinically. These organisms may occur singly, in pairs, tetrads, or short chains, but usually occur in clusters. They are facultatively anaerobic and strongly gram-positive and catalase-positive. Terminology has changed several times over the years, but only two species are accepted at present (R. Hugh and M. A.

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Ellis, Bacteriol. Proc., p. 62, 1967). *S. aureus* includes the traditional yellow organisms, as well as the former "*S. citreus*" and colorless organisms. *S. epidermidis* includes the former "*S. albus*," as well as organisms with colorless colonies. Speciation is no longer acceptable on the basis of pigmentation, which appears to depend upon the fatty acid content of the medium (22), but is now being made on the basis of other physiological characteristics. Speciation of staphylococci is of considerable clinical importance, as traditionally *S. aureus* has been considered pathogenic and *S. epidermidis* nonpathogenic. That this is not always true is now well known; nevertheless, *S. aureus* is almost always more virulent than *S. epidermidis*, and the physician is rightly more concerned when *S. aureus* is reported. Therefore, it is important that the speciation be as accurate as possible.

Both species of staphylococci ferment glucose anaerobically, usually reduce nitrates to nitrites, and grow well in media containing 10% NaCl. Only *S. aureus*, however, ferments mannitol anaerobically and is coagulase-positive. Unfortunately, neither of these criteria is absolute: approximately 1% of *S. aureus* are coagulase-positive but do not ferment mannitol, and approximately 8% ferment mannitol but are coagulase-negative (12, 13).

If either criterion is positive, however, the organism is *S. aureus;* if both are negative, the organism is *S. epidermidis*. It must be remembered that some strains of *S. epidermidis* oxidize mannitol aerobically and that the proper criterion is anaerobic fermentation. This can be ascertained with a 1% mannitol agar stab, or, as recommended by the international Subcommittee on Taxonomy of Staphylococci and Micrococci (20), a mannitol agar stab tube incubated under paraffin seal. Mannitol salt agar incubated aerobically does not suffice (12). It is entirely possible that many serious infections reported to be caused by *S. epidermidis* were, in fact, caused by coagulase-negative *S. aureus*, as mannitol fermentation is not usually tested.

Although coagulase production alone is not an accurate criterion for speciation, it probably correlates better with virulence than does any other single characteristic (except perhaps mannitol fermentation). A *Staphylococcus* may also properly be considered virulent if any one of the following is positive: hemolysis, pigment, egg yolk precipitation, tellurite reduction, or production of gelatinase, deoxyribonuclease, or phosphatase. These characteristics correlate more or less well with coagulase production (4, 9, 11, 13, 15–18; P. A. Moskal, M. K. Moskal, and L. G. Herman, Bacteriol. Proc., p. 68, 1966). In addition, most coagulase-positive staphylococci are phage-typable, grow on nutrient agar at *p*H 8.5 (12), and do not require biotin (7, 10), in contrast to coagulase-negative staphylococci.

Gaffkya tetragena. This is isolated occasionally from clinical material, especially sputum and skin. It usually occurs as tetrads in the animal body and in media containing animal products (e. g., blood agar), but on nutrient agar the cells usually occur in pairs and irregular masses. Glucose is fermented anaerobically, but nitrates are not reduced; the latter is the only means of separating a nontetrad *Gaffkya* from *S. epidermidis*, and as nitrate reduction by staphylococci has been reported (5) to be weak and variable, it is not a satisfactory criterion.

Peptococcus. These organisms are part of the normal flora of the intestinal and genital tracts. They are isolated only rarely, from cases of puerperal sepsis and peritonitis. They occur in pairs, tetrads, or masses. Their biochemical characteristics vary with species, including their ability to ferment glucose, but all are obligate anaerobes and can be confused only with anaerobic species of *Sarcina*. *Peptococcus* species produce catalase (8), which distinguishes them from the more frequently isolated *Peptostreptococcus*.

Micrococcus. Species of this genus occasionally are isolated from clinical material, usually sputum, skin, and contaminated urine. They occur in masses but not in packets and tend to be smaller and to decolorize more easily than staphylococci. They do not ferment glucose anaerobically (4), which distinguishes them from *Staphylococcus* and *Gaffkya*.

Sarcina. These may be isolated from skin and mucous membranes, and may present a problem in identification. Glucose is not fermented anaerobically by the aerobic species, and the cells tend to decolorize more readily than staphylococci, but other characteristics vary with the species. Anaerobic sarcinae occur, and their differentiation from *Peptococcus* may be impossible since glucose fermentation is variable for both genera. Sarcinae tend to occur in packets of eight or more, but if they are in masses instead of packets, aerobic sarcinae are indistinguishable from *Micrococcus*, and anaerobic sarcinae are indistinguishable from *Peptococcus*. (The anaerobic species of *Sarcina*, however, are all described in *Bergey's Manual* as packet-formers.) Confusion of the aerobic genera (*Micrococcus* and *Sarcina*) is unimportant clinically, but confusion of the anaerobic genera (*Sarcina* and *Peptococcus*) would be unfortunate, for *Peptococcus* is much

more likely to be pathogenic than is *Sarcina*. The international Subcommittee may eventually resolve this problem: this Subcommittee has agreed (21) that the genus *Sarcina* should include only anaerobic species, differentiating it from *Micrococcus*. If *Sarcina* is also to include only packet-forming species, the differentiation of *Sarcina* and *Peptococcus* is also accomplished.

EXPERIMENTAL

All 220 *Micrococcaceae* isolated from 21 September to 31 December 1966 in the laboratory of a 350-bed private general hospital were identified and tabulated (Table 1) according to their probable virulence, that is, whether they were apparently the primary pathogens or probably represented contamination. The criterion for "infecting" was the organism's occurrence in substantial numbers either alone or predominating. No attempt was made to correlate results of culture with clinical symptoms because in our hospital there is very little indiscriminate culturing; the majority of our cultures were from patients with clinical symptoms compatible with bacterial infection. The category "skin," for example, was composed of cultures from skin lesions such as leg ulcers, infected abrasion, pustules, infected skin graft, nail infection, infected blister, and burn infection.

All cultures were done in the usual manner; we were not looking specifically for staphylococci. All specimens were inoculated onto rabbit blood agar, EMB Agar, azide blood agar, and MacConkey Agar. All specimens except throat swabs and sputum were also inoculated into brain broth. All specimens from spinal fluid, eyes, and genital tract were also inoculated onto Thayer-Martin medium which was incubated in 5% carbon dioxide. All intestinal tract specimens, including gallbladder and appendix, were also inoculated into GN (Difco) selective enrichment broth and Brilliant Green and Bismuth Sulfite Agars. During the period of this survey, in addition to the *Micrococcaceae* isolated, many other pathogens and contaminants were isolated.

Of all *Micrococcaceae* isolated, 58.1% were judged to be causing infection. Almost 18% of *S. aureus* isolates were coagulase-negative. The majority (77.4%) of *S. aureus* isolates were judged to be the cause of infections, but 15.4% of the infection-causing *S. aureus* isolates were coagulase-negative. Coagulase-negative *S. aureus*

TABLE 1. *Micrococcaceae isolates*

Source	*Staphylococcus aureus*				*Staphylococcus epidermidis*		*Gaffkya*		*Micrococcus*		Total
	Coagulase-positive		Coagulase-negative								
	I[a]	C[a]	I	C	I	C	I	C	I	C	
Urine											
Catheterized	3	0	0	0	6	4	1	0	1	1	16
"Clean voided"	6	3	6	2	25	28	1	1	0	2	74
Wounds	20	0	2	0	16	7	0	0	0	1	46
Sputum	4	4	0	1	0	9	0	0	0	0	18
Throat	5	4	0	0	1	2	0	0	0	0	12
Skin	8	0	0	0	2	1	0	0	0	0	11
Genital tract	0	0	0	0	2	4	0	1	0	1	8
Stool	2	3	0	0	0	2	0	0	0	0	7
Nose	1	0	0	1	0	3	0	0	0	0	5
Eye	0	0	0	0	2	2	0	0	0	0	4
Abscess	2	0	0	1	1	0	0	0	0	0	4
Synovial fluid	1	0	0	0	1	1	0	0	0	0	3
Chest	1	0	2	0	0	0	0	0	0	0	3
Peritoneum	2	0	0	0	0	0	0	0	0	0	2
Bile	0	0	0	0	1	1	0	0	0	0	2
Ear	0	0	0	0	1	1	0	0	0	0	2
Blood	0	0	0	0	1	0	0	0	0	0	1
Pilonidal cyst	0	0	0	0	1	0	0	0	0	0	1
Lung (autopsy)	0	0	0	0	0	0	0	0	0	1	1
Total	55	14	10	5	60	65	2	2	1	6	220

[a] I, infecting; C, contaminating.

isolates were almost as often the cause of infection (66.7%) as were coagulase-positive isolates (79.7%) and significantly more so than other coagulase-negative *Micrococcaceae* (46.3%). No *S. aureus* isolates, even coagulase-negative isolates, were found in wounds unless they were present either alone or predominating, although 30% of the *S. epidermidis* isolates in wounds were judged to be contaminants. *S. epidermidis*, the most common isolate, was judged to be infecting 48% of the time. Two of the four *Gaffkya* and only one of the seven *Micrococcus* isolates were infecting. No *Sarcina* or *Peptococcus* were identified in this 15-week period.

DISCUSSION

A key to the identification of the human *Micrococcaceae* (Fig. 1) permits the construction of flow charts for aerobic (Fig. 2) and anaerobic catalase-positive gram-positive cocci. These flow charts can be amended to serve the needs of any laboratory. If a report of "anaerobic *Staphylococcus*" serves the needs of the clinicians, then the Gram stain, catalase test, and oxygen requirements are all that need to be done. If desired, *Gaffkya* and *S. epidermidis* may be differentiated on the basis of morphology alone, as may be *Sarcina* and *Micrococcus;* by this method some *Gaffkya* and some *Sarcina* will be misidentified, but this is rarely if ever of clinical importance. On the basis of the results of this survey, the use of the following modified scheme of identification seems justified:

(i). The first day, catalase and slide coagulase tests are done. All slide coagulase-positive *Micrococcaceae* are reported as "*Staphylococcus aureus*, coagulase-positive."

(ii). All catalase-positive, slide coagulase-negative gram-positive cocci are inoculated into 1% mannitol and 1% glucose tryptic soy agar (Difco) stab tubes with phenol red as the indicator, and a tube of dehydrated rabbit coagulase plasma (Hyland Laboratories, Los Angeles, Calif.). The preliminary report for these organisms is "Staph_____, coagulase_____." The tube coagulase test is checked at 2–4 hr, and all three tube tests are read at 20–24 hr.

(iii). If the tube coagulase test is positive, the report is completed to read "*Staphylococcus aureus*, coagulase-positive."

(iv). If the tube coagulase test is negative but the mannitol agar is fermented (at the bottom of the tube), the report is completed to read "*Staphylococcus aureus*, coagulase-negative."

I. Aerobic, microaerophilic, or facultatively anaerobic:	
A. Glucose fermented anaerobically and/or	
1. Mannitol fermented anaerobically: coagulase produced	*S. aureus*
2. Mannitol not fermented anaerobically; coagulase not produced:	
a. Nitrates reduced; no tetrads	*S. epidermidis*
b. Nitrates not reduced; may be tetrads	*Gaffkya*
B. Glucose not fermented anaerobically; readily discolorized:	
1. Cells in packets	*Sarcina*
2. Cells in masses	usually *Micrococcus* (rarely *Sarcina*)
II. Obligately anaerobic:	
A. Cells in packets	*Sarcina*
B. Cells singly, in pairs, tetrads, short chains, or irregular groups	*Peptococcus*

FIG. 1. *Key to the identification of human Micrococcacaee.*

I. Slide coagulase-positive	*S. aureus*
II. Slide coagulase-negative:	
A. Tube coagulase-positive	*S. aureus*
B. Tube coagulase-negative:	
1. Anaerobic mannitol-positive	*S. aureus*
2. Anaerobic mannitol-negative:	
a. Anaerobic glucose-positive:	
1. NO_3-reduced; no tetrads	*S. epidermidis*
2. NO_3-negative; may be tetrads	*Gaffkya*
b. Anaerobic glucose-negative; readily decolorized:	
1. Cells in packets	*Sarcina*
2. Cells in masses	probably *Micrococcus* (rarely *Sarcina*)

FIG. 2. *Key to identification of human aerobic Micrococcaceae.*

(v). If both tube coagulase and mannitol agar stab are negative, but the glucose agar stab is positive, the report is completed to read "*Staphylococcus epidermidis*, coagulase-negative," unless the cells are in tetrads, in which case the correction is made that "The '*Staphylococcus*' is *Gaffkya*."

(vi). If the glucose agar stab is also negative, the report is corrected to read "The '*Staphylococcus* is *Micrococcus*," unless the cells are in packets, in which case "The '*Staphylococcus*' is *Sarcina*."

This scheme gives our physicians four choices instead of the usual two ("coagulase-positive *Staphylococcus*" or "coagulase-negative *Staphylococcus*"). It takes into account the fact that coagulase-negative *S. aureus*, while less apt to be virulent than is coagulase-positive *S. aureus*, is more apt to be virulent than is *S. epidermidis*, which in turn is more apt to be virulent than is *Micrococcus*. On the other hand, *Gaffkya* is isolated so rarely that apparently there is no justification for using the nitrate reduction test, especially as *Gaffkya* appears to have the same ratio of infection-to-contamination as *S. epidermidis*. Similarly, anaerobic *Micrococcaceae* are reported as "anaerobic staphylococci (*Peptococcus*)" unless the cells are in packets, in which case the report is "anaerobic *Sarcina*."

For the bacteriology laboratory reports to be most useful to the clinician, they must give him information as accurate as possible in the shortest possible time. Clinically, the physician is much more interested in the antibiogram than in the accurate identification of the organism, but identification may be important from the standpoints of prognosis and intensity of therapy, as well as epidemiologically. Because of the well-known close correlation of coagulase production with virulence, the use of coagulase production as the only criterion for speciation of staphylococci is common practice. Similarly, many laboratories call all aerobic coagulase-negative *Micrococcaceae* "coagulase-negative *Staphylococcus*." This is not only inaccurate but it may mislead the physician.

The ability to ferment glucose anaerobically, the accepted criterion for separating *Staphylococcus* and *Gaffkya* from *Micrococcus* and *Sarcina*, "shows a gradual transition in a series of strains and lacks precision as a criterion for differentiation" (6) and does not correlate perfectly with deoxyribonucleic acid base ratios due to differences in both speed and strength of production of acid (15). It may, therefore, be impossible to differentiate *Staphylococcus* and *Micrococcus* with certainty on the basis of anaerobic fermentation of glucose, but it is the best criterion available, and the physician should have whatever benefit accrues from the attempt. He should know whether "*S. aureus*" means only coagulase-positive *S. aureus*, and whether "coagulase-negative *Staphylococcus*" or "*S. epidermidis*" includes *Micrococcus* and coagulase-negative *S. aureus*. This knowledge may affect his treatment of the patient.

The physician depends upon the laboratory to be accurate, not to decide for him whether or not it is important to be accurate. The ultimate academic goal is complete accuracy in the same length of time required for an educated guess, and the scheme presented here increases accuracy of identification without delaying the report. The time and media required for complete accuracy may be wasted; clinical expediency may suffice, but this decision should not be made without the knowledge of the physician caring for the patient.

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