Comparison of techniques for distinguishing staphylococci and micrococci

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In the past a variety of tests have been proposed for the differentiation of the genus *Staphylococcus* from *Micrococcus*. The most fundamental division between these two genera of Gram-positive cocci is in the DNA base composition: staphylococci have a guanine and cytosine (GC) content of about 30 to 40% and micrococci of about 50 to 75% (Kocur, Bergan, and Mortensen, 1971). The determination of the percentage GC content is, however, too tedious and time consuming for epidemiological work involving many specimens. Other methods used have consisted mainly of demonstrations of the ability of *Staphylococcus* species to grow and ferment glucose anaerobically; *Micrococcus* species do not have this ability; they are oxidative.

The principal taxonomic scheme for the *Micrococcaceae* is that of Baird-Parker (1963, 1965); this scheme uses the ability of *Staphylococcus* species to ferment glucose anaerobically and has been the basis of epidemiological studies in this department. During the course of these studies other methods, principally the thioglycollate medium of Evans and Kloos (1972) and the novobiocin sensitivity method of Jeffries (1968), were also used. This paper reports a comparison of these methods.

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Materials and Methods

Strains of Gram-positive, catalase-positive, coagulase-negative cocci isolated during epidemiological studies were used. *Staphylococcus aureus* phage propagating strains were used as control 'positive' organisms and Sarcina (tetrad forming, nonfermentative, aerobic cocci with a typical colony and yellow pigment) were used as 'negative' controls. Anaerobic glucose medium was prepared according to Baird-Parker (1963), thioglycollate according to Evans and Kloos (1972), and novobiocin sensitivity tests were carried out according to Jeffries' disc method on DST agar (Oxoid).

Results

The results are shown in table I. It can be seen that novobiocin was of no value in this series of strains; no explanation can be found. There was good agreement between the other two methods for strains classified as staphylococci (SII-SVI) by Baird-Parker's technique but not for those classified as micrococci (M1-M7). The distribution of strains from various surgical lesions was changed by substituting the results obtained from the thioglycollate medium of Evans and Kloos for the anaerobic glucose medium of Baird-Parker; the percentage of M3 strains was reduced but the differences are not statistically different (table II). M3 strains were obtained significantly more frequently from sites below the waist when grouped according to Baird-Parker ($P < 1\%$, $\chi^2 = 10{\cdot}3$) but not when grouped according to Evans and Kloos ($P > 5\%$).

Results According to Baird-Parker Scheme	*Anaerobic Growth (Evans and Kloos, 1972)*		*Novobiocin*[1]		*Total Strains Tested*
	Staphylococcus	*Micrococcus*	*Sensitive*	*Resistant*	
Staph. aureus	23	0	22	1	23
SII	123	2	115	10	125
SIII	9	0	7	2	9
SIV	1	0	1	0	1
SV	14	0	14	0	14
SVI	30	4	34	0	34
M1	14	1	15	0	15
M2	16	5	21	0	21
M3	11	19	29	1	30
M4	0	1	1	0	1
M5	5	4	8	1	9
M6	0	1	0	1	1
Sarcina	0	18	17	1	18

Table I *Distinguishing staphylococci from micrococci*

[1]Jeffries (1968) found that staphylococci and M7 were sensitive whilst micrococcus species M1 to M6 were resistant.
S = Staphylococcus M = Micrococcus

Organism	*Percentage Distribution by Site*							
	Abdomen		*Leg*		*Perineum*		*Other*	
	Anaerobic Glucose Medium							
	Baird-Parker[1]	*Evans and Kloos*	*Baird-Parker*	*Evans and Kloos*	*Baird-Parker*	*Evans and Kloos*	*Baird-Parker*	*Evans and Kloos*
SII	53	57	34	41	42	48	51	56
SIII	4	4	3	3	2	2	4	4
SIV	1	4	0	9	0	0	0	11
SV	3	3	6	3	6	4	7	9
SVI	17	16	6	22	20	28	7	11
M1	5	0	9	0	4	2	6	0
M2	6	3	9	3	4	0	12	2
M3	7	10	22	13	20	10	7	6
M4	1	1	0	0	0	4	0	0
M5	1	1	9	6	0	0	6	0
M6	0	0	0	0	0	0	1	1
Total strains	94		32		49		85	

Table II *Distribution of strains according to sites of lesions*

[1]Baird-Parker = Anaerobic glucose according to Baird-Parker classification.
[2]Evans and Kloos = Anaerobic glucose (thioglycollate) medium of Evans and Kloos.
S = Staphylococcus
M = Micrococcus

Comment

Evans and Kloos reported their thioglycollate medium to give results which more closely approached the percentage GC content than did others. In the present series the *Staphylococcus aureus* and *Sarcina* sp gave results according to expectation. *St. epidermidis* strains (Baird-Parker's SII, III, IV, V, VI) gave agreement in all except six (3%) strains. The equivocal groups were those identified as *Micrococcus saprophyticus* (M1, 2, and 3) and *M. lactis* (M5 and 6) according to Baird-Parker, for over 60% of these strains were *Staphylococcus* species using the medium of Evans and Kloos (1972). This is not surprising for it has long been known that such strains, whilst lacking or having a poor ability to ferment glucose anaerobically, may nevertheless have a low GC value (Silvestri and Hill, 1965; Auletta and Kennedy, 1966). This survey has identified S II and S VI as the most common strains in wounds with M 3 strains common in lesions of the legs and perineum but not of the abdomen or other sites. This is in agreement with the studies of others. Holt (1969) found S II to be the organism responsible for infection of ventriculoatrial shunts in babies with hydrocephalus. Coagulase-negative staphylococci isolated from blood stream infections are also of this type (Rames, Wise, Goodman, and Piel, 1970). Urinary tract infection in patients outside the hospital environment is most frequently with type M 3 (Mitchell, 1968). Few other studies have been reported of complete characterization of cocci from surgical lesions.

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