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# The cultural and biochemical characters of *Streptococcus milleri* strains isolated from human sources

BY LYN C. BALL AND M. T. PARKER
*Cross-Infection Reference Laboratory, Colindale, London NW9 5HT*

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SUMMARY

A collection of 346 strains of *Streptococcus milleri* from a variety of human sources was examined culturally and biochemically, and for the presence of Lancefield group antigens. Most of the strains were non-haemolytic and ungroupable, but 25% were $\beta$ haemolytic and 19% were $\alpha$ haemolytic; 28% possessed a group antigen (A, 5%; C, 6%, F, 14%, G, 3%). These antigens were present in 69% of $\beta$-haemolytic but in only 13% of $\alpha$-haemolytic or non-haemolytic strains; $\beta$ haemolysis occurred in 82% of group-F strains, 43% of other groupable strains and 11% of ungroupable strains.

The following reactions were given by > 80% of *S. milleri* strains: hydrolysis of arginine and aesculin, a positive Voges–Proskauer reaction, and acidification of trehalose, lactose, salicin and sucrose. A minority of strains showed enhancement of growth by $CO_2$, bile tolerance, NaCl tolerance, and ability to acidify other sugars, notably mannitol, raffinose and melibiose. Departures from the modal pattern of biochemical reactions showed a weak correlation with the type of haemolysis and the presence or absence of a group antigen but were not sufficiently systematic for clear-cut subdivisions to be recognized within the species.

*S. milleri* therefore appeared to comprise a 'central' group of non-haemolytic strains that rarely formed a Lancefield-group antigen, in which the aesculin reaction was nearly always positive, lactose was usually acidified, and a considerable minority showed enhancement of growth by $CO_2$ and bile tolerance. Deviations from this pattern were of two main types. (1) 'Loss' of one or more of these reactions, which tended to be associated with $\beta$-haemolysis and presence of a group antigen. In these respects, $\alpha$-haemolytic strains tended to occupy an intermediate position. (2) 'Gain' of the ability to acidify additional sugars, notably raffinose and melibiose or mannitol; this occurred mainly among otherwise typical non-haemolytic strains that were rarely groupable.

Only 12% of isolations from the bloodstream of patients suffering from systemic infections were $\beta$ haemolytic and only 18% possessed a group antigen, but a considerably greater proportion of those from visceral abscesses were $\beta$ haemolytic (28%). Among isolations from superficial lesions in some body sites there were considerably greater proportions of $\beta$-haemolytic and groupable strains; thus, nearly one-half of those isolated from the abdomen other than the female genital tract were $\beta$-haemolytic and over one-half were groupable. On the other hand, strains from the teeth and gums were nearly always non-haemolytic and

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ungroupable, and most vaginal isolations were of non-haemolytic strains with a wide sugar-fermentation pattern.

## INTRODUCTION

Guthof (1956) proposed the name *Streptococcus milleri* for a group of non-haemolytic streptococci isolated from dental abscesses and other inflammatory lesions in or around the mouth, and characterized by uniform cultural and biochemical reactions and the absence of a Lancefield group antigen. Ottens & Winkler (1962) examined non-haemolytic streptococci from the dental root-canal, and found that a number of them possessed the Lancefield-group antigen C, F or G. These workers recognized that the non-haemolytic members of groups C and G differed from the familiar 'pyogenic' $\beta$-haemolytic members of these groups, and also detected the type antigens of the group-F streptococcus (Bliss, 1937) in many of the non-haemolytic streptococci of the dental root-canal, whether or not a group antigen was present. Colman & Williams (1965, 1972; see also Colman, 1968) compared representative strains from Guthof (1956) and Ottens & Winkler (1962) with minute-colony forming $\beta$-haemolytic streptococci of groups F and G (Long & Bliss, 1934; Bliss, 1937) and with the type strain of *Streptococcus* MG (Mirick *et al.* 1944). They concluded that all of them should be included in the species *S. milleri*, but characterized it by a somewhat different set of biochemical characters from those given by Guthof (1956), by the sugar components in the cell wall, and by the frequent possession of one of a series of antigens that included the type antigens of group-F streptococci (the so-called Ottens antigens). Only a minority of the strains were $\beta$-haemolytic or had a Lancefield-group antigen; when this was present it might be the group-A, -C, -G, or -F polysaccharide.

Liu (1954) and Diebel & Niven (1955) observed that the growth of some strains of group-F streptococci was enhanced by $CO_2$. It was, however, not generally realized until quite recently that this character was shared by many other *S. milleri* strains. The fact that a number of them grew poorly or not at all on solid media in air, but grew more profusely in an anaerobic jar, led to their being described as 'micro-aerophilic' or even 'anaerobic' streptococci.

In a study of the streptococci (other than pneumococci) associated with systemic disease in man (Parker & Ball, 1976) we drew attention to the fact that *S. milleri* was the most common cause of streptococcal abscesses in internal organs. This appeared to be the first general account of the role of this organism as a cause of serious disease in man, though there are records of single cases of abscess formation in viscera and of purulent meningitis due to $\beta$-haemolytic, groupable streptococci that can in retrospect be allocated to the species.

There has been some reluctance among medical microbiologists to the inclusion, in a single species, of streptococci that differ widely in characters they have been accustomed to use for the primary subdivision of the streptococci (haemolysis, presence of Lancefield-group antigen and ability to grow in air). We now report on the examination of a larger series of *S. milleri* cultures, reconsider the evidence for including them in the one species, and discuss the difficulties in differentiating them from the 'pyogenic' streptococci of Lancefield's groups A, C and G.

Table 1. *Origin of* 346 *strains of* S. milleri *from human sources*

| Source | No. of strains | |
|---|---|---|
| (A) Blood, or internal organs* | | |
| Blood: 'endocarditis'† | 36 | |
| Blood: febrile illness‡ | 48 | |
| Purulent lesion§ | 90 | |
| Blood: bacteriaemia after dental extraction | 9 | |
| Blood: no clinical history | 17 | |
| Subtotal | | 200‖ |
| (B) Other | | |
| Mouth or respiratory tract | 41 | |
| Vagina | 28 | |
| Other abdominal | 55 | |
| Limbs | 14 | |
| Other or unspecified | 8 | |
| Subtotal | | 146¶ |
| Total | 346 | |

* In abdomen, thorax, central nervous system or joint.
† Clinical diagnosis: endocarditis 26; probable endocarditis 10.
‡ Clinical evidence of purulent lesion in internal organs: 16.
§ At operation 78; at necropsy 12.
‖ From 199 patients.
¶ From 145 patients.

## MATERIALS AND METHODS

### *Streptococci*

We examined 346 cultures of *S. milleri*, isolated from 344 patients, that had been sent to us for identification in the years 1970–6. On many occasions two or more isolations from the same patient had been sent, but only one of these was included unless members of two Lancefield groups were present; if some of the cultures were groupable and others ungroupable, a single groupable culture was included.

The 200 isolations from the blood or an internal organ (Table 1) included 81 that had been isolated in Britain in 1972–4 (Parker & Ball, 1976), a further 116 British strains received before 1972 or in 1975–6 and three strains from other countries. The clinico-pathological definitions of diseases in the patients from whom the streptococci had been isolated are those used by Parker & Ball (1976); of 174 cultures associated with specified systemic infections, 36 were from the blood of patients suffering from definite or suspected endocarditis, 48 from the blood of patients with fever and bacteraemia (in 16 of whom there was clinical evidence of a purulent lesion in an internal organ), and 90 from a purulent lesion in an abdominal or thoracic viscus, a joint, or the central nervous system.

In addition to these, there were 146 cultures from lesions that were open to the body surface or in superficial soft tissues, or from carrier sites. Among the 41 oral and respiratory-tract cultures there were 22 from the teeth or gums of patients who were undergoing or had recently undergone dental extraction (Phillips *et al.* 1976); the remainder comprised miscellaneous cultures from the throat, paranasal

sinuses or tissues around the upper respiratory tract. Cultures from abdominal sites other than the vagina were mainly from wound swabs taken after operations on the bowel, or from inflamed appendices, ischiorectal abscesses or bile. The isolations from the limbs were from a variety of superficial septic infections or ulcers, but two were from abscesses associated with a hip prosthesis.

This collection of *S. milleri* strains formed a part of a larger series of 1826 streptococcal strains that had been examined by similar methods. The results obtained with a selection of these will be quoted for comparison.

### *Bacteriological methods*

These were described by Parker & Ball (1976). The following tests were made on all the strains. Broth cultures were examined microscopically and for catalase production, and cultures on blood agar for the amount of growth and the type of haemolysis after incubation aerobically, anaerobically and in air with the addition of $CO_2$. Until the end of 1974 the effect of $CO_2$ on growth was investigated by growth in a candle jar; in the last two years of the study a $CO_2$ incubator was used ($CO_2$ 5 %, v/v). A 'requirement' for $CO_2$ was recorded when visible colonies appeared on blood-agar plates after incubation for 48 h in the presence of added $CO_2$ but not in its absence; 'enhancement' by $CO_2$ was recorded when larger colonies appeared in the presence of $CO_2$ than in its absence, or when there was evidence of 'requirement'.

Lancefield-grouping tests were made with sera for groups A–H and K–S. Other tests used were: for growth in the presence of 40 % and 10 % bile, 6·5 % and 4·0 % NaCl, at 45 °C and at pH 9·6; for survival at 60 °C for 30 min; for the hydrolysis of arginine and aesculin; for the production of dextran or laevan from sucrose; the Voges–Proskauer (VP) reaction; and for acid production from trehalose, lactose, salicin, sucrose, mannitol, raffinose, inulin, melibiose, melezitose, sorbitol, arabinose and dulcitol.

All members of Lancefield-group A were tested for sensitivity to bacitracin with a 0·1 unit disk; resistance was indicated by an inhibition zone of diameter $< 12$ mm around a paper disk of diameter 6 mm.

## RESULTS

### *Cultural and biochemical characters of* S. milleri

The streptococci classified as *S. milleri* formed small colonies on blood agar (usually $< 0·5$ mm in diameter at 18 h) even in the presence of added $CO_2$ and usually possessed the following biochemical characters: hydrolysis of arginine and aesculin, a positive VP reaction, and acid production from trehalose, lactose, salicin and sucrose; other reactions were either usually negative or variable (see below).

### *Haemolysis and presence of a Lancefield-group antigen*

Of the 346 cultures 87 (25 %) were $\beta$ haemolytic, 67 (19 %) were $\alpha$ haemolytic and 192 (56 %) were non-haemolytic; only 94 (27 %) of the total were groupable

Table 2. *Haemolysis and production of Lancefield-group antigens by cultures of* S. milleri

| Grouping reaction | No. of strains that were non-haemolytic | α haemolytic | β haemolytic | tested |
|---|---|---|---|---|
| None | 166 | 59 | 27 | 252 |
| A | 7 | 3 | 7 | 17 |
| C | 8 | 4 | 8 | 20 |
| F | 9 | 0 | 41 | 50 |
| G | 2 | 1 | 4 | 7 |
| Performed | 192 | 67 | 87 | 346 |

Table 3. *Other cultural and biochemical characters of* 346 *cultures of* S. milleri

| Character | % Frequency of the stated character | Character | % Frequency of the stated character |
|---|---|---|---|
| $CO_2$: requirement | 20 | Acid from | |
| $CO_2$: enhancement | 36 | trehalose | 90 |
| Bile tolerance: 40 % | 16 | lactose | 82 |
| Bile tolerance: 10 % | 22 | salicin | 95 |
| NaCl tolerance: 6·5 % | 6 | sucrose | 97 |
| NaCl tolerance: 4·0 % | 13 | mannitol | 4 |
| Growth at 45 °C | 9 | raffinose | 11 |
| Growth at pH 9·6 | 1 | inulin | 2 |
| Arginine hydrolysis | 95 | melibiose | 11 |
| Aesculin hydrolysis | 81 | melezitose | 2 |
| Voges–Proskauer reaction | 93 | | |

All cultures grew in air with the addition of $CO_2$; none survived 60 °C for 30 min or acidified sorbitol and arabinose; one culture failed to form chains in broth, one gave a weakly positive catalase reaction, one acidified dulcitol, and one formed dextran from sucrose.

(group A 5 %, group C 6 %, group F 14 % and group G 2 %). The majority (225 strains; 65 %) were not β haemolytic and were ungroupable; of the rest, 60 (17 %) were β haemolytic and groupable, 34 (10 %) were not β-haemolytic but were groupable, and 27 (8 %) were β haemolytic but ungroupable (Table 2). No streptococcus classifiable as *S. milleri* possessed a group antigen other than A, C, F or G, and all the group-F streptococci in the 1826 human streptococcal strains of which the present collection forms a part were considered to belong to the species *S. milleri*.

There was a tendency for β haemolysis and presence of a group antigen to be associated; thus, 60 of 87 β-haemolytic strains (69 %), but only 34 of 259 strains that did not exhibit β haemolysis (13 %), possessed the antigen A, C, F or G. Over one-half of all groupable strains were members of group F, in which β haemolysis predominated (41 of 50; 82 %) and none was α-haemolytic; but β-haemolysis was also more frequent among other groupable strains (19 of 44; 43 %) than among non-groupable strains (27 of 252; 11 %).

*Other characters*

As in most other streptococcal taxa, few of the cultural or biochemical characters of *S. milleri* strains were entirely uniform (Table 3). All except one of the cultures formed chains in broth, and only one gave a positive catalase reaction. None failed to grow in air with the addition of $CO_2$. Enhancement of aerobic growth by the addition of $CO_2$ was observed in 36 % of the cultures, and requirement for added $CO_2$ in 20 %. In 1974 we performed extensive parallel tests of the growth of *S. milleri* strains in a candle jar and in the $CO_2$ incubator, and obtained virtually identical results. These satisfied us that growth was enhanced by the presence of the gas and not by reduction of oxygen tension or increased humidity in the candle jar. Separate analysis of results obtained before the end of 1974 and subsequently gave similar percentages of *S. milleri* strains that showed enhancement by $CO_2$. All tests with $CO_2$ were done on receipt of the cultures, but some of the cultures had been subcultured several times before we received them.

Of the 'Sherman' characters (Sherman, 1938), survival at 60 °C for 30 min was invariably and growth at pH 9·6 almost invariably absent. Ability to grow at 45 °C and in 6·5 % NaCl were present in less than 10 % of strains, but considerable minorities exhibited tolerance for bile and for 4 % NaCl. Nearly as many grew in the presence of 40 % as of 10 % bile (16 % and 22 % of strains respectively). A characteristic finding was that when a strain grew poorly on 40 % bile agar it also grew poorly on 10 % bile agar.

The seven 'positive' characters – arginine and aesculin hydrolysis, the VP reaction, and acidification of trehalose, lactose, salicin and sucrose – were each present in more than 80 % of strains. Most of the departures from this common pattern occurred singly in strains that behaved characteristically in other respects, with the exception of an association between a negative aesculin test and failure to ferment lactose. A minority of strains acidified other sugars – notably raffinose, melibiose and mannitol; as we shall see, their other characters left little doubt that they should be included in the species. The single strain that formed dextran from sucrose on repeated testing was an otherwise typical $\beta$-haemolytic group-F streptococcus.

*Relation of haemolysis and the production of a Lancefield-group antigen to other characters*

Conformity to this general description by strains that exhibited different types of haemolysis or formed particular Lancefield antigens, or were ungroupable, is shown in Table 4.

On the whole, requirement for and enhancement by $CO_2$, bile tolerance, aesculin hydrolysis and the acidification of lactose, raffinose, melibiose, and possibly mannitol, were less common among $\beta$-haemolytic than non-haemolytic strains; in most of these respects $\alpha$-haemolytic strains resembled non-haemolytic strains or occupied an intermediate position. On the other hand, non-haemolytic strains were less often tolerant of 4 % NaCl than were $\beta$- and $\alpha$-haemolytic strains.

Differences between groupable and ungroupable strains were generally in the

Table 4. *Other characters of* S. milleri *cultures in relation to haemolysis and production of group antigen*

| Character | % Frequency of the stated character among | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | non-haemolytic strains (192)* | α haemolytic strains (67) | β-haemolytic strains (87) | un-groupable strains (252) | groupable strains (94) | group-A strains (17) | group-C strains (20) | group-F strains (50) | group-G strains (7) |
| $CO_2$: requirement | 26 | 20 | 9 | 23 | 11 | 0 | 15 | 14 | 0 |
| $CO_2$: enhancement | 40 | 37 | 28 | 39 | 28 | 24 | 40 | 22 | 43 |
| Bile tolerance: 40 % | 21 | 18 | 2 | 18 | 10 | 12 | 20 | 4 | 14 |
| Bile tolerance: 10 % | 30 | 22 | 6 | 24 | 17 | 24 | 30 | 10 | 14 |
| NaCl tolerance: 6·5 % | 6 | 3 | 10 | 6 | 9 | 0 | 5 | 14 | 0 |
| NaCl tolerance: 4·0 % | 9 | 18 | 16 | 13 | 13 | 6 | 10 | 18 | 0 |
| Growth at 45 °C | 8 | 12 | 8 | 9 | 9 | 6 | 10 | 8 | 14 |
| Growth at pH 9·6 | 2 | 1 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| Arginine hydrolysis | 94 | 96 | 94 | 94 | 95 | 100 | 90 | 94 | 100 |
| Aesculin hydrolysis | 87 | 79 | 68 | 83 | 74 | 71 | 90 | 66 | 100 |
| Voges–Proskauer reaction | 95 | 96 | 89 | 96 | 88 | 94 | 85 | 86 | 100 |
| Acid from | | | | | | | | | |
| trehalose | 90 | 88 | 93 | 89 | 87 | 100 | 85 | 82 | 100 |
| lactose | 90 | 96 | 52 | 87 | 66 | 76 | 100 | 44 | 100 |
| salicin | 95 | 94 | 94 | 95 | 94 | 100 | 90 | 92 | 100 |
| sucrose | 96 | 96 | 98 | 96 | 97 | 94 | 95 | 98 | 100 |
| mannitol | 7 | 1 | 0 | 5 | 1 | 0 | 0 | 2 | 0 |
| raffinose | 14 | 7 | 6 | 12 | 9 | 0 | 15 | 4 | 43 |
| inulin | 2 | 1 | 1 | 1 | 3 | 0 | 5 | 2 | 14 |
| melibiose | 14 | 10 | 3 | 13 | 5 | 0 | 10 | 4 | 14 |
| melezitose | 2 | 3 | 0 | 2 | 1 | 0 | 5 | 0 | 0 |

* In parentheses, number of cultures examined.

Table 5. *Cultural characters of* S. milleri *strains with wide fermentation patterns*

| | % with the stated character among strains with pattern no. | |
|---|---|---|
| | 1* (48 strains) | 2† (30 strains) |
| Haemolysis | | |
| None | 71 | 77 |
| $\alpha$ | 19 | 13 |
| $\beta$ | 10 | 10 |
| Group antigen | | |
| None | 81 | 80 |
| A | 0 | 0 |
| C | 8 | 7 |
| F | 6 | 7 |
| G | 4 | 7 |
| Require $CO_2$ | 25 | 27 |
| Enhanced by $CO_2$ | 46 | 50 |
| Bile tolerance: 40% | 15 | 10 |
| Bile tolerance: 10% | 17 | 13 |
| NaCl tolerance: 6·5% | 8 | 13 |
| NaCl tolerance: 4·0% | 12 | 20 |
| Arginine hydrolysis | 90 | 97 |
| Aesculin hydrolysis | 94 | 93 |
| Voges–Proskauer reaction | 96 | 97 |
| Acid from | | |
| trehalose | 90 | 90 |
| lactose | 98 | 100 |
| salicin | 100 | 100 |
| sucrose | 98 | 100 |
| mannitol | 29 | 43 |
| raffinose | 75 | 100 |
| inulin | 10 | 10 |
| melibiose | 78 | 97 |
| melezitose | 9 | 10 |
| dulcitol | 2 | 20 |

* Acid from mannitol, raffinose, inulin, melibiose, melezitose or dulcitol.
† Acid from raffinose and melibiose or mannitol, or both.

same direction but were smaller, and it was clear that the groupable streptococci did not form a homogeneous group. The lower frequency of lactose fermentation among groupable than among ungroupable strains was accounted for by the fact that only 44% of group-F streptococci but 91% of other groupable strains had this character. There was a similar but less striking difference between the frequency of aesculin hydrolysis by group-F streptococci (66%) than by other groupable streptococci (84%). The numbers of strains that belonged to each of the other Lancefield groups were too small to reveal any other patterns of characters associated with the presence of individual antigens.

The minority of strains that acidified sugars other than trehalose, lactose, salicin and sucrose were in most other respects characteristic members of the species (Table 5). However, only 10% of them were $\beta$-haemolytic, and they included

Table 6. *Other characters of* S. milleri *cultures in relation to requirement of* $CO_2$ *for growth and enhancement of growth by* $CO_2$

| Character | % Frequency of the stated character among cultures requiring $CO_2$ for growth (69)* | showing enhancement of growth† by $CO_2$ (125) | not showing enhancement of growth by $CO_2$ (221) |
|---|---|---|---|
| Haemolysis: | | | |
| None | 71 | 61 | 52 |
| α | 17 | 20 | 19 |
| β | 12 | 19 | 29 |
| Group antigen | | | |
| None | 86 | 80 | 69 |
| A | 0 | 3 | 6 |
| C | 4 | 6 | 5 |
| F | 10 | 9 | 18 |
| G | 0 | 2 | 2 |
| Bile tolerance: 40% | 7 | 8 | 20 |
| Bile tolerance: 10% | 9 | 10 | 29 |
| NaCl tolerance: 6·5% | 6 | 6 | 6 |
| NaCl tolerance: 4·0% | 9 | 12 | 13 |
| Growth at 45 °C | 0 | 2 | 13 |
| Growth at pH 9·6 | 3 | 3 | $< 0·5$ |
| Arginine hydrolysis | 93 | 94 | 95 |
| Aesculin hydrolysis | 77 | 80 | 81 |
| Voges–Proskauer reaction | 97 | 96 | 92 |
| Acid from | | | |
| trehalose | 84 | 87 | 92 |
| lactose | 81 | 80 | 82 |
| salicin | 90 | 91 | 96 |
| sucrose | 93 | 94 | 98 |
| mannitol | 9 | 7 | 2 |
| raffinose | 13 | 14 | 9 |
| inulin | 0 | 0 | 3 |
| melibiose | 16 | 15 | 8 |
| melezitose | 1 | 2 | 1 |

* In parentheses, number of cultures examined.
† Includes cultures that grew only in the presence of $CO_2$.

none of the group-A and few of the group-F strains. Among the 48 strains that acidified mannitol, raffinose, inulin, melibiose, melezitose or dulcitol (fermentation-pattern no. 1), the most frequent association was of acidification of raffinose and melibiose or mannitol (fermentation-pattern no. 2); of 30 strains with the latter pattern, 29 fermented melibiose and 13 fermented mannitol.

*Enhancement of growth by* $CO_2$

The *S. milleri* strains that grew better – or only – in the presence of added $CO_2$ did not differ greatly from the rest (Table 6). As expected (see Tables 4 and 5),

Table 7. *Haemolysis by and Lancefield-grouping reactions of strains of* S. milleri *from different sources*

| Source | No. of strains examined | No. of strains that were | | | No. of strains with grouping reaction | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | non-haemolytic | α-haemolytic | β-haemolytic | negative | A | C | F | G |
| Blood or internal organs | *200* | *116* | *44* | *40* | *162* | *7* | *10* | *17* | *4* |
| Blood: systemic disease* | 84 | 51 | 23 | 10 | 69 | 3 | 6 | 3 | 3 |
| Pus: systemic disease | 90 | 50 | 15 | 25 | 71 | 2 | 4 | 12 | 1 |
| Blood: other† | 26 | 15 | 6 | 5 | 22 | 2 | 0 | 2 | 0 |
| Other clinical material | *146* | *76* | *23* | *47* | *90* | *10* | *10* | *33* | *3* |
| Teeth or gums‡ | 22 | 12 | 9 | 1 | 16 | 1 | 3 | 2 | 0 |
| Other oral or respiratory | 19 | 5 | 2 | 12 | 8 | 2 | 4 | 4 | 1 |
| Female genital tract | 28 | 24 | 2 | 2 | 24 | 1 | 1 | 0 | 2 |
| Other abdominal | 55 | 22 | 6 | 27 | 26 | 1 | 2 | 26 | 0 |
| Limbs | 14 | 10 | 2 | 2 | 11 | 2 | 0 | 1 | 0 |
| Other or unknown | 8 | 3 | 2 | 3 | 5 | 3 | 0 | 0 | 0 |

* Febrile illness, 48; 'endocarditis', 36.
† No clinical history, 17; dental bacteriaemia, 9.
‡ See Phillips *et al.* (1976).

Table 8. *Sites of isolation of* S. milleri *strains with a wide fermentation pattern*

| | No. of *S. milleri* strains from the stated site | % of *S. milleri* strains from the stated site that had wide-fermentation pattern no.* | |
|---|---|---|---|
| | | 1 | 2 |
| Blood or internal organs | 200 | 7·5 | 3·5 |
| All other sites | 146 | 23 | 16 |
| Other sites in mouth or respiratory tract | 40 | 25 | 12·5 |
| Other sites in female genital tract | 28 | 68 | 61 |
| Other sites in abdomen | 55 | 7 | 2 |
| Other sites in limbs | 14 | 0 | 0 |
| Other than above, or not known | 8 | 0 | 0 |

* See Table 5.

a rather greater proportion of them were non-haemolytic and fermented 'extra' sugars, and a rather smaller proportion were $\beta$ haemolytic, groupable, and bile tolerant, than of cultures that grew as well with $CO_2$ as without it.

### *Site of isolation of* S. milleri *strains in relation to haemolysis, presence of Lancefield-group antigen, and other characters*

Table 7 gives the frequency of haemolysis and of the presence of Lancefield-group antigens among *S. milleri* strains isolated from the blood or internal organs of patients with systemic infections and from a variety of other more superficial sites. It shows that $\beta$ haemolysis and group antigens were infrequent among the 84 isolates from the blood of patients with febrile illnesses (12 % and 18 % respectively), and that $\beta$ haemolysis was considerably more common among the 90 cultures from visceral pus (28 %). Both $\beta$ haemolysis (32 %) and the presence of a group antigen (38 %) were much more common among the 146 cultures from 'other clinical material' (almost all collected from superficial sites) than among those from the blood. However, the subgroup of 22 cultures from 'teeth and gums' included very few $\beta$-haemolytic streptococci (4 %); these came from a study (Phillips *et al.* 1976) in which all colonial types of streptococci on the primary plates were subject to detailed examination. On the other hand, the 19 strains labelled 'other oral and respiratory', in which haemolytic strains predominated (63 %), had been submitted by colleagues interested in the identification of potential pathogens in cases of respiratory and oral infection. The 28 cultures from the female genital tract, mainly from patients with suspected local inflammation or about to undergo gynaecological operations, included only 7 % of $\beta$-haemolytic and 14 % of groupable streptococci. In contrast, the 55 'other abdominal' cultures, predominantly from wound infections and other local septic conditions, included 49 % of $\beta$-haemolytic, 53 % of groupable strains and 47 % of group-F streptococci.

The *S. milleri* strains with an unusually wide fermentation pattern (see Table 5) also appeared to be somewhat localized in their distribution (Table 8), particularly the strains that acidified raffinose and melibiose or mannitol (fermentation pattern

no. 2). These were found much less often among cultures from the blood and internal organs (3·5 %) than from superficial sites (16 %). Among the latter, they formed 61 % of isolations from the female genital tract, 12·5 % from the mouth and respiratory tract, but only 2 % from abdominal sites other than the female genital tract. Two of the three isolates from systemic diseases that followed a gynaecological operation or an abortion had this fermentation pattern.

## DISCUSSION

The streptococci that we have described as *S. milleri* conformed to the amended description of the species given by Colman & Williams (1972), but our strains, and those of Colman and Williams, differed from Guthof's (1956) original description in that only a minority of them grew at 45 °C or were bile resistant. Our methods of testing conformed closely to those of Colman (1970), and these two tests are ones in which small differences in technique greatly affect the results. For example, we used a blood-containing bile-aesculin 'ditch plate' to test for bile tolerance but Guthof (1956) used bile-blood agar. In our laboratory, both *S. milleri* and *S. mutans* appear to be bile resistant considerably less often on a 40 % bile-aesculin ditch plate containing ferric citrate than they do on a 40 % bile-agar plate made from the same nutrient-agar base (Sheena A. Waitkins, personal communication), though the results obtained with other streptococcal species are in close conformity.

The decisions to recognize *S. milleri* as an aggregate species of haemolytic and non-haemolytic streptococci, and to include in it a number of strains that form Lancefield-group antigens hitherto thought to characterize other well-recognized streptococcal taxa, have been convincingly argued by Colman & Williams (1965, 1972) but are not yet universally accepted. Bergey's Manual (Buchanan & Gibbons, 1974), for example, does not recognize the existence of *S. milleri*, but continues to apply the name *S. anginosus* to a species comprising Lancefield's group F and members of serotype 1 of group G. It holds that non-haemolytic and ungroupable strains 'known as *Streptococcus* MG' are distinct from *S. anginosus* despite similarities in physiological characters, and lists enhancement of growth by the addition of $CO_2$ as a diagnostic character of the latter.

The specific name *anginosus* was originally applied by Andrewes & Horder (1906) to haemolytic streptococci that are now difficult to identify from the description given. Smith & Sherman (1938) used the name for minute-colony-forming $\beta$-haemolytic streptococci of group G, and an earlier edition of Bergey's Manual (Breed, Murray & Smith, 1957) extended the definition to include similar group-F streptococci. Mirick *et al.* (1944) gave a good description of a non-haemolytic, ungroupable streptococcus that is recognizable in retrospect as *S. milleri*, but names it *Streptococcus* MG, unfortunately not an acceptable epithet under the rules of bacteriological nomenclature. Whether specific status should be accorded to the $\beta$-haemolytic strains or to strains possessing the group-F antigen, the group-F and -G antigens, or any Lancefield antigen will depend on the ease with which they can be demarcated from the rest of the *S. milleri* strains.

Table 9. *Cultural and biochemical characters of 'pyogenic' members of Lancefield-groups A, C and G*, and of strains of* S. milleri *with corresponding group antigens*

| Character | % with the stated character among 'pyogenic' members of group | | |
|---|---|---|---|
| | A (27 strains) | C (21 strains) | G (34 strains) |
| Haemolysis: | | | |
| None | 0 (41)† | 5 (40)‡ | 0 (29)§ |
| α | 0 (18) | 10 (20) | 6 (14) |
| β | 100 (41) | 86 (40) | 94 (57) |
| $CO_2$ requirement | 0 (0) | 0 (15) | 0 (0) |
| Enhanced by $CO_2$ | 0 (24) | 5 (40) | 0 (43) |
| Bile tolerance: 40% | 0 (12) | 0 (20) | 0 (14) |
| Bile tolerance: 10% | 7 (24) | 0 (30) | 0 (14) |
| NaCl tolerance: 6·5% | 41 (0) | 14 (5) | 21 (0) |
| NaCl tolerance: 4·0% | 70 (6) | 48 (10) | 53 (0) |
| Arginine hydrolysis | 100 (100) | 100 (90) | 100 (100) |
| Aesculin hydrolysis | 26 (71) | 29 (90) | 21 (100) |
| Voges–Proskauer reaction | 11 (94) | 0 (85) | 0 (100) |
| Acid from | | | |
| trehalose | 100 (100) | 90 (85) | 94 (100) |
| lactose | 100 (76) | 71 (100) | 65 (100) |
| salicin | 100 (100) | 86 (90) | 68 (100) |
| sucrose | 100 (94) | 95 (95) | 100 (100) |
| mannitol | 18 (0) | 0 (0) | 0 (0) |
| raffinose | 0 (0) | 10 (15) | 3 (43) |
| inulin | 4 (0) | 14 (5) | 3 (14) |
| melibiose | 4 (0) | 19 (10) | 6 (14) |

* From a study of 1826 streptococcal strains from human sources (unpublished).
† In parentheses: percentage among 17 *S. milleri* strains with group-A antigen.
‡ In parentheses: percentage among 20 *S. milleri* strains with group-C antigen.
§ In parentheses: percentage among 7 *S. milleri* strains with group-G antigen.

Our examination of 346 strains of *S. milleri* led us to the conclusion that subdivision of the species on the grounds of dissimilarity in cultural and biochemical characters was not possible. There was considerable variability in single characters and in small groups of characters, but little or no evidence of sharp lines of demarcation. There was a strong but far from invariable association between *β* haemolysis and the presence of a Lancefield-group antigen, particularly the group-F antigen, and a much weaker association of *β* haemolysis with indifference to $CO_2$, bile sensitivity, salt tolerance, and absence of attack on aesculin and lactose. The differences between groupable and ungroupable strains were in the same direction, but were smaller. The reactions given by members of individual Lancefield groups, though too small for definite conclusions to be drawn, appeared to be far from homogeneous and to deviate from the modal *S. milleri* pattern in different directions. Strains that acidified sugars other than trehalose, lactose, salicin and sucrose, on the other hand, conformed well in other respects to the modal pattern for non-haemolytic *S. milleri* strains.

These deviations cast little doubt on the unity of the species, but may occasionally lead to practical difficulties in the classification of individual strains, especially those possessing the group antigen A, C or G. A formal distinction between *S. milleri* and the 'pyogenic' streptococci (Sherman, 1938) of groups A, C and G (*S. pyogenes*, group A; 'human' *equisimilis*-like streptococci of group C; the 'large-colony-forming', β-haemolytic group-G streptococci) is usually made on the results of the aesculin-hydrolysis and VP tests (Colman & Williams, 1972). In our experience, however, about one-quarter of 'pyogenic' members of these three Lancefield groups hydrolyse aesculin, and an occasional group-A strain gives a positive VP reaction (Table 9). Absence of β haemolysis suggests that a groupable strain is not a 'pyogenic' streptococcus, but there are occasional exceptions to this rule. The formation of 'minute' colonies on blood agar, and the presence of a characteristic 'honey-like' odour carry a similar implication, but the former is somewhat dependent on the medium used and the latter is more easily detected by some observers than others. Colman & Williams (1972) drew attention to the salt sensitivity of most *S. milleri* strains and the salt tolerance of many of the 'pyogenic' group-A, -C and -G streptococci. The presence of salt sensitivity and, though less often, of bile tolerance and enhancement of growth by $CO_2$, sometimes provide useful additional evidence for exclusion from the 'pyogenic' streptococci. The distinction between an aesculin-positive *S. pyogenes* strain and a β-haemolytic *S. milleri* strain with the group-A antigen is not always easy to make on strictly biochemical grounds. However, *S. pyogenes* strains are almost invariably bacitracin sensitive and possess T and M antigens (or M-associated protein; Widdowson, Maxted & Pinney, 1971); *S. milleri*, on the other hand, is resistant to bacitracin (Colman & Williams, 1972) and does not form surface-protein antigens characteristic of *S. pyogenes*.

Thus, although none of the single tests we employed could be relied upon to distinguish *S. milleri* from the 'pyogenic' members of groups A, C and G, this could nearly always be done with confidence by a complete cultural, biochemical and serological examination.

The formation of large abscesses in a wide range of visceral organs (Parker & Ball, 1976; Parker, 1978) appears to be a unique character of *S. milleri* strains, whether or not they are haemolytic or possess a group antigen. This provides another strong indication of the unity of the species, though the mechanism of pathogenesis responsible for it is not yet understood. The distribution of abscesses suggests that many and perhaps most of the lesions follow penetration of tissues from a carrier site in the same region of the body, at which local inflammatory disturbances may have been minimal or absent.

Information about the characters of *S. milleri* strains present at carrier sites is rather scanty. It appears, however, that few of the strains from teeth and gums are β haemolytic or groupable. Mejàre & Edwardsson (1975) observed β haemolysis in only 5 of 91 dental strains, and we found only one β-haemolytic and 6 groupable strains (two of group F) among 22 unselected strains from teeth and gums. The situation may, however, be quite different in the gut. Poole & Wilson (1977) found 41 β-haemolytic and 70 groupable strains (35 of group F) among 116 isolated from

the appendix. Our own observations (Table 6) suggest that *S. milleri* strains with 'wide' fermentation patterns may tend to be associated with the female genital tract.

We observed that our isolations from the bloodstream included only a small proportion of $\beta$-haemolytic and of groupable strains, and that these percentages increased progressively through the series from visceral pus, and all superficial lesions, to the isolations from superficial lesions in the abdominal region, among which about half were $\beta$ haemolytic and half groupable (Table 7). It would, however, be unsafe to assume that our collections of strains were all representative of the streptococci present at the sites sampled. The two main variable characters of *S. milleri*, haemolysis and the presence of a group antigen, are used by clinical microbiologists to select streptococci for further examination, and most of our streptococci had been through this selective process before they reached us. We believe, nevertheless, that the isolations from the bloodstream of patients with febrile diseases were reasonably unselected, because nearly all of them were present in pure culture and most were of obvious clinical significance. There may have been some selection in favour of $\beta$-haemolytic strains in the cultures from visceral pus, particularly when *S. milleri* was not present in pure culture, and obvious selection was apparent in some of the series of cultures from local superficial sites. This was particularly so among the 'other oral and respiratory' isolations which came from sites at which $\beta$-haemolytic 'pyogenic' streptococci were being sought actively. However, the difference in the characters of the *S. milleri* strains from the vagina and from other abdominal sites was striking, and lends colour to the suggestion that there may indeed be regional differences in the distribution of cultural and serological varieties of *S. milleri*. The distribution of characters among our 'other abdominal' strains did not differ greatly from that observed by Poole & Wilson (1977) among strains from the appendix. Whether *S. milleri* strains with 'wide fermentation' patterns predominate in the normal vagina remains to be seen. If further studies substantiate the suggestion that the different varieties of *S. milleri* are irregularly distributed in the flora of various regions of the body, this may assist in establishing the common sites from which this streptococcus invades the tissues. This possibility will be discussed in a subsequent communication.

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