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The isolation of streptococci from human faeces*

By P. F. UNSWORTH†
Division of Hospital Infection, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT

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SUMMARY

Three selective media were designed for isolation of streptococci from faeces. Samples of faeces from twelve normal adults were suspended and serially diluted in saline or broth saline, and equal volumes of each dilution were spread and incubated on the media. The number of colonies of each different type which developed was counted and one colony of each type was subcultured and identified.

Altogether, streptococci of 13 taxa were found. *S. faecium*, *S. mutans*, *S. milleri*, *S. faecalis* and *S. mitior* were each found in over half the samples. Lancefield group B and G streptococci, *S. bovis* II, an atypical strain of *S. bovis* I, *S. cremoris*, *S. durans* and a dextran-positive strain of *S. mitior* were each present in 1 or 2 samples. Individual samples contained 2–7 (mean 4·6) streptococcal taxa, and total viable counts of streptococci of 3×10^3–3×10^8 (geometric mean 7×10^5) per g. The significance of these findings is discussed.

INTRODUCTION

Advances in classification of α- and non-haemolytic ('viridans') streptococci (Colman & Williams, 1972) have led to better understanding of their clinical importance. *Streptococcus milleri* emerged as the predominant streptococcus associated with deep abscesses, many of them intra-abdominal, from over 700 streptococci isolated from the blood or internal organs of patients (Parker & Ball, 1976). The same study showed the marked propensity of several streptococci, particularly *S. mutans* and *S. bovis* biotype I (*S. bovis* I), for causing bacterial endocarditis. *S. milleri* has been isolated from the appendix (Poole & Wilson, 1977) and dental root canal (Ottens & Winkler, 1962) and group F strains of *S. milleri* from faeces (Hare & Maxted, 1935). It seemed that faeces might be an important reservoir for *S. milleri* and *S. bovis*, and for Lancefield group-B streptococci which occur in the vagina of 4–20% of pregnant women and cause serious disease in about 0·2% of newborn infants (Parker, 1977). Hence this study of the numbers and frequency of these and other streptococci in human faeces.

* An abbreviated account of these results has already appeared in *Pathogenic streptococci* (ed. M. T. Parker), Reedbooks, Chertsey, Surrey (1979) pp. 239–40.

† Present address: Tameside General Hospital, Ashton-under-Lyne, Lancashire, OL6 9RW.

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MATERIALS AND METHODS

Selective media

Thirty-six media, including that of Kidson (1967), were tested semi-quantitatively for their ability (*a*) to support growth of strains of streptococci identified in this laboratory, by the criteria of Parker & Ball (1976), as *S. pyogenes*, group B streptococcus, *S. pneumoniae*, *S. sanguis*, *S. mitior* (dextran-producing strain), *S. salivarius*, *S. milleri* (of Lancefield groups A, C, G, F and none), *S. mutans*, *S. bovis* (biotypes I and II), *S. faecalis*, *S. faecium* and *S. durans*, and two strains of 'unclassified viridans' streptococci; and (*b*) to inhibit growth of ten other organisms obtained from the National Collection of Type Cultures at Colindale: namely *Pseudomonas aeruginosa* (NCTC 10299), *Proteus mirabilis* (6369), *Klebsiella aerogenes* (5055), *Escherichia coli* (10538), *Lactobacillus acidophilus* (4504), *Corynebacterium hoffmanii* (8634), *Staphylococcus aureus* (8766), *Staphylococcus epidermidis* (10519), *Aerococcus viridans* (8251) and *Neisseria catarrhalis* (3622).

Hence three media were chosen for the study. Medium (i) was intended to isolate all species of streptococci from faeces, medium (ii) to indicate the presence of group B streptococci and medium (iii) the presence of *S. bovis* and *S. milleri* in primary cultures. The media contained: (i) 5 % horse-blood agar [Hartley digest (Parker & Ball, 1976) or Columbia agar [Oxoid] with (final concentrations) polymyxin B 50000 u./l, nalidixic acid 20 mg/l and crystal violet 0·8 mg/l; (ii) Islam's (1977) medium with serum 5 % v/v, and neomycin 30 mg/l, nalidixic acid 15 mg/l and metronidazole 50 mg/l; (iii) Carlsson's (1967) medium without tellurite or sulphasomidine, but with sulphadimidine 1 g/l and nalidixic acid 20 mg/l. Medium (i) was also test-inoculated with mixed cultures of *S. milleri* or *S. bovis* with *E. coli*, *P. mirabilis* and *P. aeruginosa*. Medium (ii) was tested, anaerobically with CO_2 (Gaspak, BBL Ltd), for growth and pigment production of the streptococci listed, for inhibition of faecal anaerobes, but not for inhibition of the non-streptococci listed. The latter was tested, in air + 5 % CO_2, on blood agar with neomycin 30 mg/l and nalidixic acid 15 mg/l. Batches of the three media were controlled for semi-quantitative growth of strains of *S. bovis*, *S. milleri* and group B streptococci.

Faeces

Faeces samples were from 12 adults from a South Wales valley who did not complain of any gastrointestinal or other disease, nor receive antibiotics. The donors' ages and sex and stool weights were noted. Samples were chosen from a larger collection, at random except that stools less than 40 g or from donors in the same house were excluded.

Processing of faeces

Samples were stored at − 20 °C, thawed and diluted 10^{-1} w/v with 0·85 % saline (faeces 1–9) or 0·85 % saline with 5 % nutrient broth (Oxoid Ltd), vigorously hand shaken with glass beads for 2 min, then immediately serially diluted tenfold v/v to 10^{-6}. Media were inoculated and spread with each dilution (0·02 ml) and with the 10^{-1} dilution (0·2 and 1·0 ml). Samples 1–9 were inoculated on medium (i) made

with Columbia agar and samples 7–12 on (i) made with Hartley agar. Media (i) and (iii) were incubated at 37 °C in air + 5 % CO_2 and medium (ii) anaerobically with CO_2 (Gaspak, BBL Ltd), and examined after 24, 48 and 72 h. From each sample, colonially distinguishable organisms on each medium were counted separately and one colony of each sort subcultured in air + CO_2 in Todd–Hewitt broth (Cowan & Steel, 1974), then on blood agar to check purity, then in nutrient broth with glycerol 16 % v/v and horse blood 4·2 % v/v for storage at −20 °C.

Identification of strains from faeces

A Todd–Hewitt broth culture of each strain was Gram-stained, catalase-tested on nutrient agar (Cowan & Steel, 1974, method 1) and incubated for 24 h on a bile–aesculin/blood agar ditch plate (Parker & Ball, 1976). Gram-positive, catalase-negative cocci grown overnight on blood agar at 37 °C in air + 5 % CO_2, were divided by eye into 'large' (> 1 mm) colony forms, which grew on 40 % bile agar and hydrolysed aesculin; and 'small' (< 1 mm) forms, mostly with no or poor growth on 40 % bile agar. The 'large' forms were tested for survival at 60 °C for 30 min and for Lancefield group D antigen (methods as in Parker & Ball, 1976); for pyruvate fermentation (Waitkins, 1978); and for growth on 6·5 % salt agar and, if pyruvate-negative, for arabinose fermentation (Waitkins, Ball & Fraser, 1980). Strains of *S. bovis*, *S. durans* and 'pyogenic' group B and G streptococci also had the full range of tests described below for 'small' colony forms. Group B streptococci were serotyped in the Streptococcal Reference Laboratory, Colindale.

The 'small' colony forms were first screened by four tests: colonial morphology on blood agar (Parker & Ball, 1976), and arginine hydrolysis, mannitol fermentation and aesculin hydrolysis or fermentation (by the agar-replication methods of Waitkins, Ball & Fraser, 1980). From each sample, all strains distinguishable by the four tests were tested further. The tests already described for 'large' colony forms were used. Additional tests were growth on 10 % bile agar, Voges-Proskauer (VP), starch hydrolysis and fermentation of sorbitol, lactose and raffinose (agar replication, Waitkins, Ball & Fraser, 1980); and effect of CO_2 on growth, production of dextran or laevan from sucrose and testing formamide extracts for group antigens A–S (Parker & Ball, 1976). Extracts in 0·07 M-HCl of strains thought possibly to be *S. mitior* were made and tested with sera to groups M and O (Parker & Ball, 1976). Group D antigen was extracted from selected strains of *S. bovis* and other organisms with phenol (Elliot, McCarty & Lancefield, 1977), after growth in glucose broth. Precipitin tests were done by double gel diffusion in agarose 1 % (Koch–Light Ltd, Colnbrook, UK).

When, in a few instances, unexpected results were obtained by the agar-replication methods, the test was repeated by an orthodox method and, when there was a discrepancy, the latter result was accepted. VP-negative strains of *S. mutans* were grown for 5 days in a medium containing Oxoid tryptone 1 %, Oxoid yeast extract powder 0·5 %, K_2HPO_4 0·5 % and glucose 0·5 %, pH adjusted to 7·3. Then, to 2 ml of culture fluid was added 1 ml 6 % α-naphthol in absolute ethanol and 0·4 ml of 40 % potassium hydroxide, shaking the tube and looking for pink coloration after 15 min (Colman, 1970). Strains of *S. milleri* and *S. mitior* which

failed to hydrolyse arginine were re-tested with Niven's broth and by method 1 as in Cowan & Steel (1974). A few strains of various species which unexpectedly fermented sorbitol, mannitol, raffinose and, particularly, arabinose were re-tested (Parker & Ball, 1976).

A 'small' colony strain of *S. cremoris* was also tested for growth in 6·5% salt broth (Parker & Ball, 1976), at about 20 °C and at 45 °C, and in 0·1% methylene blue milk (Deibel & Seeley, 1974).

A few 'large' colony streptococci from sample 1 were discarded after only partial testing.

Classification of streptococci

This was done by the criteria of Parker & Ball (1976) except for *S. durans* (Cowan & Steel, 1974) and *S. faecium* and *S. cremoris* (Deibel & Seeley, 1974).

Mean colony counts of streptococci on individual media

In calculating the geometric mean count of a streptococcus present in several faeces samples at different concentrations, a medium failing to yield the organism from a sample known to contain it was 'credited' with the minimum count (10/g) detectable by the methods used.

RESULTS

Preliminary testing of selective media

All streptococci, except one 'unidentified viridans' species, grew on medium (i) with Columbia agar base, and mostly about as well as on Columbia blood agar. Of non-streptococcal organisms, only *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Aerococcus viridans* grew on (i). The first and second grew only very scantily, without spreading. Only streptococci grew from mixed cultures inoculated on medium (i). Medium (ii) supported growth of *S. faecalis*, *S. faecium*, *S. durans* and the group B streptococcus. The latter was reduced in quantity but produced strong orange pigment. No other organisms produced pigment on this medium, and growth of faecal anaerobes was completely inhibited. On blood agar with neomycin and nalidixic acid as in medium (ii), incubated in air + CO_2, no non-streptococci grew except *Pseudomonas aeruginosa*, which is completely inhibited by anaerobic conditions. *S. milleri* and group D streptococci grew on medium (iii), but group B and most other streptococci were strongly inhibited. So were all non-streptococci tested, except *Pseudomonas aeruginosa* which grew well.

Most streptococci grew well on Kidson's (1967) medium, but group B streptococci, *S. faecalis* and *S. salivarius* were inhibited and lactobacilli and *P. mirabilis* grew, the latter with some spreading.

Identification of streptococci from faeces

Most streptococci were easily allocated to one of the taxa described by Parker & Ball (1976). The 'large' colony forms were mostly *S. faecium*, *S. faecalis*, *S. durans*, *S. bovis* and 'pyogenic' Lancefield group B and G streptococci. All strains of *S. faecium* and *S. faecalis* survived 60 °C for 30 min, grew on 6·5% salt, and had group D antigen. All strains of *S. faecalis* fermented pyruvate, and those of

S. faecium fermented arabinose but not pyruvate. Thirteen discarded strains were probably duplicates of *S. faecium* and an 'unidentified' streptococcus. The strain of *S. durans* conformed to the test results listed by Cowan & Steel (1974), except that it was α-haemolytic. It was VP-positive, starch- and pyruvate-negative, and did not produce dextran or laevan.

Thirteen strains of *S. mutans*, from eight samples, were VP-positive on re-testing. Four strains of *S. milleri* and 2 of *S. mitior* were arginine-positive on re-testing. Unexpected positive fermentation reactions were negative when re-tested. The strain of *S. cremoris* had group N antigen, grew at about 20 °C but not in methylene blue milk, salt broth nor at 45 °C, and was arginine-negative (Deibel & Seeley, 1974). The one streptococcus about which serious doubt remained was classified tentatively as *S. bovis* biotype I; it was a group-D streptococcus that was VP-, starch-, 40% bile-, raffinose- and mannitol-negative but appeared to form laevan from sucrose.

Strains isolated on each selective medium

Of the 279 strains processed 250 were streptococci. Table 1 shows the organisms isolated on each medium. Medium (i) yielded most taxa of streptococci and most non-streptococci, namely 14 micrococcaceae and 7 Gram-positive bacilli. Medium (iii) yielded most duplicate isolations of streptococci, i.e. colonial variants of the same taxon, and some non-streptococci, mostly Gram-positive bacilli.

Strains of most taxa grew best on medium (i) and, of 3 taxa, only on this medium, which by itself was sufficient to give maximum yields of 7 of 13 taxa (Table 2). The extra yield of other taxa gained by using a second medium was small, except for *S. faecium* and *S. durans* on medium (iii). Colonial morphology gave more help in identification of strains grown on (i) than (ii) or (iii), except for *S. mutans* on (iii) (Carlsson, 1967) and group B streptococci from sample 6, which yielded orange colonies on (ii). No other organism produced pigment on (ii).

The effect of using different bases in medium (i) is shown in Table 3. Four of eight taxa grew as well with either base and two grew only with Columbia base. For various taxa, this base yielded larger colonies than Hartley base, but less colonial differentiation.

Data referring to faeces samples

Donors, 5 men and 7 women, were aged 45–72, mean 56 yr. Stools weighed 40–200, mean 102 g. The (wet) weight sampled was 1·5–2·1, mean 1·9 g. Storage at −20 °C was for 14–21 weeks.

Streptococcal flora of faeces

Each sample contained 2–7, mean 4·6, taxa of streptococci and a total per g of 3×10^3–3×10^8, mean 7×10^5, streptococci (Table 4). Enterococci were present in all samples but predominated in only one-half. Streptococci of mainly α- and non-haemolytic taxa were found in 11 samples and predominated in one-half. The streptococcal flora of faeces is strikingly inconstant. Also striking is the wide range of counts of individual taxa (Table 5). Only group D streptococci reached

Table 1. *Organisms isolated from 12 faeces samples on each of three media*

Media	Total no. of strains of all species	Strains of streptococci isolated: From 12 samples: Total no.	From 12 samples: (% of all organisms)	From individual samples: No. of Taxa: Range	No. of Taxa: (Mean)	No. of duplicate isolations: Range	No. of duplicate isolations: (Mean)	No. of duplicate isolations: (% of all strains)
(i)	122	101	(83)	2–7	(3·9)	1–10	(4·5)	(54)
(ii)	53	51	(96)	1–5	(2·3)	1–4	(1·9)	(45)
(iii)	104	98	(94)	2–4	(3·2)	2–9	(4·8)	(61)
(i), (ii) and (iii)	279	250	(90)	2–7	(4·6)	8–25	(16·2)	(78)

Table 2. *Streptococci isolated from 12 faeces samples on each of three media*

Taxa of streptococci	No. of samples from which each streptococcus was grown (and geometric mean colony count per g) on medium: (i)	(ii)	(iii)	any	Media sufficient to detect the organism in maximum no. of samples
'Pyogenic', group B	2 (2×10^4)	1 (7×10^3)	—	2 (2×10^4)	(i)
'Pyogenic' group G	1 (5×10^2)	—	—	1 (5×10^2)	(i)
S. mitior	6 (3×10^2)	1 (3×10^1)	1 (2×10^1)	7 (7×10^3)	(i) and (ii)
S. mitior (dextran pos.)	1 (5×10^5)	—	—	1 (5×10^5)	(i)
'unclassified viridans'	1 (2×10^1)	—	2 (8×10^1)	3 (8×10^2)	(iii) and (i)
S. milleri	7 (5×10^4)	4 (4×10^2)	7 (2×10^3)	8 (3×10^4)	(i), and (iii) or (ii)
S. mutans	8 (7×10^3)	7 (1×10^3)	8 (6×10^3)	9 (1×10^4)	(i) and (iii)
S. cremoris (group N)	1 (5×10^2)	—	1 (6×10^4)	1 (6×10^4)	(iii) or (i)
S. bovis (biotype I)	1 (1×10^3)	—	—	1 (1×10^3)	(i)
S. bovis (biotype II)	2 (1×10^6)	2 (2×10^5)	1 (5×10^4)	2 (1×10^7)	(i)
S. faecalis	7 (6×10^3)	5 (1×10^3)	6 (7×10^3)	7 (1×10^4)	(i)
S. faecium	8 (6×10^3)	7 (5×10^2)	9 (1×10^4)	11 (2×10^4)	(iii) or (i), and (ii)
S. durans	—	—	1 (1×10^3)	1 (1×10^3)	(iii)

Table 3. *Streptococci isolated from three faeces samples on medium (i) made from different bases*

Taxa of streptococci	No. of samples yielding each streptococcus (and geometric mean colony count per g) on medium (i) made from: Columbia agar base	Hartley digest agar
S. mitior	1 (1×10^2)	2 (2×10^4)
S. milleri	3 (3×10^4)	3 (2×10^4)
S. mutans	2 (6×10^4)	2 (3×10^4)
S. cremoris (group N)	1 (5×10^2)	—
S. bovis (biotype I)	1 (1×10^3)	—
S. bovis (biotype II)	1 (2×10^2)	1 (5×10^3)
S. faecalis	2 (8×10^2)	2 (3×10^2)
S. faecium	3 (3×10^4)	2 (1×10^4)

counts of 10^7–10^8 per g. *S. faecalis* was only the fourth most frequently isolated streptococcus. Both group B strains were of serotype Ib.

Eight samples yielded *S. milleri* strains with no Lancefield group antigen (5 non- and 4 α-haemolytic). Three samples yielded group F strains (β-, α- and non-haemolytic); two, group G strains (β- and α-haemolytic); and one, an α-haemolytic group C strain.

Sample 10 contained *S. milleri* with group G antigen, 1×10^3 per g, and a 'pyogenic' group G streptococcus, 5×10^2 per g. Both were β-haemolytic, and differed only in colony size, VP (*S. milleri*, positive), mannitol fermentation (*S. milleri*, negative) and growth requirement for CO_2 (*S. milleri*, absolute requirement; 'pyogenic' streptococcus, merely enhanced by CO_2).

DISCUSSION

Saline dilution may have reduced colony counts of streptococci, more than dilution in broth saline, though exposures were brief. Moore & Holdeman (1974) state that 1 g samples of the same stool commonly vary in bacterial composition, but this refers particularly to very oxygen-sensitive, non-sporing anaerobic species (M. J. Hill, personal communication). No significant differences were found between counts of streptococci or other organisms in duplicate 1 g samples collected from the same subjects over a 2-week interval (Gorbach, *et al.* 1967). On the other hand these authors reported great variation in the numbers of bacteria in the faeces of different people of the same age, which was not due to errors or difficulties in sampling. This accords with the inconstancy of the streptococcal flora found in these 12 subjects' faeces.

General considerations of the effects of freezing and thawing on micro-organisms (Smith, 1961) suggest that storage of faeces at -20 °C for several months would not reduce streptococcal colony counts by more than 10- or 100-fold. However, it is preferable to dilute faeces 10% (w/v) in broth containing glycerol 10% (v/v), to minimize losses during frozen storage (Crowther, 1971).

To isolate streptococci from faecal-type sources, Pavlova, Brezenski & Litsky

Table 4. *Colony counts of each streptococcal taxon found in each faeces sample*

Taxa of streptococci	Colony counts of streptococci from sample											
	1	2	3	4	5	6	7	8	9	10	11	12
'Pyogenic' group B	—	—	—	—	—	6×10^6	—	—	—	—	5×10^1	—
'Pyogenic' group G	—	—	—	—	—	—	—	—	—	5×10^2	—	—
S. mitior	2×10^2	—	—	—	2×10^2	5×10^5	—	1×10^4	5×10^4	—	2×10^3	5×10^4
S. mitior (dextran pos.)	—	—	—	—	—	—	—	—	—	—	—	5×10^5
'unclassified viridans'	5×10^2	—	—	—	—	—	—	—	—	—	1×10^2	1×10^4
S. milleri	—	—	1×10^5	4×10^5	—	—	2×10^4	1×10^4	3×10^5	1×10^3	1×10^3	4×10^5
S. mutans	1×10^2	—	5×10^2	9×10^5	2×10^4	—	4×10^5	6×10^3	—	5×10^1	3×10^4	2×10^6
S. cremoris (group N)	—	—	—	—	—	—		—	6×10^4	—	—	—
S. bovis (biotype I)	—	—	—	—	—	—	—	—	1×10^3	—	—	—
S. bovis biotype II)	—	—	—	—	—	—	—	—	5×10^5	—	—	3×10^8
S. faecalis	—	1×10^7	—	4×10^3	—	1×10^8	—	5×10^2	1×10^3	1×10^2	—	1×10^3
S. faecium	2×10^2	1×10^5	1×10^7	1×10^2	7×10^4	1×10^8	4×10^4	1×10^2	6×10^6	5×10^1	2×10^4	—
S. durans	—	—	—	—	—	—	—	—	—	1×10^3	—	—
Probable enterococci	3×10^3	—	—	—	—	—	—	—	—	—	—	—
All taxa (total)	4×10^3	1×10^7	1×10^7	1×10^6	9×10^4	2×10^8	5×10^5	3×10^4	7×10^6	3×10^3	5×10^4	3×10^8

— = $< 5 \times 10^1$ organisms per g.

Table 5. *Frequency and range of counts of streptococci found in 12 faeces samples*

Taxa of streptococci in rank order of frequency	Number of samples yielding colony counts per g in the range 1–9 × 10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^8	Any count ⩾ 5×10^1	Geometric mean colony count per g
S. faecium	2	2	—	3	1	1	1	1	11	2×10^4
S. mutans	1	2	1	2	2	1	—	—	9	1×10^4
S. milleri	—	—	2	2	4	—	—	—	8	3×10^4
S. faecalis	—	2	3	—	—	—	1	1	7	1×10^4
S. mitior	—	2	1	3	1	—	—	—	7	7×10^3
'unclassified viridans'	—	2	—	1	—	—	—	—	3	8×10^2
S. bovis II	—	—	—	—	1	—	—	1	2	1×10^7
Group B	1	—	—	—	—	1	—	—	2	2×10^4
S. mitior (dex. pos.)	—	—	—	—	1	—	—	—	1	5×10^5
S. cremoris	—	—	—	1	—	—	—	—	1	6×10^4
Probable enterococci	—	—	1	—	—	—	—	—	1	3×10^3
S. durans	—	—	1	—	—	—	—	—	1	1×10^3
S. bovis I	—	—	1	—	—	—	—	—	1	1×10^3
Group G	—	1	—	—	—	—	—	—	1	5×10^2

— = zero.

(1972) list 68 media, but many will inhibit 'viridans' streptococci. Medium (i) will selectively yield a wide range of streptococci from faeces, with little reduction in colony counts. Columbia agar base may be preferred for more fastidious species. The greater colonial variation with Hartley Digest base may help in identification. Medium (iii) has advantages for certain enterococci and *S. mutans*. Medium (ii) is a useful selective-indicator medium for group B streptococci, though not all strains produce pigment on it (Waitkins & Unsworth, in preparation).

Similar total counts of streptococci were found in six other studies, with geometric mean counts of 10^4–10^7 per g faeces (Drasar & Hill, 1974). Hare & Maxted (1935) found 'pyogenic' group G streptococci in the faeces of 2% of women in labour, and group A streptococci in the faeces of 20% of scarlet fever patients, but no group B streptococci. Faeces may be the source of vaginal 'contamination' and thence 'early-onset' infection of the newborn infant with group B streptococci (Parker, 1977).

Drasar, Shiner & McLeod (1969) found only 10^2–10^6, geometric mean 3×10^3, enterococci per g in adults' faeces, and Noble (1978) found *S. faecium* in only 15 of 39 adults' faeces. The differences from this study may be due to the different selective media used. *S. faecium* seems to occur commonly in faeces and yet is rarely associated with serious disease in man (Parker & Ball, 1976). Hence this enterococcus appears to be intrinsically less pathogenic for man than *S. faecalis*.

Drasar *et al.* (1969) found 10^2–10^6, mean 10^4, 'viridans' streptococci and 0–10^7, mean 3×10^2, *S. salivarius* per g in adults' faeces. Similar counts were found in this study, except for 3×10^8 *S. bovis* II in one sample; and the absence of *S. salivarius*, perhaps due to storage losses. *S. mutans* was found previously in only 23 of 108 specimens of faeces (Kilian, Theilade & Schiøtt, 1971). The present findings of *S. bovis* II confirm the carriage of *S. bovis* in about 10% of normal faeces (Klein *et al.* 1977).

Parker & Ball (1976) showed five streptococci to be particularly associated with endocarditis in man: *S. mutans*, *S. bovis* I, *S. sanguis* and dextran-positive and -negative *S. mitior*. Four of these were found in the mouth, but not *S. bovis* (Phillips *et al.* 1976). Only two of these, *S. mitior* (dextran-negative) and *S. sanguis*, were found to cause bacteraemia following dental extraction, among a small series (Phillips *et al.*, 1976), and at least 75 of 80 strains in Porterfield's (1950) series belonged to these two species. Thus, the bowel might be an important source of streptococci causing endocarditis, particularly *S. bovis* (Klein *et al.* 1977), *S. mutans* and dextran-positive *S. mitior*. As *S. bovis* I is associated with endocarditis most frequently in those aged $\geqslant$ 55 years (Parker & Ball, 1976), faecal carriage rates may be higher in older subjects.

This limited study suggests that *S. milleri* is common in normal adults' faeces. Most strains isolated from the blood or internal lesions of patients (Ball & Parker, 1979), from the dental root canal (Ottens & Winkler, 1962) and in this study are α- or non-haemolytic and have none or group F Lancefield antigen. Finer markers for distinguishing strains may establish how *S. milleri* reaches deep abscess sites. Strains causing intra-abdominal and intra-cranial abscesses may originate in the gut and respiratory air sinuses, respectively (Parker & Ball, 1976; de Louvois,

Gortvai & Hurley, 1977). The relative importance of extension from adjacent foci and of metastatic blood spread, due to bacteraemia or septic embolism (Melo & Raff, 1978) is not clear. Consistent with the latter, Phillips *et al.* (1976) found five strains of *S. milleri* among 21 incidents of streptococcal bacteraemia, mostly following dental extraction, and Melo & Raff (1978) found evidence of liver abscess in two of three patients with brain abscess.

There are interesting taxonomic similarities between strains of classical 'pyogenic' streptococci of Lancefield groups A, C and G, and strains of *S. milleri* with the same antigen (Ball & Parker, 1979). The occurrence of both sorts of 'group G streptococci' in one subject's faeces might indicate some relationship between the strains.

There has been little information about the numbers of different sorts of streptococci in the faeces of normal persons. This study provides information about selective media for streptococci in faeces or 'contaminated' sources and preliminary data on faecal carriage of various pathogenic streptococci.

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