Streptococcus milleri in the appendix

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SUMMARY The appendix was investigated as a possible habitat of *Streptococcus milleri*. Both normal and inflamed appendices were examined and the isolation rates compared. *S. milleri* was present in a quarter of the normal appendices and more than half of those associated with appendicitis—a difference that was statistically highly significant. The isolation rates throughout were independent of age. There was a pronounced connection between the presence of *S. milleri* in the appendix and the purulent manifestations of appendicitis. *S. milleri* was isolated from other abdominal sites associated with appendicitis. The frequency of isolation was increased by culture in an enrichment broth containing nalidixic acid and sulphadimidine.

The species *Streptococcus milleri* comprises a group of streptococci distinguishable by their biochemical and physiological similarities (Colman, 1968; Colman and Williams, 1972). It includes all group F streptococci and some streptococci that contain antigen of groups A, C, or G. Most contain no recognised group antigen. Strains may be β -haemolytic, α -haemolytic, or non-haemolytic. Characteristically *S. milleri* grows poorly or not at all in air. Growth is better anaerobically and is enhanced by the addition of 10% CO₂ to the atmosphere. Most strains hydrolyse aesculin and arginine, are Voges-Proskauer positive, and ferment carbohydrates predictably. Haemolytic strains are less active in the fermentation of lactose. Many strains, mostly non-haemolytic or α -haemolytic, grow in the presence of bile salts. No extracellular polysaccharides are produced from sucrose. All strains are sensitive to penicillin, erythromycin, and trimethoprim and resistant to sulphamethoxazole. They are usually sensitive to tetracycline and resistant to bacitracin and nitrofurazone. Occasional strains behave aberrantly.

S. milleri is notably associated with purulent disease. Guthof (1956) originally described it in abscesses of the oral cavity. It has been found in liver abscesses (Bateman *et al.*, 1975; Reid and Davidson, 1976), intracranial and intraspinal abscesses (De Louvois *et al.*, 1976), and in septic lesions of these and other internal organs (Parker and Ball, 1976). Poole and Wilson (1976) described haemolytic strains in purulent lesions of the chest and abdomen as well as in more superficial abscesses.

The presence of group F streptococci has been recorded on many occasions in meningitis, empyema, peritonitis, and other pus-forming lesions (Thomas, 1939; Rantz, 1942; Wheeler and Foley, 1943; Foley, 1947; Koepke, 1965; Wort, 1975).

The normal habitat of the streptococci now included in the species *S. milleri* is being gradually revealed. They have been described on the teeth (Mejare and Edwardsson, 1975), in dental root canals (Winkler and Amerongen, 1959; Ottens and Winkler, 1962), in the nasopharynx (Hare, 1935), in the throat (Long *et al.*, 1934), in the vagina (Lancefield and Hare, 1935; Wort, 1975), and in faeces (Hare and Maxted, 1935). The observation of Parker and Ball (1976) that infection with *S. milleri* was often associated with a preceding abdominal infection or operation drew attention to the gastrointestinal tract as a possible source of infection. Likewise, the presence of group F streptococci (Rogers, 1957) in 11% of appendices indicated that further investigation of this organ in both the normal and diseased state might be worthwhile.

Material and methods

One hundred and eight complete appendices obtained at necropsy and portions about 2 cm long of 164 appendices removed at operation were received unfixed in dry sterile containers. The lumen of each appendix was exposed and the mucosa rubbed with a cotton-wool swab.

Swabs from various abdominal sites in addition to the appendix were received from 17 patients with appendicitis.

The impregnated swabs were inoculated on to two-

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layered 10% horse-blood agar plates, two neomycin blood agar plates containing neomycin 50 mg/l, one plate each of MacConkey (Oxoid) and deoxycholate citrate agar, and into a tube of selenite F broth. Finally, the tip of the swab was broken off into an enrichment medium consisting of Todd-Hewitt broth containing 7% horse blood, 30 mg/l nalidixic acid, and 50 mg/l sulphadimidine. The enrichment broth and one of each pair of blood and neomycin blood agar plates were incubated in 10% CO₂ in air. The remaining plate of each pair was incubated anaerobically. All other media were incubated aerobically. The temperature of incubation was 37°C throughout.

The enrichment broth was subcultured after 24 hours to blood agar and neomycin blood agar incubated in 10% CO₂ in air and to blood agar anaerobically. The selenite F broth was subcultured to deoxycholate citrate agar. All plates were examined after 24 hours and a record made of any bacteria present. Plates containing blood were reincubated for a further 24 hours. *S. milleri* appeared as tiny (<0.5 mm at 24 hours) convex colonies with an entire edge, sometimes non-haemolytic, sometimes surrounded by a zone of α -haemolysis with greenish discoloration of red blood cells around or under the colony, and sometimes β -haemolytic with a clearing of the medium around the colony. Colonies resembling *S. milleri* were subcultured for purity and submitted to characterisation tests.

CHARACTERISATION TESTS

Acid production from lactose, sucrose, salicin, raffinose, trehalose, and sorbitol were investigated by growth in peptone water sugars containing 10% horse serum. Examination for the production of extracellular polysaccharide was by growth in Bailey and Oxford's (1958) medium for five days followed by differential precipitation with ethanol for the detection of dextran or levan (Hehre and Neill, 1946).

Comparison of growth anaerobically, aerobically, and in the presence of 10% carbon dioxide in air was tested by culturing similar inocula on blood agar plates overnight in the three atmospheres. Tests for the hydrolysis of aesculin and arginine, the Voges-Proskauer test, and bile tolerance were carried out by methods previously described (Poole and Wilson, 1976), as were also tests for sensitivity to penicillin, tetracycline, erythromycin, bacitracin, nitrofurazone, sulphamethoxazole, and trimethoprim.

Polysaccharide group antigens of group A, C, F, and G were prepared by the enzyme method of Maxed (1948) and identified with Wellcome antisera.

Results

One hundred and sixteen strains of streptococci biochemically consistent with *S. milleri* were isolated (Table 1). These included six double isolates from appendices and three additional strains from abdominal sites which differed from the finding in the relevant appendix. Multiple isolates of similar strains from the same patient were treated as single strains. They comprised 1 group A, 18 group C, 35 group F, and 6 group G streptococci, the remaining 56 being ungroupable. Forty-one strains showed β haemolysis. The remainder were either α -haemolytic or non-haemolytic. 55.7% of all strains isolated were present in direct culture, often in heavy growth. The proportion of such heavy growths was similar in appendices from all sources. The remainder were obtained by subculture of the enrichment broth.

Table 2 shows the number and percentage of isolates of *S. milleri* by age and sex from 108 appendices obtained at necropsies and 33 histologically normal appendices removed at operation. In this table the figures for necropsy material from persons aged over 60 years are given separately in order to record that *S. milleri* is indeed present in the appendix of elderly people. Apart from making this observation, the results from all persons aged over 40 years are combined into a single category, there

Table 1 Cultural features of 116 strains of *S. milleri*: numbers and percentages of strains that gave the stated reaction

	No.	%
β -haemolytic	41	35.3
α -or non-haemolytic	75	64.7
Lancefield group		
A	1	0.9
C	18	15.5
F	35	30.2
G	6	5.2
Ungroupable	56	48.3
Enhanced by CO ₂	95	82.0
Growth in 10% bile	58	50.0
Growth in 40% bile	48	41.4
Aesculin hydrolysis	76	65.5
Arginine hydrolysis	110	94.8
Positive VP reaction	106	91.4
Polysaccharide from glucose	—	—
Acid production from carbohydrates		
Lactose	80	68.9
Sucrose	115	99.0
Salicin	107	92.2
Raffinose	3	2.6
Trehalose	76	65.6
Sorbitol	—	—
Sensitivity to anti-bacterial agents		
Penicillin	116	100
Erythromycin	116	100
Tetracycline	113	97.4
Trimethoprim	116	100
Sulphamethoxazole	—	—
Bacitracin	4	3.4
Nitrofurazone	6	5.2

Table 2 Number and percentage of isolates of *S. milleri* from 108 appendices obtained at necropsies and from 33 histologically normal appendices by age and sex

Necropsy						Histologically normal					
Age (years)	Male		Female		Total	Age (years)	Male		Female		Total
	No. in group	No. (%) <i>S. milleri</i>	No. in group	No. (%) <i>S. milleri</i>	No. (%) <i>S. milleri</i>		No. in group	No. (%) <i>S. milleri</i>	No. in group	No. (%) <i>S. milleri</i>	No. (%) <i>S. milleri</i>
>20	8	5 (62.5)	7	2 (28.6)	15 (46.7)	<20	3	2 (66.6)	10	2 (20)	13 (30.7)
20-39	22	4 (18.2)	4	2 (50)	26 (23.1)	20-39	1	—	10	2 (20)	11 (18.2)
40-59	15	3 (20)	10	4 (40)	25 (28)	≥40	2	—	7	2 (28.6)	9 (22.2)
≥60	30	8 (26.6)	12	—	42 (19)	Total	6	2 (33.3)	27	6 (22.2)	33 (24.2)
Total	75	20 (26.6)	33	8 (24.2)	108 (25.9)						

being too few appendices removed surgically above this age to merit further subdivision. The total isolation rates from necropsy (25.9%) and histologically normal (24.2%) appendices were similar, and these two groups together form an 'all normal' group for comparison with the results found in 121 appendices showing evidence of mild (35) or acute (86) appendicitis (Table 3).

Table 3 shows isolation rates of *S. milleri* in the 'all normal' group (25.5%) and in the appendicitis group (57.8%). For each age/sex combination the isolation rate in the appendicitis group was higher than in the 'all normal' group. Taking age and sex into account, this difference was highly significant overall at the 0.1% level. In the 'all normal' group the isolation rate in males aged under 20 years was higher than in the other groups and was significant at the 0.5% level. There were no significant dif-

ferences in the isolation rates among the other five age/sex divisions of this group. In the appendicitis group the total isolation rate for males is significantly higher than for females at the 5% level, but no significant difference was found within individual age groups. *S. milleri* was isolated from one of the remaining 10 appendices removed surgically, which were variously described as fibrosed or chronically inflamed. These were not considered further.

The percentage isolation rates for other bacteria found in the appendix are shown in Table 4. Lactose-fermenting coliform bacilli and *Bacteroides* sp. predominated throughout all groups. The incidence of enterococci was less in appendicitis (37.2%) than in normal material ('all normal' 54.6%). This difference was significant at the 1% level. A similar trend was observed with other undifferentiated streptococci not shown in the Table. *Proteus* sp. and

Table 3 Number and percentage of isolates of *S. milleri* from 'all normal' and 'appendicitis' appendices by age and sex

Age (years)	Normal					Appendicitis				
	Male		Female		Total	Male		Female		Total
	No. in group	No. (%) <i>S. milleri</i>	No. in group	No. (%) <i>S. milleri</i>	No. (%) <i>S. milleri</i>	No. in group	No. (%) <i>S. milleri</i>	No. in group	No. (%) <i>S. milleri</i>	No. (%) <i>S. milleri</i>
<20	11	7 (63.6)	17	4 (23.5)	28 (39.3)	48	34 (70.8)	31	14 (45.2)	79 (60.7)
20-39	23	4 (17.4)	14	4 (28.6)	37 (21.6)	14	7 (50)	14	9 (64.3)	28 (57.1)
≥40+	47	11 (23.4)	29	6 (20.7)	76 (22.4)	5	4 (80)	9	2 (22.2)	14 (42.8)
Total	81	22 (27.2)	60	14 (23.3)	141 (25.5)	67	45 (67.2)	54	25 (46.3)	121 (57.8)

Table 4 Percentage of isolation rates for other bacteria isolated from appendices obtained at necropsy and appendectomy

Bacteria	Necropsy (n = 108)	Histologically normal (n = 33)	All normal (n = 141)	Appendicitis (n = 121)
Coliforms	91.7	87.8	90.7	83.5
<i>Bacteroides</i> sp.	70.1	69.7	70.9	62.8
Enterococci	53.7	57.6	54.6	37.2
<i>Proteus</i> sp.	9.3	9.1	9.2	0.8
<i>Pseudomonas</i> sp.	9.3	12.3	10.0	2.5
<i>Clostridium</i> sp.	19.6	9.1	15.0	7.4

Pseudomonas sp. were seldom present in appendicitis but occurred in about 10% of normal appendices. *Clostridium* sp. were found more often in necropsy than in surgical material. No salmonella or shigella organisms were isolated.

Table 5 records specific clinical features in patients with appendicitis in relation to the isolation of *S. milleri* from the appendix. Pus in the lumen and in the wall of the appendix as well as free fluid in the peritoneum were more commonly associated with the presence of *S. milleri*. Purulent exudate was noted on the peritoneal surface of 10 (14.3%) appendices that yielded *S. milleri* compared with two (3.9%) in which no isolation was made. In all five cases of peritonitis and five of pelvic abscess *S. milleri* were present in the appendix.

Notably, 10 (16.9%) out of 59 primarily sutured wounds in cases in which *S. milleri* was present in the appendix broke down and discharged whereas only three (6.8%) did so in 44 cases in which *S. milleri* was absent.

Table 6 shows isolations of *S. milleri* from related abdominal sites in 17 patients with appendicitis. Swabs were received from 14 of the 70 patients whose appendix had yielded the organism. Two peritoneal swabs yielded no growth. *S. milleri* was isolated from one or more sites in the remaining 12 cases. One patient yielded three strains, one similar and two distinct from the original isolate. Swabs were received from three of the 51 patients whose appendix

had not yielded *S. milleri*. *S. milleri* was grown from the discharging wound of one of these. Altogether 13 (76%) of the 17 patients yielded *S. milleri*, invariably mixed with faecal bacteria.

Discussion

We examined all of the 164 appendices removed surgically during the first six months of 1976. In 10 the histological appearances were indefinite and they were not considered further. Just under a quarter of the remainder were histologically normal. Some had been removed during operation for other surgical conditions. The remainder were from patients with appendicitis: 65% of them were under 20 years of age. Owing to the large number of necropsies of elderly people it was impracticable to study all appendices from this source during the same six months. We decided to examine a sample, otherwise unselected, according to age. Originally we intended to include appendices from 25 people in each age subgroup. In the event we investigated rather more than this number from persons aged over 60 years, and somewhat less from persons under 20 years.

The age disparity between the persons whose appendices were obtained at operation and those in whom it was obtained at necropsy was not detrimental in analysing the results since the isolation of *S. milleri* in each category (histologically normal, appendicitis, and necropsy) was unaffected by age. The results for the histologically normal and necropsy material were combined to form a single normal group. A quarter of all normal appendices yielded *S. milleri*, confirming that it is a commensal at this site and suggesting that it may be part of the flora of the ileocaecal region. This finding supports the conclusion of both Wort (1975) working with group F streptococci and Parker and Ball (1976) that the source of a considerable number of infections may well be in the abdomen.

If the occurrence of *S. milleri* in normal appendices is compared with that in appendices removed from

Table 5 Number (%) of clinical features observed in the presence or absence of *S. milleri* in the appendix

Clinical feature	<i>S. milleri</i> present (n = 70)	<i>S. milleri</i> absent (n = 51)
Pus in lumen	42 (60)	24 (47)
Pus all coats	35 (50)	20 (39.2)
Fluid in peritoneal cavity	15 (21.4)	8 (15.6)
Exudate on surface of appendix	10 (14.3)	2 (3.9)
Peritonitis	5 (7.1)	—
Pelvic abscess	5 (7.1)	—

Table 6 Isolation of *S. milleri* from lesions in various abdominal sites in 17 patients with appendicitis related to presence or absence of *S. milleri* in appendix

Site of lesion	<i>S. milleri</i> present in appendix (n = 14)		<i>S. milleri</i> absent from appendix (n = 3)	
	<i>S. milleri</i> present in lesion	<i>S. milleri</i> absent from lesion	<i>S. milleri</i> present in lesion	<i>S. milleri</i> absent from lesion
Wound	4	—	1	2
Peritoneum	3	2	—	—
Wound + peritoneum	2	—	—	—
Pelvic abscess	1	—	—	—
Wound + pelvic abscess	2	—	—	—
Total	12	2	1	2

patients with appendicitis a highly significant increase in isolation rate is observed in the presence of inflammation. The association of these streptococci with appendicitis was first noted by Rogers (1957), who isolated group F strains from appendices of children. Wort (1975), also working with group F streptococci, isolated one-third of his strains from patients with acute appendicitis. Poole and Wilson (1976) reported 18 haemolytic isolates in similar circumstances. They described two isolates occurring in pure culture. Generally, however, these streptococci form part of a mixed faecal flora.

Parker and Ball (1976) emphasise the association of *S. milleri* with purulent disease. This corresponds well with its presence in appendicitis, where pus formation is common. Likewise, peritonitis, pelvic abscess, and purulent discharge from wounds are not unusual. Although the number of our patients showing such individual features was often small they tended to be associated with *S. milleri* in the appendix. Furthermore, swabs from other abdominal sites were received five times more often from patients harbouring *S. milleri* in the appendix than from patients who were not, and three-quarters of these swabs yielded *S. milleri*.

In respect of the other common bowel bacteria in the appendix the only outstanding difference between normal and diseased appendices was the low isolation rate of some bacteria, notably enterococci, in appendicitis. We have identified one site in the abdomen where *S. milleri* forms part of the normal flora. Its occurrence in the gut is unlikely to be limited to that site. There is some evidence that it is present, albeit transiently, in the large intestine, since Hare and Maxted (1935) isolated group F streptococci from normal faeces. It has been implicated in sepsis after colectomy (Poole and Wilson, 1976).

From the experience of Bateman *et al.* (1975) and Reid and Davidson (1976), neither the ileo-caecum nor the large intestine seem likely to be implicated commonly in the occurrence of *S. milleri* in liver abscess, although blood-borne infection from any site is possible. It may well be that *S. milleri* is more widely distributed throughout the alimentary tract or adjacent sites. Its presence may not always be easily recognised if it is mixed with either an aerobic or anaerobic faecal flora, and this may account for its not being recorded by other workers. Incubation in CO₂ in air facilitates its isolation, as does also the use of an enrichment medium incorporating sulphadimidine in addition to nalidixic acid. The enrichment medium we used enabled us to distinguish *S. milleri* from other streptococci as well as from Gram-negative bacteria. We used sulphadimidine in this way because we had found (Poole and Wilson, 1976)

that 131 haemolytic strains of *S. milleri* were uniformly resistant to sulphonamides, as were all other strains, both haemolytic and non-haemolytic, encountered by us in routine clinical bacteriology. Carlsson (1967) similarly had observed resistance to sulphonamide in *Streptococcus MG ATCC 9895*, which is identical with *S. milleri* (Mejare and Edwardsson, 1975). Other strains of streptococci resistant to sulphonamide did not present much problem. Besides enterococci, five resistant strains of *Streptococcus sanguis* and one of *Streptococcus mitior* were identified.

Our work indicates that *S. milleri* may be an important pathogen in appendicitis and its complications. Whether it plays an initial role in the aetiology of this condition remains uncertain.

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