REVIEW ARTICLE

Lancefield group F and related streptococci

J. M. WHITWORTH

The Dental Hospital, Richardson Road, Newcastle upon Tyne

Introduction

Improvements in methods of isolation and identification, in combination with changing patterns of disease, have brought into question the classical concepts of pathogenic and non-pathogenic micro-organisms. An example is the Lancefield group F and related streptococci (*Streptococcus milleri*) which has emerged in recent years as a group of organisms associated with purulent disease in man. In this review, an overview is given of the literature regarding the often confusing taxonomy, clinical significance, and cell-surface and potential virulence characters of these important streptococci.

The group F streptococci are gram-positive, facultatively anaerobic or capnophilic cocci, that form pairs or chains. Although the early isolates formed minute, haemolytic colonies on blood agar,^{1–3} colonies may display considerable diversity,⁴ and all types of haemolysis may be seen. A distinctive but variable feature is the production of a caramel-like odour in culture.

Taxonomy

According to European views of the taxonomy of this group of micro-organisms, all group F streptococci belong to the broader *S. milleri* group.⁵ In the following section, a historical review is given of the often confusing taxonomy of the *S. milleri* group, in an attempt to clarify current views. Organisms belonging to this taxon will be introduced by their original title, and in chronological order.

The earliest reference to an organism of the *S. milleri* group is believed to be the *S. anginosus* of Andrewes and Horder in 1906;⁶ this was a variant of *S. pyogenes* associated particularly with sore throat (angina). Despite questionable results when properties of the current *S. anginosus* type strain were compared with those of Andrewes and

Horder,⁷ association with the *S. milleri* group is believed to be valid. None of the original isolates is now available for examination.

Group F streptococci were first described in 1934² by Long and Bliss amongst the “minute haemolytic streptococci”. Serological grouping^{3,8} showed that 91% of these throat isolates were group F and the remainder group G. Serological types were also identified and Bliss’s serotype I was found in some group F and group G strains, whereas serotype II was found only among group F strains.⁸ Similar organisms were isolated from human faeces by Smith and Sherman,⁹ who retained the title “minute haemolytic streptococci” to describe only their group F strains. They preferred the name *S. anginosus* for their group G strains which were said to agree entirely with the original description of Andrewes and Horder. On the other hand, Niven,¹⁰ in 1957, included haemolytic streptococci of groups F and G under the heading *S. anginosus*.

Streptococcus MG, a non-haemolytic isolate, was isolated from the respiratory tract of patients with primary atypical pneumonia.¹¹ Serological examination^{12,13} led to its classification as a separate genus related only to *S. salivarius* type 1,¹⁴ but later examination of this relationship¹⁵ revealed a cross-reaction with typing sera. *Streptococcus MG* was found to be a group F, Ottens type III strain (see later), and cross-reactivity was explained by the similarity of the “salivarius” antigen to the type III antigen of the *Streptococcus MG* strain.¹⁶

The title “*S. milleri*” was introduced by Guthof in 1956¹⁷ to describe a physiologically distinct group of non-haemolytic streptococci isolated from dental abscesses and other suppurative lesions around the mouth. Lancefield grouping was applied unsuccessfully to these strains, but in their study of similar organisms isolated from dental root canals, Winkler and van Amerongen¹⁸ reported that many of their isolates were group F.

Classification of group F isolates across haemolytic boundaries was suggested by Ottens and Winkler^{19,20} in 1962; they noted only minor

differences in biochemical activity and identity of the group F antigen in haemolytic and non-haemolytic strains. They also showed²⁰ that group F streptococci could possess one of five (I–V) carbohydrate typing antigens (antigens of types I and II were as described by Bliss⁸). The distribution of typing antigens was subsequently observed outside group F, as follows:

Type I in haemolytic and indifferent (non-haemolytic) group G strains;²⁰

Type II in group T²¹ and group A strains;²²

Type III antigen in indifferent group C,²⁰ and in group L strains.¹⁵

These typing antigens were also present in strains which lacked a recognised grouping antigen.^{20,23,24}

The group F and related streptococci thus presented problems of classification resulting from variables in colony morphology and haemolytic activity, the presence of a series of typing antigens shared with other serological groups, and a range of specific names used over the years to describe various members of the group.

The problems of classification within the non-haemolytic streptococci were addressed in a series of studies by Colman and Williams in the 1960s,^{25–27} the results of which were summarised in 1972.⁵ Six species were recognised among the viridans streptococci from man: *S. pneumoniae*, *S. salivarius*, *S. mitior*, *S. milleri*, *S. sanguis* and *S. mutans*. Each species was serologically heterogeneous but possessed a distinctive combination of characters (see below).

Within the *S. milleri* cluster were included the following organisms:

- (i) *S. milleri*;¹⁷
- (ii) *Streptococcus MG*;¹¹
- (iii) all group F strains of streptococci;
- (iv) certain non-haemolytic streptococci of groups A, C, and G (comparison being made with a variant group A strain described by Michel and Gooder);²⁸
- (v) strains possessing an Ottens and Winkler²⁰ typing antigen, but no Lancefield group antigen.
- (vi) the “minute haemolytic streptococci”,^{1,2,29} the inclusion of which was tentative at that stage, but was later supported by the work of Mejre and Edwardsson,³⁰ Poole and Wilson,³¹ and Ltticken *et al.*³²

Therefore, considerable clarity was achieved by the Colman and Williams⁵ scheme, but acceptance was not universal, particularly in the USA, where

preference was given to other names for members of this group.

In his study of the physiological differentiation of viridans streptococci, Facklam³³ showed identity between *Streptococcus MG* and *S. intermedius*,^{34–36} (a non-haemolytic, lactose-fermenting organism often isolated in anaerobic culture), and between *S. constellatus*^{37–40} (a variably haemolytic, lactose non-fermenting strain also isolated in anaerobic culture) and *S. anginosus*. Considerable physiological and serological similarity was also noted between the two clusters. Despite agreeing with the Colman and Williams⁵ description of *S. milleri*, and accepting close relationships amongst these strains, Facklam³³ differentiated between them for epidemiological purposes. Non- β -haemolytic strains were divided on the basis of lactose fermentation into: *S. MG-intermedius* (lactose fermenter) and, *S. anginosus-constellatus* (lactose non-fermenter). Many of these strains belonged to Lancefield group F. β -Haemolytic group F streptococci were termed *S. anginosus*. This system of nomenclature was adhered to in the 1980 Approved List of Bacterial Names,⁴¹ in which *S. milleri* was given no official status.

Major differences in the American and British streptococcal taxonomic schemes, with special reference to *S. milleri*, were discussed further by Facklam in 1984,⁴² and are summarised in the table. All of these organisms may be considered as *S. milleri* according to the Colman and Williams⁵ scheme. A universally accepted taxonomy has not emerged for the *S. milleri* group of streptococci, and has prompted the search by several workers for alternative or additional means of establishing systematic relationships within this taxon.

Alternative techniques for analysing and clarifying these organisms have included gas chromatographic analysis of cellular fatty-acid

Table. Facklam’s proposed nomenclature⁴² for streptococci of the *S. milleri* group

Description of strains	Name (Facklam ⁴²)
β -haemolytic streptococci	
Group F	<i>S. anginosus</i> group F
Group A, minute	<i>S. anginosus</i> group A
Group C, minute	<i>S. anginosus</i> group C
Group G, minute	<i>S. anginosus</i> group G
Ungroupable	<i>S. anginosus</i> (no group)
Non- β -haemolytic strains (serological group not applicable)	
<i>S. MG-intermedius</i>	<i>S. intermedius</i>
<i>S. anginosus-constellatus</i>	<i>S. constellatus</i>

composition,^{43,44} and whole-cell trimethylsilyl-sugar profiles,⁴⁵ and SDS-PAGE examination of cellular proteins.^{46,47} Each method showed a general degree of homogeneity within the *S. milleri* taxon, and provided no definitive indication of subgroups within it. Recently, the techniques of genetic analysis (C + G content and DNA-DNA homology) have been applied in attempting to clarify taxonomic designations within the *S. milleri* group.

Examination of the C + G content of *S. milleri* strains by Drucker and Lee,⁴⁸ suggested that some degree of genetic heterogeneity may exist. However, DNA-DNA hybridisation studies by Welborn *et al.*⁴⁹ indicated that certain strains of *S. intermedius*, *S. constellatus*, *S. mitis*, *S. MG-intermedius*, and group F strains were closely related to each other genetically, and should be considered to belong to the same species. Although the group was physiologically heterogeneous, the division of strains on the basis of their ability to produce acid from lactose³³ was considered to be unjustified. Similar findings of genetic similarity were reported by Farrow and Collins⁵⁰ and Ezaki *et al.*⁵¹

More stringent hybridisation conditions were used by Kilpper-Bälz *et al.*,⁵² who demonstrated clear separation of the type strains of *S. constellatus*, and *S. anginosus*. *S. intermedius* was also shown to be clearly distinct although it was more closely related to *S. constellatus* than to *S. anginosus*. Two distinct groups were demonstrated within eight strains of "*S. milleri*" tested, one showing high homology with *S. constellatus*, and one with *S. anginosus*, suggesting their correct taxonomic designation to one of these groups. However, no reliable phenotypic markers were available to reflect the DNA-homology findings. In an extensive study by Coykendall *et al.*⁵³ embracing "*S. milleri*" isolates of all biotypes, haemolytic types, and serotypes, in addition to the type strains *S. anginosus*, *constellatus*, *intermedius* and group F streptococcus, considerable genetic similarity was noted, supporting taxonomic unification of these strains. Since, in his opinion *S. constellatus* and *S. milleri* were later synonyms of *S. anginosus*, and *S. intermedius*⁵⁴ was also a later synonym of *S. anginosus*, Coykendall⁵³ recommended the adoption of *S. anginosus* as the unifying title. More recently, however, Knight and Shlaes,⁵⁵ using highly stringent hybridisation conditions, found evidence of at least three genetically and phenotypically distinguishable groups within 18 representatives of the *S. intermedius* taxon. No formal proposals were made on the basis of their limited data.

To date, even the powerful techniques of genetic

analysis have been unable to reach unequivocal, definitive answers on the precise taxonomical position of a collection of organisms clustered within the *S. milleri* group by Colman and Williams.⁵

Comments on S. milleri taxonomy

Despite continued academic discussions on the details of streptococcal taxonomy, organisms of clinical significance must have names which convey clear meaning to those involved in the management of infected patients. The name *S. milleri* has become meaningful because of its association with deep-seated sepsis in man. Until clear evidence emerges to justify the use of more than one title to describe this group of related organisms, *S. milleri* will continue to be used by European taxonomists to describe a cluster of organisms listed in the 1986 edition of Bergey's Manual⁷ thus: *S. anginosus*;⁶ *Streptococcus MG*;¹¹⁻¹³ *S. constellatus*;^{37,40} *S. intermedius*;^{34,36} "*S. milleri*";¹⁷ minute β -haemolytic streptococci of groups F and G.^{2,8}

Common worldwide usage of the name *S. milleri*, it was noted, would make it likely that admission to the Approved List would be sought in the near future.

Throughout this review, the European system of classification will be used. Streptococci of Lancefield's group F will generally be referred to as *S. milleri* regardless of haemolytic activity or behaviour in biochemical tests. However, in reviewing the published reports of other workers, the titles used in the original communication will be used.

Isolation and identification of *S. milleri* in the clinical laboratory

Cultural requirements

Early group F isolates were described as "minute",^{1,2} reflecting both their microscopic appearance (50–60% the size of "ordinary" β -haemolytic streptococci), and their appearance on routine aerobic culture. Other strains have been considered anaerobic because of their improved growth under anaerobic conditions. Growth is enhanced by the addition of CO₂ to the atmosphere, or by the addition of oleic acid to the medium.^{29,56-58} Specimens in which *S. milleri* is suspected should be cultured routinely in an atmosphere containing CO₂ 5–10% for periods up to 48 h, and sometimes for greater periods in cases of intracranial sepsis.⁵⁹ Culture of samples under increased CO₂ tension is now routine practice in many service laboratories, and this may have contributed to the increased

recognition of *S. milleri* in clinical specimens in recent years.

Selective media have not been developed for the isolation of *S. milleri* from mixed cultures. However, the sulphonamide-containing MC agar of Carlsson,^{30,60,61} originally described for the isolation of *S. mutans* from the oral cavity, is useful.

Identification of S. milleri in the clinical laboratory

Streptococcal identification in the clinical laboratory is dichotomous. Classically, only β -haemolytic varieties were considered as noteworthy pathogens, and Lancefield⁶² grouping provided a simple and accurate means for their identification. The viridans streptococci cannot be reliably classified by Lancefield grouping, and despite numerous attempts to develop an alternative serological scheme,⁶³⁻⁷¹ no satisfactory system has emerged. Viridans streptococci are necessarily classified by physiological and biochemical tests.

S. milleri represents a special case, in that its members traverse traditional haemolytic boundaries, and are known to be capable of possessing C antigens A, C, F, or G. Isolates may be classified as *S. milleri* on the basis of characteristic biochemical tests, or, as with the group F streptococci, on the basis of Lancefield grouping. Behaviour in biochemical and physiological tests has been described by many workers as relatively homogeneous.^{5,30,32,72-74} Strains brought together as *S. milleri* by Colman and Williams,⁵ with few exceptions, fermented lactose, sucrose, trehalose and salicin, formed acetoin from glucose, hydrolysed arginine and aesculin, and were resistant to bacitracin and nitrofurazone. Their cell walls contained rhamnose and, usually, glucose, galactose, and galactosamine. Few grew at 45°C, or in NaCl 4% broth, fermented raffinose or inulin, or hydrolysed starch. None formed extracellular polysaccharide from sucrose, survived 60°C for 30 min, or hydrolysed hippurate.

Behaviour is not, however, entirely uniform. Considerable variation in single or small groups of characters was demonstrated by Ball and Parker,⁷⁵ who described a central group of *S. milleri* isolates displaying typical biochemical reactions, in addition to two major deviations from this pattern: (1) "loss" of one or more reactions; and (2) "gaining" the ability to acidify additional sugars, notably raffinose, melibiose, or mannitol. Characteristically, though not exclusively, isolates displaying wide carbohydrate-utilisation profiles have been associated with the female genital tract.^{75,76}

Before the advent of commercially-available galleries of pre-formed tests, the identification of

viridans streptococci to species level was a laborious and time-consuming process involving the application of large batteries of tests,^{5,32,33,73,74,77,78} that were impractical for routine use by service laboratories. Identification of these isolates of questionable clinical significance was limited to laboratories with a special interest, and resulted in widespread use of such descriptions as "viridans streptococci", or "normal commensal flora". Some laboratories developed short series of tests for the presumptive identification of isolates which carry inherent problems of inaccuracy.^{72,79-81}

Commercial kits (e.g., the API-20 Strep system) have revolutionised the identification of the viridans streptococci to species level, and have been favourably reviewed for the identification of *S. milleri* isolates,⁸²⁻⁸⁵ accommodating the different sugar-fermentation patterns known to exist.⁸⁴ Three biotypes are currently described within the group. Rapid systems are also readily available for the serological grouping of streptococci, and the majority now contain reagents for the identification of group F streptococci.

The clinical significance of *S. milleri*

S. milleri as a commensal in man

In common with other species of viridans streptococci, *S. milleri* has been regarded in the past as a member of the complex resident microflora of a number of mucosal sites, and as a pathogen of note only in infective endocarditis. Before discussing the current status of *S. milleri* as a pathogen in man, the literature will be reviewed concerning sites in which this organism may be considered to be part of the resident flora.

The oral cavity. *Streptococcus* sp. MG was regarded as a normal resident of the human mouth^{86,87} and *S. milleri* has been isolated from the healthy mouths of children,⁸⁸⁻⁹⁰ and adults.^{30,91-93} *S. milleri* appears to have a predilection for sheltered areas on hard surfaces within the mouth, notably the gingival crevice³⁰ and the fitting surface of dentures.⁹⁴ As a proportion of the total oral streptococcal flora, the contribution of *S. milleri* appears to increase with the number of deciduous teeth in children.⁹⁰

The upper respiratory tract. The original descriptions of "minute haemolytic streptococci",^{1,2} group F streptococci,^{3,95} and *Streptococcus* MG,¹¹ included isolates from the healthy upper respiratory tract. Although colonisation of children appears to be slow,⁹¹ *S. milleri* is a resident of the healthy pharyngeal mucosa of adults.^{91,96} α -Haemolytic

and micro-aerophilic streptococci are also components of the flora of normal maxillary sinuses,⁹⁷ and it is speculated that *S. milleri* may contribute to the flora of this site.

The gastrointestinal tract. Numerous studies have demonstrated *S. milleri* group streptococci as components of the normal faecal flora;^{9,95,98-101} one study found a particular association with the appendix.¹⁰² Again, levels appear to be higher in adults than in children.

Quantitatively, *S. milleri* has been demonstrated in moderate numbers (10^3 – 10^6 /g of faeces) in the stools of healthy adults,¹⁰⁴ suggesting that the gut is an important reservoir of this organism.

The urogenital system. Group F streptococci and *S. milleri* have been isolated from the male urinary tract, the vagina and from urine samples, possibly as vaginal or perineal contaminants.^{3,4,58,75,105-108} No pathogenic role was suggested in these sites, and, recently, Rabe *et al.*,¹⁰⁹ found *S. intermedius* to be the species of viridans streptococcus isolated most frequently from the healthy vagina.

S. milleri as a pathogen

Virtually all infections due to viridans streptococci arise endogenously. Traditionally, the aerobic streptococci of the mouth have been regarded as a rather homogeneous group of low-grade pathogens. Today this belief is no longer valid,¹¹⁰ and *S. milleri* is becoming recognised as an important cause of pyogenic infection at a range of sites. Full appraisal of the pathogenic role of *S. milleri* is made difficult by inaccurate speciation in the past, and no attempt is made in the current review to convert such descriptions as *S. viridans* into modern equivalents.

The mouth and peri-oral tissues. Dental caries. *S. milleri*, unlike *S. mutans*, is not recognised as an agent of primary aetiological importance in dental caries, despite its reportedly high levels in deep areas of carious dentine.¹¹¹ Animal experiments have indicated varying degrees of cariogenicity for organisms of the *S. milleri* group,¹¹²⁻¹¹⁶ all but one of which,¹¹² demonstrated the capacity of *S. milleri* to produce caries in experimental animals, though less severely than *S. mutans* strains. The involvement of *S. milleri* as a primary agent in the initiation and progression of dental caries has not been substantiated, and would seem unlikely. Hardie *et al.*¹¹⁷ isolated *S. milleri* from 45% of proximal plaque samples from sites which had not developed carious lesions over a 2-year period, and recent *in vitro* studies have also shown that *S. intermedius* cannot reduce the pH at a tooth surface to the same extent as *S. mutans*.¹¹⁸

Periodontal disease. *S. milleri* group streptococci have been isolated frequently, and in high numbers in association with experimental gingivitis,⁴⁷ early periodontitis,¹¹⁹ severe periodontitis,⁵⁴ and severe generalised periodontitis.¹²⁰ Implication of a pathogenic role for individual bacterial species within the mixed flora of periodontal sites is always difficult, and no specific roles were suggested. It was suggested by Moore *et al.*⁵⁴ that *S. anginosus* may be implicated in a model of episodic destructive activity in periodontal disease, caused by periods of aggressive pyogenic activity. However, Crawford and Russell⁹³ found no significant differences in the numbers of viridans streptococci isolated from sites described as "healthy", "gingivitis", "early or moderate periodontitis", or "severe periodontitis". *S. milleri* was identified in low numbers, and no pathological significance could be attributed to it.

Haffajee *et al.*¹²¹ observed that the proportions of *S. intermedius* were increased in both active and inactive sites in groups of patients who responded poorly to periodontal therapy, compared to a similar group who responded well. Such an observation, if confirmed, could be useful as a bacteriological prognostic indicator before commencing periodontal therapy.

Although associations are rarely clear-cut, it is possible that, in certain circumstances, members of the *S. milleri* group, within a mixed flora, could contribute to the pathogenic processes of periodontal disease.

Odontogenic abscesses. The association of *S. milleri* with purulent lesions of odontogenic origin was described by Guthof in 1956.¹⁷ More recently, reports of *S. milleri* group isolates from dental abscesses, often in pure culture, have confirmed a strong association with such lesions,^{32,58,91,106,107,122-129} being the commonest facultative organisms isolated from dental abscesses.^{130,131} Their pathogenic importance in abscess formation remains unclear, but a role in autogenic succession within a developing lesion has been suggested.^{124,132,133} Lewis *et al.*¹³³ showed that *S. milleri* was often isolated in pure culture from lesions aspirated on the first day of clinical symptoms, whereas lesions symptomatic for 2–3 days tended to yield a predominantly anaerobic flora. Synergic interactions between *S. milleri* and anaerobic gram-negative bacilli injected subcutaneously into mice have recently been demonstrated,¹³⁴ which may be important in the development of dentoalveolar and other abscesses in man.

Paranasal sinusitis. Close proximity of the root apices of the maxillary dentition to the floor of the

maxillary antrum makes possible the direct spread of purulent material from maxillary periapical lesions to the sinus. The bacterial flora of the infected sinus in these circumstances is likely to reflect that of the discharging dental lesion. *S. milleri* may be involved in a process of autogenic succession in sinusitis of non-dental origin, similar to its suggested role in dental abscesses. Closure of the sinus ostium by mucosal hyperaemia leads to a fall in oxygen tension within the sinus, which initially favours *S. pneumoniae* and *Haemophilus influenzae*. A continued fall in oxygen tension, coupled with a rise in CO₂ tension, encourages the growth of micro-aerophiles such as *S. milleri*, and ultimately strict anaerobes. Chronic sinusitis is commonly associated with anaerobic and micro-aerophilic streptococci.¹³⁵ Organisms of the *S. milleri* group have been isolated from cases of sinusitis, sometimes in pure culture, and from complicating lesions such as subdural empyema and periorbital cellulitis.^{106,107,135-138} Other recognised complications include meningitis and intracranial abscess.

Intracranial and spinal infection. Reports of *S. milleri* in infections of the central nervous system and meninges are numerous.^{33,59,74,101,135,137,139-153} Streptococci form the largest group of organisms isolated from intracranial pus, and, of these, *S. milleri* is the commonest.¹⁵⁴ In one major series of intracranial abscesses⁵⁹ all 20 of the *S. milleri* isolates encountered belonged to Lancefield group F, and possessed the Ottens type III antigen. Furthermore, animal experiments¹⁵⁵ have shown that *S. milleri* has a well-defined affinity for the central nervous system of young mice. *S. milleri* has been isolated from purulent lesions in a range of intracranial sites, but abscess of the frontal lobe secondary to sinusitis is observed most frequently.⁵⁹ Intracranial sepsis due to *S. milleri* may also arise following dental infection,¹⁴⁵ traumatic injury,¹⁴⁸ or metastatic spread from a distant purulent focus.¹⁴⁴

Infections of the cardiovascular system. Bacteraemia. Transient bacteraemia is probably common and unsuspected in most cases. Viridans streptococci may enter the circulation following vigorous toothbrushing, and occasionally intestinal bacteria enter the portal circulation. More significant bacteraemia may follow trauma, surgical manipulation, or the development of a neoplastic or focal purulent lesion in various body sites. Bacteraemia due to *S. milleri*, often following dental extraction, has been documented frequently.^{93,156-160} However, bacteraemic infections due solely to *S. milleri* are so infrequent that the isolation of *S. milleri* from

the blood of a febrile patient should always stimulate a search for pus in an internal organ.^{128,161,162} In 1988, Minault *et al.*¹⁶³ described a case of septic shock due to *S. milleri* following endoscopic sclerosis of oesophageal varices.

Infective endocarditis. *S. milleri* is not a common cause of endocarditis,⁷⁴ even in elderly males, accounting for only 4-15% of α -haemolytic streptococcal endocarditis.^{33,74,164} It has been suggested¹⁶⁵ that dextran production may aid the establishment of streptococci on heart valves, by virtue of its stickiness. The inability of *S. milleri* to produce such extracellular polysaccharides was suggested by Parker and Ball⁷⁴ as a possible reason for the low incidence of endocarditis due to this organism. Although *S. milleri* is an infrequent cause of endocarditis, Murray *et al.*¹⁰¹ reported its association with an unusually high frequency of metastatic suppurative complications, but evidence for local cardiac tissue destruction could not be found, despite the nature of the extracardiac complications. On the other hand, Sussman *et al.*¹⁶⁶ were firmly of the opinion that inaccuracies in the speciation of viridans streptococci rendered reports of species meaningless, and that no well-characterised species was associated with an outcome more serious than any other. Isolated reports have implicated *S. milleri* in cases of myocardial abscess associated with endocarditis,^{167,168} or as a metastatic lesion secondary to a primary purulent focus;¹⁶⁹ mitral valve aneurism secondary to endocarditis;¹⁷⁰ and mycotic aneurism of the aorta.¹⁷¹ *S. milleri* has also been isolated from cases of purulent pericarditis.^{126,172-174}

Infection of the respiratory tract. Pharyngitis. *S. anginosus*,^{175,176} and group F streptococci^{3,105} have been isolated from infected throats. More recent surveys have demonstrated extremely low (0.04-1%) isolation rates of β -haemolytic group F streptococci from cases of acute pharyngitis,^{106,177,178} although other reports have indicated higher pharyngeal isolation rates for *S. milleri*.^{107,179} Association with pharyngitis however, was not always clear. Poole and Wilson³¹ showed a trend towards heavier growths of *S. milleri* from the throats of patients with symptoms than from asymptomatic carriers. Of 25 patients complaining of sore throat or tonsillitis, 21 (84%) yielded the organism in heavy growth compared with 5 (33%) of 16 symptomless carriers. *S. milleri* is not generally regarded as an important pathogen in the throat, and recent studies have indicated the importance of differentiating between *S. milleri* isolates and their "large-colony-forming" counterparts in throat cultures.^{179,180} *S. milleri* is not associated with the

serious post-streptococcal complications seen in group A streptococcal infection.

Pleuropulmonary disease. In recent years, *S. milleri* has been recognised as a cause of purulent pleuropulmonary disease, notably pleural empyema.^{74,181-185} The mouth and upper respiratory tract have frequently been regarded as the source of *S. milleri*, and it is thought that infections are preceded by aspiration.^{126,182,186} Cases have also followed pneumoniae,¹⁸⁴ and hepatic abscess.¹⁸⁷ The isolation of *S. milleri* in pure culture from pulmonary abscesses and empyema is not uncommon, particularly when sepsis is confined to the pulmonary cavity, whereas the presence of gastrointestinal fistulae often gives rise to a mixed flora.¹⁸⁶ A preponderance in the distribution of empyemas caused by *S. milleri* in males has been noted.^{126,184}

Abdominal infection. Surgical sepsis. Recognition of *S. milleri* in abdominal sepsis including acute appendicitis, peritonitis, pelvic abscess, and purulent wound discharge is increasing.^{31,58,101,106,107,129,152,161,188-191} Poole and Wilson¹⁰² recognised a highly significant increase in the isolation rate in the presence of inflammation, and a pronounced correlation between the presence of *S. milleri* in the appendix and the purulent manifestations of appendicitis. 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*. It has been suggested that the prophylactic use of antibiotic combinations such as gentamicin and metronidazole in patients undergoing colorectal surgery may be a possible cause of the emergence of *S. milleri* as an abdominal pathogen.^{189,190} Recommendation was made by both groups that *S. milleri* should be considered in the formulation of prophylactic and therapeutic antibiotic regimens for patients undergoing abdominal surgery.

Hepatic abscess. Anaerobic and micro-aerophilic streptococci are the commonest isolates from pyogenic liver abscesses.¹⁹² Cases of hepatic abscess due to *S. milleri*, often carrying the group F antigen, and often in pure culture, have appeared frequently in the literature of this rare condition.^{58,74,101,126,193-201} Moore-Gillon *et al.*¹⁶² described 16 cases of pyogenic liver abscess over a 10-year period. In 10 instances, *S. milleri* was isolated in pure culture, and in mixed culture in a further three. Of the 13 *S. milleri* isolates, 12 belonged to Lancefield group F. Hatoff¹⁹⁸ also recognised *S. milleri* as the most frequent isolate from liver abscesses, and as in surgical sepsis,¹⁸⁹ suggested overgrowth of this organism during metronidazole

therapy as a possible factor in the development of *S. milleri* liver abscesses. In most cases the source of *S. milleri* was probably abdominal, via the portal circulation, though a dental origin was suggested in one case.¹⁹⁵

Other infections. Neonatal infection. *S. milleri* has been associated only infrequently with intrauterine pneumonia,^{202,203} fulminant sepsis,^{204,205} and septicaemia of the newborn.²⁰⁶ In most cases, infection was associated with premature rupture of the membranes and consequent ascending infection.

Infection of bone and joints. There have been sporadic reports of the association of *S. milleri* with septic arthritis^{128,207-209} and osteomyelitis.^{126,169} Such infections have usually been in an immunocompromised host with alcoholism or diabetes, or receiving immunosuppressive treatment.

Infection of skin and subcutaneous tissue. Miller *et al.*²¹⁰ reported a very high incidence of subcutaneous sepsis caused by *S. milleri* following human bites. A similarly high incidence of small distal extremity abscesses secondary to trauma was reported by Libertin *et al.*¹²⁸ Rarer conditions have included necrotising fasciitis,^{126,211} and hydradenitis suppurativa.²¹²

Summary of clinical significance

S. milleri has in recent years been associated increasingly with serious purulent infection at various sites. Infection is usually endogenous, and preceded by disease or trauma to a mucosal surface. Often, a systemic condition which compromises the immune system predisposes the host to endogenous infection. However, unlike many opportunist infections, no significant increase in incidence is noted in the elderly.²¹³ Another notable feature is the preponderance of reported cases of infection in men, and much smaller numbers in women after the first decade of life.²¹³

Management of infection caused by *S. milleri*

Surgical drainage remains central to the management of abscesses, and is often augmented by antibiotics. The antibiotic of choice for infections caused by *S. milleri* is penicillin, to which all but a few strains are very sensitive.^{126,157,214,215} In the case of liver abscesses, and a small number of patients with persistent surgical sepsis, courses of 28 days or longer have been recommended.^{189,194,195} Suitable alternatives to penicillin include erythromycin, clindamycin, and a cephalosporin. Sensitivity to tetracyclines is variable.^{126,194,215} Sulphonamides are inactive against

S. milleri and have been used as a selective agent for the isolation of this organism from the oral cavity.^{30,61}

The cell surface and potential virulence characters of group F and related streptococci

It is clear that *S. milleri* is capable of producing infection in a range of sites, and appears to be unusual amongst the viridans streptococci in its capacity to produce purulent disease. Whether the ability to produce purulent infection is a capacity possessed uniformly by all members of this taxon, or whether there are defined sub-groups with regard to clinical significance remains unclear. In the following section, the literature regarding the cell-surface characteristics, and potential virulence factors in *S. milleri* disease is reviewed with special reference to group F streptococci.

The bacterial cell surface and its relationship with pathogenicity

Structural components of the bacterial cell envelope are often fundamental to pathogenicity,

and structural components may be primary factors in producing disease. A diagram illustrating the basic components of the gram-positive cell envelope is shown in the figure.

Capsule. Encapsulation is a well-established bacterial virulence character, important in conferring various degrees of resistance to phagocytosis by inflammatory cells. The presence of capsules in *S. milleri* strains has rarely been reported.

Some *S. milleri* group streptococci were shown by Brook and Walker²¹⁶ to possess a polysaccharide capsule, demonstrated by Hiss's and ruthenium red stains. Only capsule strains were able to produce subcutaneous abscesses when injected alone into mice. However, passaging of non-capsulate strains with other capsule organisms often restored encapsulation and pathogenicity. Lewis *et al.*¹³⁴ also demonstrated the capacity of *S. milleri* to produce experimental abscesses in pure culture, and speculated that encapsulation may be a possible virulence character. The nature of such capsular material is unknown, but the typing antigens present in group F and related streptococci have often been regarded as microcapsular structures, capable of preventing phagocytosis.²¹⁷ The typing

Figure. The gram-positive cell envelope. P, protein; PL, phospholipid; H = cross-linking peptide chain (courtesy of J. Wiley & Co.).

antigens of group F streptococci will be discussed more fully below.

Wall-associated proteins. Lütticken *et al.*³² demonstrated the presence of one or two protein antigens ("sm" antigens) in HCl extracts of many *S. milleri* strains. These protein antigens were believed to be located at or near the cell surface, and a role in virulence analogous to that of *S. pyogenes* M protein was suggested. At least three serologically distinct protein antigens were also isolated from group F reference strain O'Mahoney (Colindale) by Nakayama and Maekawa.²¹⁸

Surface appendages. The term "fibrillae" has been used to describe fine wisps of M protein on the surface of *S. pyogenes*.²¹⁹ Fine fimbrial structures have been demonstrated on the surface of oral streptococci, including *S. milleri*,^{220,221} which may be important in adherence to surfaces, and in the inter-species interactions common in the mixed flora of mucosal surfaces, dental plaque, and purulent lesions. Little work has been done on the possible role of such structures in *S. milleri* disease.

Accessory carbohydrate polymers of the group F streptococci. Accessory wall polymers in the form of group and type antigens have been described in group F strains. The group F C-substance was described by Lancefield and Hare,³ and was believed to be carbohydrate, in common with the group antigens of other streptococcal strains. Ottens and Winkler²⁰ also described five independent carbohydrate type antigens in group F and related strains. Both the group and type antigens were regarded as cell-wall components located in or on the cell wall, but the presence of some antigenic material within the cell could not be excluded.²⁰ Considerable interest was shown in the biochemical nature of group and type antigens contained in group F and related streptococci by Willers's group in Utrecht during the 1960's and early 1970's. The most probable structure of the group-specific oligosaccharide contained in group F strains was believed to be 3- β -D-glucopyranosyl-N-acetyl-D-galactosamine.²²²⁻²²⁴

Type antigens of group F streptococci. *Type I:* contains galactose, glucose, rhamnose and galactosamine in the approximate molar ratio 4:2:2:1; the determinant group probably contains N-acetyl-galactosamine.^{222,225} *Type II:* contains galactosamine, galactose, rhamnose, and glucose in the molar ratio 1:1:2:2;^{224,226} the antigenic determinant consists of β -(N-acetyl-D-galactosaminyl)-(1-2)- α -D-galactosyl-(1-2)- α -L-rhamnosyl-(1-4)- β -D-glucosyl-(1-4)-D-glucose and N-acetyl-galactosamine is the terminal sugar. *Type III:* a linear polymer containing mainly rhamnose, glucose and galactose

in the approximate molar ratio 1:2:3;²²⁷ the most probable structure of the determinant group is suggested to be β -glucosyl-(1-6)-galactosyl-(1-6)-galactosyl-(1-3)-rhamnose, but the presence of a second determinant group was also considered to be possible. *Type IV:* contains rhamnose, glucose, galactose and N-acetyl-glucosamine in the molar ratio 4:4:4:1;²²⁸ inhibition reactions indicate that both of the trisaccharides β -galactosyl-glucosyl-galactose and β -galactosyl-glucosyl-rhamnose, are determinant groups; no clear information was provided on the linkages within these trisaccharides, nor on the role of N-acetylglucosamine in the antigen molecule. *Type V:* although the type V antigen was described by Ottens and Winkler²⁰ and partially characterised by Willers *et al.*,²²² it was later found not to be a distinct type.²²⁹

Most of the analysis described above was on material purified from formamide extracts of whole cells,^{15,223,227} or of cell walls purified by incubation with proteolytic enzymes,²²² a method known to be capable of leaving substantial amounts of cytoplasmic debris in association with cell walls.²¹⁷ There is no evidence to indicate a direct role of the group- or type-specific antigens of group F streptococci in pathogenicity.

Distribution of group F and type antigens within the bacterial envelope. Group and type antigens were both believed to be cell-wall components that could be extracted from cell walls by a variety of methods. A superficial position in the wall was proposed for type antigens, based on the following evidence:

- (i) the release of type antigen into the culture medium during growth;
- (ii) the production of antibodies directed only against type antigen when strains containing group and type antigens were used to raise antisera in rabbits;²¹⁷ and
- (iii) the localisation of fluorescence on streptococci carrying both group and type antigen after treatment with fluorescent anti-type or anti-group sera²³⁰—weaker fluorescence was observed with anti-group serum, suggesting a sub-surface location.

A surface, microcapsular location was proposed for the type antigen, though this "capsule" was not demonstrable by negative staining with India ink. However, electronmicroscopy of cell sections treated with ferritin-labelled anti-type sera by Huis in't Veld and Linssen²³¹ showed that type antigen was present in abundance throughout the thickness of the cell envelope. Furthermore, very dense localisation of ferritin particles was seen in the cytoplasm, close to the cytoplasmic membrane.

This was suggested as a site of antigen synthesis or storage. Negative staining with India ink again did not demonstrate the presence of a capsule.

Recently, Yakushiji *et al.*²³² demonstrated at least 10 distinct serotypes within oral *S. milleri* strains. Carbohydrate typing antigens, which are said to be distinct from Lancefield grouping antigens, were extracted from whole cells by the Rantz and Randall²³³ autoclaving method. Strains belonging to serotypes a, c, and f corresponded strictly with those of Lancefield groups A, C, and F respectively. Only the type b antigen has been investigated in any detail;²³⁴ it contained rhamnose and glucose in the molar ratio 1.7:1.0, with a trace of galactosamine (0.1). Glycerol and ribitol were not detected, and the amounts of protein and phosphorus were negligible. Quantitative inhibition tests suggested that rhamnose was structurally involved in the immunodeterminant epitope.

The cytoplasmic membrane. The isolation and analysis of membrane, or lipoteichoic acids, from group F and related streptococci has not been described, although a hexosamine-free component with the properties of a lipoteichoic acid was demonstrated in a z₃III strain by Huis in't Veld and Linssen.²³¹

Plackett and Shaw²³⁵ isolated immunologically active diglucosyl diglycerides from *Streptococcus MG* (NCTC 8037) that cross-reacted with antisera to *Mycoplasma laidlawii* (*pneumoniae*). Similar glycolipids were found in the cell walls of z₃III and F III strains by Huis in't Veld and Willers.²¹⁷ The complexing of glycolipids with teichoic acids in the walls of gram-positive organisms had been described earlier by Wicken and Knox,²³⁶ and it could not be excluded that this may occur in the cell walls of streptococci.²¹⁷

It is interesting to speculate from the behaviour and distribution of type antigens within group F strains that these polymers may be lipoteichoic acids or analogues thereof. Little work has been done to investigate this possibility, although recent and hitherto unpublished results of the authors lend further support to this possibility.

Extracellular products of S. milleri

Enzymes. *Hyaluronidase.* Some *S. milleri* group streptococci, especially β -haemolytic varieties (many belonging to group F), produce hyaluronidase.^{5,91,237} Four distinct serotypes of *S. milleri* hyaluronidase were identified by Unsworth *et al.*,²³⁸ which correlated with the source of the strain. Isolates from dental plaque, and from purulent lesions predominantly produced type IV hyaluron-

idase, suggesting an oral origin for "abscess strains". High titres of antibodies in blood-donor sera to type III and type IV hyaluronidases additionally suggested a greater invasiveness for strains producing these serotypes of enzyme. Recently, Unsworth²³⁷ demonstrated a strong correlation between hyaluronidase production and isolation from internal abscesses. Conversely, *S. milleri* isolates from non-abscess sites tended not to produce hyaluronidase. Therefore, hyaluronidase production may be important in pathogenicity, and its detection could be helpful in predicting the likelihood of deep sepsis in isolates from blood cultures.

Nucleases. Production of RNAase and DNAase has been observed in some strains of *S. intermedius*²³⁹ and *S. milleri*.⁴⁸ No correlation has been shown between pathogenicity and production of nucleases in these strains.

Proteolytic enzymes. The release of extracellular proteins, including potentially damaging proteolytic enzymes, was demonstrated in an endocarditis strain of *S. MG-intermedius* by Strauss *et al.*²⁴⁰ When grown in conditions of essential amino-acid deprivation, to mimic the conditions in a fibrotic heart lesion, the release of proteins as a proportion of the dry weight of bacteria increased 4–8-fold. Therefore, it was suggested that in infected sites where nutritional conditions may not be optimal, certain bacterial strains may still be able to damage host tissues by the continued release of destructive enzymes.

Rutinase. Some strains of *S. milleri* (though not *S. salivarius* or *S. mutans*) from the mouths of healthy individuals are capable of releasing carcinogenic substances by the hydrolysis of common foodstuffs.²⁴¹ Hydrolysis of rutin, a common component of foods and beverages, liberated quercetin, a genotoxic substance which may be involved in the production of epithelial carcinoma. It was speculated that certain oral microbial populations may be instrumental in carcinogenesis in the mouth, and a possible correlation between poor oral hygiene and oral cancer was suggested.

Bacteriocins. The production of substances that are antagonistic to other micro-organisms has important implications for colonisation ability in a mixed flora. Bacteriocin-like activity was demonstrated in 78% of α -haemolytic streptococci by Dajani *et al.*²⁴² Antagonistic activities of α -haemolytic streptococci in the urogenital tract have also been recognised.⁹⁶ Drucker and McKillop²⁴³ described the widespread production of antagonistic substances in the form of H₂O₂ production and bacteriocin-like activity amongst *S. milleri*

strains. It was also observed that all *S. milleri* isolates were sensitive to the bacteriocin-like activity of *S. mutans* (NCTC 10832). However recent work²⁴⁴ has suggested a low rate of bacteriocin-like activity among α -haemolytic *S. milleri* strains.

Interaction with the host immune system

Higherd *et al.*²⁴⁵ found that the crude extracellular products liberated by gentle washing of *S. intermedius* cells (CEP-Si) suppressed fibroblast proliferation and altered lymphocytic immunological responses *in vitro*. Subsequent investigation detected a strongly immunosuppressive, non-cytotoxic substance in this crude extract.²⁴⁶ Purification revealed a 90 Kda protein, designated f3'EP-Si, which was further shown to induce T-suppressor lymphocytes, and to have B-cell mitogenic activity.²⁴⁷⁻²⁴⁹ The extent of production of this immunosuppressive and fibroblast-inhibiting substance in *S. milleri* strains has not been established, but it is possible that it may emerge as an important virulence factor for this species.

Comments on the cell-surface and virulence characters of S. milleri

There can be little doubt that certain organisms belonging to the *S. milleri* group are capable of causing infection in man. It is unclear from the literature whether the capacity to produce infection is, in the right circumstances, a quality possessed uniformly by all members of this diverse group, or whether it is a property of certain members only. The work done hitherto on the potential virulence mechanisms involved in *S. milleri* disease has revealed several potentially important agencies including: the capacity to tolerate conditions of low Eh; possession of antiphagocytic capsular material; possession of antiphagocytic surface-protein structures; the release of material which interacts with the host immune system; and the release of hyaluronidase. The relative importance of these and other factors and their distribution within the *S. milleri* group as a whole have yet to be clearly established. Pathogenic mechanisms within the *S. milleri* group remain largely unclear.

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