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Group Q Streptococci

I. Ecology, Serology, Physiology, and Relationship to Established Enterococci

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The group Q streptococci possess unique serological and physiological characteristics which differentiate them from established enterococci. The group Q antigen was not demonstrable in all strains; however, all possessed the group D antigen. All group Q strains were physiologically similar regardless of whether or not they possessed the group Q antigen. These strains differed from the established enterococcal species, as they neither hydrolyzed arginine nor initiated growth in 1.0% methylene blue-milk. They also differed radically in the fermentation of various carbohydrates, especially the polyhydric sugar alcohols. The results indicate that the group Q streptococci constitute a unique taxonomic entity; the species designation *Streptococcus avium* sp. n. is suggested, owing to their characteristic occurrence in chicken fecal specimens.

Although the streptococcal flora of the mammalian intestinal tract has been the subject of many previous investigations, this segment of the intestinal flora has not been characterized completely. It is generally accepted that the predominant streptococci occurring in the intestinal tract belong to Lancefield's serological group D and that the species incidence varies with the mammalian host animal. However, even within this serological group, confusion still exists regarding speciation as only too frequently strains have been isolated which do not conform to the described and established species.

While studying the streptococcal flora of human feces, the occurrence of isolates was noted which resembled the enterococci in some respects but differed significantly in other key characteristics. A collection of these strains shared a common antigen, distinct from the group D antigen, and creation of a new serological entity, group Q, was suggested (6). Subsequently, Smith and Shattock (20) demonstrated the occurrence of the group Q antigen in the cell wall of these organisms and, in addition, they detected the group D antigen between the wall and the membrane, where it occurs in the established group D species (7, 18).

It was demonstrated that the group Q streptococci grow at 10 and 45 C, at *p*H 9.6, in 6.5% sodium chloride, and resist heating at 62.5 C for 30 min (6, 20). These are the classical tests for the delineation of the enterococci (17). However, the group Q strains could be distinguished from the enterococci in that they are unable to initiate growth in 0.1% methylene blue-milk, and, unlike most streptococci, they characteristically ferment sorbose.

The close physiological relationship between these two serological groups is evident, and the possibility exists that the group Q streptococci may account partially for the occurrence of so-called "intermediate strains" of group D streptococci. On this premise a more detailed study of the ecology, serology, and physiology of the group Q streptococci was undertaken.

MATERIALS AND METHODS

Rabbits were immunized with group Q strains by the method of Lancefield (10). These strains were obtained from D. G. Smith, University of London, England. In addition to these experimentally prepared antisera, a commercial group Q antiserum was purchased from Burroughs Wellcome Co., Tuckahoe, N.Y. The group D antiserum was also obtained from the latter source.

Antigens were extracted from the cells by the hot acid (11) and formamide (5) procedures. A modification of the Lancefield method (13) for growing cells was used to obtain superior yields of the group D antigen in extracts. Antigen extracts evidencing weak reactions in tube precipitin tests were concentrated by the alcohol precipitation method (16).

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The double-diffusion precipitin technique (14) was employed with the diffusion gel suggested by Wilson and Pringle (21). The plates were incubated at room temperature for 2 weeks and checked daily for bands of precipitate.

The physiological methods used to characterize the strains have been presented previously (4).

RESULTS

Isolation and ecology. In preliminary experiments it was observed that pure cultures of the group Q streptococci could initiate growth in a medium adjusted to *p*H 10 (2) with glucose as the energy source. These organisms could also grow in this medium if the glucose was replaced with sorbose, and characteristically pure cultures of *Streptococcus faecalis* and *S. faecium* (3) were unable to grow in the *p*H 10 medium with sorbose as the energy source. Therefore, to enhance the enrichment of group Q streptococci, the *p*H 10 medium was prepared with 1.0% sorbose as well as 0.02% sodium azide. Fecal specimens from various animals and food samples were cultured in this medium (48 hr at 37 C) and then streaked on a sorbose-bromocresol purple-nutrient agar for isolation. The isolates were identified by precipitin and physiological tests, as shall be discussed subsequently. A high incidence of group Q streptococci was detected in chicken feces, and a diminished incidence was observed in specimens from dogs, pigs, and humans (Table 1). No strains were isolated from cow, horse, mouse, rabbit, or sheep specimens or from a wide variety of various food samples.

By use of the most probable numbers technique, coupled with the *p*H 10 medium in which glucose (*S. faecalis* and *S. faecium* count) or sorbose (group Q streptococcus count) was used as the energy source, the relative incidence of the enterococcal species in chicken fecal matter was quantitated. Subcultures from all tubes evidencing growth were performed and the isolates were identified by physiological tests. In all instances, *S. faecalis* or *S. faecium* (or both) predominated; diminished, but significant, numbers of the group Q streptococci were present (Table 2).

Serology. The 17 strains isolated in this study and the 5 strains obtained from previous investigators were subjected to a detailed study to detect the group Q and D antigens. Nine of the strains (eight from this study) either failed to react with various preparations of group Q antisera in the precipitin test or they gave a positive reaction only after a protracted period of time. Both the hot acid and formamide antigen-extraction procedures were used. The commercial antiserum as well as the two experimentally prepared antisera were employed in an effort to obtain a positive

TABLE 1. *Incidence of group Q streptococci in fecal specimens from various animals*

Animal source	Samples investigated	Samples containing sorbose-fermenting streptococci	Group Q strains isolated
Chicken	13	12	11
Cow	12	1	0
Dog	9	7	2
Horse	19	1	0
Human	14	4	3
Pig	22	1	1
Rabbit	3	0	0
Sheep	5	0	0
Total	97	26	17

TABLE 2. *Quantitation of various enterococci in chicken feces*

Specimen	Most probable number count/g	
	S. faecalis and *S. faecium*	Group Q streptococci
A	9.2×10^5	2.8×10^5
B	1.3×10^5	7.9×10^3
C	1.3×10^5	4.2×10^4

reaction. In addition, the simple diffusion test plate (15) was inoculated with isolates which gave weak or negative reactions in the tube precipitin tests. For these cultures, 5 ml of sterile, high-titer group Q antiserum was added to 100 ml of the basal medium just prior to pouring the plates. Each strain was inoculated with the tip of a needle, and care was exercised not to penetrate completely the agar which would permit growth of the strain in the water of syneresis.

Four strains were apparently devoid of the group Q antigen, whereas five gave an extremely weak reaction under one or more of the test conditions. The other 13 strains gave a relatively rapid precipitin test, although some variation was noted with the commercial group Q antiserum as exemplified with strains Q2, E1551, and SS1 (Table 3). For convenience of discussion, the strains that gave a prompt reaction with the group Q antisera are referred to as Q_r. The strains that failed to give a reaction or that evidenced a weak reaction are denoted as Q_w.

When rabbits were immunized with Formalin-killed cells (Lancefield, *personal communication*) of Q_w strains D3P1 and CBP1 (which failed to give positive tube precipitin tests with group Q antisera), each of the resulting immune sera formed a precipitate with its homologous antigen

TABLE 3. *Precipitin reactions of group Q streptococci*

Group Q strain	Tube precipitin test				Petrie-gel technique (antiserum Q2)
	Lancefield HCl extraction			Fuller formamide (antiserum Q_{BW})	
	Q_{BW}[a]	Q2[b]	E1551[b]		
Q2	5[c]	1	1	1	5[d]
E1551	10	1	1	1	5
SS1	1	1	1	1	5
E6556	—[e]	15	60	—	7
P1H3	60	30	—	—	5
CCH6	—	10	30	—	16
HMH3, CCP1	—	—	—	—	—
CBP1	—	—	—	—	10
Herr	60	—	—	—	—

[a] Group Q antiserum obtained from Burroughs Wellcome Co.

[b] Group Q antiserum experimentally produced in rabbits with corresponding group Q strains.

[c] Positive reaction observed after indicated time in minutes.

[d] Band of precipitate or halo observed after indicated time in days.

[e] Indicates a negative result.

within 1 min. However, the immune sera did not react with extracts from other group Q strains, with the exception that CBP1 antiserum gave a rapid precipitin reaction with the antigen from another group Q_w strain, CCP1. Thus, it appears that these group Q_w strains lack the group-specific antigen, but it is possible that they (at least strains CBP1 and CCP1) may possess a type-specific antigen which would account for the rapid reaction with CBP1 immune serum.

Antigen extracts from group Q_r strains, when tested with group Q antiserum in seven-well, agar-gel double-diffusion plates, formed bands of precipitate in the pattern of identity as defined by Ouchterlony (14). Between each peripheral well (containing antigen) and the central one (containing group Q antiserum), the bands of precipitate formed straight lines, indicating that the diffusion coefficient of the antigens was similar. Since the proximal band tips coalesced in a hexagonal pattern without spur formation, the antigens were presumed to be homologous. Extracts of group Q_w strains formed no bands of precipitate on the double-diffusion plates. Group Q antigens and antiserum, when reacted in a five-well double-diffusion plate, formed single bands of precipitate, indicating that there was only one precipitating element common to extracts and serum.

Extracts of Q_r strains were treated with periodate by the method of Bobbitt (1). These extracts failed to form precipitates with group Q antiserum, whereas the untreated preparations gave a rapid reaction. The results indicate that the active site responsible for antigenic specificity is probably carbohydrate in nature. It has been demonstrated that the group Q antigen is in the cell wall (20) and that rhamnose, glucose, and galactose constitute the major sugars in the wall (19). Thus, it would appear that the group Q antigen consists of a polymer of one or more of these sugars.

All but 1 of the 22 group Q strains gave a precipitin reaction with group D antiserum. In some instances the reaction was weak and delayed. Essentially, these results confirm those of Smith and Shattock (20).

Physiology. All group Q strains were gram-positive, homofermentative, catalase-negative cocci that tended to form chains. The physiological results obtained with the Q_r and Q_w strains were not significantly different; consequently, they are not listed separately (Table 4). The ability to grow at 10 and 45 C, as well as the tolerance to salt and alkaline conditions of growth, tend to establish these streptococci as enterococci. However, in contrast to *S. faecalis* and *S. faecium*, the group Q strains do not tolerate 0.1% methylene blue.

All of the group Q strains fermented mannitol, xylose, glucose, fructose, galactose, maltose, mannose, lactose, sucrose, trehalose, melizitose, cellobiose, inulin, and dextran. As a group, these organisms have a marked fermentative ability in comparison to the established enterococcal species. Characteristically, the group Q strains fermented a wide variety of polyols, a property not shared with other enterococci.

In contrast to *S. faecalis* and *S. faecium*, none of the group Q strains hydrolyzed hippurate or gelatin. Significantly, none of the group Q strains hydrolyzed arginine, this property being commonly associated with the enterococci.

In toto, the temperature limits of growth and tolerance to sodium chloride and alkaline growth conditions warrant the inclusion of the group Q streptococci with the enterococci. However, they differ significantly from established enterococci in their fermentation pattern, inability to tolerate 0.1% methylene blue, and failure to hydrolyze arginine.

Growth responses on enterococcal isolation media. In years past, many media have been developed for the selective isolation of enterococci. Since the group Q streptococci are similar in many respects to these organisms, their growth on various selective media was compared to determine any similarities between the two and, hence, the possibility of confusing group Q streptococci

TABLE 4. *Physiological reactions of group Q streptococci and their comparison with those of other enterococci*

Reaction	Group Q streptococci (22 strains)	*S. faecalis*	*S. faecium*
Growth			
10 C	+[a]	+	+
45 C	+	+	+
6.5% NaCl	21+; 1−[b]	+	+
0.04% Tellurite	−	+	−
0.1% Methylene blue-milk	−	+	+
Fermentation			
Ascorbate	+	−	−
Glycerol, anaerobic	21+; 1−	+	−
Arabitol	+	−	0[c]
Dulcitol	21+; 1−	−	−
Ribitol	+	−	0
Sorbitol	+	+	−
Xylitol	+	−	0
Sorbose	+	−	−
Arabinose	+	−	+
Tetrazolium reduction	15+; 7−	+	−
Lysozyme sensitivity	−	−	V[d]
Litmus milk reduction (8 hr)	15+; 7−	+	−
Energy utilization			
Pyruvate	−	+	−
Serine	−	+	−
Citrate	−	+	−
Gluconate	20+; 2−	+	−
Malate	−	+	−
Arginine	−	+	−

[a] Indicates a positive result.
[b] Indicates a negative result.
[c] No data available.
[d] Strain variation.

with the established enterococci. The 12 media tested were prepared according to the procedures suggested by the various authors, and strains of *S. faecalis* and *S. faecium* were cultured on each medium for comparative purposes.

In general, growth of the group Q streptococci resembled and paralleled that of *S. faecalis*. In media containing tetrazolium salts, however, the growth response was similar to that of *S. faecium*, as neither of these enterococci reduces tetrazolium compounds to the same extent as does *S. faecalis*. In ethyl violet-azide agar (12), the growth response of *S. faecalis*, *S. faecium*, and the group Q streptococci was indistinguishable.

This phase of the study indicated that group Q streptococci were able to grow on media used for the isolation and enumeration of enterococci. Since, in most cases, growth was identical to either *S. faecalis* or *S. faecium*, it is possible that group Q streptococci could easily be mistaken for enterococci. Thus, this may explain, in part, the "atypical" enterococci which have often been reported in isolation studies.

DISCUSSION

Throughout the literature one can find references to fecal streptococci which resemble the enterococci in most reactions; however, they differ in one or more of the commonly accepted critical characteristics used to classify the enterococci. These organisms have been grouped under the broad heading of enterococcus biotypes, and little progress has been made regarding their classification.

The group Q streptococci, characterized in this study, resemble the enterococci in numerous reactions and have probably been designated previously as atypical enterococci or biotypes. The isolation of group Q streptococci from the feces of several domestic animals as well as from humans indicates that their distribution parallels that of the enterococci. Group Q streptococci were most prevalent in chicken feces, with strains being isolated from almost every sample examined. Kenner et al. (8) observed enterococcus biotypes to occur more commonly in the feces of fowl than of pigs, sheep, cows, and humans. In fact, they observed that two-fifths of the streptococci in the feces of fowl were enterococcus biotypes, with the remainder belonging to the enterococcus group. It seems likely that some of the biotypes which they described were actually group Q streptococci.

The media used in the initial isolation of group Q streptococci from feces contained sodium azide and sorbose. Sorbose was added as the prime source of energy to produce a selective environment for the group Q streptococci. It was subsequently observed that other sorbose-fermenting organisms could grow in this medium. These organisms had physiological characteristics typical of enterococci, and they differed from the group Q streptococci by their ability to grow in 0.1% methylene blue-milk or by the ability to hydrolyze arginine with the production of ammonia. Thus, although this isolation medium was not without shortcomings, it provided a reasonably selective means of isolating group Q streptococci.

Serological examination of the group Q streptococci revealed that almost one-half of the isolates apparently lacked the group-specific antigen. The group Q_w strains, whose antigen extracts failed to react with the group Q antisera, came from the same sources as the group Q_r strains, which gave a rapid precipitin reaction. Thus, the

difference in antigenic makeup did not appear to be host-specific.

Some consideration was given to the possibility that the group Q streptococci are a type-specific variety of the group D streptococci. Although the group Q strains do not always possess the group Q antigen, they characteristically have the group D antigen. However, the apparent absence of the Q antigen may represent a loss in its group antigen. Changes in the antigenic specificity of group A streptococcal strains has been reported and thoroughly investigated (9). It is difficult to reconcile the host of varying physiological characteristics between the group Q streptococci and the established group D streptococci on the basis of a variation in type-specificity. Type-specific varieties of both *S. faecalis* and *S. faecium* have been characterized; however, there is no analogous and gross divergence of physiological characteristics among these type-specific variants.

As a consequence of these considerations, we propose that the group Q streptococci constitute a valid species which should be included in the serological group D streptococci. Thus, in the physiological divisions of Sherman (17), the group Q streptococci would be included in the enterococcal division. Furthermore, in view of their characteristic occurrence in chicken feces, we propose the designation, *Streptococcus avium* sp. n., for these organisms. The following description of the species is based on results obtained in this and previous (6, 20) investigations.

Streptococcus avium sp. n. L. noun *avis* bird; *avium* of birds.

Morphology: Gram-positive, ovoid cells occurring in pairs or short chains.

Serology: Constitutes Lancefield's group Q. Cellular location of the group antigen is associated with the cell wall. This antigen is not demonstrable in all strains; when the antigen is not demonstrable, identification rests on physiological characteristics. Serologically, these streptococci are peculiar in that the majority of the strains encountered also contain the group D antigen; as in the established group D species, the antigen is not an integral part of the cell wall, and it is located between the wall and the membrane.

Tolerance tests: Growth occurs at 10 and 45 C, in media containing 6.5% sodium chloride and in media adjusted to *p*H 9.6. No growth occurs in milk containing 0.1% methylene blue or in media containing 0.04% potassium tellurite.

Physiology: Acid produced from ascorbate, arabinose, xylose, glucose, fructose, galactose, maltose, mannose, lactose, sucrose, trehalose, melizitose, cellobiose, inulin, dextran, glycerol (aerobically and anaerobically), arabitol, dulcitol, ribitol, sorbitol, xylitol, and sorbose. Neither starch nor gelatin is hydrolyzed. Ammonia is not produced from arginine.

Distinctive characters: This species is similar to *S. faecium* and *S. faecalis* but may be differentiated in that it does not grow in milk containing 0.1% methylene blue and ammonia is not produced from arginine. Sorbose and a wide variety of polyols are fermented, in further contrast to other enterococci.

Habitat: Fecal matter of human and warm-blooded animals, especially chickens.

As yet, these organisms have not been deposited with the American Type Culture Collection.

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