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Carriage of group D streptococci in the human bowel

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SUMMARY Sixty faecal samples, 39 from adults and 21 from neonates, were investigated by means of a simple scheme to isolate and identify any group D streptococci present. A number of differences were found between the two groups. Group D streptococci were absent from 43% of the neonates compared with only 13% of the adults; *Streptococcus bovis* was commoner in the neonates (23·8%) than in the adults (5%), and *Streptococcus faecium* was not isolated from any of the infant samples although it was common in the adult samples (25%). The viable counts of *Strep. faecium* were found to be, on average, 100-fold lower than those of *Streptococcus faecalis*.

The methods and results are discussed with reference to the clinical significance of group D streptococci, especially in bacterial endocarditis and carcinoma of the colon.

Group D streptococci form part of the normal gut flora of man and animals, but the distribution of the various species within the group is still imperfectly understood (Medrek and Barnes, 1962). Most studies show that *Streptococcus faecalis* and *Streptococcus faecium* are common human gut commensals, while *Streptococcus bovis* and other species are less frequently present. Recently, attention has been focused on *Strep. bovis*, which has been shown to be an important cause of endocarditis. In a three-year study of streptococcal isolates, Parker and Ball (1976) showed that *Strep. bovis* was associated with 17·3% of 317 cases of endocarditis, and that in the over-55 age group this organism was associated with nearly one-quarter of the cases.

More recently, Klein *et al.* (1977) have shown that the incidence of gut carriage of *Strep. bovis* is greatly increased in patients with carcinoma of the colon. Gross *et al.* (1975) devised a relatively simple scheme for identifying and speciating group D streptococci, which was shown by Facklam (1976) to give an accuracy of speciation of 98·5% in one large series.

The present study is an investigation of group D streptococci isolated from faecal samples from three groups of patients—hospital neonates, adult inpatients, and adult outpatients—at Dulwich Hospital. The streptococci were identified by means of the above-mentioned scheme, and viable counts were performed.

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Material and methods

Sixty faecal samples were examined: 21 were obtained from hospital neonates aged 6-7 days, 10 from adult inpatients, and 29 from adult outpatients.

The transit time for domiciliary specimens was a maximum of five hours, but hospital specimens were received more promptly. On arrival in the laboratory all specimens were refrigerated at 4°C and processed within three hours. A pea-sized lump of each sample was placed in a weighed sterile container of glycerol broth, homogenised on a vortex mixer, and stored for up to one month at −20°C. Frozen specimens were thawed at room temperature, and serial dilutions were made in Ringer's solution up to a dilution of 10^{-4} (Hewitt and Rigby, 1976). All the dilutions were immediately inoculated on to *M. enterococcus* Agar, a selective medium for group D streptococci (Becton, Dickinson & Co Ltd) in 0·1-ml volumes, by means of Pasteur pipettes calibrated to deliver 0·02 ml drops, and were spread with a glass rod. All dilution plates were incubated aerobically at 37°C for 48 hours when counts of each colonial type were made at the most convenient dilution. The minimum count detectable with this system is 100 organisms per gram of faeces. Counts were made on the

assumption that each sample weighed 1 g, and a correction was made later for the actual weight of sample taken.

Single colonies were picked and inoculated on Horse Blood Agar plates (Oxoid) for purity. Subcultures were made from the pure cultures on to 40% bile aesculin slopes (prepared in the laboratory from Oxoid constituents) and to Robertson's Cooked Meat Broths (laboratory prepared from Lab M constituents).

Strains that grew on 40% bile aesculin, blackened the medium after 48 hours' incubation, were catalase-negative, and were Gram-positive oval cocci in pairs or chains in broth culture, were presumed to be group D streptococci and were submitted to further tests. Strains that failed to satisfy these criteria were discarded. All isolates were tested for their ability to (1) utilise pyruvate, (2) hydrolyse arginine, and (3) grow in 6·5% sodium chloride broth. The scheme of identification is set out in the Figure and is that described by Gross *et al.* (1975) and Facklam (1974).

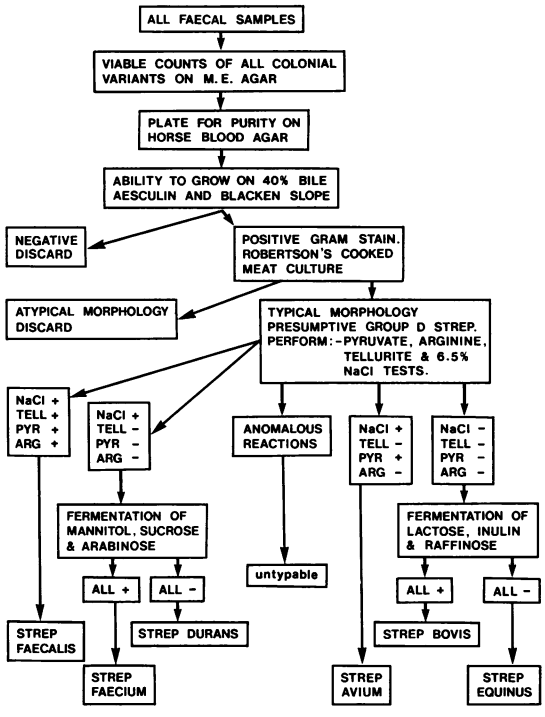

Figure *Scheme for identifying and speciating group D streptococci.*

UTILISATION OF PYRUVATE

Formula of medium:

Difco yeast extract 2·5 g
Difco tryptone 5·0 g
Dipotassium hydrogen phosphate 2·5 g
Sodium of pyruvate salt 5·0 g
Bromothymol blue 0·02 g
Agar 6·0 g
Distilled water 500 ml.

The medium was prepared as a slope in a bijou bottle. A positive reaction was indicated by the development of a yellow coloration after 48 hours' incubation at 37°C (Facklam, 1976).

SALT TOLERANCE

Five millilitres of broths containing 6·5% sodium chloride were inoculated heavily and incubated at 37°C for 48 hours. They were examined for turbidity with the naked eye; broths that were not turbid were Gram stained and, if no organisms were seen, the test was repeated (Cowan, 1974).

HYDROLYSIS OF ARGININE

Ammonia production was indicated by the development of a brown coloration on the addition of 0·25 ml of Nessler's Reagent to 5 ml of medium after five days' incubation at 37°C (method of Niven *et al.* (1942), see Cowan (1974)).

In addition to the tests described above, carbohydrate fermentation tests were performed on those isolates thought to be either *Strep. faecium* or *Strep. bovis*. Hiss' 1% Serum Sugars with Andrade's Indicator were inoculated and incubated for 48 hours at 37°C. Fermentation of mannitol, sucrose, and arabinose distinguishes *Strep. faecium* from *Strep. faecium var durans*, and fermentation of lactose, inulin, and raffinose distinguishes *Strep. bovis* from *Streptococcus equinus*.

Finally, all the presumptive group D isolates were submitted to Lancefield grouping. The isolates were cultured in 50-ml volumes of glucose broth (Southern Group Laboratory) at 37°C for 18 hours. The supernatant was removed and the deposit autoclaved to extract the group D antigen (Hamilton, 1972). Group D antiserum was obtained from Wellcome Biological Reagents, Beckenham, Kent. Precipitation tests were performed by the layering method (Williams, 1958) in capillary tubes, and positive reactions occurring within five minutes were recorded. Extracts that failed to group were retested at a dilution of 1 in 2, 1 in 4, and 1 in 8 in order to overcome any prozone effect due to antigen excess. A minority of isolates failed to group by this procedure and were submitted to counter-current immunoelectrophoresis by the method of McIntyre (1978).

A number of lyophilised strains of group D streptococci were obtained from the National

Table 1 *List of control strains*

NCTC strains	
8133	*Strep. bovis*
8307	*Strep. faecium var durans*
775	*Strep. faecalis*
2705	*Strep. faecalis var liquefaciens*
5957	*Strep. faecalis var zymogenes*
7171	*Strep. faecium*
Streptococcal Reference Laboratory strains	
A	*Strep. bovis*
B	*Strep. faecalis*
C	*Strep. faecium*
D	*Strep. bovis*

Collection of Type Cultures and some further isolates from the Streptococcal Reference Laboratory. These strains were used to provide positive and, where appropriate, negative controls for the biochemical tests (Table 1).

Results

All the control strains were tested alongside the faecal isolates and gave appropriate results for (1) hydrolysis of aesculin in 40% bile, (2) tolerance of 6·5% sodium chloride, (3) pyruvate reduction, and (4) arginine hydrolysis. One control *Strep. bovis* failed to ferment inulin on repeated testing. Serological grouping was performed with the following results:

Number of strains tested	*Group D status*
5 controls	Confirmed
46 faecal strains	43 confirmed

Three faecal isolates failed to group with the D antiserum by precipitation or immunoelectrophoresis.

On the basis of the scheme described under Material and methods, isolates were speciated into *Strep. faecalis*, *Strep. faecium*, *Strep. avium*, *Strep. bovis*, and *Strep. equinus*. No *Strep. faecium var durans* was isolated, and strains that could not be identified by this method were classified as unidentified group D streptococci.

Table 2 shows the relative frequency of isolation of the various species of group D streptococci from the three groups of patients, and Table 3 shows the distribution of viable counts in the 43 specimens examined. The viable counts for the neonates and adult groups are combined, as insufficient data are available for separate tables.

One or more species of group D streptococci were isolated from 46 of the 60 samples studied. Seventeen samples yielded two different species, and two samples yielded three species.

Table 4 shows the selectivity of *M. enterococcus* Agar for group D streptococci.

Table 3 *Viable counts of various species of group D streptococci from all groups of patients (expressed as organisms per gram of faeces)*

Species	*No. of isolates falling in each log range*							*No. of samples studied*
	10^8	10^7	10^6	10^5	10^4	10^3	10^2	
Faecalis	1	5	4	5	1	2	1	19
Faecium	0	1	0	3	2	2	2	10
Avium	0	0	0	1	1	0	0	2
Bovis	0	1	2	1	0	0	0	4
Equinus	0	0	0	1	1	0	0	2
Unidentified	0	2	0	1	1	2	0	6

NB 1 *Strep. faecium* not isolated from neonates.
2 *Strep. bovis* counts *all* on neonates.
3 *Strep. faecalis* counts for adults and neonates not significantly different.

Table 4 *Selectivity of* M. enterococcus *Agar for group D streptococci*

Total number of isolates from all specimens	80
Total number of group D isolates	67
Total number of non group D isolates	13
% of non group D isolates	16·25

Discussion

A variety of group D streptococci may be isolated from human faeces (see Table 2). In this study, one or two species were commonly isolated from a

Table 2 *Species of group D streptococci isolated from faeces of hospital neonates, adult inpatients, and adult outpatients*

Patient group	*No. of faeces examined*	*No. of faecal specimens containing various species of group D streptococci*						
		Strep. faecalis	Strep. faecium	Strep. avium	Strep. bovis	Strep. equinus	*Not identified*	*Any group strep.*
Neonates	21	10 (47·6)	0	0	5 (23·8)	0	1 (4·8)	12 (57·1)
Adult inpatients	10	8 (80)	3 (30)	1 (10)	0	0	1 (10)	9 (90)
Adult outpatients	29	14 (48·2)	12 (41·3)	2 (6·9)	2 (6·9)	2 (6·9)	6 (20·7)	25 (86·2)

Percentages are given in parentheses.

single sample. The predominant species was *Strep. faecalis*, which was cultured from 53% of the samples studied. This was followed by *Strep. faecium* (25%) and *Strep. bovis* (11·6%). Other group D streptococci were much less common. Thirteen per cent of the samples produced strains that could not be identified by means of the scheme described. Of these eight unidentified strains, all but one belonged to Lancefield group D. Five of them gave a positive arginine reaction but failed to grow in 6·5% sodium chloride broth or to utilise pyruvate.

In this study, 16% of all the isolates from *M. enterococcus* Agar were presumed not to be group D streptococci because they failed to blacken bile aesculin medium. The bile aesculin test is a reliable presumptive test of group D status, as shown by Facklam *et al.* (1974), who tested 920 strains of group D streptococci and found that 99% blackened the medium. Gross *et al.* (1975) found that 99·7% of 844 strains of group D streptococci were bile aesculin positive.

Where mixed cultures of group D and non group D isolates occurred, the presence of the latter did not make counting difficult. All colonial variants were submitted to bile aesculin testing, as it is not possible to distinguish group D streptococci on *M. enterococcus* Agar by colonial morphology alone. These findings are in agreement with those of Pavlova *et al.* (1972), who found that 18·4% of their isolates from faeces, sewage, and food samples, plated on *M. enterococcus* Agar, were not group D streptococci, 6·2% being either *Streptococcus mitis* or *Streptococcus salivarius*. Raibaud *et al.* (1961) reported overgrowth of lactobacilli when pig caecal samples were plated on this medium.

According to Pavlova *et al.* (1972), the yield of group D streptococci from faecal samples is less with *M. enterococcus* Agar than with the thalleous acetate medium of Barnes (1956), $3{\cdot}3 \times 10^5$ organisms per gram as compared with $8{\cdot}3 \times 10^6$ organisms per gram. However, Burkwall and Hartman (1964) found that the range of percent recovery of group D streptococci from frozen foods plated on *M. enterococcus* Agar was 70-500%, assuming 100% recovery from Barnes' medium. Pavlova *et al.* (1972) used small inocula, 0·1-ml volumes of 10^{-4} faecal dilutions, and did not report any relative inhibition.

My findings are broadly in agreement with those of previous studies (Sabbaj *et al.*, 1971; van der Wiel-Korstanje and Winkler, 1975). There were, however, several differences between the flora of the neonates and of the adults that have not previously been recorded. *Strep. faecium* was isolated from 15 of the 39 adult faeces (38·5%) whereas it was not isolated from any of the 21 neonatal samples. *Strep. bovis* was more frequently isolated from the neonatal group, 5 out of 21 (23·8%) compared with 2 out of 39 (5%) for the adults. Nine of the neonatal samples produced no group D streptococci (43%) compared with only five of the adult samples (13%). The adult group produced a wider range of streptococci, but this may have been merely a reflection of the larger number of adult specimens examined (39:21).

In over half of the samples yielding *Strep. faecalis*, viable counts of the organism were within the range 10^6-10^9 organisms per gram, whereas in the samples yielding *Strep. faecium*, viable counts of the organism were in all cases below 10^6 organisms/g, and two-thirds of samples had counts below 10^5 organisms/g.

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