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Streptococci of Lancefield's Group L Isolated From Human Mouths

by

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SUMMARY The properties of Group L Streptococci from the normal human mouth are discussed. It was found that they differed in some respects from earlier human and animal isolates, and frequently gave rise to bizarre bacillary forms.

INTRODUCTION

Group L streptococci are well recognised animal pathogens, which may occasionally cause disease in man. The group was first differentiated by Fry (1941), who obtained his isolates from the genito-urinary tract of dogs and subsequently pigs. Laughton (1948) described 12 strains of Group L streptococci isolated from the genital tract of dogs, six of them from cases with clinical disease. Ernst (1942), quoted by Skadhauge, (1954) categorised eight isolates of a haemolytic streptococci from milk into a new group N, but these subsequently proved to be group L strains. They have been frequently isolated from infected milk and mastitis in cattle (Bruhn 1944, Hansen 1945; as cited by Skadhauge, 1954). McLean (1955) found 16 Group L strains among 58 isolates from the carcasses of slaughtered pigs. Olsen (1957) isolated this group from the vagina of diseased pigs and considered that infection might be transmitted from swine to cattle by the intermingling of herds. As may be expected, they have been repeatedly isolated from abattoirs (Boissard, 1975).

Recorded cases of human isolates of this group are few. The first strains were obtained by White, Rudd and Ward (1939) from the throats of two patients with scarlet fever in Sydney, Australia. Subsequently, they have been isolated from cases of parotitis, (Feingold, Stagg and Kunz, 1966) tonsillitis and cellulitis (Duma, Weinberg, Medrek and Kunz, 1969).

In the course of a survey on viridans streptococci from normal mouths and from dental abscesses Wickremasinghe and Rusell, 1976), it was observed that some of the former isolates possessed the Group L antigen and also showed distinct bacillary forms. As α haemolytic isolates of this group from the healthy mouth have not previously been reported and their bacillary morphology was unusual, it was decided to investigate these strains further.

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METHODS AND MATERIALS

Specimens were taken from the mouths of healthy dental students. Sterile cotton wool swabs were rubbed over the dorsum and ventral aspects of the tongue, gums, soft palate and buccal mucosa. These were streaked on 5% horse blood agar and incubated aerobically at 36°C. Greening streptococcal colonies were picked on to blood agar and stored at + 4°C after incubation. They were maintained by monthly sub-culture on blood agar.

Lancefield's grouping was performed in the following manner; cultures were grown in Hartley's digest broth with 0.5% glucose for 48 hours at 36°C. Extracts were made by Lancefield's technique (1933). Each extract was tested against group A, B, C, D, E, F, G, H, K, L and O antisera. The antisera were drawn up into 1 mm diameter capillary tubes followed by an equal quantity of extract. The tubes were stuck in plasticine and examined periodically for 30 minutes. In doubtful cases, the tests were repeated, employing standard precipitation tubes. Four strains of 200 isolates namely 12a, 17b, 38d, 39a were shown to possess Group L antigen. These cultures were further examined as described elsewhere (Wickremasinghe and Russell, 1976) for the following properties:- colonial characteristics on mitis-salivarius agar, on blood agar and in pour plates of blood agar after 48 hours incubation, at 36°C; Gram reaction, cell morphology and motility; presence of catalase and oxidase; growth at 45°C; production of extra-cellular polysaccharide in 5% sucrose broth; growth in 4% and 6.5% NaCl broth and on 10% and 40% bile blood agar; acid production in 1% raffinose inulin, mannitol, sorbitol and trehalose; arginine and aesculin hydrolysis; gelatinase production, optochin resistance; with one strain (17b), bacitracin sensitivity, employing 0.1 unit discs; terminal pH in 1% glucose, sucrose and lactose broths. The production of a soluble haemolysin was tested for by the method of Collins and Taylor (1967). Finally, the presence of T agglutinins was examined by treating the deposit of a 24 hour culture in Todd Hewitt broth with 2% trypsin and testing the suspension with T antisera.

MICROCULTURE OF SINGLE CELL ISOLATES

It was observed that three Group L cultures gave bacillary forms together with chains of cocci. To exclude the possiblity of the occurrence of any contaminating bacilli and to investigate morphological stability, it was decided to employ a single cell culture technique on one of these strains (isolate 17b).

The method used was that of Johnstone (1969). A drop of diluted broth culture was spread on a non-nutrient gel on a glass slide and a chain of cocci was transferred to a separate uninoculated part of the agar using a phase contrast microscope, a glass micro-needle and a micro-manipulator. The agar was dissected and the block of agar with the single chain of organisms was transferred into a tube of broth which was subsequently incubated at 36°C for 24 hours. Gram stained films were then made and the broth subcultured on to blood agar; colonies on these plates were examined microscopically after 24 and 48 hours incubation.

RESULTS

There were four Group L strains of streptococci isolated from a total of 200 cultures. All strains were non-motile, resistant to optochin and the single tested culture was sensitive to bacitracin, Gram reaction at 48 hours was variable, some strains being strongly Gram-positive while others were entirely Gram-negative. Three of the cultures showed a mixture of coccal and bacillary forms, the bacilli occurring singly or in chains of varying length. The appearance on mitis-salivarius agar was variable but in general all four organisms resembled the Strep. mitis strains of Carlsson (1967). Three showed smooth colonial forms and the fourth was matt on blood agar. All cultures showed α-haemolysis on repeated culture. Although clearer haemolysis was shown in poured blood agar plates, none produced a soluble haemolysin. No isolate fermented mannitol sorbitol or trehalose, liquefied gelatine or produced extracellular polysaccharide. None grew on 10% or 40% bile blood agar, in 4% or 6.5% NaCl broth or at 45°C; No strain was agglutinated by Group A, T antisera. Further results are shown in Table 1.

Table 1 - Biochemical properties of Group L Streptococci

Isolat Number	Lactose	Sucrose	Insulin	Raffinose	TERMINAL pH in 1%			Aesculin	Aginine
					Glucose Broth	Lactose Broth	Sucrose Broth		
12a	A	A	—	—	5.05	5.2	5.2	+	+
17b	A	A	—	—	4.9	5.6	5.2	—	+
38d, 39a	A	A	A	A	5.05	5.7	5.4	—	+

A = Acid only : + = Hydrolysis

MICROCULTURE

The cell forms produced from a chain of cocci (Fig. 1) in a culture of strain 17b are shown in Fig. 2. There is a mixture of bacilli and cocci with bacillary forms predominating in the latter. The Gram reaction ranges from strongly Gram positive to entirely Gram negative.

DISCUSSION

Isolation of Group L streptococci from human sources has been rare, and in all cases associated with a diseased condition. Thus, in addition to instances of recovery from pathological lessions and sore throats mentioned earlier, Ellner (1970) recorded a patient with sub-acute bacterial endocarditis from whose blood Group L streptococci were isolated on several occasions, and Dwyer (1975) observed a similar patient in Cambridge from whom these organisms were isolated on two occasions. To the present author's knowledge, this is the first time Group L strains have been isolated from the healthy mouth.

Human strains have not been fully characterised before although the type of haemolysis has been noted. The isolates in the present series show certain features of interest. All four strains were α-haemolytic on horse blood agar which is contradictory to the experience

of Fry (1941), Laughton (1948) and Skadhauge (1954) who observed that all their animal strains showed prominent β-haemolysis. McLean (1955), however, noted that seven Group L strains obtained from the carcasses of pigs were α-haemolytic. None of the present strains formed a soluble haemolysin thereby differing from the organisms examined by Fry (1941), Ernst (1942) quoted by Skadhauge (1954) and Skadhauge (1954) who found that all the dog, pig and cattle strains produced a soluble haemolysin of the S type. Laughton (1948) observed that although all her strains produced β-haemolysis, only one produced a soluble haemolysin and McLean (1955) found that none of his seven α-haemolytic strains produced a soluble haemolysin.

Varied reactions on horse blood agar have been obtained with human strains. The isolates of White, et al. , (1939), and Ellner (1970) showed β-haemolysis whereas those of Feingold, et al., (1966) and Duma, et al., (1969) produced greening. The production of a soluble haemolysin by human strains has not been recorded.

Colonies on blood agar from each strain in the present work were either matt or smooth and of uniform size. This is at variance with the findings of Laughton (1948) and Skadhauge (1954), who observed that each strain yielded a mixture of colonial types which also differed in size. No mention is made by those authors of the occurrence of any bacillary forms.

Most workers agree that lactose, sucrose and trehalose are fermented and arginine hydrolysed; although Fry's original strains gave variable results with lactose. Our results were generally in accordance with their findings but were unusual in that trehalose was not fermented. The terminal pH in 1% lactose, sucrose and glucose broths was in the range of 4.9-5.7; Laughton (1948) noted that the terminal pH in 1% glucose broth of her strains was 4.7-5.2. AlthoughWilson and Miles (1975) and others state inulin and raffinose are not fermented, McLean (1955), found that four of seven haemolytic Group L strains attacked these sugars. Two of our strains fermented inulin and raffinose which agree with McLean's (1955) findings. The present isolates did not ferment mannitol or sorbitol, in agreement with the findings of Laughton (1948), Skadhauge (1954) and McLean (1955). One strain hydrolysed aesculin, which was unusual.

Most workers state that their strains were bile resistant, but none of our cultures grew on 10% or 40% bile agar and in this resembled Fry's original strains. One of our strains was bacitracin sensitive in accord with the findings of Jelinkova and Rotta (1967) who showed that a proportion of Group L strains were sensitive to this antibiotic. Since these organisms are also sensitive to penicilin, a strain showing β-haemolysis could be mistaken for *Strep. pyogenes* in the absence of serological testing. None of our strains yielded T agglutinins which was at variance with the experience of Perch and Olsen (1964) who found that many of their strains contained T8 and T20 antigens.

Colman and Williams (1972) showed that among the viridans streptococci *Strep. milleri* may carry Lancefield's group antigens and we have found this to be so occasionally among *Strep. sanguis* and *Strep. mitis* Wickremasinghe and Russell, (1976). Although some of their 42 isolates carried Lancefield's Group A, C, F or G antigens, none was Group L. According to Colman and Williams (1972) *Strep. milleri* generally hydrolysed aesculin and

arginine, but did not ferment inulin or raffinose and usually was bile resistant. Only one of our present strains, 12a, had this correlation of properties (see Table 1). Furthermore, all isolates in the present series produced greening on blood agar whereas 39 of 42 of their strains were nonhaemolytic. Although these strains, in general, resembled the *Strep. mitis* strains of Carlsson (1967), they differed in some respects.

Our organisms, therefore, seem to be Group L strains that differ in many respects from earlier human and animal isolates. Whether they could be classified as variants of *Strep. milleri* (Colman and Williams 1972) or *Strep. mitis* (Carlsson, 1967) is uncertain.

There does not appear to be any previous record of Group L streptococci giving rise to bacillary forms. Although aberrant forms were also found among Groups C, F and a few ungroupable organisms, they were most frequently found among Group L strains. It has been shown by Tomley and Russell (1977) that prolonged incubation and repeated sub-culture of some viridans Streptococci lead to increased rod formation due to the failure of the dividing cocci to separate. It may be that, unknowingly, conditions employed during the present tests had a similiar effect on cell structure.

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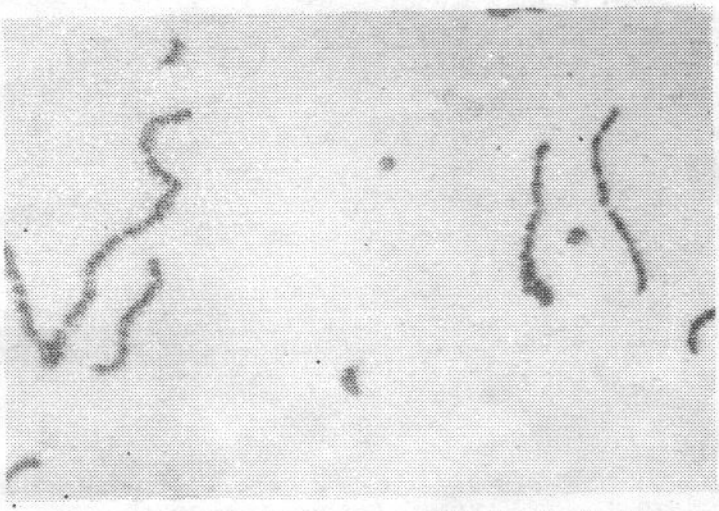

FIGURE 1: x 1000. Group L Streptococci Gram Stain Cocci in chains.

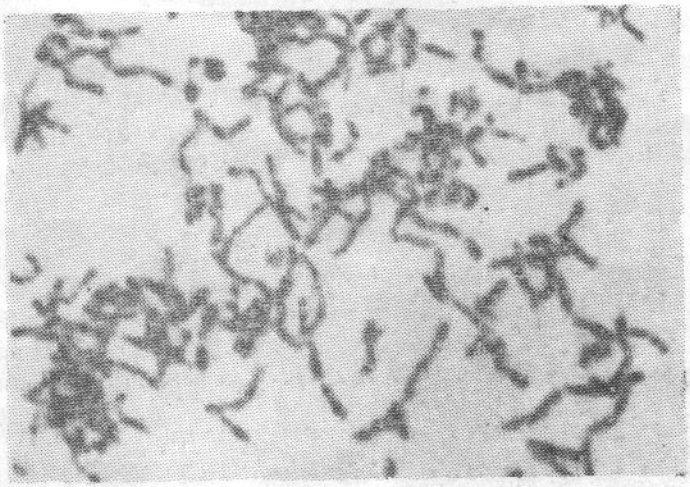

FIGURE 2: x 850. Group L Streptococci. Gram Stain Microculture of a coccal chain. Bacilli predominant.