A BACTERIOLOGICAL STUDY OF STREPTOCOCCI ISOLATED FROM THE GENITO-URINARY TRACT

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Although comprehensive investigations of the streptococci from almost every other source have been made, those occurring in genito-urinary infections have received relatively little study from the point of view of the recent advances in bacteriological techniques now available for the more accurate identification of these organisms.

While under certain conditions sulfanilamide and its derivatives have afforded a marked advance in the treatment of genito-urinary infections, it is well known that certain organisms are resistant to these methods of chemotherapy. We have the opportunity in this Clinic to follow closely the shifts of the bacterial flora which may be observed in surgical patients undergoing treatment with these drugs. Our attention has been focussed on the striking way in which the streptococci and aerobacters tend to persist, while other organisms disappear, often with great rapidity. This observation led us to undertake as thoroughly as possible the exact differentiation of these streptococci for the purposes of establishing a rationale for choice of chemotherapeutic agent, gaining further knowledge concerning the rôle of these organisms in genito-urinary infection, and finally determining their importance as compared to that of other organisms commonly found under these circumstances.

As a preliminary survey we have undertaken a study of 100 strains of streptococci isolated at random from urine specimens. No attempt has been made to correlate the findings with the case histories of the patients. We have centered our interest on

determining what type or types of streptococci predominate in these cases, and whether or not we can find any bacteriological basis for their remarkable resistance to treatment.

Until the introduction by Lancefield of serological methods for the grouping of streptococci, such organisms isolated from urine could be routinely identified only by their type of hemolysis on blood agar and by their carbohydrate reactions. By such means, the majority of streptococci recovered from the genito-urinary tract were found to belong in the rather all-inclusive group, labeled gamma *Streptococcus fecalis*. Alpha streptococci have been isolated in a number of cases, but beta streptococci from this source have been extremely rare. Employing the methods recently made available, we have organized our more detailed investigation for the purpose of determining what percentage of these alpha and gamma streptococci would come under the classification of enterococci.

Until recently the term "Enterococcus" has been ill defined. It was originally introduced by Thiercelin (1899) to describe one type of streptococcus isolated from the intestinal tract, which differed morphologically from the other streptococci by being primarily a diplococcus and less often occurring in short chains. A few years later Andrewes and Horder (1906) studying streptococci from the same source gave the name *Streptococcus fecalis* to one of the six groups they differentiated. This group may or may not have been identical with the Enterococcus of Thiercelin. Consequently, much confusion has evolved over the use of these two terms. The two have been used by some to denote separate and distinct groups of streptococci; others have considered them synonymous. The more recent literature, however, (Sherman, 1937, 1938; Graham, 1939; etc.) tends toward the opinion that the two are not interchangeable but suggests that the term "Enterococcus" should be accepted as the general name for fecal streptococci possessing the morphological and biological properties of *Streptococcus fecalis*, and at the same time including other strains which differ slightly in their fermentation reactions from *Streptococcus fecalis*.

The fact that enterococci are distributed over the surface of

the skin and often occur in the normal genital tract may account for their frequent presence in infections of various types. They are generally considered as saprophytes or facultative parasites since they exhibit little or no pathogenicity for laboratory animals. They do, however, seem to show some degree of invasiveness when the resistance of the host becomes lowered from some other cause.

METHODS

The method of collecting the cultures and isolating the individual organisms was carried out according to the procedure outlined in Young's *Practice of Urology*.

For the purpose of this study we limited our examination to those tests which seemed to have been most generally accepted as criteria for true enterococci.

1. Hemolysis. Twenty-four hour broth cultures were plated in deep infusion agar to which was added 0.5 ml. defibrinated rabbit's blood. The plates were incubated at 37°C. for 48 hours and then placed in the icebox overnight. All plates were examined under the microscope with the low-power lens. Those colonies which showed no clear zone of hemolysis, although some produced a green discoloration of the agar around the colony, were classified as gamma;[1] those which did show a definite zone of hemolysis beyond the region of fixed cells were classified as alpha.

2. Fermentation reactions. Twenty-four hour cultures were inoculated into sugar-free infusion broth to which had been added the carbohydrates, lactose, salicin, mannitol, and inulin to a final dilution of 1 per cent. The cultures were examined every day and the results read at the end of 7 days.

3. Hydrolysis of aesculin the presence of bile salt. The aesculin bile broth recommended by Meyer and Schonfeld (1929) was

[1] A few of these strains produced a green discoloration of the red blood cells around the agar, although they showed no hemolysis at the end of the three days. This type of colony has been described by Bryant (1925) as being distinct from either the alpha or gamma colony types. The introduction of the term "delta" was suggested to distinguish this type of streptococcus colony from the others. For the purposes of this study, however, the few delta strains were included and studied together with the gamma strains.

selected since it not only tests the organism's ability to split aesculin but at the same time its resistance to bile salts. Twenty-four hour broth cultures were inoculated into the medium and observed for 10 days. A positive reaction is indicated by the broth turning black, since the presence of a ferric salt in the medium acts as an indicator for the end-products of the hydrolysis.

4. Reduction of methylene blue. To sterile fat-free milk was added sterile 0.5 per cent aqueous methylene blue to a final concentration of 1–10,000. Twenty-four hour broth cultures were inoculated in 0.1 ml. amounts and the cultures held for 7 days.

5. Heat resistance. Approximately 1 ml. of 24-hour infusion broth cultures was sealed off in small glass ampoules and immersed in a water bath at a temperature of 60°C. for one-half hour. The ampoules were then opened and the contents subcultured into infusion broth enriched with a small amount of ascitic fluid. After 24 hours incubation at 37°C. the cultures were examined for growth and checked for purity by the gram stain.

6. Serological grouping. Antigens were prepared from all the strains according to the method of Brown (1938) and tested with Lederle commercial sera for the Lancefield groups A, B, C and D. Observations were made immediately upon setting up the tests; at the end of the first half hour, again at 1 hour and finally after 24 hours at room temperature.

Discrepancies in the results of any of these examinations were checked by repeating the test before making the final report.

RESULTS

The results of the biochemical and precipitin tests are recorded in Table I. No beta hemolytic streptococci were found in this series. Twenty-four, or 24 per cent, of the strains examined were alpha streptococci. The remaining 76, or 76 per cent, produced no hemolytic change on blood agar and were classified as gamma streptococci.

In keeping with the findings of Dible (1921), Sherman (1937), Houston (1934), Archer (1938), *et al.*, the alpha streptococci grew in broth forming a flocculent sediment and leaving the upper

portion of the tube clear, whereas the gamma strains produced a uniform turbidity. When examined microscopically in broth cultures, the alpha streptococci appeared very pleomorphic; the chains were sometimes short, sometimes long and tangled, and the individual cocci varied in size and shape from small cocci to large bacillary forms. The gamma strains, however, generally appeared as elongated diplococci, and less often were seen in short chains made up of diplococcal segments. On solid media the alpha colonies were uniformly small, shiny and opaque, frequently exhibiting a small zone of hemolysis around the edge of the colony.

TABLE 1

Characteristics of 100 streptococci isolated from genito-urinary tract infections

ORGANISM	NUMBER OF STRAINS	GROUP	LACTOSE	SALICIN	MANNITOL	INULIN	AESCULIN-BILE	METHYLENE BLUE-MILK	HEAT RESISTANCE
Streptococcus fecalis gamma and delta..	70	D	+	+	+	−	+	+*	+
Streptococcus liquefaciens gamma.....	3	D	+	+	+	−	+	+†	+
Streptococcus fecalis gamma.....	2	−	+	+	+	−	+	+*	+
Streptococcus fecalis gamma	1	−	+	+	+	−	+	+*	−
Streptococcus salivarius alpha.....	2	−	+	−	−	−	−	−	−
Streptococcus mitis alpha.....	4	B	+	+	−	−	−	−	−
Streptococcus mitis alpha.....	3	−	+	+	−	−	−	−	−
Streptococcus equinus alpha.....	10	B	−	+	−	−	−	−	−
Streptococcus fecalis alpha.....	5	−	+	+	+	−	−	−	−

* Coagulated.

† Coagulated, digested.

The gamma colonies ranged in size from that of the alpha type to those resembling staphylococci. Gram stains made from these colonies often showed the organisms in staphylococcus-like arrangement rather than the diplococcoid or chain formation characteristic in broth media.

On the basis of the fermentation reactions the alpha streptococci were divided into 4 types: *Streptococcus equinus*, 10 strains; *Streptococcus mitis*, 7 strains; *Streptococcus fecalis*, 5 strains; *Streptococcus salivarius*, 2 strains. The gamma streptococci all fermented lactose, salicin and mannitol. No strain fermented inulin.

A sharp differentiation between alpha and gamma strains could be noted in the results of the hydrolysis of aesculin in a bile salt broth. The alpha strains failed to grow in 7 days, whereas all of the gamma streptococci grew luxuriantly and produced the change overnight.

In the presence of 0.1 per cent methylene blue, the alpha streptococci exhibited some variation in their ability to survive and reduce the dye. The two *Streptococcus salivarius* strains produced no change. The other types failed to curdle the milk but a few scattered ones reduced the dye to a slight extent in 4 to 5 days. The gamma streptococci were uniform in their ability to reduce the methylene blue completely within 24 to 48 hours. All of them curdled the milk overnight and 3 of the strains peptonized the clot within the week.

The results of the heat resistance tests paralleled those on hemolysis and the hydrolysis of aesculin. The alpha streptococci were killed by the end of 30 minutes exposure to 60°C.; the gamma streptococci, with the exception of 1 strain, survived and grew within 24 hours following transfer to a fresh broth medium.

The precipitin test, using the Lancefield grouping sera further differentiated the alpha from the gamma strains. Although little has been reported in the way of serological study of the alpha streptococci as a group, we did find some serological relationship of the alpha *Streptococcus equinus* and *Streptococcus mitis* strains to the Lancefield hemolytic group B. All 10 of the *Streptococcus equinus* strains were precipitated within 30 minutes by the B serum; 4 of the 7 *Streptococcus mitis* strains likewise reacted with the same serum. The remaining *Streptococcus mitis* strains failed to react with any sera. The alpha *Streptococcus fecalis* and *Streptococcus salivarius* strains were not precipitated by any of the group sera.

It has been reported that the group B of Lancefield does contain both beta hemolytic and non-hemolytic types. It is interesting to note that those streptococci which did react with the group B serum differed from one another only in respect to the fermentation of lactose. All of them fermented salicin; none of them fermented mannitol or inulin. This bears some relationship to the

fact that the beta strains of human origin belonging to group B also ferment salicin but are variable on lactose. Why there should be a variation in the ability of the different *Streptococcus mitis* strains to react with the B serum is a problem yet to be investigated.

Of the gamma streptococci, 73 of the 76 strains were precipitated immediately in the group D serum. None of the strains showed any reaction with any of the other sera tested. These results bear out the findings previously reported by Sherman (1938) and Graham (1939) that the *Streptococcus fecalis* and *Streptococcus liquefaciens* are enterococci and do belong to the Lancefield group D.

DISCUSSION AND CONCLUSION

The enterococcus as defined in the more recent literature is a gram-positive coccus characteristically occurring in pairs and less often in short chains; it is resistant to bile and bile salts; it can survive in milk containing 0.1 per cent methylene blue, curdle the milk and completely reduce the dye; it can withstand heating at 60°C. for 30 minutes; and it is serologically related to the streptococcus group D of Lancefield. Hemolysis on blood agar, proteolysis, and fermentation reactions may be variable since it has been shown by Sherman (1938) and Graham (1939) that the beta *Streptococcus zymogenes*, *Streptococcus liquefaciens*, and some alpha strains should be included within the group of enterococci since they have in common all of the characteristics outlined above.

In consideration of the foregoing facts, the results of the microscopic, biochemical, and serological examinations of 100 strains of streptococci isolated from the genito-urinary tract infections lead us to conclude that enterococci of the gamma *Streptococcus fecalis* type are the predominating streptococci associated with these infections. The few proteolytic enterococci recovered might otherwise be classified as *Streptococcus liquefaciens*. Alpha streptococci of all species do occur in these infections but according to our results none of them could be considered as members of the enterococcus group.

Therefore, referring to the fact that the streptococci seem to be the most resistant to treatment of all the various types of bacteria known to invade the genito-urinary tract, a partial explanation may be afforded by these findings. Since the enterococcus group as a whole consists of streptococci possessing unusual resistance to physical and chemical factors generally injurious to other types of organisms, it seems to follow that they should also exhibit a more marked resistance to treatment with drugs. To further substantiate these conclusions, an analysis should be made of a large series of cases in order to determine the incidence of enterococci and other streptococci in genito-urinary tract infections as correlated with the results of treatment.

That these streptococci occur in genito-urinary tract infections, quite often in pure culture and more frequently as persistent invaders after the other associated organisms have been eliminated, indicates that they may have some pathogenic significance and may be definitely related to the clinical picture.

SUMMARY

One hundred strains of streptococci isolated from genito-urinary tract infections have been studied culturally and serologically.

1. A total of 70 cultures were found to be true enterococci of the gamma *Streptococcus fecalis* type.

2. Three strains were enterococci of the gamma *Streptococcus liquefaciens* type.

3. The remaining 24 strains were alpha streptococci, including the types *Streptococcus mitis*, *Streptococcus salivarius*, *Streptococcus fecalis* and *Streptococcus equinus*. All of these were clearly differentiated from the gamma strains and none of them could be classified as enterococci.

4. Seventy-three of the 76 strains of gamma streptococci were serologically members of the streptococcus group D of Lancefield. Of the 3 remaining strains of gamma streptococci, none was precipitated in any of the group sera, 2 were heat-resistant, and 1 was heat labile. Otherwise these 3 strains were similar to the other strains of gamma *Streptococcus fecalis*.

5. All of the alpha *Streptococcus equinus* strains were shown by the precipitin test to belong to the Lancefield serological group B. Four of the 7 alpha *Streptococcus mitis* strains were likewise precipitated by group B serum, whereas the remaining 3 strains were serologically inert.

6. The alpha *Streptococcus fecalis* and *Streptococcus salivarius* strains failed to react with any of the Lancefield grouping sera, A, B, C or D.

7. Enterococci are the predominant group of streptococci recovered from the genito-urinary tract and may, therefore, be of pathogenic significance in infections of this nature. Their unusual resistance to treatment and the persistence of the clinical symptoms in infections associated with streptococci would further support this conclusion.

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