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STUDIES ON THE GENUS STREPTOCOCCUS

A Thesis Presented to

The Graduate Faculty

of the University of the Pacific

In Partial Fulfillment of the Requirements for the Degree

Master of Science

by

Janet Lee Storey

May 1977

This thesis, written and submitted by

_____
Janet Lee Storey
_____

is approved for recommendation to the Committee
on Graduate Studies, University of the Pacific.

Department Chairman or Dean:

_____
F. R. Hunter
_____

Thesis Committee:

_____ Chairman

_____
Alice S. Hunter

_____

_____

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Dated   14 April 1977

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I. Introduction and Historical Review

The streptococci, as a group, are Gram positive cocci which occur in
pairs or chains; they are nonmotile, nonsporing, and catalase negative.
Most species are facultative anaerobes, and a few are obligate anaerobes.
They are chemorganotrophs producing lactic acid as an end product of
glucose metabolism. The G + C (guanine + cytosine) content reported for
15 species is 33-42% (Diebel and Seeley, 1974).

The genus Streptococcus includes a large number of saprophytic,
pathogenic and nonpathogenic species. Many of these bacteria are members
of the normal human body flora. However, man is very susceptible to the
pathogenic members, and no organ in the body is completely immune to
streptococcal infections. As a result, streptococci cause a greater
variety of clinical manifestations than any other genus of bacteria.

Brown (1919) described the hemolytic action of streptococci on
red blood cells and divided them into three groups; 1. alpha-partial
lysis of the erythrocytes and a green zone around the colony, 2. beta-
complete lysis of red blood cells and a clear zone around the colony and,
3. gamma-no lysis and therefore no zone of hemolysis. This method of
classification is based on the macroscopic and microsopic examination of
subsurface colonies on sheep blood agar pour plates. The reaction depends
on the type of animal blood used, type and pH of the medium, presence or
absence of oxygen, and amount of glucose present. Different strains
within the same group may produce variable reactions, and under different
conditions a single strain may act differently. Table I shows the variety
and overlap of hemolytic characteristics of the various streptococci.

Lancefield (1933) developed a serological method of classification.
Based on differences in the terminal groups of the carbohydrate antigen

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in the streptococcal cell wall (C-substance), the streptococci were classified into groups A to N and other groups were added later. (For example the terminal group for Group A streptococci is rhamnose-N-acetylglucosamine, for Group B rhamnoseglucosamine, and for Group C rhamnose-N-acetylgalactosamine.) Twenty-one species are now recognized, five do not fit into any serologic group, and a few have not been assigned specific names (Table II). Although this method of classification is the most reliable, it is time consuming, expensive, and rarely resorted to in the clinical laboratory.

Identification of streptococci is routinely based on biochemical and physiological tests including: growth at 10 and 45 C, growth in media containing 6.5% NaCl, hydrolysis of hippurate and esculin, production of pigment in DMS agar, growth in selective media such as ethyl violet azide (EVA), and sensitivity to the antimicrobial substances ethylhydrocupreine hydrochloride (optochin) and bacitracin.

The importance of classification is related to the varied antibiotic resistance of streptococci, different clinical manifestation, and consequently, treatment. Although 95% of human streptococcal infections are caused by Group A, other groups, especially B and D and to a lesser extent C, G, and O are also important pathogens of man (Evans and Chinn, 1947; Reinarz and Sanford, 1965; Feingold et al., 1966; Blazevic et al., 1972; Wilkinson et al., 1973; Wannamaker, 1975; Finch et al., 1976; Reiner et al., 1976; and Ablow, 1976).

The application of antimicrobial susceptibility patterns as an aid in classification of bacteria of other genera has been used by several investigators (Kock and Rose, 1966; Lerner and Weinstein, 1967; Ramirez, 1968; von Graevenitz and Redys, 1968; Gilardi, 1971). Maxted (1953)

reported a reliable presumptive identification of Group A streptococci

by bacitracin susceptibility. Jones et al. (1957) studied the

susceptibility patterns of streptococci other than Group D, and found

them uniformly susceptible to eleven individual antibiotics, with

bacitracin susceptibility in Group A being the outstanding exception.

They also suggested group-specific differentiation by susceptibility of

the non-Group D strains to erythromycin and penicillin. Toala et al.

(1969) tested Group D streptococci to determine their susceptibility to

21 antibiotics and found that various species differed quantitatively

in their susceptibility to the individual drugs, namely tetracycline,

chloramphenicol, lincomycin and clindamycin. Lee (1972) studied the

antibiogram patterns of Group D streptococci as a diagnostic aid in

differentiating them from other "Viridans" streptococci. Thornsberry

et al. (1974) compared the antibiotic susceptibility patterns of 74

strains of S. bovis, a non-enterococcal Group D, with 35 enterococcal

strains and reported that antibiotic susceptibility varies among strains

of Group D, with S. bovis exhibiting more uniform susceptibility than

the enterococci. Karchmer et al. (1975) reported high susceptibility of

serotypes A, B, C, F, G, H, L, and M to clindamycin and lincomycin,

resistance in Group D enterococci but susceptibility in the Group D

non-enterococci. Gunn (1976) using the letters S and R for sensitivity

and resistance respectively, and referring to bacitracin (Taxo A) and

sulfamethoxazole-trimethoprim (SXT), in that order, reported an SR

pattern for Group A with 98.8% accuracy, RR for Group B with 97.1%

accuracy, and RS pattern for beta hemolytic streptococci other than

Groups A or B with 100% accuracy.

As far as is known, no biochemical, physiological, or antibiogram pattern studies have been done on streptococci from Stockton or other parts of California. Therefore it is the purpose of this investigation to biochemically and physiologically characterize primarily extra-respiratory streptococcal isolates, study their antibiogram patterns, and relate these characteristics to each other and to other patterns reported in the United States.

II. Materials and Methods

One hundred and thirteen strains of streptococci were obtained from cultures isolated primarily from extra-respiratory materials from patients at Dameron Hospital (77), Saint Joseph's Hospital (31), and Spencer Laboratories (5), in Stockton, California between August 1976 and February 1977. The cultures were received on blood agar plates, bile esculin slants or plates, or blood Mueller-Hinton plates. Table III is a summary of the number of strains and site of isolation.

Each strain was subcultured on a blood agar plate to determine purity, Gram-stained, and tested for catalase production. A representative colony was isolated and inoculated on a chocolate agar or brain heart infusion (BHI) slant and stored at 2-8 C. Each strain was tested for growth at 45 C, growth in 6.5% NaCl, hydrolysis of hippurate and esculin, production of pigment in modified Columbia agar (DMS), growth in ethyl violet azide (EVA), and sensitivity to Taxo P (optochin) and Taxo A (bacitracin). After classification by physiological characteristics and biochemical reactions (Table IV), the bacteria were tested using the paper disc method for susceptibility to the following 13 antimicrobial substances (all Difco preparations): penicillin G., ampicillin, methicillin, cephalothin, vancomycin, erythromycin, clindamycin, gentamicin, kanamycin, sulfamethoxazole-trimethoprim (SXT), tetracycline, chloramphenicol, and nitrofurantoin.

All media were autoclaved for 15 minutes at 15 lbs. pressure and 121 C.

Growth at 45 C

Bile esculin slants were inoculated and incubated at 45 C for 72 hours. A positive test is indicated by a blackening of the slant.

Negative strains were tested again on BHI slants and observed for growth after 72 hours.

BHI Slants

These were used for stock cultures and occasionally growth at 45 C. The medium was prepared by dissolving 37 gm of dehydrated brain heart infusion (Difco) and 15 gm of Bacto-agar (Difco) in one liter of demineralised water, dispensed as 7 ml aliquots in screw cap tubes, autoclaved, and slanted.

6.5% NaCl Broth

This medium was prepared from 37 gm of dehydrated BHI (Difco) dissolved in one liter of demineralised water and the addition of 60 gm of NaCl. Ten ml aliquots were dispensed into tubes and autoclaved. Positive test is evident by growth.

Hydrolysis of Hippurate

Sodium hippurate broth was prepared as a 1% aqueous solution by adding 10 gm of sodium hippurate to one liter of BHI broth. Seven ml aliquots were dispensed into tubes and the level marked on the tube with a wax pencil. The tubes were autoclaved, and prior to testing sterile distilled water was added to each tube to bring it up to the original mark and compensate for any evaporation or alteration in concentration. Eight-tenths ml of inoculated broth was mixed with 0.2 ml ferric chloride reagent (12 gm ferric chloride dissolved in 100 ml 2% aqueous HCL). A precipitate formed in each tube and then redissolved in less than 10 minutes. A permanent percipitate, however, did not dissolve after 10 minutes indicating the presence of benzoic acid and the hydrolysis of

hippurate. Accurate concentrations are crucial or false positive results

may occur.

Hydrolysis of Esculin

Bile esculin agar was prepared by dissolving 64 gm of dehydrated

media (Difco) in one liter of demineralized water. It was found to be

easier and less expensive to prepare the medium without the addition of

serum, and the results were adequate. Seven ml aliquots were dispensed

into tubes, autoclaved, and slanted. A positive test is indicated by a

blackening of at least one half of the tube. Results are considered

negative only after 72 hours of incubation.

DMS Agar

Introduced by Merritt et al. (1976) this modification of Columbia

agar (named DMS after Dartmouth Medical School) was prepared by

dissolving 37 gm of GC basal medium (Difco) and 10 gm of proteose

peptone 3 (Difco) in one liter of demineralized water, dispensed as 7 ml

aliquots into tubes, autoclaved, and cooled as agar deeps. Inoculation

is by stab technique and a positive test is evident as an orange pigment

along the stab line after 24-48 hours of incubation.

Ethyl Violet Azide (EVA) Broth

This medium was prepared by dissolving 35.8 gm of dehydrated media

(Difco) in one liter of demineralized water, dispensed in 7 ml aliquots

and autoclaved. A positive test is evident by growth in 24-48 hours.

Taxo P Sensitivity (optochin)

This test determines the susceptibility of the organisms to low

concentrations of ethylhydrocupreine hydrocholoride (optochin),

incorporated into filter paper discs in a concentration of 1: 4,000. A

positive test is indicated by a zone of inhibition of 15-30mm in diameter.

The discs were purchased from Baltimore Biological Laboratories (BBL),

Baltimore, Maryland.

Taxo A Sensitivity (bacitracin)

The bactracin test presumptively identifies Group A streptococci.

The discs, purchased from BBL, have a potency of 0.04 units of bacitracin.

A positive test is a zone of inhibition, usually 12mm or more, around the

disc.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was conducted on Mueller-Hinton

medium (Difco) with 5% defibrinated sheep blood added, using the Bauer-

Kirby standardized disc method (Bauer et al. 1966). Mueller-Hinton

plates were prepared by dissolving 38 gm of dehydrated media (Difco) in

one liter of demineralized water and dispensing 60 ml into small capped

bottles. After autoclaving the bottles were cooled to 50 C in a

waterbath. To each bottle 3 ml of blood (defibrinated sheep blood) was

added, mixed thoroughly, and poured into sterile 150mm plastic petri

dishes. Susceptibility testing procedures were conducted according to

the Bauer-Kirby technique as follows: The organisms were introduced

into BHI broth (Difco) and incubated until the density of the bacteria

was observed to be approximately equal to that of a 1% $BaCl_2$ in H_2SO_4

(0.5ml of a 1% $BaCl_2$ solution in 99.5 ml of 0.36 N H_2SO_4). The

suspension was streaked with a sterile cotton swab in three directions to

insure even distribution of bacteria. The surface was allowed to dry for

at least 5, but not longer than 20 minutes and then antibiotic discs were

placed on the surface of the plate using a 12-magazine-150mm dispenser

(Difco). The plates were incubated in an inverted position at 37 C for

24 hours and the diameter of each zone measured to the nearest mm and

compared to the standard chart for interpretation of results. Table V

lists the antibiotics and concentrations used and the standard disc zone

interpretation.

III. RESULTS

The 113 streptococcal isolates were found to belong to three Groups: Group A (7), Group B (6), Group D (100).

The results (Table IV) indicate that all 7 members of Group A were sensitive to bacitracin (Taxo A), resistant to Taxo P, negative for growth at 45 C and growth in 6.5% NaCl, failed to hydrolyse hippurate and esculin, and did not produce pigment in DMS agar deeps. One strain (14.2%) grew in EVA broth. This strain was recovered from blood and except for this characteristic no other unusual features were noted. Its antibiogram shows resistance to kanamycin only. The other six strains were obtained from urine (3), wounds (2), and cervix (1).

All six strains of Group B gave positive reactions for hippurate hydrolysis and pigment production in DMS agar, and negative reactions for the other tests. These strains were obtained from urine (2), cervico-vaginal (2), endotracheal (1), and blood culture (1).

The largest number of strains (100) belonged to Group D. All strains gave positive reactions for growth at 45 C, esculin hydrolysis, and growth in EVA broth, but negative reactions to pigment production in DMS agar and sensitivity to Taxo P and Taxo A.

Ninety-eight strains of the Group D were positive for growth in 6.5% NaCl and 2 strains were negative. On the basis of this test the 2 strains were identified as non-enterococci and the other 98 as enterococci.

Hippurate was hydrolysed by 7.1% of Group D and all strains hydrolysed esculin. All 100 isolates were resistant to optochin (Taxo P) and bacitracin (Taxo A).

Antibiotic Susceptibility Patterns (Table VI)

Group A strains were 100% susceptible to penicillin G, ampicillin, methicillin, cephalothin, vancomycin, erythromycin, clindamycin, and nitrofurantoin, 85.7% to chloramphenicol, 71.3% to gentamicin, 48.1% to SXT, and 42.9% to tetracycline. All strains were resistant to kanamycin.

Group B strains were 100% susceptible to penicillin G, ampicillin, methicillin, cephalothin, vancomycin, and nitrofurantoin, 83.3% to erythromycin and clindamycin, 66.7% to gentamicin and 50% to chloramphenicol. Resistance to kanamycin was 100%, SXT 83.3% and tetracycline 50%.

All 98 strains of Group D enterococci were susceptible to ampicillin and vancomycin, 99% to nitrofurantoin, and 74.5% to gentamicin. Group D enterococci were less susceptible to penicillin (93.9% showing intermediate susceptibility) compared to ampicillin, and resistant to methicillin (96.9%), clindamycin (100%), SXT (98%), cephalothin (95.9%), kanamycin (87.8%), tetracycline (78.6%), chloramphenicol (65.3%), and erythromycin (40.8%).

The Group D non-enterococci had patterns similar to those of Groups A and B showing 100% susceptibility to penicillin G, ampicillin, cephalothin, vancomycin, erythromycin, clindamycin, gentamicin and nitrofurantoin, and 50% susceptibility to SXT and cloramphenicol. The two strains were resistant to tetracycline, one strain exhibited resistance to kanamycin, and both showed intermediate susceptibility to methicillin.

VI. DISCUSSION

The most reliable method for the identification of streptococci is
to grow pure cultures, extract the group specific antigen, and classify
them according to specific serologic reactions. However, this method is
expensive and time consuming. Alternate methods of identifying
streptococci are based on biochemical tests. More recently various
studies (Jones et al., 1957; Toala et al., 1969; Lee, 1972; Thornsberry
et al., 1974; Karchmer et al., 1975; and Gunn, 1976) suggested the
possibility that antibiogram patterns may also be used in the
identification of streptococcal groups, and even species within a group
Lee, 1972 and Karchmer et al., 1975). For routine processing of
clinically isolated specimens identified as streptococci by colonial
morphology, Gram stain and catalase test, group differentiation may be
based on a combination of biochemical tests (type of hemolysis,
sensitivity to bacitracin and optochin, hydrolysis of esculin and
hippurate, growth in 6.5% NaCl, and pigment production in DMS agar) and
antibiogram patterns.

These biochemical tests and antibiograms, in combination, can aid in
the presumptive identification of streptococci and subsequent treatment
of streptococcal infections.

Identification of hemolytic patterns are, by definition, carried out
on blood agar pour plates and examined macroscopically and microscopically.
Since, in part, hemolysis is due to oxygen labile streptolysin O,
anaerobic incubation of sheep blood agar plates (when pour plates are
impractical) is recommended. Determination of hemolysis is important in
the presumptive identification of Group A streptococci with bacitracin;
however, hemolytic patterns in other groups tend to overlap to such an

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extent that they are not as useful as a characteristic for further differentiation of streptococci into other groups (Table I).

Bacitracin sensitivity is characteristic of Group A streptococci. Several investigators have reported susceptibility among "Viridans" streptococci (Maxted, 1953 and Facklam et al., 1974) and beta hemolytic streptococci other than Group A (Facklam et al., 1974; Merritt et al., 1976; and Gunn, 1976) indicating the importance of further biochemical testing to insure reliable identification for extra-respiratory streptococci.

Optochin susceptibility is characteristic of $\underline{S}$. pneumoniae, which is sensitive to concentrations of 1:500,000-1:100,000, whereas other streptococci require 1:5,000 or greater to inhibit growth (McFaddin 1976). This test is useful in differentiating $\underline{S}$. pneumoniae from other alpha hemolytic streptococci.

Bile esculin medium is a reliable presumptive method of identification for Group D streptococci as evidenced by the data of this investigation and supported by those of Facklam (1972, 1973) and Facklam et al. (1974) who reported 99.6% of the enterococci and 99.3% of the non-enterococci produced positive BEM reactions. Growth in 6.5% NaCl is positive for Group D enterococci, whereas only 2.2% of the non-enterococci can tolerate the salt concentration (Facklam et al., 1974). This test, when combined with BEM can be used to differentiate within the Group D enterococci (BEM +, NaCl +) and non-enterococci (BEM +, NaCl -).

Facklam et al. (1974) also reported that nearly 80% of the Group B grew in 6.5% NaCl. This is neither supported by limited data of this investigation nor by Bailey and Scott (1974) or Deibel and Seeley (1974).

The sodium hippurate test, as developed by Ayers and Rupp (1922) for the presumptive identification of Group B is prone to many inherent errors. Although Facklam et al. (1974) reported the use of this medium with reliable results, there are many complications for the clinical lab employing this test. As reported in the data, 7.1% of Group D hydrolysed hippurate. This is in close agreement with those of Facklam et al. (1974) who reported a positive hippurate test for 6.9% of Group D. Correct concentrations are essential for proper results (Bailey and Scott, 1974). In this investigation some of the hippurate media were purchased from a local supply laboratory (Microbiological Media, Concord, California), others were prepared and marked as to original level on each individual tube. The prepared media provided numerous false positive results due to evaporation prior to delivery. Even when these were locally prepared, marked, and properly adjusted as to concentration, centrifugation of the broth was necessary for reliable results. We also discovered that results were not generally reliable if the test was conducted on cultures younger than 48 hours.

The new presumptive test by Merritt et al. (1976) and Merritt and Jacobs (1976) based on the observations of Fallon (1974), testing for pigment production in DMS agar, proved to be simple and accurate. This test has not received wide enough usage to replace hippurate as a presumptive test for Group B. However, with increasing employment of this medium its reliability and ease should make it the preferred test. Another important factor favoring DMS agar is that a positive test can be seen within 24 hours. The implication of Group B in neonatal sepsis (Ablow, 1976) accompanied by high mortality rate, necessitates rapid presumptive identification. As far as is known this is the first

investigation where large numbers of Group D were tested in this medium.
Merritt et al. (1976) tested only 4 strains.

Antibiogram patterns are characteristic of certain species of
bacteria. In the absence of any genetic mutations, these patterns may
be used as an aid in the identification of these organisms. In the case
of streptococci some very distinct patterns were found in this study that
appear to be similar to those reported from other geographical areas, as
cited by literature (Hartman and Weinstein, 1948; Jawetz et al., 1950;
Kenney et al., 1953; Finland, 1955a, 1955b and 1955c; Jones et al., 1957;
Toala et al., 1969; Moellering et al., 1970; Lee, 1972; Thornsberry et
al., 1974; Karchmer, 1975; and Gunn, 1976).

Groups A and B are readily differentiated from Group D by
antibiogram patterns (Table VI). Groups A and B have similar antibiograms
which include susceptibility to penicillin G, ampicillin, methicillin,
cephalothin, vancomycin, erythromycin, clindamycin, cloramphenicol, and
nitrofurantoin, and resistance to kanamycin, SXT, and tetracycline.
These results are supported by those of Jones et al. (1957) who
reported that penicillin and erythromycin were the most effective
antiobiotics against non-group D streptococci, followed by decreasing
susceptibility to tetracycline and chloramphenicol. Karchmer et al.
(1975) reported that Groups A and B, unlike D, tended to be susceptible
to clindamycin. Gunn (1976) reported supportive data that all 166 Group
A and 34 Group B in his study were sensitive to methicillin. Gunn (1976)
also reported that 99.4% Group A were sensitive and 100% Group B were
resistant to SXT, as compared to only 48.1% sensitive Group A and 83.3%
resistant Group B in this investigation. On the basis of his study
Gunn (1976) concluded that this antimicrobial substance may be used in

the differentiation of Groups A and B. It is evident that our results do not support this conclusion.

Group D enterococci exhibit susceptibility to ampicillin and vancomycin, with intermediate susceptibility to penicillin and a high degree of resistance to methicillin (96.9%), cephalothin (95.9%), clindamycin (100%), kanamycin (87.8%), and to a lesser extent, tetracycline (78.6%), chloramphenicol (65.3%) and erythromycin (40.8%) (Table VI). These data are in agreement with those of Toala et al. (1969) who reported ampicillin, penicillin G, and vancomycin as the antibiotics most effective against enterococci, and erythromycin active against approximately half the strains, with an increasing resistance to tetracycline and chloramphenicol. Lee (1972) reported supportive data regarding resistance to clindamycin and cephalothin, and Karchmer et al. (1975) to clindamycin.

Group D can be further differentiated between enterococci and non-enterococci on the basis of non-enterococcal susceptibility to clindamycin, cephalothin, and to a lesser extent, methicillin. Toala et al. (1969), Lee (1972), Thornsberry et al. (1974), and Karchmer et al. (1975) reported that the non-enterococci were significantly more susceptible to penicillin, ampicillin, methicillin, cephalothin, erythromycin, and clindamycin, than the Group D enterococci.

There is a low incidence of non-enterococcal Group D in the present collection and the two strains represented by this study provide only limited data. Further testing with a larger sample is necessary.

This study suggest that presumptive identification of Group A, Group B, Group D enterococci, and Group D non-enterococci may be made on the basis of a few biochemical tests (6.5% NaCl, bile esculin, DMS agar,

sodium hippurate and Taxo A) and susceptibility to the antiobiotics

methicillin, cephalothin, and clindamycin (Table VII).

V. SUMMARY

One hundred and thirteen strains of streptococci isolated from clinical material and representing 3 serologic groups: Group A (7), Group B (6) and Group D (100) were studied with respect to their biochemical activities and antibiogram patterns. The study suggests that the use of 6.5% NaCl, bile esculin, DMS agar sodium hippurate, bacitracin (Taxo A) and the antibiotics methicillin, cephalothin and clindamycin are sufficient to identify the three serologic groups and distinguish between enterococci and non-enterococci among Group D.

TABLE I

Hemolytic Patterns in Various Groups of Streptococci

Serological Group	Hemolytic Characteristics
A	beta
B	alpha, beta, gamma
C	beta
D	alpha, beta, gamma
E	beta
F	alpha, beta, gamma
G	beta
H	alpha, gamma
N	gamma
Ungrouped Viridans	alpha, gamma

TABLE II

Antigenic Classification of Streptococci

Serological Group	Species	Habitat
A	S. pyogenes	Man
B	S. agalactiae	Cattle
C	S. equi S. equisimilis S. zooepidemicus S. dysagalactiae	Horses Man Lab animals Man
D	S. durans S. liquefaciens S. faecalis S. bovis	Man and cattle Man Man and animals Man and bovine
E	Streptococcus sp.	Milk and swine
F	Streptococcus sp.	Man
G	S. anginosus	Man and dog
H	S. saquis	Man
K	Streptococcus sp.	Man
L	Streptococcus sp.	Pigs, dogs, and man
M	Streptococcus sp.	Dogs and man
MG	S. acidominus	Cattle
N	S. cremoris S. lactis	Cattle Cattle
O	Streptococcus sp.	Man
Viridans*	S. equimus S. mitis S. salivarus S. thermophilus S. uberus	Horse and man Man Man Cattle Cattle

* Not defined serologically

TABLE III

Clinical source, Site of Isolation, and Number of Isolates

Clinical Source	Urine	Wound	Blood	Cervico-vaginal	Burn	Respiratory Tract	Peritoneal Fluid	Total
Dameron Hospital	33	16	6	18	2	2	0	77
Saint Joseph's Hospital	12	14	4	0	1	0	0	31
Spencer Laboratories	2	0	0	1	0	1	1	5
Total	47	30	10	19	3	3	1	113

TABLE IV

Per Cent of Positive Isolates for Biochemical and Physiological Tests

Streptococcal Group	Group A	Group B	Group D enterococci	Group D non-enterococci
Growth at 45 C	0	0	100%	100%
Growth in 6.5% NaCl	0	0	100%	0
Hippurate Hydrolysis	0	100%	7.1%	0
Esculin Hydrolysis	0	0	100%	100%
Pigment in DMS agar	0	100%	0	0
Growth in EVA broth	14.2%	0	100%	100%
Taxo P (optochin) Sensitivity	0	0	0	0
Taxo A (bacitracin) Sensitivity	100%	0	0	0

TABLE V

Zone Size Interpretation Chart for Antibiograms

by Bauer-Kirby Technique

Antibiotic	Disc Content	ZONE DIAMETER (to nearest mm)		
		Resistant	Intermediate	Susceptible
Penicillin G	10 units	11	12–21	22
Ampicillin	10ug	11	12–13	14
Methicillin	5ug	9	10–13	14
Cephalothin	30ug	14	15–17	18
Vancomycin	30ug	9	10–11	12
Erythromycin	15ug	13	14–17	18
Clindamycin	2ug	14	15–16	17
Gentamicin	10ug	12	----	13
Kanamycin	30ug	13	14–17	18
Sulfamethoxazole-trimethoprim 1:19 (SXT)	25ug	10	11–15	16
Tetracycline	30ug	14	15–18	19
Chloramphenicol	30ug	12	13–17	18
Nitrofurantoin	300ug	14	15–18	19

TABLE   VI

Antibiogram Patterns

Streptococcal Group	Interpretation	Penicillin G	Ampicillin	Methicillin	Cephalothin	Vancomycin	Erythromycin	Clindamycin	Gentamicin	Kanamycin	Sulfamethoxazole-trimethoprim (SXT)	Tetracycline	Chloramphenicol	Nitrofurantoin
Group A (7)	R	0	0	0	0	0	0	0	28.7%	100%	42.9%	48.1%	0	0
	I	0	0	0	0	0	0	0	–	0	0	0	14.3%	0
	S	100%	100%	100%	100%	100%	100%	100%	71.3%	0	48.1%	42.9%	85.7%	100%
Group B (6)	R	0	0	0	0	0	16.7%	16.7%	33.3%	100%	83.3%	50%	16.7%	0
	I	0	0	0	0	0	0	0	–	0	0	16.7%	33.3%	0
	S	100%	100%	100%	100%	100%	83.3%	83.3%	66.7%	0	16.7%	33.3%	50%	100%
Group D Enterococci (98)	R	1%	0	96.9%	95.9%	0	40.8%	100%	25.5%	87.8%	98%	78.6%	65.3%	0
	I	93.9%	0	3.1%	4.1%	0	12.3%	0	–	4.1%	0	3.1%	7.1%	1%
	S	5.1%	100%	0	0	100%	46.9%	0	74.5%	8.1%	2%	18.3%	27.6%	99%
Group D non-Enterococci (2)	R	0	0	0	0	0	0	0	0	50%	50%	100%	0	0
	I	0	0	100%	0	0	0	0	0	50%	0	0	50%	0
	S	100%	100%	0	100%	100%	100%	100%	100%	0	50%	0	50%	100%

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TABLE VII
Identification Chart for Streptococci

Streptococcal Group	6.5% NaCl	Bile Esculin	Pigment in DMS	Sodium Hippurate	Taxo A Sensitivity	Methicillin	Cephalothin	Clindamycin
Group A	-	-	-	-	+	S	S	S
Group B	-	-	+	+	-	S	S	* S
Group D enterococci	+	+	-	d	-	R	R	R
Group D non-enterococci	-	+	-	d	-	I	S	S

Key: + = 90% or more strains positive   - = 90% or more strains negative   d = less than 90% positive

S = 90% or more sensitive   R = 90% or more resistant   I = intermediate susceptibility
(*S = only 83.3% in this study although this represents 5/6)

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