

# *Aerococcus viridans* in the Hospital Environment<sup>1</sup>

MILDRED A. KERBAUGH<sup>2</sup> AND JAMES B. EVANS

Department of Microbiology, North Carolina State University, Raleigh, North Carolina 27607, and Laboratory Division, North Carolina State Board of Health, Raleigh, North Carolina

Received for publication 18 December 1967

*Aerococcus viridans* has been described as an airborne organism prevalent in occupied rooms. It has also been described as an organism having many characteristics that might cause it to be confused with streptococci or staphylococci, and this may account for the fact that the presence of *A. viridans* has not been reported in the hospital environment or in clinical specimens. Swab specimens were taken from 47 objects in 11 different areas in a local hospital, cultured overnight in Trypticase Soy Broth, and streaked on blood-agar and on a selective serum agar containing potassium tellurite and crystal violet. Of 85  $\alpha$ -hemolytic cultures isolated, 11 proved to be typical *A. viridans* based on diagnostic tests that also were applied to a collection of gram-positive cocci, including authentic strains of *A. viridans*. These organisms are gram-positive cocci with a strong tendency toward tetrad formation in broth cultures. They are predominantly aerobic, have a very weak catalase activity, and lack porphyrin respiratory enzymes. Three similar cultures also were obtained from routine clinical specimens.

In an extensive study of the incidence of "mouth streptococci" in air samples from occupied schoolrooms, factories, offices, and dust from yards, streets, and on clothing, Williams and Hirsch (10) found their investigation complicated by the presence of a large number of  $\alpha$ -hemolytic cocci that did not form chains and differed in other respects from streptococci. Shaw, Stitt, and Cowan (8) included a collection of these bacteria in their taxonomic study of staphylococci and micrococci and concluded that these organisms were quite distinct. Unlike the staphylococci and micrococci, they grew poorly in air, produced small translucent or semi-opaque colonies, and were very feebly catalase-positive. In addition, these  $\alpha$ -hemolytic cocci produced acid from glucose and a number of other sugars and failed to reduce nitrates.

A more detailed study of this group of bacteria by Williams, Hirsch, and Cowan (11) resulted in the proposal of new genus and species names, *Aerococcus viridans*, to describe them. These organisms, in addition to previously mentioned characteristics, were able to tolerate 40% bile, 0.001% potassium tellurite, and 0.00025% crystal violet. Anaerobic incubation was reported

to decrease growth slightly if at all. A variety of characteristics differentiated these organisms from streptococci and from pediococci, with which it was suggested they may have been confused by earlier workers.

Deibel and Niven (4) concluded that these organisms were indistinguishable from the lobster pathogen, *Gaffkya homari*, and from some pediococci that are common in meat-curing brines. These investigators suggested that the organisms should be called *Pediococcus homari*. Other workers have also recognized the similarity of these organisms to pediococci but have suggested retention of the genus *Aerococcus* (1, 2, 9). Most of these workers recognized the frequent production of tetrads in suitable broth media in contrast to the scarcity of such morphological grouping reported by Williams et al. (11).

Possible clinical significance of aerococcus-like bacteria is suggested by reports of their occurrence in "fluid medicaments" (1) and in the lung of a patient suspected of having tuberculosis (5). However, their principal significance in the clinical laboratory lies in the possible confusion of these bacteria with streptococci and staphylococci. Despite the original report of the frequent occurrence of aerococci in the air of occupied rooms, no reports have appeared indicating their occurrence in the hospital environment. The present study was designed to investigate the incidence of aerococci in a variety of areas and on numerous items in a hospital.

<sup>1</sup> Paper no. 2531 of the Journal Series of the North Carolina State University Agricultural Experiment Station, Raleigh.

<sup>2</sup> Recipient of a graduate scholarship from the North Carolina Public Health Association. This study was conducted as part of an M.S. thesis.

## MATERIALS AND METHODS

**Control cultures.** A variety of cultures was obtained from other laboratories, and these cultures were used for comparative purposes and in the evaluation of media and methods. The cultures included three *Aerococcus* strains (301, 302, and 303) from Baird-Parker (Unilever Research Laboratory, Bedford, England), Williams' type culture of *A. viridans* (ATCC 11563), two cultures identified as *A. viridans* ( $C_2$  and  $C_7$ ), and two cultures designated *A. catalasicus* ( $C_6$  and  $C_9$ ) from Clausen (University of Oslo, Norway), two strains labeled *Staphylococcus salivarius* (FDC-3 and FDC-7) from Gordon (Forsyth Dental Center, Boston), and a culture of *Pediococcus cerevisiae* (ATCC 8081).

**Isolation methods.** Cotton-tipped swabs moistened with broth were used to collect specimens. After swabbing a small area on the object to be sampled, the swabs were incubated in Trypticase Soy Broth (BBL) overnight at 37 C. These enrichment cultures were streaked on Neopeptone Blood Agar Base (Difco) with 5% defibrinated sheep cells and on the selective serum agar of Williams and Hirsch (10) containing 0.001% potassium tellurite and 0.00025% crystal violet. (The levels of tellurite and crystal violet were inadvertently reversed on page 507 of the original article.) All  $\alpha$ -hemolytic colonies on the blood-agar were restreaked on blood-agar, and an isolated  $\alpha$ -hemolytic colony was picked from each. All typical bacterial colonies on the Williams and Hirsch medium were streaked on blood-agar, and all colonies that were  $\alpha$ -hemolytic were picked for further study. The areas and items sampled are listed in Table 1.

**Morphological examination.** All isolates were

inoculated into APT Broth (Difco) and incubated overnight at 32 C; then Gram-stained preparations were made. APT Broth was reported to favor tetrad formation by aerococci and chain formation by streptococci (4).

**Physiological studies.** All cultures were tested against TAXO Pneumococcus Discs (BBL) on blood-agar. Bile tolerance and esculin hydrolysis were determined according to the method of Williams and Hirsch (10). Viridans streptococci did not grow on this medium, enterococci grew and hydrolyzed esculin, and aerococci grew but produced little or no esculin hydrolysis. Catalase tests were performed by adding 3% hydrogen peroxide to cultures on slants of a low glucose medium (Trypticase Soy Agar, BBL) and observing the cultures for the evolution of oxygen. The benzidine test for porphyrins was conducted on APT Agar (Difco) according to the method of Deibel and Evans (3). Relation to oxygen was determined by use of agar shake cultures in Trypticase Soy Broth (BBL) plus 1.5% agar. To test anaerobic fermentation of glucose, Purple Broth Base (Difco) with 1.0% glucose was inoculated and incubated at 37 C in a Gas Pak Anaerobic Jar (BBL).

**Serological tests.** Precipitin tests with streptococcal group D antisera were performed according to the technique of Lancefield (6). Organisms that gave negative results were cultured in Todd Hewitt broth with 1.0% glucose and were retested as recommended by Medrek and Barnes (7).

## RESULTS

A total of 249 swab samples were taken as part of this study.  $\alpha$ -Hemolytic colonies were found on 31 of the primary blood-agar plates. Typical

TABLE 1. Areas and items from which specimens were collected

Areas in hospital		
Premature nursery <sup>a</sup>	Recovery room <sup>a</sup>	Operating room scrub area
Obstetric nursery <sup>a</sup>	Operating room <sup>a</sup>	Pediatrics ward <sup>a</sup>
Intensive care unit <sup>a</sup>	Labor room	Cystoscopic room <sup>b</sup>
Treatment room <sup>a</sup>	Delivery room <sup>b</sup>	
Items within the areas		
Counter	Faucet	Fire extinguisher <sup>a</sup>
Desk	Trash can	Delivery table
Door <sup>a</sup>	Air vent	Addressograph
Mask	Teletalk	Resuscitator
Scales <sup>a</sup>	Knee press	Pencil sharpener
Incubator	Glove rack	Orange stick
Chair	Crib	Water fountain
Telephone <sup>a</sup>	Charts	Soap dispenser
Sink	Isolette <sup>a</sup>	Door handle
Bed <sup>b</sup>	Respirator	Bulletin board
Wall	Oxygen mask	Towel dispenser
Light	Tape dispenser <sup>a</sup>	Catheter tray
Table <sup>a</sup>	Sponge rack <sup>a</sup>	Light switch
Stool	Oxygen tank	Stethoscope
Shelf <sup>a</sup>	Saline bottle	Window frame
Stirrup	X-ray view box	

<sup>a</sup> An area or item from which a typical *Aerococcus* was subsequently isolated.

<sup>b</sup> An area or item from which two cultures were isolated.

bacterial colonies were found on 119 of the plates of Williams and Hirsch medium, but only 54 of these plates yielded  $\alpha$ -hemolytic colonies when restreaked on blood-agar. The morphology of the 85  $\alpha$ -hemolytic cultures was examined (Table 2). Of these 85 cultures, 35 produced long chains of cocci and were considered to be Viridans streptococci, and two of these cultures were considered to be rods. The 48 cultures that were morphologically similar to micrococci, enterococci, or tetracocci represented 37 different original samples. Of these 37 samples, 11 yielded this type of bacterium on both the blood-agar and on the Williams and Hirsch medium. Two samples yielded this type of bacterium on blood-agar only, and 24 samples yielded it only on the Williams and Hirsch medium. Preliminary tests indicated that the 11 pairs of cultures from the same sources were physiologically similar; hence only the organisms isolated from these sources and cultured on the Williams and Hirsch medium were used in subsequent studies.

These 37 cultures from separate original sources all gave negative tests against the TAXO Discs for pneumococci. However, six of these cultures proved to be sensitive to 40% bile, were catalase negative, and failed to give a precipitin test with group D antisera. These six were considered to be Viridans streptococci that had not been recognized on morphological examination. Seventeen cultures hydrolyzed esculin rapidly, precipitated group D antisera, and demonstrated rapid and vigorous anaerobic fermentation of glucose. Three other cultures possessed these characteristics, except for a failure to react with group D antisera. These 20 cultures apparently were enterococci. This left 11 cultures that appeared to be typical aerococci. These were isolated from 9 of the 11 areas that were sampled in the hospital. The areas and items from which these 11 cultures were isolated are noted in Table 1.

Although a systematic search was not conducted, three strains that appeared to be aerococci

TABLE 3. *Physiological characterization of aerococci*<sup>a</sup>

Culture no.	Catalase <sup>b</sup>	Esculin hydrolysis	Relation to oxygen <sup>c</sup>	Anaerobic glucose <sup>d</sup>
<i>Hospital strains</i>				
32	±	±	MA	—
53	+	—	FA	±
55	±	±	FA	—
80	—	±	FA	—
83	++	—	MA	—
95	++	—	F	±
132	++	—	F	±
146	++	—	F	—
165	±	±	MA	—
188	±	±	FA	—
201	++	—	F	—
<i>Clinical strains</i>				
3	++	—	FA	—
767	++	—	FA	—
1486	++	—	FA	—
<i>Controls</i>				
C <sub>2</sub>	+	±	FA	—
C <sub>6</sub>	+	±	FA	±
C <sub>7</sub>	+	±	FA	—
C <sub>9</sub>	+	—	FA	—
301	+	—	FA	—
302	+	—	FA	—
303	+	+	FA	—
FDC-3	+++	NT	A	—
FDC-7	+	±	FA	±
11563	+	—	FA	—
8081	++	+	F	+++

<sup>a</sup> All of the organisms in this table were gram-positive cocci with a strong tendency to produce tetrads. All except FDC-3 gave a negative benzidine test and were able to grow in the presence of 40% bile.

<sup>b</sup> Strains designated as ± failed to show evolution of oxygen from peroxide but, after overnight storage, showed gas bubbles in the medium. Other strains evolved gas following peroxide addition, the number of pluses indicating the relative vigor of this evolution.

<sup>c</sup> MA strains produced numerous discrete colonies in the top few millimeters of the shake culture but showed no anaerobic growth even after 48 hr. FA strains grew as MA strains for the first 24 hr, but, within 48 hr, had produced a few scattered colonies deep in the tube. F strains showed some growth throughout the tube within 24 hr. The A strain grew on the surface and for a few millimeters beneath the surface.

<sup>d</sup> Strains designated as ± produced slight acidification of the bromocresol purple indicator within 36 hr.

were isolated from blood-agar plates of clinical specimens. Strains 3 and 767 were from urine specimens, and strain 1486 was from a sore on a child's leg. No clinical significance was attached

TABLE 2. *Morphological examination of  $\alpha$ -hemolytic isolates*

Morphological type	Cultures isolated on Williams-Hirsch media	Cultures isolated on Primary Blood Agar plates
Gram-positive cocci in long chains.....	19	16
Gram-positive rods....	0	2
Gram-positive cocci in pairs, short chains, and clusters.....	24	11
Gram-positive cocci in tetrads.....	11	2

to these cultures, but they were included in the physiological studies of this project.

Results of the physiological screening experiments on 11 cultures from the hospital environment, 3 cultures from clinical specimens, and 11 control cultures are summarized in Table 3. Based on this limited array of diagnostic tests, the aerococci seem to be a relatively homogeneous and distinct group that differs significantly from the other genera with which they may have been identified. Strain FDC-3 seemed to be an aerobic *Micrococcus*, and strain 8081 (the *P. cerevisiae* control) showed a capability for anaerobic fermentation quite different from that of the aerococci.

#### DISCUSSION

The present study has shown that bacteria indistinguishable from reference cultures of *A. viridans* can be isolated from numerous items in many areas in a hospital. The enrichment technique employed allowed no interpretation as to the relative or absolute numbers of these organisms in the environment. In fact, the technique may have decreased the chances of detecting the bacteria, since these organisms grow relatively slowly in Trypticase Soy Broth used for enrichment and frequently may have been overgrown by Viridans streptococci and enterococci. Direct streaking on the plating media, particularly the Williams and Hirsch selective medium, might have led to a higher proportion of aerococci among the  $\alpha$ -hemolytic isolates.

This study emphasizes the confusion that is sometimes created by conflicting interpretations of results obtained by different experimental methods. For example, Williams et al. (11) described *A. viridans* as an organism that was catalase-negative, benzidine-positive, and lacking the tendency toward tetrad formation. Deibel and Niven (4) showed that tetrad formation occurred frequently when a suitable broth medium was used and that slight catalase activity was frequently demonstrable on low glucose media. Our study confirms these latter findings and, in addition, indicates that the aerococci are benzidine-negative when the taxonomically more useful technique of Deibel and Evans (3) is used.

The present study also raises questions concerning the oxygen relationship of the aerococci. Williams et al. (11) indicated that anaerobic growth of *A. viridans* is essentially equivalent to aerobic growth, and Deibel and Niven (4) also classified these organisms as facultative with respect to oxygen, although they reported microaerophilic growth in some media. The agar shake cultures used in the present study generally

showed very numerous discrete colonies in a narrow zone just beneath the surface and far fewer, if any, colonies deeper in the tube. This suggests that most of the organisms are inocula incapable of initiating anaerobic growth. When broth cultures were incubated under anaerobic conditions, good anaerobic growth was observed.

Failure to demonstrate anaerobic glucose fermentation in our present study presumably resulted from our use of bromocresol purple as the indicator and the relatively high final pH (usually above 5.0) reported for these organisms by other investigators (2, 4, 9, 11).

Until more detailed physiological and taxonomic studies are conducted, it seems desirable to retain the name *Aerococcus viridans* for this apparently widespread and distinctive group of organisms. A detailed investigation of the pathways of energy metabolism in these organisms would be of particular interest since, despite appearing to have a defective fermentative system, they seem to lack an orthodox respiratory system.

#### ADDENDUM IN PROOF

Colman (J. Clin. Pathol. 20:294, 1967) has now reported the isolation of 10 strains of *A. viridans* from human infections such as endocarditis and urinary-tract infections.

#### ACKNOWLEDGMENTS

This investigation was supported by U.S. Public Health Service research grant AI-07693 from the National Institute of Allergy and Infectious Diseases.

We thank Edna Maness and the administration of Rex Hospital, Raleigh, North Carolina, for their help and cooperation in obtaining specimens and Max D. Moody of the U.S. Public Health Service Communicable Disease Center, Atlanta, Georgia, for the streptococcal Group D antiserum.

#### LITERATURE CITED

1. CLAUSEN, O. G. 1964. The discovery, isolation, and classification of various alpha-hemolytic micrococci which resemble aerococci. *J. Gen. Microbiol.* 35:1-8.
2. COSTER, E., AND H. R. WHITE. 1964. Further studies on the genus *Pediococcus*. *J. Gen. Microbiol.* 37:15-31.
3. DEIBEL, R. H., AND J. B. EVANS. 1960. Modified benzidine test for the detection of cytochrome-containing respiratory systems in microorganisms. *J. Bacteriol.* 79:356-360.
4. DEIBEL, R. H., AND C. F. NIVEN, JR. 1960. Comparative study of *Gaffkya homari*, *Aerococcus viridans*, tetrad-forming cocci from meat curing brines, and the genus *Pediococcus*. *J. Bacteriol.* 79:175-180.
5. DEIBEL, R. H., J. H. SILLIKER, AND P. T. FAGAN.

1964. Some characteristics of an oleate-requiring, hemolytic *Pediococcus*. *J. Bacteriol.* **88**: 1078-1083.
6. LANCEFIELD, R. C. 1928. The antigenic complex of *Streptococcus haemolyticus*. *J. Exptl. Med.* **47**:91-103.
7. MEDREK, T. F., AND E. M. BARNES. 1962. The influence of the growth medium on the demonstration of a group D antigen in faecal streptococci. *J. Gen. Microbiol.* **28**:701-709.
8. SHAW, C., J. M. STITT, AND S. T. COWAN. 1951. Staphylococci and their classification. *J. Gen. Microbiol.* **5**:1010-1023.
9. WHITTENBURY, R. 1965. A study of some pediococci and their relationship to *Aerococcus viridans* and the enterococci. *J. Gen. Microbiol.* **40**:97-106.
10. WILLIAMS, R. E. O., AND A. HIRCH. 1950. The detection of streptococci in air. *J. Hyg.* **48**: 504-524.
11. WILLIAMS, R. E. O., A. HIRCH, AND S. T. COWAN. 1953. *Aerococcus*, a new bacterial genus. *J. Gen. Microbiol.* **8**:475-480.