

Distribution of Hemolytic Streptococci in Respiratory Specimens

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One hundred thirty-seven isolates of beta-hemolytic streptococci were recovered from 623 pharyngeal cultures. Twenty-nine percent of these were group A, 10% were group B, 31% were group C, 11% were group F, 12% were group G, and 7% could not be grouped. The significance of non-group A isolates in pharyngitis could not be evaluated in the absence of viral and serological studies. Hemolytic streptococci were recovered from 9% of 799 lower respiratory cultures. All except one were non-group A, and other potential respiratory pathogens were also present in these specimens. It is our impression that the presence of hemolytic streptococci in lower respiratory tract specimens usually represents pharyngeal contamination.

Streptococcal pharyngitis is generally thought to be due to group A organisms. Previous studies of the bacteriology of respiratory specimens have reported the recovery of non-group A streptococci, without defining their serological group (6, 11, 13, 14).

Non-group A streptococci have been implicated in a variety of extra-respiratory infections (1, 4, 7, 9) and, in at least one instance, as a cause of pharyngitis (3). The present study was undertaken to determine the distribution of the various serological groups in the respiratory specimens commonly received in a clinical laboratory.

MATERIALS AND METHODS

Specimens were obtained from adult patients at the Columbia-Presbyterian Medical Center over a 43-day period during October and November 1978, and sent to the Clinical Microbiology Service for culture.

Pharyngeal swabs were immediately placed in Amies medium (Scott Laboratories, Inc., Fiskeville, R.I.) for transport to the laboratory. These swabs were rolled on a portion of colistin-nalidixic acid agar plates containing 5% sheep blood (BBL Microbiology Systems, Cockeysville, Md., or Scott). The inoculum was further distributed by streaking with a loop, which was also used to cut the agar in the area of heavy inoculation.

Sputa and other lower respiratory specimens were received in the laboratory in the Sputum Collection System (BBL Microbiology Systems) or in Lukens traps. These specimens were diluted with an equal volume of sterile 10% dithiothreitol solution (Sputolysin; Calbiochem, La Jolla, Calif.), homogenized on a Vortex mixer, and permitted to stand at room temper-

ature for 15 min. A smear of the diluted sputum was prepared, Gram stained, and examined at $\times 100$ magnification for the presence of polymorphonuclear leukocytes. Specimens showing inflammatory cells were cultured on colistin-nalidixic acid agar and other media.

The colistin-nalidixic acid agar plates were incubated at 35°C in an atmosphere of 4 to 7% CO₂. Plates were examined at 18 to 24 h for the presence of beta-hemolytic colonies; negative plates were reincubated for an additional 24 h and reexamined.

Gram stains were prepared from representative hemolytic colonies, which were also tested for catalase production. Streptococcal growth on primary plates was scored as "few" (less than 10 colonies), "moderate" (10 to 100 colonies), "many" (more than 100 colonies), or "heavy" (confluent growth). Hemolytic streptococci were subcultured to bile-esculin agar (Scott) and examined microscopically for fluorescence after staining with group A conjugate (BBL Microbiology Systems). Streptococci producing blackening on bile-esculin agar were considered to be group D; isolates giving a positive fluorescent antibody reaction were identified as group A. Non-group A or D streptococci were tested by a modified Lancefield technique (16) using antisera to groups B, C, F, and G (BBL Microbiology Systems). Non-streptococcal isolates other than commensal flora were identified by conventional methods (8).

The charts of patients from whom hemolytic streptococci were recovered were reviewed, and the clinical findings were noted.

RESULTS

Pharyngeal cultures. Single swabs were cultured from 623 patients; beta-hemolytic streptococci were recovered from 137 (22%) of these. Twenty-nine percent of the streptococcal iso-

lates were group A, 10% were group B, 31% were group C, 11% were group F, and 12% were group G. Seven percent of the isolates could not be grouped with the available sera. No hemolytic group D streptococci were recovered.

Outpatients were the source of 88% of the streptococcal isolates. The age and sex distribution of the patients and the associated clinical findings are shown in Table 1. The quantitation of streptococcal growth obtained on the primary plates is given in Table 2.

Lower respiratory cultures. Specimens were processed from 799 patients. Eighty percent of these specimens were expectorated sputa, 15% were obtained by tracheal suction, 4% were bronchial washings, and 1% were obtained by transtracheal aspiration.

Seventy-one isolates of hemolytic streptococci were recovered. Fifty-eight of these were from expectorated sputa, 11 from tracheal suction specimens, and 2 from bronchial washings. The distribution of the streptococcal groups is shown in Table 3. No hemolytic group D streptococci were recovered. The majority of the patients were over 30 years of age and were equally divided by sex. Eighty-two percent were inpatients. As shown in Table 3, other potential bacterial respiratory pathogens were recovered from 77% of the patients from whom non-group A streptococci were isolated.

TABLE 1. *Patients with throat cultures positive for beta-hemolytic streptococci*

Streptococcal group	Total patients	Patients with pharyngitis ^a	Age distribution ^b			Sex	
			20-30 yr	31-50 yr	>50 yr	M	F
A	39	35	25	9	3	10	29
B	13	8	10	3	0	5	8
C	43	29	31	8	3	16	27
F	15	10	10	3	2	7	8
G	17	15	10	3	3	6	11
Undetermined	10	7	8	1	1	3	7

^a Patients with symptoms and/or physical findings consistent with pharyngitis (fever, sore throat, erythema, exudate, tonsillar involvement).

^b Age not available on all patients.

TABLE 2. *Quantitation of streptococcal growth on primary plates*

Streptococcal group	Growth		
	Few	Moderate	Many/heavy
A	2	7	24
B	3	1	4
C	10	8	7
F	3	4	3
G	5	4	5
Undetermined	2	2	3

TABLE 3. *Patients with hemolytic streptococci in lower respiratory specimens*

Streptococcal group	No. of patients	Clinical evidence of lower respiratory infection ^a	Other potential respiratory pathogens present ^b
A	1	1	0
B	11	4	8
C	18	12	17
F	22	13	17
G	11	9	7
Undetermined	8	5	5

^a Fever; pulmonary physical and/or X-ray findings.

^b *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter* spp., *Serratia marcescens*, *Escherichia coli*, *Proteus* spp.

DISCUSSION

The recovery of beta-hemolytic streptococci from 22% of the pharyngeal cultures is consistent with the 25 to 29% recovery rate reported in other studies of adults (10, 13). Sixty-nine percent of the streptococcal isolates were recovered in the 20- to 30-year age group; this age group accounts for only 10% of our patient population. The apparent predominance of females is not significant and reflects the 61% of female patients.

The recovery of group A streptococci has been shown to vary markedly with patient age; several studies have demonstrated that streptococcal pharyngitis is most common between the ages of 2 and 29 years, with the highest incidence during the school years (12). Sixty-four percent of the group A isolates were from the 20- to 30-year age group. It has also been shown that the recovery of group A streptococci fluctuates with the seasons, with the highest incidence during the winter and spring months (13). Almost 75% of the group A isolates showed relatively heavy growth on the primary plates, extending the observation of Quinn and Lowry (14) that patients with pharyngitis have higher numbers of hemolytic streptococci than carriers.

Seventy-one percent of the streptococcal isolates from pharyngeal cultures were non-group A. Margileth et al. (11) found the prevalence of non-group A to be highest during the summer months, whereas Murray et al. (13) found the recovery of these organisms to be constant throughout the year. These workers also found the recovery of non-group A streptococci to be two times higher in adults than in pediatric patients.

The significance of non-group A streptococci in pharyngeal cultures is still unclear. In one study (6), non-group A streptococci were recovered from 24% of routine throat cultures and

19% of throat cultures from symptomatic children.

Group C streptococci were responsible for at least one outbreak of pharyngitis in children (3), and Rantz et al. (15) reported that groups C and G accounted for 1 to 2% of patients with non-group A pharyngitis. Quinn and Lowry (14) reported recovering many colonies of group C (2 cases) and a group G streptococcus from patients with clinical evidence of pharyngitis. These three patients subsequently showed a marked rise in their anti-streptolysin O titers. Group F streptococci, normally present in the respiratory tract, can cause sinusitis and empyema (1).

The pathogenicity of non-group A streptococci is well documented; extra-respiratory infections by groups B, C, F, G, and others have been reported (4, 7, 9). The significance of non-group A isolates in our study cannot be evaluated since concomitant viral and mycoplasmal cultures were not performed, nor were anti-streptolysin O titers determined. Murray et al. (13) noted that a significantly higher recovery of non-group A streptococci occurred if the plates were incubated anaerobically or in an atmosphere of CO₂.

The absence of group D streptococci among our isolates is probably due to the fact that these organisms are usually nonhemolytic on sheep blood (9), and sheep blood agar is routinely employed in our laboratory.

In contrast to pharyngeal specimens, only 9% of lower respiratory specimens contained hemolytic streptococci.

Eighty-one percent of the isolates came from expectorated sputa, 16% from tracheal suction specimens, 3% from bronchial washings, and none from transtracheal aspirates. These recovery rates very closely approximate the relative distribution of these specimen types. Studies of the microbiology of transtracheal aspirates have not shown hemolytic streptococci to be a common isolate (2). Almost 80% of the specimens from which non-group A streptococci were recovered also contained at least one other bacterial species with the potential of being a respiratory pathogen. It is our impression that the presence of hemolytic streptococci in lower respiratory tract specimens usually represents pharyngeal contamination. Pneumonia due to he-

molitic streptococci has become a rare disease in adults (5), and the diagnosis of this clinical entity can more reliably be established by isolation of the organism from blood or pleural fluid.

LITERATURE CITED

1. Bannatyne, R. M., and C. Randall. 1977. Ecology of 350 isolates of Group F streptococcus. *Am. J. Clin. Pathol.* **67**:184-186.
2. Bartlett, J. G. 1977. Diagnostic accuracy of transtracheal aspiration bacteriologic studies. *Am. Rev. Respir. Dis.* **115**:777-782.
3. Benjamin, J. T., and V. A. Perriello, Jr. 1976. Pharyngitis due to Group C hemolytic streptococci in children. *J. Pediatr.* **89**:254-256.
4. Braunstein, H., E. Tucker, and B. C. Gibson. 1971. Infections caused by unusual beta hemolytic streptococci. *Am. J. Clin. Pathol.* **55**:424-430.
5. Briggs, D. D., Jr. 1977. Pulmonary infections. *Med. Clin. North Am.* **61**:1163-1183.
6. Cornfeld, D., and J. P. Hubbard. 1961. A four-year study of the occurrence of beta-hemolytic streptococci in 64 school children. *N. Engl. J. Med.* **264**:211-215.
7. Duma, R. J., A. N. Weinberg, T. F. Medrek, and L. J. Kunz. 1969. Streptococcal infections: a bacteriologic and clinical study of streptococcal bacteremia. *Medicine* **48**:87-127.
8. Ellner, P. D. 1978. Current procedures in clinical bacteriology. Charles C Thomas, Publisher, Springfield, Ill.
9. Feingold, D. S., N. L. Stagg, and L. J. Kunz. 1966. Extrarespiratory streptococcal infections: importance of the various serologic groups. *N. Engl. J. Med.* **275**:356-361.
10. Freedman, P., H. P. Meister, H. J. Lee, E. C. Smith, B. S. Co, and G. D. Nidus. 1970. The renal response to streptococcal infection. *Medicine* **49**:433-463.
11. Margileth, A. M., G. W. Mella, and E. E. Zilvetti. 1971. Streptococci in children's respiratory infections: diagnosis and treatment. Clinical characteristics of pharyngitis related to Group A streptococci and practical utility of nose and throat bacteriology in office practice. *Clin. Pediatr.* **10**:69-77.
12. Moffet, H. L. 1975. Pediatric infectious diseases: a problem-oriented approach, p. 23. J. B. Lippincott Co., Philadelphia.
13. Murray, P. R., A. D. Wold, and J. A. Washington II. 1977. Recovery of Group A and nongroup A beta-hemolytic streptococci from throat swab specimens. *Mayo Clin. Proc.* **52**:81-84.
14. Quinn, R. W., and P. N. Lowry. 1970. The anatomical area of involvement in streptococcal infections and the carrier state. *Yale J. Biol. Med.* **43**:1-10.
15. Rantz, L. A., P. J. Boisvert, and W. W. Spink. 1946. Hemolytic streptococcal and nonstreptococcal diseases of the respiratory tract. *Arch. Intern. Med.* **78**:369-386.
16. Rantz, L. A., and E. Randall. 1955. Use of autoclaved extracts of hemolytic streptococci for serological grouping. *Stanford Med. Bull.* **13**:290.