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Significance of Hemolytic Colonies in Throat Cultures

ROBERT W. QUINN AND P. NYE LOWRY

Department of Preventive Medicine and Public Health, Vanderbilt University School of Medicine, Nashville, Tennessee 37203

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These studies indicate that a single strain of hemolytic streptococci almost exclusively predominates the bacterial flora in patients with streptococcal infections and in the carrier state. One can proceed with confidence that, in isolating streptococci from throat swabs cultured on blood-agar plates, only a single hemolytic colony need be picked for serological grouping and typing.

The material obtained by means of a throat swab from patients with streptococcal infections or from carriers yields β-hemolytic colonies of streptococci when cultured in 5% sheep blood in agar. More definitive identification of the hemolytic colonies may be secured by serological grouping and typing of streptococci from a hemolytic colony. The usual procedure is to select a single hemolytic colony to be grouped and typed. The questions often asked when there are hundreds or thousands of hemolytic colonies on the blood-agar plate are: "what assurance is there that the single colony picked to be grouped and typed is the strain etiologic for the patient's infection," or, "is the strain being carried?" Might there be more than one serological group or type of streptococcus in the patient's throat flora and, if so, which one is the cause of the infection? It is the purpose of this communication to report the results of studies designed to answer these questions.

MATERIALS AND METHODS

The diagnostic criteria for a streptococcal infection (72 subjects) were the usual signs and symptoms of streptococcal pharyngitis tonsillitis (or both), a throat culture positive for group A hemolytic streptococci, and a significant rise in antistreptolysin O or antihyaluronidase titers (or both). A subject with a throat culture positive for hemolytic streptococci but who had no clinical or serological evidence of infection was considered to be a carrier (22 subjects). Twenty-one hemolytic colonies were selected randomly from the blood-agar plate on which each subject's throat swab had been cultured. In the few instances in which fewer than 21 hemolytic colonies were on a plate, all were selected. Each colony was touched with a wire which was then used to inoculate 15 ml of Todd-Hewitt broth. After incubation for 18 hr, each isolate was grouped and typed serologically by the precipitin method (7). Nontypable strains of group A from 23 patients with infection were sent to the National Communicable Disease Center (NCDC) in Atlanta for further attempts to group and type them. ["Nontypable" refers to strains of group A β-hemolytic streptococci which could not be typed with any of the typing sera available. Typing sera for types 1, 2, 3, 4, 5, 6, 8, 11, 12, 13, 14, 15, 17, 18, 19, (22-26), (28-33), (36-44), 46, 47, and 51 were utilized during the study period.]

RESULTS

Patients with infection. All of the β-hemolytic streptococci (1,305 strains) cultured from 72 patients with infections were group A, with the exception of a single colony of group C (Table 1). Forty (55.6%) of the patients had typable strains of group A, the most common being types 3, 1, 6, 12, and 5, in declining frequency. The 31 remaining patients (43.1%) had nontypable strains of group A streptococci. More than one strain of group A were isolated from patients on four occasions. The mixtures consisted of A-NT [19] and A-12, [2], A-NT [3] and A-12 (18), A-NT [8] and A-11 [5]. (The number of each strain picked is in brackets.) With the exception of these three patients, and one in which a single colony of group C was found, the 21 colonies selected from each plate yielded the same group and type. It can not be stated with certainty that all of the nontypable strains of group A were the same. It is possible they might possess different types of M protein which could not be identified serologically for lack of appropriate typing sera. However, based on studies of nontypable group A streptococci now in progress, the possibility of recovering different nontypable strains of group A from a single individual does not seem likely.

Carriers. The results for carriers were in marked contrast to those for patients with infection. Over half of the 377 strains isolated (57.6%) belonged to serological groups other than A, namely, B, C, and G. Half of the 22 carriers were carrying non-group A streptococci. Sixty-five (40.7%) of the 160 strains of group A from carriers were typable,

TABLE 1. *Groups and types of streptococci isolated from 72 patients and 22 carriers*

Source of cultures	No. with A-NT[a] strains	Per cent with A-NT strains	Group A types												Total with typable strains	Per cent typable strains	Total with group A strains	Per cent with group A strains	Non-group A					
			1	2	3	4	5	6	11	12	18	31	41	43					B	C	G	Total non-A	Per cent non-A	Total
Patients with streptococcal infections	31	43.1	7	1	11	1	3	5	1	6	1	1	1	2	40	55.6				1		1	1.3	72
No. of patient cultures	533	40.9	147	20	225	21	53	103	5	98	16	21	20	42	772	50.1	1,304	99.9		1		1		1,305
Carriers of streptococci	7				1		1	2							4		11	50	2	1	3	11	50	22
No. of carrier cultures	95				3		21	24							65	40.7	160	42.4	41	113	63	217	57.6	377

[a] NT = nontypable with available typing sera.

the remainder nontypable. The types of group A recovered most frequently were 6, 5 and 1. A mixed flora of β-hemolytic streptococci was found in only three carriers, A-NT [8] and C [12]; A-NT [17] and A-3 [3]; and A-NT [1] and C [20]. As in the infection group, one strain was usually predominant in carriers.

The possibility of there being more than one serological group or type of β-hemolytic streptococcus on material cultured from a single swab taken from a single site from carriers and individuals with streptococcal infections was 23 times in 1,682 isolates, a ratio of approximately 1:73.

In an effort to determine whether strains of group A which we were unable to type might actually possess M protein, 418 of these so-called nontypable strains of group A from 23 patients were sent to the Streptococcus Unit, Bacteriology Section, NCDC, where sera for most types of group A are available. The NCDC was able to type 154 strains of group A which had been nontypable in our laboratory; 98 of these were types for which our laboratory did not possess typing sera, types 49 and 52. Mixed strains of group A were demonstrated three times among the 23 sets of isolates sent to the NCDC, A-22 [18] and A-NT [2], A-49 [19] and A-NT [1], A-13 and A-NT [6]. The essential point is that in only 3 individuals of 23 and in only 9 of 418 isolates sent to the NCDC was a strain other than the predominant one demonstrated.

DISCUSSION

These studies show that, for diagnostic purposes, only a single colony of β-hemolytic streptococci need be picked and isolated for serological grouping and typing, and they furnish somewhat indirect evidence that a single strain of β-hemolytic streptococcus predominates in the bacterial flora in patients with infection and in the carrier state in Nashville, Tenn., at this time. These results are in marked contrast to the bacteriological findings in patients (members of the Army Air Force during World War II) in the early stages of rheumatic fever. The majority of the strains were typable, and, in over 30% of these patients, more than one type was isolated (5).

A brief summary of references which touch on the general subject of this investigation begins with a bacteriological and clinical study of 100 cases of scarlet fever by Gunn and Griffith (4) in 1928, in which there was a change of type during the course of the disease in 50 cases. Three colonies were selected for examination in their study. Allison and Brown (1) swabbed the nose and throat of 100 patients with scarlet fever on admission and discharge, and in 57 patients found a serological type of *Streptococcus pyogenes* different from that on admission. Apparently, these patients acquired different strains during their hospital stay. Throat swabs taken by De Waal (3), in 415 cases of scarlet fever in which there were 357 positive cultures, yielded only 2 cases in which different types of hemolytic streptococci were found in throat and nose cultures. Only a single type was present in 28 early cases in which "many" colonies of the primary culture were typed. Here, as in the two previous studies, more than one type was found in the throats of some of the patients after being in the hospital. In 19 of 25 cases, a new type replaced the original. Schwentker, Janney, and Gordon (6), in studies of the epidemiology of scarlet fever in Romania from 1936 to 1939, picked no more than five colonies per plate, but they found that 50% of the plates having multiple colonies yielded more than a single strain. However, they stated that patients in the acute stage of streptococcal throat infection rarely yield more than a single strain.

It would appear that the throat flora is much more likely to harbor multiple serological types of

group A streptococci in an epidemic situation where multiple types are present in hosts and the environment, as in some of the Army and Navy training centers during World War II (2), or in scarlet fever epidemics in communities, or in hospital wards where patients with scarlet fever were treated. This is in contrast to the situation in a community like Nashville, where streptococcal infections are endemic rather than epidemic, children with streptococcal infections are not hospitalized, and significant numbers of non-group A strains are prevalent.

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