

Fatal Case of Pneumonia Caused by a Nonhemolytic Strain of *Streptococcus pyogenes*

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We report a case of pneumonia with a fatal outcome caused by a nonhemolytic strain of *Streptococcus pyogenes*. This strain was isolated in pure growth from blood cultures and was fully identified biochemically. Such strains will be difficult to recognize and isolate from sites with heavy growth of normal flora. This phenomenon has been reported sporadically before, and it is unknown how common such strains may be in pharyngeal samples.

CASE REPORT

An 89-year-old Chinese man was admitted with a 2-day history of productive cough. His general condition was poor, and he had a history that included chronic obstructive pulmonary disease and stroke. On examination, he was short of breath at rest and was pyrexial (38.0°C). His blood pressure was 140/100, and the results of his cardiovascular examination were normal. A chest X ray revealed consolidation in the right lower lobe. He had a hemoglobin level of 8.5 g/dl and a total white blood cell count of 41.5×10^9 per liter, with 98% polymorphonuclear cells. He was treated with intravenous ceftriaxone and was managed without ventilation, in accordance with his family's wishes, on a medical ward. He died on the day after admission.

Blood cultures yielded a large-colony-type, nonhemolytic, catalase-negative, gram-positive coccus. Further testing revealed that this organism did not grow on bile-esculin agar and was unable to grow in a 6% salt tolerance broth. The PYR test (for the production of pyrrolidonyl arylamidase) was positive; among the streptococci, only *Streptococcus pyogenes* and certain pneumococci show this reaction (6). The isolate was sensitive to a 0.04-U bacitracin disk and possessed only the group A Lancefield antigen (Streptex test; Biomerieux). Biochemical identification with the API Strep system yielded a profile of 0161410, indicating very good identification with *S. pyogenes*. The isolate was sensitive to penicillin according to NCCLS criteria (disk testing).

Subculture of the isolate on sheep blood agar produced growth under aerobic, 5% CO₂-supplemented, and anaerobic conditions. The colonies remained non-beta-hemolytic in all three atmospheres, even after the agar was stab inoculated in the pool. Faint alpha hemolysis was visible on the anaerobic plate and was more marked after extended incubation. The colonies from a heavily inoculated blood agar plate incubated

anaerobically were suspended in phosphate-buffered saline, and after incubation with dithiothreitol and centrifugation, the supernatant was assayed for hemolysis of rabbit red blood cells (7); no activity was detected. A recently obtained beta-hemolytic clinical isolate of *S. pyogenes* from a throat swab was similarly examined, and plentiful hemolytic activity was detected. A large proportion of this activity was inhibited by cholesterol, indicating that active streptolysin O was detectable (7).

S. pyogenes usually produces two hemolysins. Streptolysin O is oxygen labile. Streptolysin S is responsible for the beta hemolysis seen on aerobically incubated blood agar plates (1). Anaerobic incubation is optimal for the observation of streptococcal beta hemolysis (6). Nonhemolytic variants of beta-hemolytic streptococci have been reported as laboratory phenomena; Rebecca Lancefield made one such observation after in vivo passage of a group B streptococcus in mice (5). Unlike the various isolates cited (5) in previous reports, the strain studied by Lancefield was demonstrated to be nonhemolytic under both aerobic and anaerobic conditions. An epidemic of pharyngitis in North America, complicated by several cases of rheumatic fever, was caused by a nonhemolytic group A streptococcus (4). This mucoid strain could be transformed into a beta-hemolytic colony type by repeated rapid broth subcultures, indicating that the ability to produce a streptolysin(s) had not been lost. A recently published study from New Zealand described nonhemolytic variants of group A, C, and G streptococci in routinely collected throat swabs (3). The authors of that study detected such variants by employing a colistin-nalidixic acid-supplemented, PIPES [piperazine-*N,N'*-bis(2-ethanesulfonic acid)]-buffered sheep blood agar medium of their own formulation (CNA-P), which they described as enhancing streptolysin O activity; they suggested that this buffered medium may protect the toxin from inactivation at low pH. Two of the 64 isolates of *S. pyogenes* in that study were hemolytic on CNA-P agar but nonhemolytic on repeated sheep blood agar subcultures. A likely explanation is that the atypical strains of *S. pyogenes* in the New Zealand study did not express streptolysin S and produced only low levels of streptolysin O. The two clinical isolates were lost, but a laboratory strain of *S. pyogenes* (streptolysin S knockout, strain 82-541) was nonhemolytic on sheep blood agar (but beta-hemolytic on CNA-P)

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under both aerobic and anaerobic conditions (3). The majority of the nonhemolytic *S. pyogenes* isolates reported in an outbreak at an English hospital in 1942 produced streptolysin O on broth subculture, but none produced streptolysin S (2).

The biological significance of streptolysin O is unclear; however, intravenous injection of streptolysin O into a variety of laboratory animals causes death within seconds through its direct action on the heart (1). It is not known whether streptolysin S is produced *in vivo* (1). Whether or not these toxins contribute to the pathogenicity of *S. pyogenes*, beta hemolysis remains the most important laboratory observation used in the preliminary identification of this species, particularly when cultures from sites with normal flora, like the pharynx, are examined. Full biochemical identification of streptococci is usually reserved for blood culture isolates, as in the present case. From most sites, large-colony-type beta-hemolytic Lancefield group A streptococci are reported as *S. pyogenes*, but in the absence of hemolysis, colonies of this species may be mistaken for unimportant commensals and further identification steps may be omitted. The rapidly fatal outcome in the present case suggests that the isolate retained at least most of its pathogenicity. There is good reason to believe that such nonhemolytic variants are as able to spread between patients as common strains are able to spread in a community (e.g., a military base) (4) or hospital (2). In the outbreak described by Leonard

Colebrook et al. that occurred during World War II on the surgical wards of a hospital in Cambridge, England, a sulfonamide-resistant type 12 beta-hemolytic strain of *S. pyogenes* caused a series of nosocomial infections before the emergence of the nonhemolytic isolates. These isolates were similarly all sulfonamide resistant and of the Griffith type 12 classification.

As James and McFarland observed in 1971 (4), “. . . without its beta-hemolytic handle, the Group A streptococcus is like a rattlesnake without a rattle.”

REFERENCES

1. Balows, A., and B. Duerden (ed.). 1998. Topley and Wilson's microbiology and microbial infections, 9th ed., vol. 2. Systematic bacteriology. Arnold, London, United Kingdom.
2. Colebrook, L., S. D. Elliott, W. R. Maxted, C. W. Morley, and M. Mortell. 1942. Non-haemolytic group-A streptococci. *Lancet* **ii**:30-31.
3. Dierksen, K. P., and J. R. Tagg. 2000. Hemolysin-deficient variants of *Streptococcus pyogenes* and *S. dysgalactiae* subsp. *equisimilis* may be overlooked as aetiological agents of pharyngitis. *J. Med. Microbiol.* **49**:811-816.
4. James, L., and R. B. McFarland. 1971. An epidemic of pharyngitis due to a nonhemolytic group A streptococcus at Lowry Air Force Base. *N. Engl. J. Med.* **284**:750-752.
5. Lancefield, R. 1934. Loss of the properties of hemolysin and pigment formation without change in immunological specificity in a strain of *Streptococcus haemolyticus*. *J. Exp. Med.* **59**:459-469.
6. Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.). 1999. Manual of clinical microbiology, 7th ed. ASM Press, Washington, D.C.
7. Ruiz, N., B. Wang, A. Pentland, and M. Caparon. 1998. Streptolysin O and adherence synergistically modulate proinflammatory responses of keratinocytes to group A streptococci. *Mol. Microbiol.* **27**:337-346.