

Isolation of an Obligately Anaerobic *Streptococcus pneumoniae* from Blood Culture

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An obligately anaerobic strain of *Streptococcus pneumoniae* was isolated from blood culture in a 14-month-old child with an upper respiratory tract infection.

Streptococcus pneumoniae currently is classified as a facultative anaerobe. The organism lacks cytochromes and utilizes oxygen through the flavoprotein system, with hydrogen peroxide as its metabolic end product. Austrian and Collins (1) reported that 5 to 10% of strains may require an increased carbon dioxide atmosphere for primary isolation on agar media, whereas in liquid media they may grow under aerobic conditions. In blood cultures the vast majority of *S. pneumoniae* strains are isolated with no apparent difficulty, assuming, of course, that the specimen is properly collected at timely intervals. However, it is reasonable to assume that some strains of *S. pneumoniae* lack detection because of their fastidious needs for growth. This case report describes the isolation of a strain of *S. pneumoniae* from blood culture that on subculture was recovered only on an anaerobic sheep blood agar plate and in an enriched thioglycolate broth.

A 14-month-old Caucasian female was brought to the UCLA Hospital and Clinics Emergency Room with the chief complaint of high fever. She had been in good health prior to her visit when the mother noted she felt warm. The patient received aspirin, but her temperature continued to rise at home to 106°F (ca. 41.1°C). Except for some lethargy, there were no other symptoms.

The past medical history of the child revealed that her immunizations had been up-to-date and that she had not received any vaccinations prior to the onset of her symptoms. She had a history of otitis media during her first year but no other infections. A physical examination revealed a well hydrated but irritable child with a temperature of 40°C, a pulse of 180, and respirations of 80/min. Her tympanic membranes were visible with normal landmarks, and bulging was not noted. The throat was clear, and her neck was supple without nuchal rigidity or irritation; no adenopathy was noted. An examination of her chest, cardiovascular system,

abdomen, and neurological system was unremarkable.

Laboratory results showed a clear urine without bacteria or leukocytes. Her hemoglobin was 12.0 g. Her leukocyte count was 18,800, with 71% segmented neutrophils, 3% bands, 21% lymphocytes, and 5% monocytes. A chest X ray was normal. Blood and throat cultures were obtained. Because there was no apparent source of infection, the child was given aspirin and sent home, with instructions to return the next day. Upon return, the child's temperature was 40.3°C, her pulse was 140, and her respiration was 30/min. According to the mother, the child's temperature remained between 104 and 105°F (40.0 and 40.6°C) after the first visit. The report of the throat culture obtained during the previous day revealed a few group A beta-hemolytic streptococci. The physical examination was within normal limits, and the child was started on penicillin VK at 250 mg orally every 6 h for 10 days. At 4 h after receiving the antibiotic, the child's temperature had returned to 98.6°F (37.2°C), and she was less irritable and without recurrence of fever.

A conventional blood culture procedure at UCLA consists of the use of a two-bottle set, each containing 100 ml, of Trypticase soy broth with 0.03% sodium polyanetholsulfonate placed under a CO₂ vacuum (Cal Labs, North Hollywood, Calif.). One bottle is vented upon receipt to the laboratory. The pediatric blood culture procedure incorporates one unvented bottle per blood specimen.

In this case the blood culture became turbid on day 2 and was Gram stained. Gram-positive cocci were seen upon smear, and the original bottle was subcultured to the following media: an enriched (normal rabbit serum) thioglycolate broth (135 C, BBL, Cockeysville, Md.), a sheep blood agar plate and a chocolate blood agar plate both incubated under 10% CO₂, and an anaerobic sheep blood agar plate (GasPak, BBL). All were incubated at 35°C. At 48 h, only

the enriched thioglycolate broth and the anaerobic blood plate showed growth that revealed gram-positive cocci in pairs. After passage 2, the organism was found to be bile esculin and 6.5% NaCl negative, whereas the ethyl hydrocuprein hydrochloride disk test on blood agar incubated under both aerobic and capneic conditions was positive at 20 mm. Repeat disk tests on several additional colonies yielded the same results. Although the quelling reaction and the bile solubility test were not performed, a careful examination of Gram stains of colonies obtained from the anaerobic blood agar plate revealed distinct capsular-like structures.

Howden (2) reported that, of 414 respiratory specimens, 65 (15.7%) yielded pneumococci and 31 (47.7%) grew both aerobically and anaerobically, but 34 (52.3%) were isolated only from anaerobic culture. The pneumococci cultured anaerobically with added CO₂ characteristically produced large mucoid colonies that were more easily distinguished from those isolated

aerobically in mixed respiratory flora. The organism that was isolated from the infant in this case was larger in colonial morphology and produced beta hemolysis (due to pneumolysin O) on anaerobic culture. On subculture under capneic conditions, the organism produced the characteristic alpha hemolysis.

Because this strain of *S. pneumoniae* was isolated on primary subculture under anaerobic conditions only, this case report reemphasizes the importance of anaerobic culture methods for the primary isolation of fastidious organisms from blood cultures.

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LITERATURE CITED

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2. Howden, R. 1976. Use of anaerobic culture for the improved isolation of *Streptococcus pneumoniae*. *J. Clin. Pathol.* 29:50-53.