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Isolation of *Actinobacillus actinomycetemcomitans* from the Blood of a Patient with Subacute Bacterial Endocarditis

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Actinobacillus actinomycetemcomitans, a rare human pathogen, was repeatedly isolated from the blood of a 20-year-old male patient with patent ductus arteriosus who developed subacute bacterial endocarditis.

Difficulties in isolating and identifying the organism are discussed. The bacterial isolate was found to be susceptible to various antimicrobial agents.

Key Words: *Actinobacillus actinomycetemcomitans*, Blood culture, Subacute bacterial endocarditis

Actinobacillus actinomycetemcomitans is an aerobic gram-negative bacillus known to be present in the oral cavity of some normal subjects (Heinrich & Pulverer, 1959). This organism was first reported by Klinger in 1912, in association with *Actinomyces israelii* in actinomycosis. However, until the time when it was recognized as a causative agent of subacute bacterial endocarditis by King and Tatum (1962), the organism was not considered to be an important primary pathogen. This infection is certainly rare considering that there were only 52 reported cases in America and Europe until 1977 (Muhle *et al.*, 1979).

This organism was isolated from blood specimens of a patient with subacute bacterial endocarditis. We consider this is the first report of *A. actinomycetemcomitans* infection in Korea.

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CASE REPORT

A 20-year-old male (unit no. 1057272) was admitted to Severance hospital on October 10, 1982 with a 20 day history of fever and anorexia. He had been admitted to this hospital 2 years previously with a 5-year history of dyspnea on exertion. He had a blood pressure of 160/60 mm Hg, a palpable thrill at the left upper sternal border, loud P2, grade III/VI continuous murmur at the pulmonic area and a grade IV/VI pansystolic murmur at the apex. The chest x-ray showed cardiomegaly and increased pulmonary vascularity. The EKG showed left axis deviation and biventricular hypertrophy. An echocardiogram showed a markedly dilated left ventricle and atrium.

He was diagnosed as having patent ductus arteriosus, pulmonary hypertension and mitral regurgitation by cardiac catheterization. An operation of ligation of the ductus arteriosus with teflon felt wrapping was performed. He was discharged with symptomatic improvement.

On 11 May 1982 he was admitted again with fever and chills, general weakness, and dyspnea of 3 months duration. Physical examination revealed a left ventricular heave, loud P2, S3 gallop, a grade II/IV continuous murmur at the pulmonic area, and a grade III/IV pansystolic murmur at the apex. Recurrent patent ductus arteriosus and mitral regurgitation were the diagnosis. Three blood cultures were done and all were negative. He received antimicrobial therapy of penicillin G and gentamicin. His condition improved and he was discharged on the 10th day in spite of inadequate antimicrobial therapy. On September 8, 1982 he was seen in the outpatient department again with the chief complaints of fever and chills. His body temperature was 40.3°C. Two blood samples were collected for culture and both of them yielded gram-negative bacilli which were later identified as *A. actinomycetemcomitans*.

On admission, physical examination revealed a blood pressure of 150/80 mm Hg and pulse rate of 120/minute. A systolic murmur was heard and the body temperature was 37.8°C. The patient was alert and oriented. Laboratory findings included a WBC count of 15,400/ul with 75% segmented neutrophils, a hemoglobin of 6.2 g/100 ml and a hematocrit of 20.3%. The test for C-reactive protein was positve. Two blood samples were taken for culture on the first hospital day and two more were taken on the next day. All of them yielded *A. actinomycetemcomitans*. He received gentamicin. On the 6th hospital day he developed hemiparesis and a stuporous mental state. His brain scan showed ventiruclar hemorrhage with hydrocephalus. The bleeding was considered to be due to the rupture of a mycotic aneurysm. He expired on the 6th day.

MATERIALS AND METHODS

Blood samples of 10 ml amount each were collected to inoculate a set of 50 ml Tryptic soy broth (TSB, Difco) and 50 ml of Brewer thioglycollate medium (BTM, Difco). TSB was supplemented with 0.025% sodium polyanethol sulfonate (Reller *et al.*, 1982). During an 1 week incubation period, daily macroscopic observation was made and gram staining or subculture was performed if considered necessary.

For the identification of the isolate, cultural and biochemical tests were performed in the conventional way. Acid production from carbohydrate was tested using Cystine tryptic agar (Difco) as the base medium. Antimicrobial susceptibility was tested by the disc diffusion method (NCCLS, 1979).

RESULTS

All six blood cultures, two of them taken on September 8 and four of them on October 10-11 yielded growth. The growth in one sample was detected after 2 days incubation while the remaining five showed growth after 3 days incubation. In some specimens the growth was first detected in TSB while in others growth was both noted in TSB and BTM at the same time. The growth appeared as small granules. The smears showed organisms on only 3 of 9 slides prepared from each bottle. The organism was a small gram-negative bacillus. Subculture onto blood agar showed pinpoint colonies of less than 1 mm diameter after 48 hours incuba-

Table 1. Cultural characteristics of the isolate

Tests	*H. aphrophilus*[a]	*A. actinomycetemcomitans*[a]	Isolate 82-9-5457
Hemolysis	–[b]	0%	–
Motility		0	–
Gas from glucose		28	–
Acid from glucose	+	83 (16)	+
xylose	–	33 (9)	+
mannitol	–	66 (16)	–
lactose	+	0	–
sucrose	+	0	–
maltose	+	81 (15)	+
Catalase	–	99	+
Oxidase	–	19 (2)	–
Growth on MacConkey	–	4 (1)	–
Simmon citrate	–	0	–
Urease	–	0	–
Nitrate reduction	+	100	+
N2		0	–
Indole		0	–
Gelatin liquefaction		0	–
TSI slant acid	+	100	+
butt acid	+ (G)	100	+
Esculin hydrolysis	–	0	–
Growth at 25C	–	–	–
37C	+	+	+
42C	–	–	–

[a] From Weaver et al., 1979 and Weaver & Hollis, 1980.
[b] –, negative or no growth; +, positive or growth.

tion in the CO_2 incubator. Growth did not appear on blood agra incubated in air for 48 hours. The colonies were grayish white in color, smooth and convex. The isolate did not require X or V factor for growth. Other characteristics observed during a week incubation are shown in table 1. The isolate was susceptible to ampicillin, cephalothin, chloramphenicol. erythromycin, gentamicin, kanamycin, tetracycline, amikacin, tobramycin and co-trimoxazole. It was resistant to clindamycin, oxacillin and penicillin G.

DISCUSSION

A. actinomycetemcomitans is listed as *Bacterium actinomycetemcomitans* in the 8th edition of Bergey's manual (Buchanan & Gibbons, 1974). Since the first report in 1912 by Klinger of isolation from a lesion of actinomycosis the organism was considered to be only one of several coinfecting bacteria. Heinrich and

Pulverer (1959) reported that 30% of actinomycotic lesions contained this organism. It was only in 1962 (King and Tatum) that this organism was reported as an important etiologic agent of endocarditis. A subsequent report disclosed details of the patient with endocarditis (Page & King, 1966).

Since that time, more cases of *A. actinomycetemcomitans* endocarditis have been reported (Mitchell & Gillespie, 1964; Goss *et al.*, 1967; Symbas *et al.*, 1967; Serra & Tonato, 1969; Meyers *et al.*, 1971; Macklon *et al.*, 1975; Affias *et al.*, 1978). It has also been reported in brain abscess (Martin *et al.*, 1967), urinary tract infection (Townsend & Gllenwater, 1969), osteomyelitis (Muhle *et al.*, 1979) and infection of the thyroid gland (Burgher *et al.*, 1973).

A. actinomycetemcomitans was found in 5-30% of normal oral cavities (Heinrich & Pulverer, 1959). The organism seems to be a periodontopathic agent (Rienzo & Spieler, 1983). The pathogenicity of the organism seems to be low considering the fact that the reported infection is rare, and it invades already damaged heart (Page & King, 1966). However there were some infections without underlying disease (Affias *et al.*, 1978). The patient reported here had patent ductus arteriosus.

The organisms is known to be difficult to culture because it requires CO_2 and a long incubation time (King & Taum, 1962; Meyer *et al.*, 1971). The growth of our isolate in the blood culture medium was slower than other commonly encountered bacteria. Except for one sample in which the growth was detected after 2 days incubation, growth was detected after 3 days incubation. Among the 9 bottles with granules, 6 failed to show bacteria in the initial smears. Subcultures on blood agar plates which were incubated in CO_2 showed pinpoint colonies after 48 hours. At first *Haemophilus influenzae* was suspected because of the poorly staining coccobacillary morphology. However, it was different from *H. influenzae* as it required neither X nor V factors. Again there was difficulty in differentiating the isolate from *H. aphrophilus* because of their many similar characteristics (Weaver *et al.*, 1979: Weaver & Hollis, 1980). Acid production from xylose, failure to produce acid from lactose and sucrose, and positive catalase differentiated the isolate from *H. aphrophilus*.

The susceptibility of our isolate was similar to that reported by others (Affias *et al.*, 1978). It was interesting that despite in vitro susceptibility of this organism to gentamicin and therapy with the antibiotic, it persisted in the patient for a month.

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