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Penicillin-resistant *Aerococcus viridans* Bacteremia Associated with Granulocytopenia

Aerococcus viridans, a catalase-negative gram-positive coccus rarely causing bacteremia, was isolated from blood cultures of a 52-yr-old man under the granulocytopenic condition. The isolate showed the typical characteristics of *A. viridans*, i.e., tetrad arrangements in gram stain, positive pyrrolidonyl aminopeptidase (PYR) and negative leucine aminopeptidase (LAP) reactions, and no growth at 45℃. The isolate was revealed to be highly resistant to penicillin, erythromycin, clindamycin, and ceftriaxone, although most strains of *A. viridans* isolated from the previously reported patients were susceptible to penicillin and other commonly used antibiotics. Even though *A. viridans* is rarely associated with human infections, it could be a potential causative agent of bacteremia, especially in immunocompromised patients.

Key Words : *Aerococcus viridans; Bacteremia; Agranulocytosis; Penicillin resistance*

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INTRODUCTION

Aerococcus viridans, first described in 1953 (1), are catalase-negative gram-positive cocci that resemble staphylococci by Gram stain, but have biochemical and growth characteristics of streptococci and enterococci (2-4). It is generally considered as a contaminant in clinical cultures, but occasional reports have noted clinically significant roles for this organism in systemic infections such as bacteremia and endocarditis (5-10). Aerococci appear to be of low virulence and may be normally pathogenic only in patients with vulnerable conditions (3, 7, 10). No details, however, were given about the underlying diseases or special risk factors for *A. viridans* bacteremia.

A. viridans was recognized as susceptible to penicillin until Christensen et al. (11) described a penicillin-resistant strain isolated from Denmark in 1989. Thereafter, *A. viridans* infections showing penicillin resistance have been infrequently reported (9-13).

The authors isolated multidrug-resistant *A. viridans* from blood cultures of 52-yr-old man under the granulocytopenic condition. As far as we could ascertain from available literatures, this appears to be the first reported case of *A. viridans* bacteremia in Korea.

CASE REPORT

A 52-yr-old man was transferred to emergency room of Wonju Christian Hospital, Wonju, from a local hospital on June 2, 1997, due to intracerebral hematoma, subarachnoid and ventricular hemorrhage, and multiple fractures due to traffic accident.

On presentation to the emergency room, his mental status was semicomatose. Hematologic findings were: hemoglobin 11.2 g/dL, hematocrit 32.5%, WBC count 11,100/μL (87% neutrophils, 7% monocytes, and 6% lymphocytes), and platelet count 186,000/μL. And no organism was detected from three sets of aerobic and anaerobic blood cultures. On the fourth day of admission, the patient underwent stereotaxic closed drainage of the intracerebral hematoma, and as of January 21, 1998, he subsequently underwent 4 episodes of major operations to correct underlying disorders as follows: internal fixation of leg (July 7, 1997), open reduction of the mandible fracture (September 5, 1997), ventriculo-peritoneal shunt for hydrocephalus (December 16, 1997), and revision of ventriculo-peritoneal shunt (January 21, 1998). Between the admission and the 492th hospital day, the patient's WBC counts were higher than 5,000/μL, even though he was intermittently treated with antimicrobial agents for pneumonia, urinary tract infection, and wound infection.

On the 497th hospital day, mild to moderate fever accompanied with leukopenia (total WBC count; 2,470/μL, differential count; 38% neutrophils, 35% lymphocytes, 10% monocytes, 10% eosinophils, 1% basophils) was noted, and then two days later three sets of blood cultures were performed due to sustained low grade fever and low WBC count (1,640/μL).

Blood cultures were done according to our routine proce-

dure, i.e., 10 mL of venous blood was drawn and inoculated into each of 40 mL of aerobic bottle (BACTEC Plus Aerobic/F, Becton Dickinson Diagnostic Instrument Systems, U.S.A.) of the BACTEC 9240 automated blood culture system (Becton Dickinson Diagnostic Instrument Systems, U.S.A.) and 50 mL anaerobic thioglycollate broth medium (Difco, U.S.A.). All of three sets for aerobic blood culture grew gram-positive cocci after 4 days of incubation, which were later identified as *A. viridans*. No anaerobic organism was detected from anaerobic blood cultures. According to the verbal report as "gram positive cocci seen on Gram stain of blood cultures", the neurosurgeon replaced ceftezole used for urinary tract infection with pefloxacin and teicoplanin. Then, total WBC count showed a increasing trend, and recovered to 6,660/μL on the 505th hospital day. At that time, the patient became afebrile. Antimicrobial susceptibility test by National Committee for Clinical Laboratory Standards (NCCLS) recommended disk diffusion method for nonpneumococcal *Streptococcus* category (14) showed that the isolate was susceptible to vancomycin and chloramphenicol, but resistant to penicillin, erythromycin, clindamycin, ceftriaxone, and ofloxacin. Minimal inhibitory concentrations (MIC) to penicillin G, erythromycin, tetracycline (Sigma Chemical, U.S.A.), ceftriaxone (Roche, Switzerland), vancomycin (Daewoong Lilly, Korea), chloramphenicol (Chongkundang, Korea), and clindamycin (Korea Upjohn, Korea) were obtained by the NCCLS agar dilution method (15). Mueller-Hinton agar (Oxoid, U.K.) with 5% defibrinated sheep blood was used for the agar dilution test. Inocula were prepared by suspending colonies in tryptic soy broth (Difco, U.S.A.) to form approximately 10^4 CFU/spot when inoculated with a Steers replicator (Craft Machine, U.S.A.). Inoculated plates were incubated at 35℃ in air for 22-24 hr and the results were as follows: penicillin G, ceftriaxone, erythromycin, and clindamycin were $\geq$256 μg/mL, vancomycin 0.25 μg/mL, tetracycline 0.5 μg/mL, and chloramphenicol was 16 μg/ mL. To determine susceptibilities to seven antimicrobial agents tested, interpretative criteria for nonpneumococcal *Streptococcus* category of NCCLS (15) were used.

For the identification of the isolate from blood culture, conventional biochemical tests (2) and API 20 Strep and API rapid ID 32 Strep (bioMérieux SA, Marcy l´Etoile, France) were used. The characteristic test results were as follows: gram-positive cocci in tetrads and pairs on Gram stain, negative catalase reaction, no agglutination against group D streptococcal antiserum, poor growth on blood agar plate by anaerobic incubation, no growth on 45℃, small α-hemolytic colonies on blood agar, growth in 6.5% NaCl, positive PYR reaction, and negative LAP reaction. The microorganism was subcultured in Todd-Hewitt broth (Difco, U.S.A.), and the repeat Gram stain demonstrated the characteristic gram-positive cocci, predominantly in tetrads and pairs. Other biochemical test results are shown in Table 1. API 20 Strep and API rapid ID 32 Strep identified the isolate as *A. viridans* (probabilities, 99.6% [bionumber, 4500410] and 99.9% [bionumber, 21630101150], respectively).

Table 1. Characteristics of *A. viridans* isolate

Tests	*A. viridans**	Isolate
Gram stain	Tetrads, pairs	Tetrads, pairs
Relation to oxygen	Microaerophilic	Microaerophilic
Hemolysis	Alpha	Alpha
Catalase	–†	–
Bile esculin	V	+
Growth in 6.5% NaCl	+	+
Pyrrolidonyl aminopeptidase	+	+
Leucine aminopeptidase	–	–
Growth at 45℃	–	–
Arginine dihydrolase	–	–
Hippurate hydrolysis	V	–
Acid from glucose	+	+
lactose	+	+
trehalose	+	+
sorbose	–	–
Voges-Proskauer reaction	–	–

*Adapted from references 2 and 3.
†–, negative or no growth; +, positive or growth; V, variable.

DISCUSSION

A. viridans is frequently isolated from two divergent sources: as a common airborne organism in hospital environments and as a marine organism causing a fatal disease in lobster (16). In human, aerococci can be found in a very small number as indigenous inhabitants in the upper respiratory tract and on the skin of normal persons (1). This organism is generally saprophytic and rarely has been encountered as a human pathogen (2, 9, 10). It was first described as a potential human pathogen in 1967 (5). Since then, it has been reported as the causative agent of meningitis (8), endocarditis (9), bacteremia (10), or of other infections such as urinary tract infection, septic arthritis, and wound infection (2).

Although the pathogenicity and virulence of *A. viridans* have not been well-established, infections due to this organism presumably seem to occur in previously damaged tissues or may be of nosocomial in association with a prolonged hospitalization, antibiotic treatment, invasive procedures, presence of foreign bodies, or neutropenic state (2, 3, 7, 10). Kern and Vanek (7) suggested that both granulocytopenia and oral mucositis be the major risk factors for aerococcal bacteremia. These observations together with our patient's underlying conditions suggest that granulocytopenia be one of the risk factors of *A. viridans* bacteremia. Recently, Swanson et al. (10) also described that *A. viridans* might be a significant pathogen in patients with functional asplenia, in that the organism is encapsulated with an acidic polysaccharide, and the strains with heavier encapsulation are more virulent (17).

Limited are the data in the literature on the antimicrobial susceptibility of *A. viridans*, because this organism has been infrequently associated with human infections, and is usually susceptible to penicillin. In addition, standardized susceptibility testing methods and interpretative criteria are not available for aerococci, although most investigators have used the nonpneumococcal *Streptococcus* category of the NCCLS.

As revealed by a cluster of sporadic reports, antimicrobial susceptibility patterns of *A. viridans* have been rapidly changed as follows; until the late 1980s, this organism had been reported as susceptible to the most commonly used antibiotics, but recent studies have documented that *A. viridans* have shown resistance not only to penicillin but also to chloramphenicol and the quinolones as well (7, 9, 12, 18). In 1987, Kern and Vanek (7) described that two aerococci isolated from blood cultures were sensitive to penicillin G and piperacillin but resistant to fluoroquinolone and netilmicin given orally for prophylaxis. In 1996, Swanson et al. (10) reported a case of penicillin-resistant *A. viridans* (penicillin MIC, 0.5 μg/mL) bacteremia in a child who was receiving prophylactic penicillin. These observations suggest that drug resistance of *A. viridans* could be induced by selective pressure by prolonged antibiotic use.

Augustine et al. (9) reported a case of endocarditis caused by *A. viridans* with multidrug resistance, i.e., resistance to penicillin, ampicillin, cefotaxime, gentamicin, and intermediate resistant to ciprofloxacin, but they did not discussed on MICs. According to the antimicrobial susceptibility data of 30 aerococcal isolates obtained from Centers for Disease Control and Prevention (12), the MICs for 9 strains were 0.5 μg or more of penicillin per mL, with MICs for 5 strains being more than 1 μg/mL; therefore, approximately 46% of aerococci tested were either relatively resistant or resistant to penicillin. Moreover, Christensen et al. (13) recently documented that penicillin resistance should be the peculiar characteristics of *A. viridans* capable of differentiating it from *Aerococcus*-like organisms.

Since *A. viridans* is usually recognized as susceptible to penicillin, the treatment protocol for aerococcal endocarditis is similar to that for endocarditis caused by penicillin-susceptible streptococci (19). Although penicillin - or multidrug-resistant *A. viridans* strains have been occasionally isolated from clinical specimens as documented by some authors and us, optimal treatment of systemic infections caused by the penicillin-resistant *A. viridans* has not been established yet.

In conclusion, even though *A. viridans* is rarely associated with human infections, it could be a potential causative agent of bacteremia, if *A. viridans* is isolated from the multiple set of blood cultures especially in immunocompromised patients, effective antibiotics on the basis of antibiogram thereof should be administered. Further investigations are needed to establish the optimal treatment for this pathogen.

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