

Neisseria subflava Infections

—Bacteriological aspects of two cases—

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ABSTRACT

Nonpathogenic *Neisseria*, normal inhabitants of the human nasopharynx, are known to cause occasional infections including such severe ones as septicemia, meningitis and endocarditis. Recently two strains of so called nonpathogenic *Neisseria*, identified as *N. subflava*, were isolated from blood specimens of two different patients. One patient had meningitis, septicemia and subacute bacterial endocarditis while the other had septicemia.

Pigment production by both of the strains was not definite and only a light yellow color was observed after prolonged incubation. However, the isolates showed bacteriological characteristics of *N. subflava*, namely gram-negative diplococci which were oxidase positive, acid production from fructose, glucose, maltose and sucrose but not from lactose or mannitol; and iodine reacting polysaccharide production from sucrose. One of the patients revealed serum agglutinin titers up to 1:640 against the isolate.

INTRODUCTION

Nonpathogenic *Neisseria* are common inhabitants of the upper respiratory tract. Though infections due to these organisms are very

rare, they do occur. Sometimes they cause serious diseases such as meningitis, endocarditis, and septicemia, some of which are even fatal (Noguchii et al., 1963). Species of nonpathogenic *Neisseria* isolated from such infections are frequently *N. subflava*, *N. flavescens* and *N. catarrhalis*. Recently we isolated two strains of *N. subflava* from blood specimens of two different patients at Yonsei Medical Center and studies were made of their bacteriological characteristics.

1. A 36-year-old male (Unit No. 564033) was hospitalized on the 17th of March 1974. He was unconscious for two days. He had aches especially in the right leg and knee joint, high fever and headache for two weeks before admission. Petechiae were found on the face, trunk and extremities. He showed negative Babinski's and positive Kernig's signs. Body temperature was 39.5°C. The clinical details were reported under a separate study by Moon et al. (1975).

The present authors isolated gram-negative diplococci from three blood cultures, identified as *N. subflava*. The patient died of an intracranial hemorrhage due to ruptured mycotic aneurysm proven by an autopsy. The clinical diagnosis was subacute bacterial endocarditis, meningitis and septicemia.

2. A 59-year-old female (Unit No. 229326) was hospitalized on the 24th of January 1975 because of palpitation, exertional dyspnea and fever. On physical examination the patient was febrile and a pansystolic murmur radiating to the axilla was heard on the apex and left sternal border. Chest P-A and EKG revealed left ventricular hypertrophy and atrial fibrillation. A clinical diagnosis of mitral insufficiency was made. From blood cultures, *N. subflava* was isolated. She was treated with ampicillin for the bacterial infection and was discharged with general improvement and subsistence of fever after 15 days hospitalization.

MATERIALS AND METHODS

Blood cultures were done by drawing 10 ml of venous blood and inoculating 5 ml each into brain heart infusion and fluid thioglycollate medium. The media were incubated at 37°C and daily observations were made to detect growth of bacteria. P-aminodimethylaniline oxalate 1% solution was used to test oxidase. Nitrate reduction was tested by culturing the organism in potassium nitrate containing broth and by adding sulfanilic acid and alpha-naphthylamine reagents. Acid production was tested using cystine tryptic agar with 1% carbohydrates (Blair et al., 1970). Iodine reacting polysaccharide formation was tested by growing test organisms on 5% sucrose-containing media and flooding the colonies with 0.2% iodine solution (Hehre and Hamilton, 1948). Pigment production was tested on nutrient agar, Loeffler's serum slant and chocolate agar at both room temperature and at 37°C. Thayer-Martin medium was prepared with the addition of antimicrobial CNV (Difco) to chocolate agar (Thayer and Martin, 1966).

To demonstrate presence of antibody in the patient's serum an agglutination test was performed. Agglutinin was prepared by culturing the isolate in brain heart infusion containing 0.5% Tween 80. After 18-hour incubation the broth was centrifuged and the sediment was washed twice with 0.5% formalin saline. The antigen was suspended to equal the turbidity of the No. 2 tube of a McFarland nephelometer. Test serum was inactivated at 56°C for 30 minutes and serial twofold dilution was made. To 0.5 ml of serum dilution 0.5 ml of antigen suspension was added and the result was read after overnight incubation at 37°C. The titer was determined as the highest serum dilution giving a ++ agglutination reaction.

Antibiotic susceptibility testing was done with a Kirby-Bauer disc diffusion method (Bauer et al., 1966).

RESULTS

Case 1.

Laboratory findings on the day of hospitalization were hemoglobin 9.3 g%, WBC 13,100/mm³ with neutrophilia and platelet count 34,000/mm³. Spinal fluid showed a hazy appearance, protein 105 mg%, sugar 56 mg%, chloride 121 mEq/l, and WBC 200/mm³ with 25% neutrophils. On the 6th hospital day the spinal fluid WBC count was 14,200/mm³ with 93% neutrophils. Cultures of both spinal fluid specimens yielded no bacteria.

Blood cultures were done on three occasions on the day of hospitalization and from all of three specimens gram-negative diplococci were isolated (Table 1). The isolate was catalase and oxidase positive, produced acid from fructose, glucose maltose and sucrose. Acid was not produced from lactose and mannitol.

Table 1. Cultural Characteristics of the Isolates

Characteristics	Isolate from	
	Patient No. 564033	Patient No. 229326
Morphology	Gram-negative diplococci	Gram-negative diplococci
Oxidase	+	+
Acid from:		
Fructose	+	+
Glucose	+	+
Maltose	+	+
Sucrose	+	+
Lactose	—	—
Mannitol	—	—
Polysaccharide production from 5% sucrose	+	+
Nitrate reduction	—	—
Pigment	Light yellow	Light yellow
Growth:		
Room temperature (blood agar)	+	+
Thayer-Martin medium	—	—
Extra CO ₂	Not required	Not required

*+: positive, production or growth.

—: negative, no production or no growth.

Nitrate was not reduced. Iodine reacting polysaccharide was produced. The organism grew on blood agar at room temperature and on nutrient agar at 37C, but not on Thayer-Martin medium. Extra carbon dioxide was not required for growth. The colonies on blood agar were nonhemolytic, greyish, smooth, convex, entire, viscid and adhering to media and attained a size of 2~3 mm after 48-hour incubation. The colonies were emulsifiable in saline. Most of the colonies became furrowed when incubation was continued. Colonies on nutrient agar were only light yellow even after 1 week of incubation at room temperature. Pigment production on Loeffler's medium was also light. Based on the cultural characteristics the isolate was identified as *N. subflava* (formerly *N. perflava*).

The organism was susceptible to ampicillin, chloramphenicol, and tetracycline, but resistant

to lincomycin, cloxacillin and penicillin. The agglutinin titers of both of the two serum samples, which were taken 10 days apart, were 1 : 640 while those of both controls were less than 1 : 20 (Table 2).

Table 2. Agglutination Test Results

Test serum and Date of collection	Agglutinin titer
Patient No. 564033	
Mar. 29, 1974	1 : 640
Apr. 8, 1974	1 : 640
Control 1*	less than 1 : 20
Control 2**	less than 1 : 20

*25 year old healthy female.

**28 year old healthy male.

Case 2.

Laboratory tests showed hemoglobin 11.4 g%, hematocrit 36%, WBC 14,600/mm³ with 88% neutrophils and 12% lymphocytes. She

was suspected of typhoid fever, but Widal test titer was not significant and from urine and stool cultures no enteric pathogen was isolated. Three blood cultures were done on the day of hospitalization and from all of them gram-negative diplococci were isolated (Table 1).

The isolate showed characteristics of *Neisseria*. Biochemical characteristics were the same as the isolate from the first case. Colonies on blood agar were not hemolytic, greyish, smooth, convex and 1~2 mm after 24-hour incubation. The colonies were viscid, adhering to media and emulsifiable in saline but agglutinated spontaneously on standing. Most colonies turned out to be deeply furrowed ones after prolonged incubation. Pigment production was not definite even after two week incubation at room temperature on nutrient agar, Loeffler's slant and blood agar. Colonies on chocolate agar remained smooth and light yellow pigment production was definite after one week of incubation at room temperature. The organism was identified as *N. subflava*.

The isolate was susceptible to chloramphenicol and tetracycline, resistant to cloxacillin and lincomycin, and intermediate to ampicillin and penicillin.

DISCUSSION

Organisms in the genus *Neisseria* are gram-negative cocci in pairs with characteristic kidney shapes. They are positive for catalase and cytochrome oxidase. Burgey's manual lists 6 species of *Neisseria*, 2 well known pathogens and 4 nonpathogens (Buchanan and Gibbons, 1974). Characteristics used to differentiate species are formation of capsule, acid production from carbohydrates, polysaccharide formation from 5% sucrose, H₂S production,

nitrate and nitrite reduction, pigment production, CO₂ requirement and ability to grow at 22°C. There have been difficulties in defining the species of chromogenic *Neisseria* and various names have been given to the same organism by different workers (Noguchii et al., 1963). The present edition of the manual combines *N. perflava*, *N. flava* and *N. subflava* and recognizes them as one species, *N. subflava*. *N. catarrhalis* became a new genus *Branhamella*.

The present isolates were easily identified as *Neisseria* as defined by the manual. But for differentiation of the species, we had difficulties. Acid production from carbohydrates indicated that the organism must belong to either *N. sicca* or *N. subflava*. The manual differentiates the two by pigment production and colony morphology. The pigment produced by the isolate from the first patient was only light yellow after several days of incubation. The colonies after 24 hour incubation were smooth and viscid. Because of these characteristics and acid production from carbohydrates the organism was identified as *N. subflava* (*N. perflava* according to the 7th edition of the manual).

Identification of the second isolate as *N. subflava* was based on pigment production, which was slow and light, but observable after several days incubation of chocolate agar at room temperature, and smooth and viscid colony formation together with the result of biochemical reactions.

Sometimes it may be difficult to identify aberrant meningococci (Kippax et al., 1968). However, our isolates were not meningococci, because they produced acid from sucrose, produced polysaccharide from sucrose, grew at room temperature and failed to grow on Thayer-Martin medium (Thayer and Martin,

1966).

When an opportunistic pathogen is isolated from blood, it is sometimes difficult to determine if the isolate is the causative agent or merely a contaminant. Repeated isolation of the same organism usually indicates it is an etiologic agent. Demonstration of antibody titer in a patient's serum against the isolate is another way of proving the etiology. From the first patient all of the 3 blood cultures yielded the same organism and high agglutinin titers were demonstrated. Also from the second patient the same organism was isolated from all of the 3 blood cultures.

Among *Neisseria* species only two are usually regarded as pathogens. *N. gonorrhoeae* in general cause venereal disease and *N. meningitidis* is usually involved in acute meningitis. However, some other than the two species are known to be involved in various infections (Branham, 1940; Sophian, 1944; Major and Johnson, 1945; Branham, 1953; Prentice, 1957; Aronson, 1962; Clark and Patton, 1968; Wertlake and Williams, 1968). Although a report of "non-pathogenic *Neisseria*" infection was seen to be made as early as 1907 (Noguchii et al., 1963) not many cases are found in the literature, indicating rarity of the infection.

Clinical features due to these organisms are septicemia, meningitis, subacute bacterial endocarditis, acute conjunctivitis and genitourinary tract infection. The infection involves children as well as adults. They are usually found in sporadic cases of meningitis (Noguchii et al., 1963). In the present cases, both patients were adults and there was one fatality. It is interesting to note that in the first patient multiple lesions developed. His history does not show any predisposing factor which could have resulted in the invasion of

this rarely pathogenic organism. For the second patient, the weakened heart may be considered to be predisposed to the infection.

N. meningitidis infection is known to be rarer now than in the preantibiotic era (Chun, 1975). Lewin and Hughes (1966), reporting 5 cases each of *N. meningitidis* and *N. subflava* infections, suggested that the latter are more commonly pathogenic than has been previously thought. Noguchii et al. (1963) showed that among chromogenic *Neisseria*, *N. subflava* is most frequently involved in infection.

In general *Neisseria* is susceptible to many antibiotics. However, the first organism was susceptible to chloramphenicol and tetracycline only and resistant to cloxacillin, lincomycin and penicillin. The patient received various antibiotics including ampicillin, chloramphenicol and penicillin but his temperature never returned to normal during his hospitalization. The second isolate was susceptible to chloramphenicol and tetracycline and resistant to cloxacillin and lincomycin. It was intermediate to ampicillin and penicillin. The second patient received ampicillin and was discharged with improvement.

We are grateful to Dr. R.E. Weaver, CDC, USPHS, for confirmation of the identification of the first organism.

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