INTERNAL MEDICINE

□ CASE REPORT □

The First Case Report of Acute Cholangitis and Bacteremia Due to *Neisseria subflava*

Yoshifumi Uwamino[1,2], Kayoko Sugita[1], Eisuke Iwasaki[3], Hiroshi Fujiwara[1], Tomoyasu Nishimura[4], Naoki Hasegawa[1] and Satoshi Iwata[1]

Abstract

We herein report a case of acute cholangitis and bacteremia caused by a commensal *Neisseria* species, *Neisseria subflava*, in an 82-year-old man with cholangiocarcinoma. Emergency endoscopic nasobiliary drainage and cefoperazone/sulbactam therapy were effective. Gram negative coccobacilli were isolated from both blood and bile cultures on 5% sheep blood agar. The isolate was identified as *N. subflava* biovar *perflava* by mass spectrometry, a sequence analysis of the 16S rRNA, and biochemical testing. Although biliary infections due to commensal *Neisseria* are extremely rare, this case demonstrates the possibility of its occurrence in patients undergoing bile duct treatment.

Key words: *Neisseria subflava* biovar *perflava*, acute cholangitis, bacteremia, endoscopic retrograde cholangiopancreatography, cholangiocarcinoma

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Introduction

Neisseria subflava is a family of the so-called commensal *Neisseria* that is a part of the normal oral flora. Commensal *Neisseria* is a rare cause of invasive diseases such as meningitis, endocarditis, bacteremia, ocular infections, pericarditis, empyema, peritonitis, septic arthritis, bursitis, and osteomyelitis (1). Reports of intraabdominal infection due to *Neisseria* species are exceedingly rare. Among these, a form of perihepatitis caused by *N. gonorrhoeae*, referred to as the Fitz-Hugh-Curtis syndrome, is well-characterized. A few cases of peritoneal dialysis-related peritonitis caused by *Neisseria* species have been reported (2, 3). However, there are no reported cases of infection of the biliary system by *Neisseria* species. We herein report a case of acute cholangitis and bacteremia due to *N. subflava*.

Case Report

An 82-year-old man with cholangiocarcinoma was admitted to the emergency room of the Keio University Hospital for acute epigastric pain and vomiting. The patient's medical history was unremarkable, except for hypertension and lumbar stenosis, until the dilation of a lower bile duct was detected on a positron emission tomography scan at an annual health check. One month prior to admission, he had been diagnosed with distal extrahepatic bile duct cancer after diagnostic endoscopic retrograde cholangiopancreatography (ERCP) was performed a second time.

At admission, the patient was febrile (38.4℃) but his other vital signs were stable. There was epigastric tenderness and a yellowish discoloration of the skin. Laboratory tests showed elevated liver enzyme levels (AST; 589 IU/L, ALT; 307 IU/L, LDH; 620 IU/L, ALP; 410 IU/L) and hyperbilirubinemia (Total bilirubin; 3.4 mg/dL). The patient's white blood cell counts were normal. Computed tomography (CT) revealed a dilated common bile duct. He was diagnosed with acute cholangitis, and cefoperazone/sulbactam was administered after obtaining two sets of blood cultures (BD, Franklin Lakes, NJ, USA).

Emergency endoscopic nasobiliary drainage was per-

[1]Center for Infectious Diseases and Infection Control, Keio University School of Medicine, Japan, [2]Department of Laboratory Medicine, Keio University School of Medicine, Japan, [3]Department of Internal Medicine, Division of Gastroenterology and Hepatology, Keio University School of Medicine, Japan and [4]Health Center, Keio University, Japan
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Correspondence to Dr. Naoki Hasegawa, n-hasegawa@z8.keio.jp

Table. Biochemical Chalacteristics of Current Strain and *Neisseria* Species[4].

Species	Acid production from:				Nitrate reduction
	GLU	MAL	SUC	FRU	
N. flavescens	-	-	-	-	-
N. mucosa	+	+	+	+	+
N. subflava					
bv. *flava*	+	+	-	+	-
bv. *subflava*	+	+	-	-	-
bv. *perflava*	+	+	+	+	-
Current strain	+	+	+	+	-

GLU: glucose, MAL: maltose, SUC: sucrose, FRU: fructose.

formed, and a bile specimen was obtained. After 7 days of antibiotic treatment, he gradually improved and was discharged. Radical pancreatoduodenectomy was performed 8 days later.

Two sets of blood cultures showed bacterial growth after 9 hours of incubation. Gram staining showed Gram negative coccobacilli. Gram negative coccobacilli were also isolated from a bile culture on 5% sheep blood agar (Nissui, Tokyo, Japan).

Samples from these positive blood cultures were subcultured on 5% sheep blood agar (Nissui) and MacConkey II agar (BD) under aerobic conditions at 35℃. The colonies were slightly yellow, smooth, opaque, and often adherent. The Gram staining of the colonies showed Gram negative diplococci.

Bacterial identification using a BBL Crystal™ *Neisseria/Haemophilus* identification system (BD) indicated *N. subflava*. Mass spectrometry using AXIMA™ Performance (Shimadzu, Kyoto, Japan), and VITEK™ MS plus systems (Sysmex bioMerieux, Lyon, France) showed a 99.9% morphology match with *N. subflava*. To confirm the identity of the isolates, genomic DNA was extracted from the colonies by heat extraction (100℃, 10 min); PCR amplification and sequencing were performed to characterize the 16S rRNA gene. An analysis using the Blast software program revealed the strong homology (>99%) of the obtained 16S rRNA gene sequences (1,220-1,331 bp) with *N. subflava* biovar *perflava*; these were found to be 99.85% identical to those of *N. subflava* biovar *perflava* U15 strain in an analysis using the EzTaxon software program (http://www.ezbiocloud.net/eztaxon/). However, the same gene sequences were found to be 99.62% identical to *N. flavescens* ATCC13120 strain, *N. mucosa* M5 strain in an analysis using the EzTaxon software program.

To distinguish between these three species, biochemical testing was performed using ID Test HN-20 Rapid tools (Nissui) according to manufacturer's instructions. The results (Table) were positive for acid production from glucose, maltose, sucrose, and fructose; however, they were negative for acid production from lactose and nitrate reduction. These results were compatible with the biochemical activity profile of *N. subflava* biovar *perflava* and different from the profiles of *N. flavescens*, and *N. mucosa* (4). Thus, the Gram negative isolates recovered from the blood and bile cultures were determined to be *N. subflava* biovar *perflava*. Based on the results, the patient was diagnosed with acute cholangitis and bacteremia caused by *N. subflava* biovar *perflava*.

Discussion

N. subflava was isolated from both blood and bile cultures from a patient with acute cholangitis with cholangiocarcinoma. *N. subflava* has low pathogenicity. A literature search of the PubMed database suggested that documented cases of *N. subflava* blood stream infection have largely been limited to cases of meningitis (5), endocarditis (6), and osteomyelitis (7). Most of the cases occurred after medical intervention or intravenous drug injections. Although further testing is needed to confirm the identification, it is not difficult to identify *N. subflava* using a widely available kit. Thus, *N. subflava* blood stream infections are not likely to be overlooked at ordinary clinical laboratories. The small number of reports on *N. subflava* blood stream infections is simply due to the rarity of *N. subflava* blood stream infections.

In the present case, the minimum inhibitory concentration (MIC) of *N. subflava* isolated from blood culture was measured using the broth microdilution method. The MICs for penicillin G and cefotaxime were 0.5 μg/mL and <0.5 μg/mL, respectively. Although antimicrobial breakpoints against *N. subflava* have not been determined, the isolate was classified as having intermediate resistance to penicillin G and susceptibility to cefotaxime according to the breakpoint criteria for *N. gonorrhoeae* in CLSI M100-S26 (8). This result is consistent with a previous domestic report that showed reduced susceptibility against penicillin G (9). In this case, cefoperazone/sulbactam, a third generation cephalosporin, was administered. Although the MIC for cefoperazone/sulbactam was not measured, the antimicrobial therapy was effective in the present case because of the susceptibility to cefotaxime.

N. subflava is a component of the normal oral flora; it is not commonly found in the bile tract or duodenum. The common pathogens causing biliary tract infection are enterobacteriaceae, enterococci, and anaerobes (10), which normally inhabit the gastrointestinal tract. In this case, it was not exactly clear why *N. subflava* caused cholangitis. We hypothesize that the diagnostic ERCP performed as a part of a diagnostic work-up for bile duct neoplasm might have played a role. The normal oral flora, including *N. subflava*, may have been displaced to the duodenum and bile duct. The biliary obstruction due to the progression of cholangiocarcinoma may have finally caused cholangitis due to presence of the *N. subflava* colonies in the duodenum. Furthermore, endoscopic sphincterotomy was performed at the second diagnostic ERCP trial, which might have rendered the biliary sphincter permanently insufficient. The loss of this physiologic barrier between the duodenum and biliary tract

results in duodenocholedochol reflux and bacterial colonization of the biliary tract (11). Colonization of the biliary tract and cholangitis were more likely to have occurred in this patient. This study is associated with two major limitations. First, there was no evidence that *N. subflava*, which was isolated from the blood, was originally derived from components of the normal oral flora because no oral cultures were obtained. However, in a previous study, 37 out of 40 healthy adults were found to carry *N. subflava* biovar *perflava* in their nasopharynx (12). Secondly, there are no documented cases of post-ERCP duodenal and biliary colonization by *Neisseria* or post-ERCP cholangitis due to oral normal flora. However, a case of post-ERCP retroperitoneal abscess caused by *Haemophilus parainfluenza*, a component of the normal oral flora is on record (13).

Further research on cholangitis due to *Neisseria* is necessary to confirm this hypothesis. To the best of our knowledge this is the first report of cholangitis due to *Neisseria* species, which demonstrates that commensal *Neisseria* can cause cholangitis in a patient after bile duct treatment.

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