

Neisseria gonorrhoeae Endocarditis Confirmed by Nucleic Acid Amplification Assays Performed on Aortic Valve Tissue[∇]

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The Aptima Combo 2 assay is proposed as a rapid method of diagnosing *Neisseria gonorrhoeae* endocarditis or other suspected disseminated gonococcal disease.

CASE REPORT

A 45-year-old Caucasian male presented with progressive pains in his left ankle, which had made it difficult for him to cope with his work as a bus driver during the last days before hospitalization. Three weeks previously, he had returned from a journey to Bangkok, Thailand, with his Thai wife. There was no history of risk behavior, trauma, fever, or genitourinary complaints and no exposition to malaria. He was otherwise well, and his past medical history only revealed slightly impaired lung function due to smoking. He took no medications.

On hospital admission, the patient had a temperature of 39.5°C, a blood pressure of 165/75 mm Hg, a pulse of 88 beats/min, and a normal electrocardiogram. An inspection of the ankle showed a fiery red, well-demarcated area of edematous skin. An auscultation of the heart revealed a systolic grade 3 ejection murmur in the aortic area with no radiation. A physical examination of the neurological, respiratory, and gastrointestinal systems was normal. Laboratory investigations showed a hemoglobin level of 13.1 g/dl, a platelet count of 185×10^9 cells/liter, a white-cell count of 14×10^9 cells/liter, and a C-reactive protein level of 184 mg/liter. A dipstick of the urine showed traces of protein. The systolic murmur led to bedside transthoracic echocardiography on the day of admission to the hospital. This did not show signs of endocarditis; a small ventricular septum defect was considered to cause the systolic murmur. A transesophageal echocardiography was scheduled 5 days later. An orthopedic surgeon evaluated the ankle and decided that there was no septic arthritis.

Thus, erysipelas was suspected, and intravenous benzylpenicillin (1.2 g/8 h) was commenced as empirical antibiotic therapy.

On day 3, one out of four blood cultures taken on the day of admission became positive with gram-negative diplococci, which 12 h later proved to be *Neisseria gonorrhoeae* in the API NH test (bioMérieux, Marcy l'Etoile, France). Antibiotic susceptibility was determined by disc diffusion (Oxoid, Basingstoke, United Kingdom) and Etests (AB Biodisk, Solna, Sweden). The characteristics of the *N. gonorrhoeae* isolate are

shown in Table 1. Notably, the isolate was penicillinase positive (i.e., penicillin resistant) and resistant to ciprofloxacin.

Meanwhile, the patient had deteriorated: the white cell count was now 27×10^9 cells/liter, the pulse had increased to 116, and an electrocardiogram showed first-degree atrioventricular block. The suspicion of infective endocarditis was strengthened, and the symptoms from the ankle were now considered to be caused by a gonococcal septic arthritis. Swab samples were taken from the pharynx, urethra, and rectum, and the antibiotic treatment was changed to ceftriaxone. The swab samples from the rectum, urethra, and pharynx were transported from the clinic to the laboratory on charcoal-impregnated sterile plastic swabs in a modified Stuart transport medium (SSI Diagnostica, Denmark). Each swab was streaked on a selective chocolate agar medium (SSI Diagnostica, Denmark) containing amphotericin B (2 µg/ml), polymyxin B (25 IU/ml), lincomycin (1 µg/ml), and trimethoprim (3 µg/ml) and cultured for 2 days. All swab samples were negative.

Repeat transthoracic echocardiography was performed due to the strengthened suspicion of infective endocarditis and now showed a mobile mass on the aortic valve and severe valve dysfunction with aortic regurgitation. Transesophageal echocardiography confirmed vegetations and also showed abscesses in the septum; this led to acute open-heart surgery. Successful abscess debridement was undertaken, and the native aortic valve was replaced with a 25-mm mechanical prosthetic St. Jude valve. At the operating theater, a sample of the excised valvular tissue was immediately put in a DNA-free and sterile container without any additives and frozen at -20°C within 45 min. Three other samples were immediately transferred to three different bottles containing 100 ml culture medium: 3a, infusion broth supplemented with 5% serum; 3b, semisolid infusion broth supplemented with saccharose; and 3c, semisolid infusion broth supplemented with thioglycolate (SSI Diagnostica, Denmark). The bottles were incubated at 35°C for 2 weeks without shaking. None of the three bottles showed any growth of bacteria.

Nucleic acids were extracted from the sample of valvular tissue using a DNeasy blood and tissue kit (Qiagen, Germany) and subjected to PCR amplification using broad-range primers targeting part of the 16S rRNA gene and subsequent sequencing as described previously (7). The nucleotide sequence was compared to the nucleotide database of NCBI using a standard BLAST approach (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Furthermore, the Ribosomal Database Project ([* Corresponding author. Mailing address: Department of Clinical Microbiology, Aarhus University Hospital Skejby, 8200 Aarhus N, Skejby, Denmark. Phone: 45 89495649. Fax: 45 89495611. E-mail: ullanise@rm.dk.](http://rdp.cme</p></div><div data-bbox=)

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TABLE 1. Characteristics of *N. gonorrhoeae* isolated from the patient's blood

Characteristic	Result ^a
Microscopy.....	Gram-negative diplococci
Oxidase.....	+
Glucose.....	+
Fructose.....	-
Maltose.....	-
Sucrose.....	-
Ornithine decarboxylase.....	-
Urease.....	-
Lipase.....	-
Alkaline phosphatase.....	-
β -Galactosidase.....	-
Proline arylamidase.....	+
γ -Glutamyl transferase.....	-
Indole.....	-
Penicillinase test.....	+
Ceftriaxone Etest.....	0.004 (S)
Ciprofloxacin Etest.....	0.500 (R)
Penicillin Etest.....	0.380 (I)

^a +, positive; -, negative; S, sensitive; R, resistant; I, intermediary.

.msu.edu/index.jsp) was interrogated for sequence homology. Both databases revealed 100% homology with *N. gonorrhoeae*. Ten microliters of the nucleic acid extracted from the valve tissue was added to 400 μ l Aptima swab specimen transport buffer and analyzed using our routine Aptima Combo 2 assay (Gen-Probe, Inc.) for the detection of *Chlamydia trachomatis* and *N. gonorrhoeae* (1). The sample was positive for *N. gonorrhoeae*.

Ceftriaxone was continued for 6 weeks (4 g daily for the first postoperative week, 2 g daily thereafter). There was no growth in subsequent blood cultures.

The patient's recovery was uneventful apart from persistent second-degree atrioventricular block, necessitating permanent pacemaker implantation on the 43rd postoperative day. After 2 weeks, the patient admitted to having had affairs with two local Thai women during his visit in Bangkok. His human immunodeficiency virus and hepatitis B and C tests came out negative, and he was discharged on the 45th postoperative day.

In the preantibiotic era, up to one-quarter of all cases of infective endocarditis were caused by *N. gonorrhoeae* (3, 5). Nowadays, gonococcal endocarditis (NGE) is a rare disease. However, in the last decade, two factors have changed, which might increase the incidence of NGE once again. First, an increasing prevalence of ciprofloxacin-resistant strains of *N. gonorrhoeae* leads to treatment failure of uncomplicated urogenital disease and hence an increased risk of an invasive infection. In Denmark, 57% of *N. gonorrhoeae* strains are now ciprofloxacin resistant (4). Second, the incidence rate of uncomplicated gonococcal disease has more than doubled since 2000 in Denmark (4) and is increasing in other countries as well (2, 3, 6).

Behavioral changes are also likely to influence the incidence of NGE in the future. Waning fear of human immunodeficiency virus is leading to more people practicing unsafe sex;

tourism to countries with high prevalences of *N. gonorrhoeae* infection has increased, and sex tourism is increasing as well.

NGE is an aggressive form of endocarditis with a mortality rate of up to 19% (5) and a worrying tendency for patients to deteriorate acutely after weeks of appropriate antibiotic treatment (6). The rapid deterioration in our patient and the development of severe cardiac pathology on echocardiography within 3 days illustrate this clearly. The disease occurs more frequently in young people, people with complement deficiencies, and males (5, 6). In two-thirds of the cases, no genitourinary symptoms have been noticed, and cultures of the urethra, pharynx, and rectum are frequently negative, as in this case. The average duration between the primary infection and endocarditis has not been established; in this case, it was probably close to 3 weeks. Arthritis and/or tenosynovitis often precede the manifestations of endocarditis and are seen in two-thirds of patients. The aortic valve is most commonly affected (50%), followed by the mitral valve (40%). More than half of the patients require valve surgery (5). Although penicillinase-producing *N. gonorrhoeae* is recognized as a cause of disseminated disease, this is infrequent relative to its occurrence in uncomplicated infections; to our knowledge, only one case of penicillinase-producing NGE (9) has been published in the English literature apart from the present case.

As shown in this case, NGE should be a differential diagnosis in sexually active patients with endocarditis. This is important, as the empirical treatment of infective endocarditis usually consists of penicillin in combination with aminoglycoside and hence does not include optimal agents against *N. gonorrhoeae*; 52% of *N. gonorrhoeae* isolates in Denmark are penicillin resistant (4).

This case also illustrates the importance of a rapid diagnosis in disseminated gonococcal disease. However, blood and valve cultures may remain negative for sustained periods of time (8, 9), and a positive culture might not be achieved at all because of the fastidious nature of *N. gonorrhoeae* or the initial antibiotic treatment.

To our knowledge, this is the first published case of NGE where nucleic acid amplification tests have been used on valve tissue. The commercial Aptima Combo 2 test is validated for the detection of urogenital gonococcal infections and provides results within 4 h. We have used purified nucleic acids from an excised heart valve in the Aptima Combo 2 assay as a complement to an in-house 16S rRNA gene PCR and proven the feasibility of both methods. Although it is not validated for tissue samples, we propose that the Aptima Combo 2 assay may be used as a simple way of obtaining a diagnosis from tissue samples within hours for cases of suspected disseminated gonococcal disease.

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