Posterior mitral valve endocarditis accompanied by bacteraemia with *Granulicatella adiacens*. A Case Report

Endocardită valvulară mitrală posterioară însoțită de bacteriemie cu *Granulicatella adiacens*. Prezentare de caz

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Abstract

Granulicatella species, formerly known as nutritionally variant streptococci (NVS) are rarely implicated in infective endocarditis (IE). We report the case of a 65-year-old woman with ischemic cardiopathy and mitral valve incompetence who developed IE. She accused progressive dyspnea and two weeks prior to admission developed vesperal fever (38–39°C) without chills and sweating. Transoesophageal echocardiography (TEE) revealed a vegetation established on the ventricular face of posterior mitral valve. G. adiacens was recovered in pure culture from five sets of consecutive blood cultures collected before antibiotic treatment. The phenotypic identification was based on morphological characteristics, special cultural requirements (satellitism, capnophilia, anaerobic atmosphere) and Vitek 2 Compact System. Molecular identification was performed by 16S rDNA gene sequence analysis. The resulting sequence was compared with sequences from the National Center for Biotechnology Information (NCBI) on-line database and was confirmed as G. adiacens. The patient was successfully treated intravenously with ceftriaxone and gentamicin. The conventional diagnosis of a G. adiacens infection relies on the bacterial polymorphism in Gram-stained smear, the discrepancy between direct smear result and the difficulty of growth using standard techniques, in association with cultural dimorphism. This case emphasizes the importance of bacteriologic identification of G. adiacens and rapid initiation of an adequate antibiotherapy.

Keywords: *Granulicatella adiacens, nutritionally variant streptococci (NVS), satellitism, infective endocarditis.*

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Rezumat

Speciile de Granulicatella cunoscute şi sub denumirea de streptococi deficienţi nutritiv sunt foarte rar implicate în etiologia endocarditei infecţioase (EI). Redăm cazul pacientei F.V. de 65 ani, care pe fondul unei cardiopatii ischemice dilatative cu insuficienţă mitrală a dezvoltat endocardită bacteriană. Pacienta a acuzat agravarea progresivă a dispneei, iar cu două săptămâni anterior momentului internării a prezentat ascensiuni febrile vesperale (38–39°C), neacompaniate de frisoane şi transpiraţii. Ecocardiografia transesofagiană a evidenţiat o vegetaţie pe faţa ventriculară a valvei mitrale posterioare. Din cinci seturi succesive de hemoculturi recoltate înaintea instituirii tratamentului antibiotic s-a izolat în cultură pură G. adiacens. Identificarea fenotipică s-a realizat pe baza caracteristicilor morfologice macro- şi microscopice, a condiţiilor speciale de cultivare (satelitism, capnofilie, anaerobioză) şi în mod automat cu Vitek 2 Compact System. Identificarea moleculară s-a realizat prin secvenţierea genei ADNr 16S. Secvenţa obţinută a fost comparată cu secvenţe existente în baza de date on-line a National Center for Biotechnology Information (NCBI), confirmându-se apartenenţa la specia G. adiacens. Pacienta a evoluat favorabil sub tratament intravenos cu ceftriaxone şi gentamicină. În diagnosticarea convenţională a unei infecţii cu G. adiacens trebuie ţinut cont de: polimorfismul bacterian din frotiul colorat Gram, discrepanţa între rezultatul frotiului direct şi dificultatea obţinerii unei culturi bacteriene prin procedee uzuale, în asociere cu dimorfismul cultural. Cazul prezentat subliniază importanţa identificării bacteriologice a G. adiacens coroborată cu instituirea precoce a unei antibioterapii adecvate.

Cuvinte cheie: *Granulicatella adiacens, streptococi deficienţi nutritiv, satelitism, endocardită infecţioasă.*

Introduction

Infective endocarditis (IE) remains a disease that is associated with a considerable morbidity and mortality (1). IE is an endovascular microbial infection of cardiovascular structures facing the bloodstream, including infections of the large intrathoracic vessels and of intracardiac foreign bodies. The early characteristic lesion is a variably sized vegetation, although destruction, ulceration, or abscess formation may be seen earlier by echocardiography (2).

A wide variety of pathogens (bacteria, fungi) are recognized in IE etiology. The condition traditionally known as "culture-negative endocarditis" can be due to many fastidious organisms such as *Haemophilus* spp., *Aggregatibacter actinomycetemcomitans,* Cardiobacterium spp., *Eikenella corrodens* and *Kingella* spp. (HACEK group of Gram negative rods), nutritionally variant streptococci (NVS), cell wall-deficient bacteria (L-forms), *Brucella* spp., *Chlamydia* spp., *Legionella* spp. and others (2).

In 1944 Lamanna mentioned that the first case of I.E. due to a streptococcus with a diphtheroid appearance was reported by Babeş and Manolescu in 1909, in a German publication (3, 4). Later, in 1961 Frenkle and Hirsch described NVS as new types of viridans streptococci exhibiting satellitism around colonies of other bacteria (5). Because of difficulties in culturing these organisms and the variety of appearances that they present on Gram stain, such strains have caused major diagnosis difficulties (6).

In 1989 Bouvet et al. demonstrated, by means of DNA-DNA hybridization studies, that NVS isolates could be divided into two groups, *Streptococcus defectivus* and *Streptococcus adiacens* (7).

In 1995, as a result of 16S rDNA gene sequence data and other phylogenetic analysis, a new genus, *Abiotrophia*, was created and consisted of two species: *A. defectiva* and *A. adiacens* (8). "Abiotrophia" means "life nutrition deficiency" and refers to the species' requirements for media supplemented with vitamin B_6 or thiol compounds for growth.

Since 1995, three new species have been added, *A. elegans*, *A. balaenopterae* and most recently *A. para-adiacens*. Phylogenetically, the

genus Abiotrophia consists of two distinct lines, *A. defectiva* and a group of other three species: *A. adiacens, A. balaenopterae*, and *A. elegans.* Therefore, Collins and Lawson have recently proposed that these last three species should be reclassified in a new genus, *Granulicatella* (9).

Abiotrophia and *Granulicatella* species are part of the normal human flora of the oral cavity (10, 11), the genitourinary and intestinal tract. *G. adiacens* is isolated more frequently from oral specimens than other NVS (11).

Bacteraemia and endocarditis are the most frequently reported clinical infections due to *Granulicatella* and *Abiotrophia* species (6). Isolated cases of keratitis, endophthalmitis, central nervous system infections, sinusitis, otitis media, prostatitis, cholangitis, peritonitis, arthritis and osteomyelitis have also been mentioned in relevant medical articles (12 - 16).

In this paper we describe the case of an adult female patient with ischemic cardiopathy and mitral valve incompetence who developed IE caused by *G. adiacens*, in an attempt to increase awareness about this microorganism and to highlight the difficulties encountered in the diagnosis and in the treatment.

Case Report

In February 2007 a 65-year old woman, F.V., was diagnosed to have ischemic cardiopathy with dilatative evolution, functional mitral valve incompetence, ventricular extrasystolic arrhythmia, left ventricular dysfunction class II NYHA (New York Heart Association), essential arterial hypertension II degree and obesity. One year later she developed an episode of bigeminism arrhythmia associated with dyspnea.

In February 2009 the patient accused progressive dyspnea increased over baseline and two weeks later she developed vesperal fever (38–39°C) without chills and sweating. The patient was treated empirically with Ciprofloxacin (1 week), and after that with amoxicillin and clavulanic acid. Nevertheless, fever and fatigue persisted.

Therefore, in March 2009 she was admitted to the Clinic of Cardiovascular Rehabilitation in Târgu-Mureş. Physical examination on admission revealed an increased systolic murmur in mitral area compared to 2007, left ventricular dysfunction class III NYHA and vesperal fever. No splenomegaly, rashes, splinter hemorrhages, Osler's nodes, Roth's spots or Janeway lesions were detected.

Laboratory parameters, at the time of admission to hospital, were as follows: leukocyte count: 6530/µL (1% myelocytes, 1% metamyelocytes, 12% unregimented neutrophils, 50% segmented neutrophils, 0% eosinophils, 0% basophils, 10% monocytes, 26% lymphocytes), hemoglobin: 11.4 g/dL, hematocrit: 34.3%, thrombocyte count: 216000/µL, erythrocyte sedimentation rate (ESR): 23 mm/h, C-reactive protein (CRP) positive, IgG 16.6 UI/mL. Peripheral blood smear showed neutrophils with pseudo-Pelger-Huet anomaly, 2-3 macrocytes/100, polychromatophilia and anisothrombocytosis. The rest of laboratory parameters were within normal limits.

The transthoracic echocardiography (TTE) visualized a "fly" prolapse of posterior mitral valve, in association with almost all posterior chordae tendineae destructed and a minimal prolapse of anterior mitral valve edge. Six days later, TEE revealed a vegetation established on the ventricular face of posterior mitral valve and on posterior chordae tendineae.

G. adiacens was recovered in pure culture from five sets of consecutive blood cultures collected before antibiotic treatment.

The patient was treated intravenously with ceftriaxone (one month) and gentamicin (two weeks), in association with diuretic, antihypertension, antiarrhythmic and symptomatic drugs. Clinical improvement and two sets of negative blood cultures were obtained in one day, respectively in nine days after the initiated antibiotic treatment. No embolic events, metastatic infections or relapses were noted.

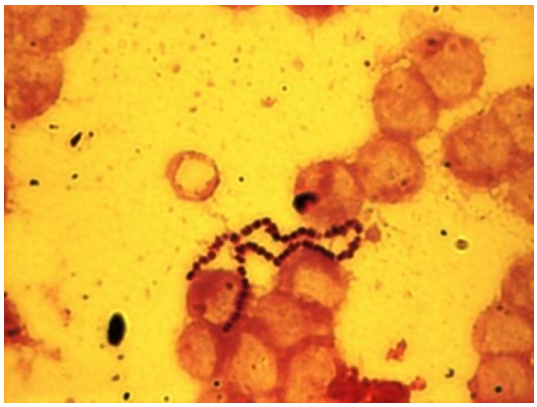

Figure 1. Direct Gram-stained smear showing "Streptococcal-like" chain (1000x)

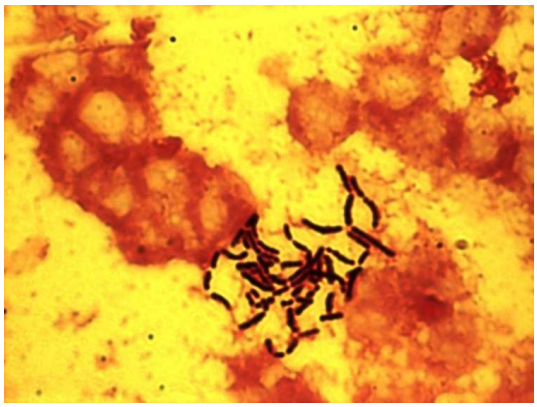

Figure 2. Direct Gram-stained smear showing Gram positive bacilli (1000x)

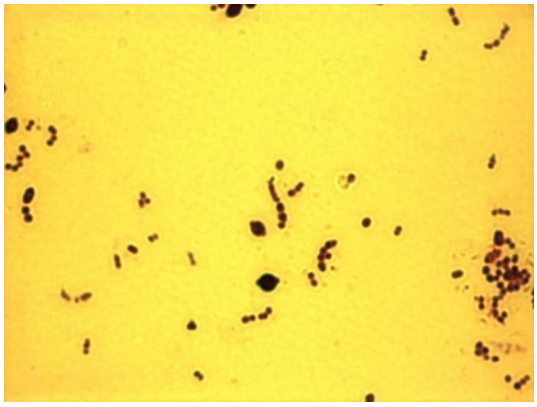

Figure 3. Gram-stained smear of 48 hours old colonies on CSBA in CO2 atmosphere incubation showing "Streptococcal-like" chains, pairs and bizarre shapes (globular forms) (1000x)

Microbiological investigations

Phenotypic characteristics

The blood cultures were processed in the Bact/Alert 120 Automatic System using aerobic and anaerobic bottles (BioMérieux).

Gram-stained direct smear from the blood cultures revealed a pleomorphic aspect with Gram positive cocci in chains, pairs, coccobacilli and bacilli (*Figures 1 and 2*).

The blood samples were cultured on routine media including 5% Columbia sheep blood agar (CSBA), chocolate agar (without antibiotics), Agar Bile Esculine (ABE) and incubated 24–48 hours at 37°C in aerobic atmosphere, 5% CO_2 and anaerobic condition.

The microorganism did not grow aerobically on CSBA and ABE. From the first set of blood cultures, α-haemolytic, tiny, grayish-white colonies (0,5-1 mm diameter) appeared on CSBA under capnophilic and anaerobic conditions (*Figure 4*). The strain showed dimorphism, with small and large colony variants. In the case of the following four sets of blood

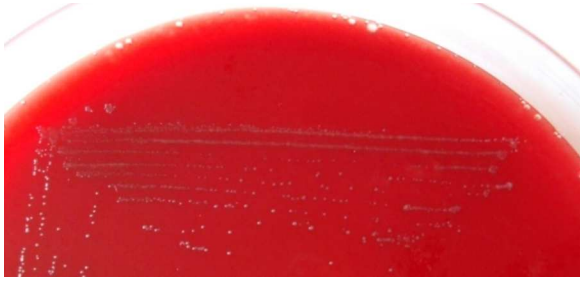

Figure 4. Colonies morphology on CSBA in anaerobic condition

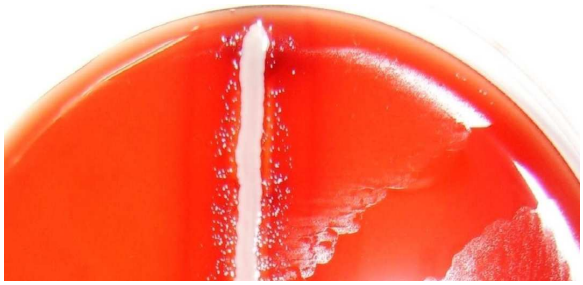

Figure 5. Satellitism test

samples, the microorganism was detected on CSBA anaerobically and around a streak of *Staphylococcus aureus* ATCC 25923 made across the plate surface, in 5% CO_2 (*Figure 5*). The isolate grew poorly on chocolate agar in capnophilic atmosphere.

Gram-stained smear from CSBA culture showed Gram stain variability and the extremely pleomorphic appearance including bizarre shapes. Variations in the size and morphology of the bacterial cell were also evident (*Figure 3*).

Catalase- and oxidase tests were negative, optochin disk resistant.

Vitek 2 Compact System (BioMérieux) identified the isolate as *G. adiacens* with 99% probability value. Pyrrolidonyl arylamidase (PYR), leucine aminopeptidase (LAP), β-glucosidase were positive and both α- and β-galactosidase were negative.

16S rDNA gene sequencing

Sequencing was performed at the Laboratory of Molecular Microbiology, "Cantacuzino" INCDMI Bucharest.

Bacterial DNA was extracted with the NucleoSpin Tissue kit (Macherey Nagel) according to the instructions of the manufacturer. 16S rRNA gene was amplified by PCR using High Fidelity PCR Master Mix kit (Roche Diagnostics) and universal primers Ad (5'-AGAGTTTG-ATCMTGGCTCAG-3', positions 8-27 according to *Escherichia coli* numbering system) and rJ (5'-GGTTACCTTGTTACGACTT-3', positions 1510 - 1492). The amplification was carried out in a 2700 GeneAmp PCR System Thermocycler (Applied Biosystem). PCR products were examined using the 1% agarose gel electrophoresis and ethidium bromide staining. The amplicons were purified using the NucleoSpin Extract II kit (Macherey Nagel). Sequencing was performed using a BigDye Terminator v3.1 kit (Applied Biosystem) and consisted of five distinct reactions of cycle sequencing. After purification with DyeEx (Qiagen), sequencing products were analyzed using a 3100 Avant ABI Prism automated sequencer with four capillaries (Applied Biosystem). Primers Ad and rJ and sequencing primers D (5'-CAGCAGCCGCGGTAATAC-3', positions 519-536), E (5'-ATTAGATACCCTGGTAGTCC-3', positions 787-806) and rE (5'-GGACTAC-CAGGGTATCTAAT-3', positions 806-787) were used. The sequences were analyzed and edited using BioEdit software. After alignment, the resulting sequence of approx. 1500 bp was compared with known sequences in the National Center for Biotechnology Information (NCBI) on-line database (GenBank) by using the BLAST (Basic Local Alignment Search Tool) algorithm available on the NCBI website (www.ncbi.nlm.nih.gov).

Identification of the studied isolate as *Granulicatella adiacens* was based on a 99% sequence similarity with sequences from NCBI GenBank.

Discussion

G. adiacens is a fastidious organism that is rarely involved in IE. *Granulicatella* species (from latin words *granulum*: small grain and *catella*: small chain) are formerly known as NVS. This group of Gram-positive bacteria has been previouslly described as satelliting, pyridoxal-dependent, or cell wall-deficient bacteria (L-forms). NVS is reported to cause approximately 5% of all cases of endocarditis (17).

Important pathogens involved in IE are microorganisms from the oral cavity. The tooth-tissue interface is a typical portal for bacteria to enter the body. Interestingly, our patient had no history of dental treatment or invasive procedures before this disease. This is in agreement with other reports which demonstrate that bacteraemias do not occur only after major procedures such as dental extraction, tonsillectomy, and bronchoscopy but also after more common events, such as toothbrushing and chewing gum (18).

NVS and viridans streptococci have low pathogenicity and they adhere frequently only to a damaged endocardium (19). During a transient bacteraemia the microorganisms can

Table 1. Principal biochemical keys for differential diagnosis between *Granulicatella* spp. and *A. defectiva*

	G. adiacens	*G. para-adiacens*	*G. elegans*	*G. balaenopterae*	*Abiotrophia defectiva*
α-galactosidase	-	-	-	-	+
β-galactosidase	-	-	-	-	+
β-glucosidase	+	-	-	-	-
Satellitism	+	+	+	+	+
Acid from sucrose	+	+	+	-	+
Acid from trehalose	-	-	-	+	+
PYR	+	+	+	+	+
LAP	+	+	+	+	+
Vancomycin susceptibility	S	S	S	S	S

adhere to non-bacterial thrombotic vegetation and fibronectin, a glycoprotein that is a major surface constituent of mammalian cells (19). Adherence tests showed a poor adherence of *Granulicatella* spp. strains to the host extracellular matrix and this could explain her lower propensity to induce IE (20). After adhesion, microorganisms can grow and induce further thrombus formation and neutrophil chemotaxis. The bacteraemia associated with IE is usually low grade and continuous.

The variability in clinical and laboratory presentation of IE requires a diagnostic strategy that is both sensitive for disease detection and specific for its exclusion across all forms of the disease (1).

Our patient presented only a few classic peripheral stigmata of IE. No immunologic vascular phenomena and no signs of renal involvement were detected. It is known that in IE deposition of circulating immune complexes accounts for diffuse or focal glomerulonephritis (2, 19). The immunologic manifestations often occur after a prolonged evolution of the disease. But, in our case the definite diagnosis of IE was made relatively rapidly and an adequate antibiotic treatment was started in only three weeks after the first febrile episode.

IE appears frequently in patients with valvular incompetence than in those with pure valvular stenosis (19). In our case report the patient had ischemic cardiopathy with dilatative evolution and functional mitral valve incompetence. TTE could not visualise the vegetation, but six days later TEE revealed an atypical establisment of a vegetation on the ventricular face of posterior mitral valve. This situation is opposite to almost all cases of IE when vegetations are attached to atrial aspects of atrioventricular valves and to ventricular sites of semilunar valves, predominantly at the valve closure line (2, 19). Also, the posterior chordae tendineae of our patient were destructed.

We identified *G. adiacens* from five sets of consecutive blood cultures collected in three different days, before antibiotic treatment. It is known that *Granulicatella* spp. have nutritional deficiencies that hinder their growth in routine laboratory culture media. Such organisms may require broth supplemented with pyridoxal hydrocloride. Addition of 0.001% pyridoxal HCl to a Todd Hewitt broth causes most isolates to convert to „streptococcus-like" cellular arrangements and Gram positivity (6, 21). These isolates can naturally exist in a cell-wall-deficient state and may be difficult to recover from clinical spe-

cimens such as blood cultures (5). Satellitism around *S. aureus* is an important phenotypic characteristic of *Haemophilus* spp., NVS and of some isolates of *Ignavigranum* (6, 22, 23).

Principal phenotypic features useful in differentiating between *Granulicatella* spp. and *A. defectiva* are mentioned in *Table 1*. Like streptococci, these two species are LAP- positive, but unlike streptococci, they are PYR-positive (23). They are also optochin-resistant and vancomycin-susceptible (23).

Granulicatella and *Abiotrophia* species are related to members of the genera *Streptococcus, Enterococcus, Aerococcus, Dolosicoccus, Dolosigranulum, Eremococcus, Facklamia, Globicatella, Gemella, Helcococcus, Ignavigranum, Lactococcus, Leuconostoc, Pediococcus, Stomatococcus* and *Vagococcus*, which also may be isolated in human infections and with whom they share various degree of phenotypic identity (6, 24).

In many cases, the species of NVS could not be determined because phenotypic characteristics did not correlate with the species descriptions published in medical literature (6). Therefore, molecular approaches to the identification of these NVS have also been described. 16S rDNA gene sequencing is considered the "gold standard" of bacterial identification to the species level (only when starting from pure bacterial culture) (22). This method is based on the amplification of bacterial 16S rDNA genes which are mosaic molecules consisting of hypervariable regions which are often flanked by conserved regions. Primers are designed to bind to conserved regions and amplify variable regions (2).

Patients with endocarditis due to NVS are more difficult to treat than those infected with viridans streptococci. As many as 41% of patients may fail to respond to antimicrobial treatment, and combination therapy is often recommended (17).

Antimicrobial susceptibility data for these nutritionally variant streptococcus-like organisms are very limited. Little is known about the genetic basis of their resistance mechanisms. Clinical and Laboratory Standards Institute (CLSI) suggested that broth microdilution with the addition of 0.001% pyridoxal HCl and minimal inhibitory concentrations (MICs) determination should be performed (25).

In a study by Tuohy et al., 41% of 27 *G. adiacens* isolates were penicillin-susceptible (MIC, $\leq$ 0.12 µg/ml), 51% were intermediate (MIC, $\leq$ 0.25-2 µg/ml), and 8% were penicillin-resistant (MIC, $\geq$ 4 µg/ml) (26). According to CLSI interpretative criteria for Streptococcus spp. other than *Streptococcus pneumoniae*, antimicrobial susceptibility of *G. adiacens* to cefazolin, ceftriaxone and meropenem was, respectively, 52%, 63% and 96% (26).

High level resistance (HLR) to penicillin or ceftriaxone (MIC, > 8 µg/ml) is rare among strains of streptococci (2). In cases of penicillin-resistant streptococci (MIC, > 8 µg/ml) the combination of vancomycin plus gentamicin for six weeks is recommended (2). Also, recent articles mentioned that all studied *Granulicatella* and *Abiotrophia* strains were susceptible to vancomycin (27, 28). Liao et al. reported that all 19 isolates of *G. adiacens* were susceptible to quinupristin-dalfopristin, linezolid, levofloxacin, moxifloxacin, and gatifoxacin (28).

No cases of NVS HLR to aminoglycosides have been reported (24). Autoradiographic studies have demonstrated homogeneous distribution of aminoglycosides into the vegetation (29).

The emergence of macrolide resistance among NVS is a great concern. Woo et al. reported that three out of nine isolates of NVS were resistant to erythromycin, clarithromycin and azithromycin (22). Also, another study demonstrated that more than 50% of isolates of *Granulicatella* and *Abiotrophia* were not susceptible to azithromycin and clindamycin.

In our case the antibiotic combination was decided according to the European Society of Cardiology guidelines on treatment in IE (regimen B for native valve endocarditis due to streptococci, including *Abiotrophia* spp., penicillin MIC between 0.1–0.5 µg/ml) (2).

In conclusion, *G. adiacens* should be considered as a possible agent of endocarditis and bacteriaemia in pacients with underlying heart disease and this microorganism should be suspected when direct Gram stains of positive blood cultures show streptococcal bacteria that fail to grow on subsequent culture or subculture. This case emphasizes the importance of a rapid diagnosis and initiation of an adequate antibiotic therapy.

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