New Tricks from an Old Cow: Infective Endocarditis Caused by Streptococcus dysgalactiae subsp. dysgalactiae

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We present a case of infective endocarditis caused by Streptococcus dysgalactiae subsp. dysgalactiae, a major cause of bovine mastitis and previously thought to be an animal-restricted pathogen. The patient reported no direct contact with animals, and the clinical course was severe and complicated.

CASE REPORT

A 65-year-old male patient was admitted to Haukeland University Hospital in western Norway with radiating pain in his left shoulder, fever, and muscle ache. One month earlier, he had been admitted to a hospital in Spain with similar symptoms but was rapidly discharged with a diagnosis of shoulder tendinitis. He had a family history of sudden cardiac death, and his previous medical history included hypertrophic obstructive cardiomyopathy and a normal coronary angiography 7 years prior to the actual admission.

Upon admission, he had a pulse rate of 100/min, a temperature of 39°C, and a respiratory frequency of 24/min, thus fulfilling the criteria of systemic inflammatory response syndrome (SIRS). He was pale, with a blood pressure of 118/59 mm Hg, and a holosystolic murmur was heard at the apex. No local signs of infection were observed over his left shoulder.

The initial blood chemistry results were as follows, with normal range values in parentheses: hemoglobin, 8.5 g/dl (13.4 to 17.0 g/dl); C-reactive protein, 277 mg/liter (<5 mg/liter); leukocytes, 20.8 × 10⁹/liter (3.5 × 10⁹ to 11.0 × 10⁹/liter); neutrophils, 18.5 × 10⁹/liter (1.7 × 10⁹ to 8.2 × 10⁹/liter); sedimentation rate, 102 mm/h (0 to 20 mm/h); procalcitonin, 12.1 μg/liter (<0.10 μg/liter); and troponin T, 896 ng/liter (<25 ng/liter). Thrombocytes were within the normal range. The electrocardiogram (ECG) demonstrated ST segment elevation in leads V₁ and V₂ and T wave inversion in leads V₃ to V₆, indicative of ischemia.

Antibiotic therapy was started on day 1 and included meropenem and vancomycin. A broader initial regimen than that recommended in the Norwegian National Antibiotic Guidelines was chosen since the patient had recently been admitted to a hospital in Spain. The following day, all four blood cultures grew nonhemolytic bacteria on blood agar. Species identification was performed using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) and showed that the isolate was Streptococcus dysgalactiae. Subsequently, group C carbohydrate specificity was documented using a slide agglutination test (Oxoid, Cambridge, United Kingdom). The antimicrobial susceptibility testing showed that the group C streptococcus (GCS) isolate was fully susceptible to all tested antibiotics, with the following MICs: penicillin G, 0.008 mg/liter; ceftriaxone, <0.016 mg/liter; clindamycin, 0.25 mg/liter; vancomycin, 0.25 mg/liter; teicoplanin, 0.25 mg/liter; and linezolid, 1 mg/liter.

A more thorough anamnestic interview revealed a history of weight loss of 6 kg, bloody stools, increasing pain in the left shoulder, and inaccuracy of vision. On examination, he had no peripheral vascular phenomena indicative of septic embolization. He was delirious and hallucinated.

Infective endocarditis (IE) with possible septic embolization to the brain and left shoulder was suspected. Since ceftriaxone penetrates the blood-brain barrier better than penicillin, the antimicrobial treatment was subsequently changed to ceftriaxone in combination with gentamicin. Transthoracic echocardiography (TTE) confirmed the clinical suspicion of IE and revealed a vegetation on the anterior mitral cusp. Magnetic resonance imaging (MRI) of the brain and left shoulder showed septic embolization to both cerebral and cerebellar hemispheres as well as fluid in the left subdeltoidal bursa. Abdominal computed tomography (CT) scan performed after 10 days confirmed embolization to the spleen, after which clindamycin was temporarily added to the antimicrobial regimen for 2 weeks to optimize abscess penetration. During the treatment course, a colonoscopy was performed due to persistent bloody stools, which revealed a malignant tumor in the rectum.

Subsequent examinations with TTE and transesophageal echocardiography revealed an increasing mitral insufficiency, a vegetation on the aortic valve, and a severe aortic insufficiency, grade III to IV. Cardiac CT showed normal coronary arteries and a significant difference in heart-minute volume (5 liters/min in the left ventricle, 7.8 liters/min in the right ventricle), confirming severe valve insufficiencies on the left side of the heart. It thus became clear that surgical replacement of the valves was needed. However, the persisting bleeding from his rectal tumor maintained anemia and inoperable conditions. Radiation therapy was successfully re-
The GCS isolate (T534) was stored on Greaves medium at 80°C until further testing. Lack of hemolysis on blood agar, 

<table>
<thead>
<tr>
<th>Primer target gene and orientation</th>
<th>Annealing temp (°C)</th>
<th>Primer sequence (5’→3’)</th>
<th>Result for S. dysgalactiae subsp. dysgalactiae</th>
<th>Result for S. dysgalactiae subsp. equisimilis</th>
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<tbody>
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- Shown are results for one nonhemolytic, emm-nontypeable GCS isolate belonging to S. dysgalactiae subsp. dysgalactiae.
- Shown are results for six beta-hemolytic group G streptococci associated with infective endocarditis in western Norway. All possessed emm genes belonging to emm types typical of S. dysgalactiae subsp. equisimilis.

Two months after admission, he suffered from a fulminating pulmonary edema and underwent acute, life-saving thoracic surgery with both biological aortic and mitral valve replacement. Aortic perivalvular abscess formation was also documented and debrided. Cultures were performed on the excised aortic and mitral valves, without bacterial growth. Unfortunately, direct 16S rRNA sequencing of the valve specimens was not performed. The patient rapidly recovered after surgery and received intravenous antibiotic therapy during the first six postoperative weeks.

Four months after admission, his rectal tumor, a differentiated adenocarcinoma, was radically resected, with acute complications causing reoperation, after which his hemoglobin level was 3.3 g/dl at the lowest. His temporary ileostomy was removed 6 months later without further complications.

He was followed on an outpatient basis for 18 months, with no clinical or echocardiographic signs of recurring endocarditis.

The GCS isolate (T534) was stored on Greaves medium at −80°C until further testing. Lack of hemolysis on blood agar, along with the MALDI-TOF MS species identification, could indicate that this strain did not belong to Streptococcus dysgalactiae subsp. equisimilis, which, together with Streptococcus spp. belonging to the anginosus group streptococci, is responsible for the vast majority of human GCS infections (1–3). In order to assign the correct streptococcal subgroup, the isolate was therefore subjected to selected molecular analyses.

First, 16S rRNA sequencing was performed with the primers 5′-GGG-GCC-AGA-CTC-CTG-GAC-GCCA-3′ (F-primer) and 5′-GCG-TGG-ACT-ACC-AGG-GTA-TCT-AAG-CC-3′ (R-primer), under previously reported PCR conditions (4). This analysis confirmed that T534 was of the species Streptococcus dysgalactiae, with 99.7% and 99.6% homology to S. dysgalactiae subsp. equisimilis and Streptococcus dysgalactiae subsp. dysgalactiae, respectively. However, neither MALDI-TOF MS nor 16S rRNA sequencing could separate S. dysgalactiae subsp. dysgalactiae from S. dysgalactiae subsp. equisimilis.

Next, we performed targeted PCR on genes or gene segments previously reported to be frequently present in S. dysgalactiae subsp. equisimilis isolates associated with human infections, and not in isolates belonging to S. dysgalactiae subsp. dysgalactiae: namely genes encoding streptokinase (ska), streptolysin O (slo), C5 a peptidase (scpA) (5), along with surface lipoprotein DppA (dppA), immunogenic secreted protein (isp), and laminin-binding protein (lmb) (6). As shown in Table 1, none of those genes was detected in T534. Six S. dysgalactiae subsp. equisimilis isolates associated with endocarditis in western Norway were also tested, and all were PCR positive for those six genes.

Based on the whole-genome sequence of an S. dysgalactiae subsp. dysgalactiae strain (ATCC 27957) associated with bovine...
udder infection (7), primers targeting the rihC gene were constructed, since a BLAST search of the rihC sequence from this particular S. dysgalactiae subsp. dysgalactiae isolate showed that it lacks significant homology with genes from streptococcal isolates associated with human infections.

T354 possessed an rihC gene, and the DNA sequence showed 100% homology to that of the rihC gene in S. dysgalactiae subsp. dysgalactiae strain ATCC 27957.

Finally, a multilocus sequence analysis (MLSA) scheme consisting of the seven housekeeping genes map, pfl, ppA, ppy, ppb, sodA, and tuf was used with the primers and thermal profiles previously described (2, 8). A BLAST search of the trimmed consensus sequences of all seven genes showed that T354 had an identical MLSA profile to Streptococcus dysgalactiae subsp. dysgalactiae strain CCUG 27439, isolated from a cow (2).

S. dysgalactiae subsp. dysgalactiae was until lately regarded as an animal-restricted pathogen and is a principal cause of bovine mastitis (9). S. dysgalactiae subsp. dysgalactiae has also shown the ability to cause severe cellulitis and toxic shock syndrome in cattle (10), septicemia in fish and dogs (11, 12), and infective arthritis in sheep (13). To our knowledge, S. dysgalactiae subsp. dysgalactiae possesses either Lancefield group antigen C or L and most often grows large colonies with surrounding alpha-hemolysis, or lack of hemolysis, on blood agar (2, 3). Human GCS infections, however, are most often caused by beta-hemolytic S. dysgalactiae subsp. equisimilis strains or, more rarely, Streptococcus isolates belonging to the anginosus group streptococci, with a wide range of clinical manifestations, including severe soft tissue infections, primary bacteremia, osteomyelitis, arthritis, pneumonia, meningitis, peritonitis, toxic shock syndrome, and endocarditis (1).

The incidence of infective endocarditis (IE) appears to be rising. In the United States, a 2.4% annual increase in admission rate of IE from 1998 to 2009 was documented, leading to an incidence of IE from 0.6 to 1.7 per 100,000 inhabitants in 2009 (14). Approximately 70% of the cases of IE occur on native valves: the mitral and aortic valves are most often affected. IE is caused by either Staphylococcus aureus or viridans group streptococci in around 50% of the cases (15), whereas IE caused by GCS belonging to S. dysgalactiae subsp. equisimilis is indeed a rarity (1, 16, 17). To our knowledge, S. dysgalactiae subsp. dysgalactiae has never been associated with human infection, and carcass soon caused by this bacterium has previously only been reported twice.

S. dysgalactiae subsp. dysgalactiae was associated with a prosthetic joint infection after total knee arthroplasty in a case report from South Korea. However, the mode of species identification of this bacterial isolate was not described (18). Furthermore, S. dysgalactiae subsp. dysgalactiae was associated with cellulitis in a patient with a poky injury of a finger after handling raw fish (19). The species identification in this study was based on the results from API Strep 20, along with partial sequencing of the 16S rRNA and sodA genes, none of which unequivocally discriminates S. dysgalactiae subsp. dysgalactiae from S. dysgalactiae subsp. equisimilis (2).

Our patient fulfilled the modified Duke criteria for definitive endocarditis (20), with one major criterion (evidence of endocardial involvement) and four minor criteria (microorganism consistent with IE, predisposing heart disease, fever, and septic embolization). The identification of this particular GCS endocarditis isolate as S. dysgalactiae subsp. dysgalactiae relied on a combination of phenotypic characteristics, MALDI-TOF MS, and 16S rRNA analysis, virtually excluding pathogens other than S. dysgalactiae subsp. dysgalactiae and S. dysgalactiae subsp. equisimilis, along with the lack of virulence genes typically present in S. dysgalactiae subsp. equisimilis, namely, ska, slo, scpA, dppA, lsp, and lmb (5, 6), the presence of rihC, previously only identified in S. dysgalactiae subsp. dysgalactiae, and finally an MLSA profile exactly matching that of an S. dysgalactiae subsp. dysgalactiae strain derived from a cow.

This case illustrates the zoonotic potential of S. dysgalactiae subsp. dysgalactiae. Despite a thorough, retrospective interview on animal exposure, the clinical history of the patient did not reveal the source and mode of transmission of the bacterium. The clinical course was complicated, and it cannot be excluded that his concurrent malignancy contributed to the severity of the infection.

We do not yet have any molecular data that can explain the virulence of our S. dysgalactiae subsp. dysgalactiae strain. A recent study comparing the genome contents of one S. dysgalactiae subsp. dysgalactiae isolate and two S. dysgalactiae subsp. equisimilis isolates showed a high degree of genetic similarity between them and the presence of two putative phages in S. dysgalactiae subsp. dysgalactiae with homology to M3 group A streptococcus (GAS) prophages, carrying possible virulence genes like those coding for hyaluronidase and streptodornase (7). That particular S. dysgalactiae subsp. dysgalactiae isolate also possessed putative integrative and conjugative elements (ICEs), which are known to be particularly abundant in Streptococcus agalactiae, another major agent of bovine mastitis. Hence, it appears that S. dysgalactiae subsp. dysgalactiae can accommodate both phages and ICEs from related streptococcal species, which might be crucial in the development of virulence attributes.

In conclusion, this first documented case of human bacteremia and endocarditis caused by S. dysgalactiae subsp. dysgalactiae calls for a broader molecular analysis of the bacterial isolate in order to explore the zoonotic potential and virulence of this pathogen.

REFERENCES
6. Rato MG, Nerlich A, Bergmann R, Beixiga R, Nunes SF, Vilela CL,


