New tricks from an old cow: Infective endocarditis caused by *Streptococcus dysgalactiae* subsp. *dysgalactiae*

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Running title: *Streptococcus dysgalactiae* subsp. *dysgalactiae* endocarditis

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New tricks from an old cow: Infective endocarditis caused by *Streptococcus dysgalactiae* subsp. *dysgalactiae*

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.

A sixty-five 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 seven 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 mmHg, 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 – 17.0), C-reactive protein 277 mg/l (<5),
leucocytes 20.8 x 10^9/l (3.5 – 11.0), neutrophils 18.5 x 10^9/l (1.7 – 8.2),

sedimentation rate 102 mm/h (0-20), procalcitonin 12.1 µg/L (<0,10) and troponin T 896 ng/l (<25). Thrombocytes were within normal range. The electrocardiogram (ECG) demonstrated ST-segment elevation in leads V1-V2 and T-inversion in leads V4-V6 indicative of ischemia.

Antibiotic therapy was started on day one, and included meropenem and vancomycin.

A broader initial regimen than recommended in the Norwegian National Antibiotic Guidelines was chosen since the patient had recently been admitted to hospital in Spain. The following day all four blood cultures grew non-hemolytic bacteria on blood agar.

Species identification was performed using 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, UK). The antimicrobial susceptibility testing showed that the GCS was fully susceptible to all tested antibiotics, with the following minimal inhibitory concentrations (mg/l): penicillin-G 0.008, ceftriaxone< 0.016, clindamycin 0.25, vancomycin 0.25, teicoplanin 0.25 and linezolid 1.

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. MRI of the brain and left shoulder showed septic embolization to both cerebral and cerebellar
hemispheres as well as fluid in left subdeltoideal bursa. Abdominal CT scan performed
after ten days confirmed embolization to the spleen after which clindamycin was
temporarily added to the antimicrobial regimen for two weeks to optimize abscess
penetration.

During the treatment course, a colonoscopy was performed due to persistent bloody
stools, and 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-IV. Cardiac CT showed normal coronary arteries and a significant
difference in heart-minute volume (5 l/min in left ventricle, 7.8 l/min 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 reducing the bleeding from his rectal tumor and treatment with
recombinant human erythropoietin helped correcting his anemia.

Two months after admission, he suffered from a fulminant 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 16rRNA sequencing on the valve specimens was
not performed. The patient rapidly recovered after surgery and received intravenous
antibiotic therapy during the first six post-operative weeks.

Four months after admission his rectal tumor, a differentiated adenocarcinoma, was
radically resected, with acute complications causing re-operation after which his
hemoglobin level was 3.3 g/dl at the lowest. His temporary ileostomy was removed six 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* (SDSE), which, together with *Streptococcus* belonging to the Anginosus group are responsible for the vast majority of human GCS infections (3,6,19). In order to assign correct streptococcal subgroup, the isolate was therefore subjected to selected molecular analyses:

First, 16S rRNA sequencing was performed with the following primers: 5’-CGG-CCC-AGA-CTC-CTA-CGG-GAG-GCW-GCA- 3’ (F-primer) and 5’-GGC-TGG-ACC-AGG-GTA-TCT-AAK-CC- 3’ (R-primer), under previously reported PCR conditions (9). This analysis confirmed that T534 was of the species *Streptococcus dysgalactiae*, with 99.7 % and 99.6% homology to SDSE and *Streptococcus dysgalactiae* subsp. *dysgalactiae* (SDSD), respectively. However, neither MALDI-TOF MS nor 16S rRNA sequencing could separate SDSD from SDSE.

Next, we performed targeted PCR on genes or gene segments previously reported to be frequently present in SDSE-isolates associated with human infections, and not in isolates belonging to SDSD; 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*) (15). As shown in table 1, none of those genes were detected in T534. Six SDSE-isolates associated endocarditis in western Norway were also tested, and all were PCR-positive for those six genes.
Based on the whole-genome sequence of a SDSD strain ATCC 27957 associated with bovine udder infection (17), primers targeting the *rihC*-gene were constructed, since a BLAST search of the *rihC*-sequence from this particular SDSD showed that it lacks significant homology with genes from streptococcal isolates associated with human infections.

T534 possessed a *rihC*-gene, and the DNA sequence showed 100% homology to that of the *rihC*-gene in SDSD from fish.

Finally, a multilocus sequence analysis (MLSA) scheme consisting of the seven housekeeping genes *map, pfl, ppaC, pyk, rpoB, sodA* and *tuf* was used, with primers and thermal profiles as previously described (1,6). A BLAST search of the trimmed consensus sequences of all these seven genes showed that T354 had identical MLSA profile with *Streptococcus dysgalactiae* subsp. *dysgalactiae* strain CCUG 27439, isolated from cow (6).

SDSD has until lately been regarded as an animal restricted pathogen, and is a principal cause of bovine mastitis (14). SDSD has also shown the ability to cause severe cellulitis and toxic shock syndrome in cattle (4), septicemia in fish and dogs (12,20) and infective arthritis in sheep (16). To our knowledge, SDSD possesses either Lancefield group antigen C or L, and most often grow large colonies with surrounding alpha-hemolysis, or lack of hemolysis, on blood agar (6,19). Human GCS infections, however, are most often caused by beta-hemolytic SDSE, or more rarely *Streptococcus* belonging to the Anginosus group, with a wide range of clinical manifestations, including severe soft tissue infections, primary bacteraemia, osteomyelitis, arthritis, pneumonia, meningitis, peritonitis, toxic shock syndrome and endocarditis (3).
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 1998-2009 was documented, leading to an incidence rate of 12.7 per 100,000 inhabitants in 2009 (2). 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 (11), whereas IE caused by GCS belonging to SDSE is indeed a rarity (3,7,18). To our knowledge, SDSD has never been associated with human carriage, and infection possibly caused by this bacterium has previously only been reported twice: SDSD was associated with a prosthetic joint infection after total knee arthroplasty in a case report from Korea. However, the mode of species identification of this bacterial isolate was not described (13). Furthermore, SDSD was associated with cellulitis in a patient with a poking injury of a finger after handling raw fish (8). 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 SDSD from SDSE (6).

Our patient fulfilled the modified Duke Criteria for definitive endocarditis (10), with one major criteria (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 SDSD relied on a combination of phenotypic characteristics, MALDI-TOF MS and 16s rRNA analysis, virtually excluding other pathogens than SDSD and SDSE, along with the lack of virulence genes typically present in SDSE, namely ska, slo, scpA, dppA, isp and lmb (5,15), the presence of rihC, previously only identified in SDSD, and finally an MLSA profile exactly matching that of an SDSD strain derived from cow.
This case illustrates the zoonotic potential of SDSD. 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 have not yet any molecular data that can explain the virulence of our SDSD-strain. A recent study, comparing the genome content of one SDSD and two SDSE showed a high degree of genetic similarity between them, and the presence of two putative phages in SDSD with homology to M3 GAS prophages, carrying possible virulence genes like hyaluronidase and streptodornase (17). That particular SDSD isolate also possessed putative integrative and conjugative (ICE) elements, which are known to be particularly abundant in *S. agalactiae*, another major agent of bovine mastitis. Hence, it appears that SDSD 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 SDSD calls for a broader molecular analysis of the bacterial isolate in order to explore the zoonotic potential and virulence of this pathogen.


### TABLE 1. Primer sequences used in PCR and presence of selected genes in SDSD and SDSE endocarditis isolates

<table>
<thead>
<tr>
<th>Primer target</th>
<th>Annealing temperature (°C)</th>
<th>Primer sequence (5’-3’)</th>
<th>Gene</th>
<th>Streptococci subspecies</th>
<th>Reference</th>
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<tbody>
<tr>
<td>ska (forward)</td>
<td>52</td>
<td>AGTCCAAAAATCAGAAAACATTT</td>
<td>ska</td>
<td>T534 - T111 - T277 - T315 - T422 - T481 - T527</td>
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</tr>
<tr>
<td>ska (reverse)</td>
<td>52</td>
<td>AAATTCTTGGACAGGGTGGG</td>
<td>ska</td>
<td>5</td>
<td></td>
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<tr>
<td>slo (forward)</td>
<td>72</td>
<td>ETTAATTTATTTGGATCGCTTTAC</td>
<td>slo</td>
<td>T534 - T111 - T277 - T315 - T422 - T481 - T527</td>
<td></td>
</tr>
<tr>
<td>slo (reverse)</td>
<td>72</td>
<td>TACCTTTAAGTTTGAAGGCC</td>
<td>slo</td>
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<tr>
<td>scpA (forward)</td>
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<td>CCCTTGAAGCTGCTTGGCGG</td>
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<td></td>
</tr>
<tr>
<td>scpA (reverse)</td>
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<td>TTAACCCTTGGCTCTTGCGC</td>
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<td>5</td>
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</tr>
<tr>
<td>dpA (forward)</td>
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<td>CCTCGTTAAGCTGCTTGGCGG</td>
<td>dpA</td>
<td>- + + + + + + + + + + + 15</td>
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</tr>
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<td>58</td>
<td>GAGCTGCTTGGCTCTTGCGC</td>
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<td>+ + + + + + + + + + + + 15</td>
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</tbody>
</table>

* One non-hemolytic group C streptococcus belonging to SDSD, **emm** - nontypeable
** Six beta-hemolytic group G streptococci associated with infective endocarditis in western Norway. All possessed **emm** genes belonging to **emm** types typical of SDSE.