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

Stina Jordal,^a Marte Glambek,^b Oddvar Oppegaard,^c Bård Reiakvam Kittang^{b,d}

Department of Microbiology, Haukeland University Hospital, Bergen, Norway^a; Department of Medicine, Haraldsplass Deaconess Hospital, Bergen, Norway^b; Department of Medicine, Haukeland University Hospital, Bergen, Norway^c; Department of Clinical Science, University of Bergen, Bergen, Norway^d

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, 20.8×10^9 /liter (3.5×10^9 to 11.0×10^9 /liter); neutrophils, 18.5×10^9 /liter (1.7×10^9 to 8.2×10^9 /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 inversion in leads V₄ 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 subdeltoid 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-

Received 27 August 2014 Returned for modification 30 September 2014

Accepted 25 November 2014

Accepted manuscript posted online 3 December 2014

Citation Jordal S, Glambek M, Oppegaard O, Kittang BR. 2015. New tricks from an old cow: infective endocarditis caused by *Streptococcus dysgalactiae* subsp. *dysgalactiae*. J Clin Microbiol 53:731–734. doi:10.1128/JCM.02437-14.

Editor: P. Bourbeau

Address correspondence to Bård Reiakvam Kittang, bki081@k2.uib.no.

Copyright © 2015, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JCM.02437-14

TABLE 1 Primer sequences used in PCR and the presence of selected genes in *S. dysgalactiae* subsp. *dysgalactiae* and subsp. *equisimilis* endocarditis isolates

			Result for <i>S. dysgalactiae</i> subsp.:							
Primer target gene and orientation	Annealing temp (°C)	Primer sequence (5'→3')	<i>dysgalactiae</i> T534 ^a	<i>equisimilis</i> ^b						Reference
				T111	T277	T315	T422	T481	T527	
<i>ska</i>										
Forward	52	AGTCCAAAATCAAAACCATTT	—	+	+	+	+	+	+	5
Reverse		AAATTCCTTGGACAGGTTGGG								
<i>slo</i>										
Forward	52	CTTATCCTATTTTCATACAC	—	+	+	+	+	+	+	5
Reverse		TACTTATAAGTAATCGAACC								
<i>scpA</i>										
Forward	52	CCATTTGATAAACTTGC	—	+	+	+	+	+	+	5
Reverse		TTAATCACCTTAGCTCCC								
<i>dppA</i>										
Forward	58	CCGTTATGGAGTCCACAATGAA	—	+	+	+	+	+	+	6
Reverse		ACTAGCTTTGAGTTTAATAGTAATC								
<i>isp</i>										
Forward	58	CAACTGAAAAAACCCAGAGCC	—	+	+	+	+	+	+	6
Reverse		GGTTGAAGTCAAAGGCACCATAA								
<i>lmb</i>										
Forward	58	AACCCCAAACAGCCTACGCAAG	—	+	+	+	+	+	+	6
Reverse		TAAAACGGGATCCGTCCAGGTAT								
<i>rihC</i>										
Forward	58	AGGTATTGATGACGCTACTGCT	+	—	—	—	—	—	—	This study
Reverse		TGAAACAGGTTTCGTAGGCTGA								

^a Shown are results for one nonhemolytic, *emm*-nontypeable GCS isolate belonging to *S. dysgalactiae* subsp. *dysgalactiae*.

^b 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*.

ducing the bleeding from his rectal tumor and treatment with recombinant human erythropoietin helped correct 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 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'-CGG-CCC-AGA-CTC-CTA-CGG-GAG-GCW-GCA-3' (F-primer) and 5'-GCG-TGG-ACT-ACC-AGG-GTA-TCT-AAK-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*), C5a 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.

T534 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*, *ppaC*, *pyk*, *rpoB*, *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 T534 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 rate of 12.7 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 carriage, and infection possibly 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 poking 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 em-

bolization). 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*, *isp*, 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

1. Broyles LN, van Beneden C, Beall B, Facklam R, Shewmaker PL, Malpiedi P, Daily P, Reingold A, Farley MM. 2009. Population-based study of invasive disease due to beta-hemolytic streptococci of groups other than A and B. *Clin Infect Dis* 48:706–712. <http://dx.doi.org/10.1086/597035>.
2. Jensen A, Kilian M. 2012. Delineation of *Streptococcus dysgalactiae*, its subspecies, and its clinical and phylogenetic relationship to *Streptococcus pyogenes*. *J Clin Microbiol* 50:113–126. <http://dx.doi.org/10.1128/JCM.05900-11>.
3. Vandamme P, Pot B, Falsen E, Kersters K, Devriese LA. 1996. Taxonomic study of Lancefield streptococcal groups C, G, and L (*Streptococcus dysgalactiae*) and proposal of *S. dysgalactiae* subsp. *equisimilis* subsp. nov. *Int J Syst Bacteriol* 46:774–781.
4. Kommedal O, Kvello K, Skjastad R, Langeland N, Wiker HG. 2009. Direct 16S rDNA sequencing from clinical specimens with special focus on poly-bacterial samples and interpretation of mixed DNA chromatograms. *J Clin Microbiol* 47:3562–3568. <http://dx.doi.org/10.1128/JCM.00973-09>.
5. Ikebe T, Murayama S, Saitoh K, Yamai S, Suzuki R, Isobe J, Tanaka D, Katsukawa C, Tamaru A, Katayama A, Fujinaga Y, Hoashi K, Watanabe H. 2004. Surveillance of severe invasive group-G streptococcal infections and molecular typing of the isolates in Japan. *Epidemiol Infect* 132:145–149. <http://dx.doi.org/10.1017/S0950268803001262>.
6. Rato MG, Nerlich A, Bergmann R, Bexiga R, Nunes SF, Vilela CL,

- Santos-Sanches I, Chhatwal GS. 2011. Virulence gene pool detected in bovine group C *Streptococcus dysgalactiae* subsp. *dysgalactiae* isolates by use of a group A *S. pyogenes* virulence microarray. *J Clin Microbiol* 49: 2470–2479. <http://dx.doi.org/10.1128/JCM.00008-11>.
7. Suzuki H, Lefebvre T, Hubisz MJ, Bitar PP, Lang P, Siepel A, Stanhope MJ. 2011. Comparative genomic analysis of the *Streptococcus dysgalactiae* species group: gene content, molecular adaptation, and promoter evolution. *Genome Biol Evol* 3:168–185. <http://dx.doi.org/10.1093/gbe/evr006>.
8. Bishop CJ, Aanensen DM, Jordan GE, Kilian M, Hanage WP, Spratt BG. 2009. Assigning strains to bacterial species via the internet. *BMC Biol* 7:3. <http://dx.doi.org/10.1186/1741-7007-7-3>.
9. Rato MG, Bexiga R, Florindo C, Cavaco LM, Vilela CL, Santos-Sanches I. 2013. Antimicrobial resistance and molecular epidemiology of streptococci from bovine mastitis. *Vet Microbiol* 161:286–294. <http://dx.doi.org/10.1016/j.vetmic.2012.07.043>.
10. Chenier S, Leclerc M, Messier S, Fecteau G. 2008. *Streptococcus dysgalactiae* cellulitis and toxic shock like syndrome in a Brown Swiss cow. *J Vet Diagn Invest* 20:99–103. <http://dx.doi.org/10.1177/104063870802000122>.
11. Netto LN, Leal CA, Figueiredo HC. 2011. *Streptococcus dysgalactiae* as an agent of septicemia in Nile tilapia, *Oreochromis niloticus* (L.). *J Fish Dis* 34:251–254. <http://dx.doi.org/10.1111/j.1365-2761.2010.01220.x>.
12. Vela AI, Falsen E, Simarro I, Rollan E, Collins MD, Dominguez L, Fernandez-Garayzabal JF. 2006. Neonatal mortality in puppies due to bacteremia by *Streptococcus dysgalactiae* subsp. *dysgalactiae*. *J Clin Microbiol* 44:666–668. <http://dx.doi.org/10.1128/JCM.44.2.666-668.2006>.
13. Rutherford SJ, Rycroft AN, Ridler AL. 2014. Sources of *Streptococcus dysgalactiae* in English and Welsh sheep flocks affected by infectious arthritis (joint ill). *Vet Rec* 174:579. <http://dx.doi.org/10.1136/vr.101753>.
14. Bor DH, Woolhandler S, Nardin R, Brusch J, Himmelstein DU. 2013. Infective endocarditis in the U.S., 1998–2009: a nationwide study. *PLoS One* 8:e60033. <http://dx.doi.org/10.1371/journal.pone.0060033>.
15. Murdoch DR, Corey GR, Hoen B, Miro JM, Fowler VG, Jr, Bayer AS, Karchmer AW, Olaison L, Pappas PA, Moreillon P, Chambers ST, Chu VH, Falco V, Holland DJ, Jones P, Klein JL, Raymond NJ, Read KM, Tripodi MF, Utili R, Wang A, Woods CW, Cabell CH. 2009. Clinical presentation, etiology, and outcome of infective endocarditis in the 21st century: the International Collaboration on Endocarditis-Prospective Cohort Study. *Arch Intern Med* 169:463–473. <http://dx.doi.org/10.1001/archinternmed.2008.603>.
16. Kittang BR, Bruun T, Langeland N, Mylvaganam H, Glambeek M, Skrede S. 2011. Invasive group A, C and G streptococcal disease in western Norway: virulence gene profiles, clinical features and outcomes. *Clin Microbiol Infect* 17:358–364. <http://dx.doi.org/10.1111/j.1469-0691.2010.03253.x>.
17. Takahashi T, Sunaoshi K, Sunakawa K, Fujishima S, Watanabe H, Ubukata K. 2010. Clinical aspects of invasive infections with *Streptococcus dysgalactiae* ssp. *equisimilis* in Japan: differences with respect to *Streptococcus pyogenes* and *Streptococcus agalactiae* infections. *Clin Microbiol Infect* 16:1097–1103. <http://dx.doi.org/10.1111/j.1469-0691.2009.03047.x>.
18. Park MJ, Eun IS, Jung CY, Ko YC, Kim YJ, Kim CK, Kang EJ. 2012. *Streptococcus dysgalactiae* subspecies *dysgalactiae* infection after total knee arthroplasty: a case report. *Knee Surg Relat Res* 24:120–123. <http://dx.doi.org/10.5792/ksrr.2012.24.2.120>.
19. Koh TH, Sng LH, Yuen SM, Thomas CK, Tan PL, Tan SH, Wong NS. 2009. Streptococcal cellulitis following preparation of fresh raw seafood. *Zoonoses Public Health* 56:206–208. <http://dx.doi.org/10.1111/j.1863-2378.2008.01213.x>.
20. Li JS, Sexton DJ, Mick N, Nettles R, Fowler VG, Jr, Ryan T, Bashore T, Corey GR. 2000. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. *Clin Infect Dis* 30:633–638. <http://dx.doi.org/10.1086/313753>.