JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2003, p. 613–618 Vol. 41, No. 2
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.2.613–618.2003

Analysis of a Viridans Group Strain Reveals a Case of Bacteremia Due to Lancefield Group G Alpha-Hemolytic *Streptococcus dysgalactiae* subsp. *equisimilis* in a Patient with Pyomyositis and Reactive Arthritis

Patrick C. Y. Woo,[1] Jade L. L. Teng,[1] Susanna K. P. Lau,[1] Peggy N. L. Lum,[1] Kit-Wah Leung,[1] Kee-Lam Wong,[2] Kin-Wah Li,[2] Kui-Chun Lam,[2] and Kwok-Yung Yuen[1,3]*

Department of Microbiology, The University of Hong Kong,[1] Hong Kong Sanatorium and Hospital,[2] and HKU-Pasteur Research Centre,[3] Hong Kong

Received 9 July 2002/Returned for modification 25 August 2002/Accepted 13 November 2002

***Streptococcus dysgalactiae* is classified by a combination of phenotypic and genotypic characteristics into Lancefield group C alpha-hemolytic *Streptococcus dysgalactiae* subsp. *dysgalactiae* and Lancefield group C, group G, and group L beta-hemolytic *Streptococcus dysgalactiae* subsp. *equisimilis*. In this study, we report the isolation of a catalase-negative, alpha-hemolytic, optochin- and bacitracin-resistant viridans group strain, which does not grow in 10 or 40% bile, on MacConkey agar or bile esculin agar, or in 6% NaCl, from the blood culture of a 73-year-old woman with pyomyositis and poststreptococcal reactive arthritis. Lancefield grouping revealed that the strain was a group G streptococcus. The Vitek system (GPI) showed that it was unidentified, and the API system (20 STREP) showed that it was 95.7% *S. dysgalactiae* subsp. *dysgalactiae*. 16S rRNA gene sequencing showed that it was a strain of *S. dysgalactiae*. Based on phylogenetic affiliation with 16S rRNA gene or GroEL amino acid (another bacterial gene, in addition to 16S rRNA gene, that is highly conserved) sequences, the strain is most closely related to Lancefield group C beta-hemolytic *S. dysgalactiae* subsp. *equisimilis*. PCR amplification and sequencing of the streptolysin S structural gene (*sagA*) and M protein gene (*emm*) hypervariable region showed the presence of these suspected primary virulence factors. Further studies would delineate whether the isolate is just a hemolysin-deficient variant of group G beta-hemolytic *S. dysgalactiae* subsp. *equisimilis* or a novel type of *S. dysgalactiae*. The present case showed that group G alpha-hemolytic *S. dysgalactiae* subsp. *equisimilis* can be associated with serious invasive infection and poststreptococcal sequelae.**

Streptococcus dysgalactiae is classified by a combination of phenotypic and genotypic characteristics into four types: Lancefield group C alpha-hemolytic *Streptococcus dysgalactiae* subsp. *dysgalactiae*, Lancefield group C beta-hemolytic *Streptococcus dysgalactiae* subsp. *equisimilis*, Lancefield group G beta-hemolytic *S. dysgalactiae* subsp. *equisimilis*, and Lancefield group L beta-hemolytic *S. dysgalactiae* subsp. *equisimilis* (19). The natural reservoirs of group C alpha-hemolytic *S. dysgalactiae* subsp. *dysgalactiae* are animals such as cows and sheep. It mainly causes mastitis in cows and suppurative polyarthritis in lambs, but it only occasionally causes infections in humans (20). On the other hand, the natural reservoirs of *S. dysgalactiae* subsp. *equisimilis* are humans, and these streptococci are major causes of streptococcal infections in humans. In a recent study of 75 group G beta-hemolytic streptococcal strains isolated from the blood cultures of 66 patients, we discovered that all 75 strains were *S. dysgalactiae* subsp. *equisimilis*, with 16S rRNA gene sequencing as the "gold standard" (22).

Hemolysin production has always been described as associated with virulence, and it was shown that the beta-hemolytic phenotype of group G *S. dysgalactiae* subsp. *equisimilis*, produced by streptolysin S and encoded by a functional homologue of the 9-gene group A streptococcus *sag* operon, contributes to the pathogenesis of streptococcal necrotizing soft tissue infection (6). On the other hand, a hemolysin-deficient variant of group G *S. dysgalactiae* subsp. *equisimilis* has recently been recovered from the throat culture of a patient with pharyngitis, and it was suggested by the authors that these hemolysin-deficient variants may be overlooked as etiological agents of streptococcal infections (4). In this study, we report the isolation of a group G alpha-hemolytic streptococcus from the blood culture of a patient with pyomyositis and reactive arthritis. The strain, named HKU7, exhibited phenotypic characteristics that do not fit into patterns of any known species. 16S rRNA gene sequencing showed that there was more than 99% base identity between the 16S rRNA gene of HKU7 and those of other strains of *S. dysgalactiae*. Further phenotypic and genotypic characterization showed that this bacterium is a strain of Lancefield group G alpha-hemolytic *S. dysgalactiae* subsp. *equisimilis*.

CASE REPORT

A 73-year-old Chinese woman was admitted to the hospital in September 2000 because of bilateral hip, knee, and shoulder pain for 2 weeks. She had well-controlled diabetes mellitus, a

* Corresponding author. Mailing address: Department of Microbiology, The University of Hong Kong, University Pathology Building, Queen Mary Hospital, Hong Kong. Phone: (852) 28554892. Fax: (852) 28551241. E-mail: hkumicro@hkucc.hku.hk.

history of carcinoma of the rectum, with abdominal perineal resection 5 years ago, and carcinoma of the cervix, with hysterectomy and radiotherapy 20 years ago. She was afebrile upon admission. An examination revealed left hip tenderness and limited range of movement in the bilateral shoulder and hip joints but no obvious swelling or effusion. Radiographs of the shoulders and hips showed only degenerative changes. The total white cell count was 11.9×10^9/liter, with a neutrophil count of 10.9×10^9/liter, a lymphocyte count of 0.7×10^9/liter, and a monocyte count of 0.3×10^9/liter. The hemoglobin level was 12.5 g/dl, the platelet count was 191×10^9/liter, the erythrocyte sedimentation rate was 81 mm/h, and the C-reactive protein level was 33.6 mg/dl. Liver and renal function tests were within normal limits, except for a low serum albumin level of 33 g/liter. She developed acute upper gastrointestinal bleeding 1 week after admission. Urgent upper endoscopy revealed multiple bleeding ulcers in the stomach and duodenum, which were related to the nonsteroidal anti-inflammatory drugs prescribed for her pain relief. The bleeding was settled, but she developed fever and septicemic shock 2 days later. Blood culture was performed, and treatment with empirical intravenous piperacillin-tazobactam was commenced. Tc-99m bone scintigraphy on the same day showed increase tracer uptake in the L1/L2, L5/S1, left sacroiliac, and left hip joints and in both elbow, knee, and ankle joints, which can be compatible with degeneration or arthritis.

On day 1 postincubation, the aerobic blood culture bottle turned positive for a gram-positive coccus (HKU7). Her fever and sepsis initially responded to piperacillin-tazobactam. Two days later, she developed progressive shortness of breath. A chest radiograph showed the new onset of a globular heart and bilateral pleural effusion. A transthoracic echocardiogram confirmed pericardial effusion with a tamponade effect. Urgent pericardiocentesis was performed, and 50 ml of blood-stained pericardial fluid was aspirated. The fluid was sent for bacterial culture and cytology, but the results were negative. The pleural and pericardial effusions resolved, but the joint pain persisted. Despite maintenance oral antibiotics, she developed a new swelling over the posterior aspect of her right thigh. There was also acute synovitis and arthritis of both shoulders and knees with prominent effusions. The total white cell count was 10.1×10^9/liter, the erythrocyte sedimentation rate was 107 mm/h, and the C-reactive protein level was 9.6 mg/dl. The levels of C3 and C4 in serum were within normal limits. The rheumatoid factor was negative, and the anti-nuclear antibody titer was 1/80 with a speckled pattern. Aspiration of the right thigh mass yielded 2 ml of thick pus. A Gram smear of the pus showed numerous white blood cells, but no bacteria, fungi, or mycobacteria were recovered. All Gram smears and cultures from left knee and left shoulder aspirates were also negative. Owing to the progressive right thigh swelling, a magnetic resonance imaging scan was performed and showed a large abscess of 15 by 7 by 6 cm, involving the muscles and subcutaneous tissue of the right buttock and thigh (Fig. 1). The abscess was drained, and intravenous azithromycin and cefazolin were administered. Although the arthritis and abscess of the right thigh gradually subsided, small subcutaneous abscesses subsequently appeared over her right buttock and left thigh. Drainage was performed, but microbiological studies of the drained pus from all abscesses were negative. The final diagnosis was streptococcal bacteremia, pyomyositis, and reactive arthritis. The patient responded to oral salazopyrin and hydroxychloroquine and remained asymptomatic, with no recurrence of abscess or arthritis, up to the time of writing, 1 year from discharge.

MATERIALS AND METHODS

Patient and microbiological methods. All clinical data were collected prospectively as described in a previous publication (14). The BACTEC 9240 blood culture system (Becton Dickinson, Sparks, Md.) was used. The bacterium was identified by standard conventional biochemical methods (15). Lancefield serogrouping was performed by using Streptex (Murex Biotech Ltd., Dartford, United Kingdom) according to the manufacturer's instructions. All tests were performed in triplicate with freshly prepared media on separate occasions. In addition, the Vitek system (GPI) (bioMerieux Vitek, Hazelwood, Mo.) and the API system (20 STREP) (bioMerieux Vitek) were used for the identification of the bacterial isolate in this study. Antimicrobial susceptibility was tested by E-test for penicillin and by the Kirby Bauer disk diffusion method for the other antibiotics, and results were interpreted according to the NCCLS criteria for alpha-hemolytic streptococci.

Bacterial DNA extraction, PCR amplification, and 16S rRNA gene sequencing. Bacterial DNA extraction, PCR amplification, and DNA sequencing of the 16S rRNA genes were performed according to methods described in previous publications (3, 10–12, 21–35). LPW200 (5′-GAGTTGCGAACGGGTGAG-3′) and LPW205 (5′-CTTGTTACGACTTCACCC-3′) (Gibco BRL, Rockville, Md.) were used as the PCR primers, and LPW200, LPW205, LPW99 (5′-TTATTGGGCGTAAAGCGA-3′), and LPW273 (5′-TTGCGGGACTTAACCCAAC-3′) were used as the sequencing primers. The sequences of the PCR products were compared with known 16S rRNA gene sequences in the GenBank database by multiple-sequence alignment with the CLUSTAL W program (17).

Streptolysin S structural gene (*sagA*) sequencing. PCR amplification and DNA sequencing of the *sagA* gene of HKU7 were performed according to the method described in a previous publication (6), with LPW614 (5′-ATKARAAAGAAAGGGTTTACAT-3′) and LPW615 (5′-CATATAGTAATTAGCAGGTAC-3′) as the PCR and sequencing primers.

M protein gene (*emm*) hypervariable region sequencing. PCR amplification and DNA sequencing of the *emm* gene hypervariable region of HKU7 were performed according to the method described in a previous publication (16), with LPW616 (5′-ATAAGGAGCATAAAAATGGCT-3′) and LPW617 (5′-AGCTTAGTTTTCTTCTTTGCG-3′) as the PCR and sequencing primers.

Cloning and sequencing of *groEL* genes. Cloning and sequencing of the *groEL* genes of HKU7, Lancefield group C alpha-hemolytic *S. dysgalactiae* subsp. *dysgalactiae* (ATCC 43078), Lancefield group C beta-hemolytic *S. dysgalactiae* subsp. *equisimilis* (ATCC 35666), Lancefield group G beta-hemolytic *S. dysgalactiae* subsp. *equisimilis* (ATCC 12394), and Lancefield group L beta-hemolytic *S. dysgalactiae* subsp. *equisimilis* (CIP55-123) were performed according to the following protocol. The bacterial DNA extracts were amplified with 0.5 μM primers (LPW368 [5′-ATTTTCAKCAGATGCNMG-3′] and LPW369 [5′-ACDACDGCTTCKGTDGTYA-3′]) (Gibco BRL). The PCR mixture (50 μl) contained bacterial DNA, PCR buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 2 mM $MgCl_2$, and 0.01% gelatin), 200 μM concentrations of each deoxynucleoside triphosphate, and 1.0 U of *Taq* polymerase (Boehringer Mannheim, Mannheim, Germany). The mixtures were amplified in 40 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min, and a final extension at 72°C for 10 min in an automated thermal cycler (Perkin-Elmer Cetus, Gouda, The Netherlands). The PCR products were gel purified and sequenced as described previously (35), with LPW368, LPW369, LPW385 (5′-ATGGTCCACAGACAATGAA-3′), LPW513 (5′-ATACTGTTGCAGTTGTGG-3′), and LPW514 (5′-GCTCTTGAGCTTGATGGC-3′) as the sequencing primers.

Phylogenetic characterization. The phylogenetic relationships between strain HKU7 and the other *Streptococcus* species and subspecies were determined by using PileUp and the neighbor-joining method with GrowTree (Genetics Computer Group, Inc.). A total of 1,373 nucleotide positions of the 16S rRNA genes were included in the analysis.

Nucleotide sequence accession number. The 16S rRNA and *groEL* gene sequences of HKU7, Lancefield group C alpha-hemolytic *S. dysgalactiae* subsp. *dysgalactiae* (ATCC 43078), Lancefield group C beta-hemolytic *S. dysgalactiae* subsp. *equisimilis* (ATCC 35666), Lancefield group G beta-hemolytic *S. dysgalactiae* subsp. *equisimilis* (ATCC 12394), and Lancefield group L beta-hemolytic *S. dysgalactiae* subsp. *equisimilis* (CIP55-123) have been logged within the GenBank sequence database under accession no. AF433167, AY121359, AY121360,

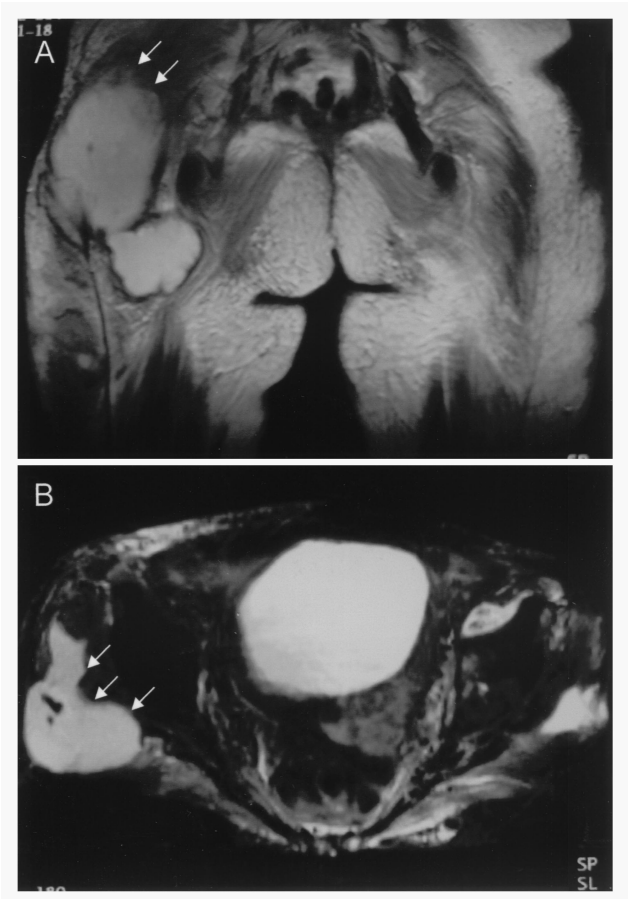

FIG. 1. Coronal (A) and axial (B) T1- and T2-weighted magnetic resonance imaging of the pelvis showing a multiloculated abscess (arrows) in the subcutaneous area of the right buttock.

AY121361, AY121362, AY121367, AY121363, AY121364, AY121365, and AY121366, respectively.

RESULTS

Phenotypic characteristics. Strain HKU7 is a gram-positive, non-spore-forming coccus arranged in chains. It grows on sheep blood agar as alpha-hemolytic, gray colonies of 0.5 to 1 mm in diameter after 24 h of incubation at 37°C in ambient air. No enhancement of growth is observed in 5% CO_2. It also grows on chocolate agar in a microaerophilic or anaerobic environment, but not in 10 or 40% bile, on MacConkey agar or bile esculin agar, or in 6% NaCl. It was catalase negative, alpha-hemolytic, and optochin resistant. Lancefield grouping of the strain showed that it belonged to Lancefield group G. It is resistant to optochin and bacitracin and is nonmotile at both 25 and 37°C. The biochemical profile of strain HKU7 is shown in Table 1. The Vitek system (GPI) showed that it was unidentified, and the API system (20 STREP) showed that it was 95.7% identical to *S. dysgalactiae* subsp. *dysgalactiae* and 4.1% identical to *S. dysgalactiae* subsp. *equisimilis*. It is sensitive to penicillin (MIC = 0.012 μg/ml), cefepime, clindamycin, erythromycin, tetracycline, and vancomycin.

Molecular characterization by 16S rRNA gene and *groEL* gene sequencing and phylogenetic characterization. PCR of the 16S rRNA gene of strain HKU7 showed a band at about 1,470 bp. There was no difference between the 16S rRNA gene sequence of strain HKU7 and that of Lancefield group C beta-hemolytic *S. dysgalactiae* subsp. *equisimilis* (GenBank accession number AY121360) or Lancefield group G beta-hemolytic *S. dysgalactiae* subsp. *equisimilis* (GenBank accession number AY121361), there were 3 base differences between the 16S rRNA gene sequence of strain HKU7 and that of Lancefield group L beta-hemolytic *S. dysgalactiae* subsp. *equisimilis*

TABLE 1. Biochemical profile of strain HKU7 by conventional biochemical tests and Vitek GPI and API 20 STREP systems

Biochemical reactions or enzyme	Result with test[a]		
	Conventional	Vitek GPI[b]	API 20 STREP[c]
Catalase	−	−	
Resistance to bacitracin	+	+	
Resistance to optochin	+	+	
Growth in 6% NaCl	−	−	
Growth in 10% bile		−	
Growth in 40% bile	−	−	
Esculin hydrolysis	−	−	−
Hippurate hydrolysis	−		−
Arginine hydrolysis	+	+	+
Lysine decarboxylase	−		
Ornithine decarboxylase	−		
Urease	−	−	
Voges-Proskauer test	−		−
Tetrazolium reduction		+	
Resistance to novobiocin	+	+	
Resistance to polymyxin B	+		
Utilization of:			
Hemicellulase		+	
Dextrose		+	
Lactose	+	+	+
Mannitol	−	−	−
Raffinose	−	−	−
Salicin	+	+	
Sorbitol	−	−	−
Sucrose	+	+	
Trehalose	+	+	+
Arabinose	−	−	−
Pyruvate		−	
Pullulan		+	
Inulin	−	−	−
Melibiose	−	−	
Melezitose		−	
Cellobiose	+	+	
Ribose	+	+	+
Xylose	−	−	
D-Glucose	+		
D-Mannose	+		
Maltose	+		
Starch			+
Glycogen			−
Pyrrolidonylarylamidase			−
α-Galactosidase			−
β-Glucuronidase			+
β-Galactosidase			−
Leucine arylamidase			+
Nitrate reduction	−		
Alkaline phosphatase			+

[a] −, negative; +, positive.
[b] The Vitek GPI System did not identify the strain.
[c] The API 20 STREP system identified the strain as 95.7% *S. dysgalactiae* subsp. *dysgalactiae* and 4.1% *S. dysgalactiae* subsp. *equisimilis.*

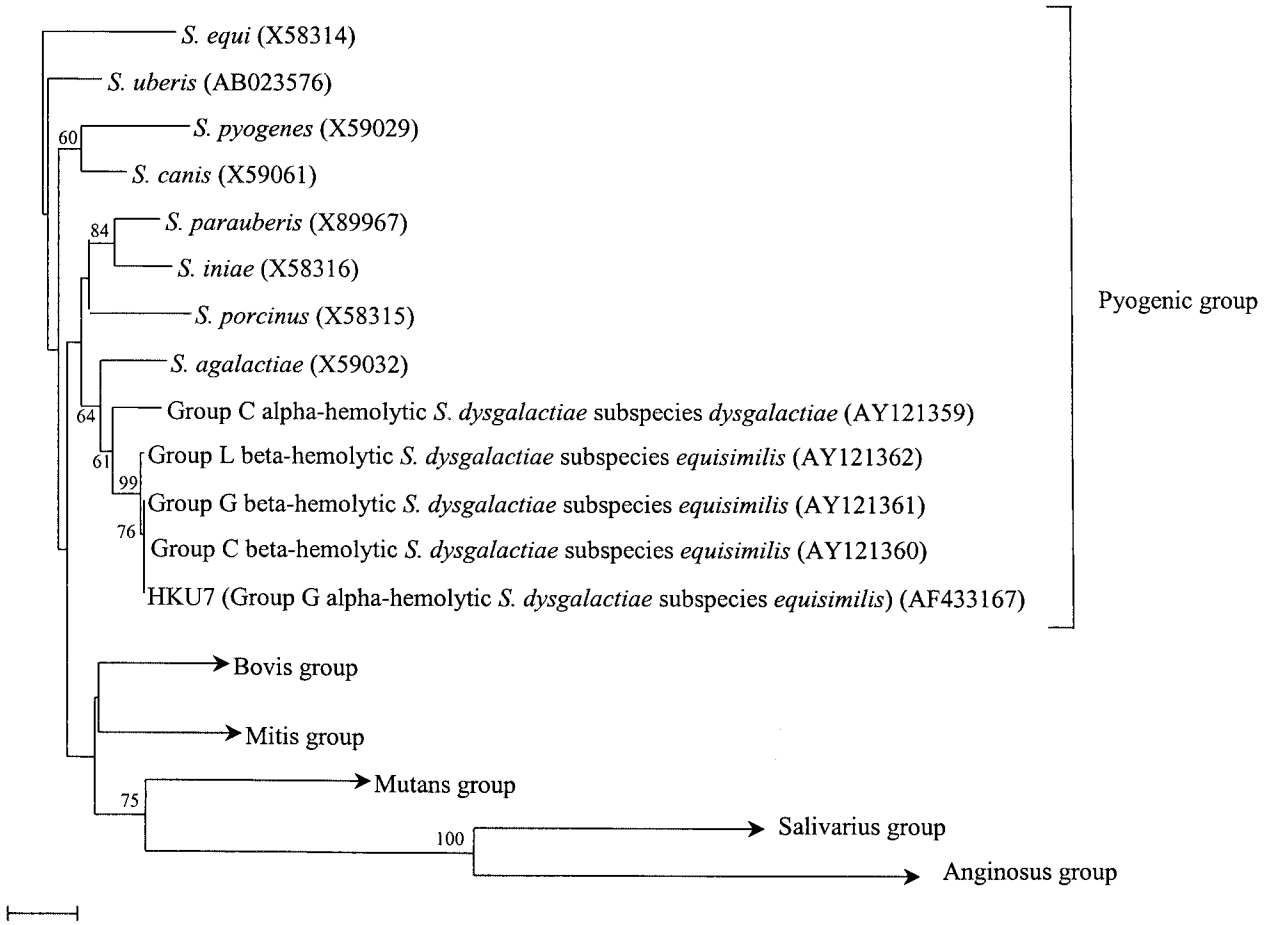

FIG. 2. Phylogenetic tree showing the relationships of Lancefield group G alpha-hemolytic *S. dysgalactiae* subsp. *equisimilis* to other types of *S. dysgalactiae* and other streptococci. The tree was inferred from 16S rRNA data by the neighbor-joining method, and bootstrap values were calculated from 1,000 trees. The scale bar indicates the estimated number of substitutions per 100 bases with the Jukes-Cantor correction. Names and accession numbers (in parentheses) are given as cited in the GenBank database.

(GenBank accession number AY121362), and there were 14 base differences between the 16S rRNA gene sequence of strain HKU7 and that of Lancefield group C alpha-hemolytic *S. dysgalactiae* subsp. *dysgalactiae* (GenBank accession number AY121359), indicating that the isolate was a strain of *S. dysgalactiae*. As for the GroEL amino acid sequences, there was no difference between the GroEL amino acid sequence of strain HKU7 and that of Lancefield group C beta-hemolytic *S. dysgalactiae* subsp. *equisimilis* (GenBank accession number AY121360) but there was 1 amino acid difference between the GroEL amino acid sequence of strain HKU7 and that of Lancefield group G beta-hemolytic *S. dysgalactiae* subsp. *equisimilis* (GenBank accession number AY121361), Lancefield group L beta-hemolytic *S. dysgalactiae* subsp. *equisimilis* (GenBank accession number AY121362), and Lancefield group C alpha-hemolytic *S. dysgalactiae* subsp. *dysgalactiae* (GenBank accession number AY121359). Based on phylogenetic affiliation with the 16S rRNA gene (Fig. 2) and GroEL amino acid sequences, HKU7 is most closely related to Lancefield group C beta-hemolytic *S. dysgalactiae* subsp. *equisimilis*.

Streptolysin S structural gene (*sagA*) sequencing. PCR of the *sagA* gene of HKU7 showed a band at about 500 bp. There was no difference between the predicted amino acid sequence of the product of the *sagA* gene of strain HKU7 and that of *S. dysgalactiae* subsp. *equisimilis* (GenBank accession number AY033399) (6).

M protein gene (*emm*) hypervariable region sequencing. PCR of the *emm* gene hypervariable region of HKU7 showed a band at about 1,600 bp. There was no difference between the nucleotide sequence of the *emm* gene hypervariable region of HKU7 and that of a novel *emm* gene described recently (GenBank accession number AF485842).

DISCUSSION

In 1936, *S. dysgalactiae* subsp. *equisimilis* was first proposed for the description of a group of Lancefield group C beta-hemolytic streptococci isolated from the pharynx, nose, skin, and vagina of humans (5). Subsequently, *S. dysgalactiae* subsp. *dysgalactiae*, which was shown to be similar to *S. dysgalactiae* subsp. *equisimilis* except for the absence of beta-hemolysis, was recovered from cattle (2). In the next 40 years, numerous strains of a combination of Lancefield serogroups C, G, and L and alpha-hemolysis, beta-hemolysis, and no hemolysis were

TABLE 2. Comparison of characteristics of HKU7, Lancefield group C alpha-hemolytic *S. dysgalactiae* subsp. *dysgalactiae,* Lancefield group C beta-hemolytic *S. dysgalactiae* subsp. *equisimilis,* Lancefield group G beta-hemolytic *S. dysgalactiae* subsp. *equisimilis,* and Lancefield group L beta-hemolytic *S. dysgalactiae* subsp. *equisimilis*

Characteristic	Results[a] for:				
	HKU7	Lancefield group C alpha-hemolytic *S. dysgalactiae* subsp. *dysgalactiae*	Lancefield group C beta-hemolytic *S. dysgalactiae* subsp. *equisimilis*	Lancefield group G beta-hemolytic *S. dysgalactiae* subsp. *equisimilis*	Lancefield group L beta-hemolytic *S. dysgalactiae* subsp. *equisimilis*
Hemolysis type	Alpha	Alpha	Beta	Beta	Beta
Lancefield group	G	C	C	G	L[b]
Hippurate hydrolysis	−	−	−	−	+
Acid from glycogen	−	−	−	−	+
Acid from sorbitol	−	+	−	−	−
β-D-Galactosidase	−	−	+	+	+
Bacitracin resistance	+	−	+	+	−

[a] −, negative; +, positive.
[b] Cross-reacts with Lancefield group A antiserum.

recovered from humans and animals. Vandamme et al. (in 1996) and Vieira et al. (in 1998) employed a combination of 16S rRNA gene sequencing, DNA-DNA hybridization, and multilocus enzyme electrophoresis to elucidate the relationships of these streptococci (18, 19). This tremendously improves our understanding of the epidemiology of this phenotypically heterogeneous group of streptococci. Using 16S rRNA gene sequence data, members of this heterogeneous group of streptococci were all classified under the species *S. dysgalactiae*, within the pyogenic group of the genus *Streptococcus* (9). With the help of additional information derived from DNA-DNA hybridization and multilocus enzyme electrophoresis, *S. dysgalactiae* was further classified into 2 subspecies and 4 types: Lancefield group C alpha-hemolytic *S. dysgalactiae* subsp. *dysgalactiae*, Lancefield group C beta-hemolytic *S. dysgalactiae* subsp. *equisimilis*, Lancefield group G beta-hemolytic *S. dysgalactiae* subsp. *equisimilis*, and Lancefield group L beta-hemolytic *S. dysgalactiae* subsp. *equisimilis* (19).

We report the isolation of HKU7, a Lancefield group G alpha-hemolytic *S. dysgalactiae* isolate, from the blood culture of a Chinese patient with pyomyositis and reactive arthritis, although the bacterium was not recovered from the abscess, probably due to the administration of antibiotics 48 h before the operative collection of the abscess pus. Further characterization showed that the suspected primary virulence factors (*sagA* and *emm* genes) were also present. Similar to other patients with group G beta-hemolytic streptococcal bacteremia (22), our patient also had major underlying diseases, including diabetes mellitus, carcinoma of the rectum, and carcinoma of the cervix. Our patient developed a nonmigratory sterile arthritis and synovitis shortly after the bacteremia, which responded poorly to nonsteroidal anti-inflammatory agents but well to more-potent antirheumatic agents, salazopyrine and hydroxychloroquine. This is compatible with the well-recognized syndrome of poststreptococcal reactive arthritis that typically follows Lancefield group A streptococcal throat infections (1, 7, 13). Although a similar syndrome has also been reported after throat infections with Lancefield group C and group G streptococci, all of the culture-proven cases were due to beta-hemolytic streptococci (8). The present report represents the first case of poststreptococcal reactive arthritis that follows an alpha-hemolytic streptococcal infection. While hemolysins of beta-hemolytic streptococci were shown to contribute to their virulence, the overwhelming clinical manifestations of the present case suggest that alpha-hemolytic streptococci are able to cause similar clinical syndromes.

Lancefield grouping of viridans group streptococci may uncover important streptococcal isolates. In clinical microbiology laboratories, for alpha-hemolytic streptococci, Lancefield grouping is only performed on *Streptococcus bovis* (Lancefield group D), *Streptococcus suis* (Lancefield group R which may cross-react with Lancefield group D), members of the *Streptococcus milleri* group (Lancefield group A, C, F, or G), and the enterococci (Lancefield group D). Lancefield grouping is not performed for other viridans group streptococci. This would miss not only the isolate described in the present study but also some other important streptococcal isolates, such as the hemolysin-deficient group A, C, and G streptococci (4). A study of Lancefield grouping of clinical isolates of alpha-hemolytic streptococci would reveal the usefulness of routine Lancefield grouping of the viridans group streptococci.

Further studies could be performed to delineate whether HKU7 is just a hemolysin-deficient variant of group G beta-hemolytic *S. dysgalactiae* subsp. *equisimilis* or a novel type of *S. dysgalactiae*. For the hemolysin-deficient group G *S. dysgalactiae* subsp. *equisimilis* described recently, the strain was identified both phenotypically by the API system (20 STREP) and genotypically by 16S rRNA gene sequencing as *S. dysgalactiae* subsp. *equisimilis*. Genotypically, the 16S rRNA gene of HKU7 exhibited more than 99% nucleotide identity with the 16S rRNA gene of all previously described bacterial strains of *S. dysgalactiae*. Based on both 16S rRNA gene sequence data and GroEL amino acid sequence data, the type most closely related to HKU7 is Lancefield group C beta-hemolytic *S. dysgalactiae* subsp. *equisimilis*, and it is clustered with the other types of *S. dysgalactiae* subsp. *equisimilis*. On the other hand, besides hemolysis on sheep blood agar and Lancefield serogrouping, HKU7 exhibited additional phenotypic characteristics that are different from the other types of *S. dysgalactiae* subsp. *equisimilis* and *S. dysgalactiae* subsp. *dysgalactiae* (Table 2). Lancefield group L beta-hemolytic *S. dysgalactiae* subsp. *equisimilis*, but not the other types, hydrolyzes hippurate and produces acid from glycogen. Lancefield group C alpha-hemolytic *S. dysgalactiae* subsp. *dysgalactiae*, but not the other types, pro-

duces acid from sorbitol. Lancefield groups C, G, and L beta-hemolytic *S. dysgalactiae* subsp. *equisimilis*, but not HKU7 or Lancefield group C alpha-hemolytic *S. dysgalactiae* subsp. *dysgalactiae*, produce β-D-galactosidase. HKU7 and Lancefield groups C and G beta-hemolytic *S. dysgalactiae* subsp. *equisimilis*, but not Lancefield group C alpha-hemolytic *S. dysgalactiae* subsp. *dysgalactiae* or Lancefield group L beta-hemolytic *S. dysgalactiae* subsp. *equisimilis*, are resistant to bacitracin. In fact, HKU7 was identified as *S. dysgalactiae* subsp. *dysgalactiae* by the API 20 STREP kit, but none of the 66 Lancefield group G beta-hemolytic *S. dysgalactiae* subsp. *equisimilis* strains that were reported previously was identified as *S. dysgalactiae* subsp. *dysgalactiae* by the API 20 STREP kit. Although whether HKU7 represents a novel type of *S. dysgalactiae* remains to be determined, the present case showed that group G alpha-hemolytic *S. dysgalactiae* subsp. *equisimilis* can be associated with serious invasive infection and poststreptococcal sequelae.

ACKNOWLEDGMENTS

This work is partly supported by the University Development Fund, University Research Grant Council, and the Committee for Research and Conference Grants, The University of Hong Kong.

REFERENCES

1. **Ayoub, E. M., and H. A. Majeed.** 2000. Postreptococcal reactive arthritis. Curr. Opin. Rheumatol. **12:**306–310.
2. **Breed, R. S., E. G. D. Murray, and A. P. Hitchens.** 1948. Manual of determinative bacteriology, 6th ed., p. 318–319. Williams and Wilkins, Baltimore, Md.
3. **Cheuk, W., P. C. Y. Woo, K. Y. Yuen, P. H. Yu, and J. K. C. Chan.** 2001. Intestinal inflammatory pseudotumor with regional lymph node involvement: identification of a new bacterium as the etiologic agent. J. Pathol. **192:**289–292.
4. **Dierksen, K. P., and J. R. Tagg.** 2000. Haemolysin-deficient variants of *Streptococcus pyogenes* and *S. dysgalactiae* subsp. *equisimilis* may be overlooked as aetiological agents of pharyngitis. J. Med. Microbiol. **49:**811–816.
5. **Frost, W. D., and M. A. Engelbrecht.** 1940. The streptococci. Willdorf Book Co., Madison, Wis.
6. **Humar, D., V. Datta, D. J. Bast, B. Beall, J. C. De Azavedo, and V. Nizet.** 2002. Streptolysin S and necrotising infections produced by group G streptococcus. Lancet **359:**124–129.
7. **Iglesias-Gamarra, A., E. A. Mendez, M. L. Cuellar, J. H. Ponce de Leon, C. Jimenez, C. Canas, J. Restrepo, M. Pena, R. Valle, and L. R. Espinoza.** 2001. Poststreptococcal reactive arthritis in adults: long-term follow-up. Am. J. Med. Sci. **321:**173–177.
8. **Jansen, T. L., M. Janssen, and A. J. de Jong.** 1998. Reactive arthritis associated with group C and group G beta-hemolytic streptococci. J. Rheumatol. **25:**1126–1130.
9. **Kawamura, Y., X. G. Hou, F. Sultana, H. Miura, and T. Ezaki.** 1995. Determination of 16S rRNA sequences of *Streptococcus mitis* and *Streptococcus gordonii* and phylogenetic relationships among members of the genus *Streptococcus*. Int. J. Syst. Bacteriol. **45:**406–408.
10. **Lau, S. K. P., P. C. Y. Woo, B. Y. L. Chan, A. M. Y. Fung, T. L. Que, and K. Y. Yuen.** 2002. *Haemophilus segnis* polymicrobial and monomicrobial bacteremia identified by 16S ribosomal RNA gene sequencing. J. Med. Microbiol. **51:**635–640.
11. **Lau, S. K. P., P. C. Y. Woo, G. K. S. Woo, and K. Y. Yuen.** 2002. Catheter-related *Microbacterium* bacteremia identified by 16S ribosomal RNA gene sequencing. J. Clin. Microbiol. **40:**2681–2685.
12. **Lau, S. K. P., P. C. Y. Woo, J. L. L. Teng, K. W. Leung, and K. Y. Yuen.** 2002. Identification by 16S ribosomal RNA gene sequencing of *Arcobacter butzleri* bacteraemia in a patient with acute gangrenous appendicitis. Mol. Pathol. **55:**182–185.
13. **Li, E. K.** 2000. Rheumatic disorders associated with streptococcal infections. Baillieres Best Pract. Res. Clin. Rheumatol. **14:**559–578.
14. **Luk, W. K., S. S. Wong, K. Y. Yuen, P. L. Ho, P. C. Y. Woo, R. A. Lee, and P. Y. Chau.** 1998. Inpatient emergencies encountered by an infectious disease consultative service. Clin. Infect. Dis. **26:**695–701.
15. **Murray, P. R., E. J. Baro, M. A. Pfaller, F. C. Tenover, and. R. H. Yolken (ed.).** 1999. Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
16. **Podbielski, A., B. Melzer, and R. Lutticken.** 1991. Application of the polymerase chain reaction to study the M protein(-like) gene family in beta-hemolytic streptococci. Med. Microbiol. Immunol. **180:**213–227.
17. **Thompson, J. D., D. G. Higgins, and T. J. Gibson.** 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:**4673–4680.
18. **Vandamme, P., B. Pot, E. Falsen, K. Kersters, and L. A. Devriese.** 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.
19. **Vieira, V. V., L. M. Teixeira, V. Zahner, H. Momen, R. R. Facklam, A. G. Steigerwalt, D. J. Brenner, and A. C. D. Castro.** 1998. Genetic relationships among the different phenotypes of *Streptococcus dysgalactiae* strains. Int. J. Syst. Bacteriol. **48:**1231–1243.
20. **Wilson, C. D., G. F. H. Salt, F. A. Skinner, and L. B. Quesnel. (ed.).** 1978. Streptococci, p. 143–156. Academic Press, New York, N.Y.
21. **Woo, P. C. Y., A. M. Y. Fung, S. K. P. Lau, and K. Y. Yuen.** 2002. Identification by 16S ribosomal RNA gene sequencing of *Lactobacillus salivarius* bacteremic cholecystitis. J. Clin. Microbiol. **40:**265–267.
22. **Woo, P. C. Y., A. M. Y. Fung, S. K. P. Lau, S. S. Y. Wong, and K. Y. Yuen.** 2001. Group G beta-hemolytic streptococcal bacteremia characterized by 16S ribosomal RNA gene sequencing. J. Clin. Microbiol. **39:**3147–3155.
23. **Woo, P. C. Y., A. M. Y. Fung, S. S. Y. Wong, H. W. Tsoi, and K. Y. Yuen.** 2001. Isolation and characterization of a *Salmonella enterica* serotype Typhi variant and its clinical and public health implications. J. Clin. Microbiol. **39:**1190–1194.
24. **Woo, P. C. Y., A. M. Y. Fung, S. K. P. Lau, E. Hon, and K. Y. Yuen.** 2002. Diagnosis of pelvic actinomycosis by 16S ribosomal RNA gene sequencing and its clinical significance. Diagn. Microbiol. Infect. Dis. **43:**113–118.
25. **Woo, P. C. Y., A. S. P. Leung, K. W. Leung, and K. Y. Yuen.** 2001. Identification of slide-coagulase positive, tube-coagulase negative *Staphylococcus aureus* by 16S ribosomal RNA gene sequencing. Mol. Pathol. **54:**244–247.
26. **Woo, P. C. Y., C. Y. Lo, S. K. Lo, H. Siau, J. S. M. Peiris, S. S. Y. Wong, W. K. Luk, T. M. Chan, W. W. Lim, and K. Y. Yuen.** 1997. Distinct genotypic distributions of cytomegalovirus (CMV) envelope glycoprotein in bone marrow and renal transplant recipients with CMV disease. Clin. Diagn. Lab. Immunol. **4:**515–518.
27. **Woo, P. C. Y., D. M. W. Tam, K. W. Leung, S. K. P. Lau, J. L. L. Teng, M. K. M. Wong, and K. Y. Yuen.** 2002. *Streptococcus sinensis* sp. nov., a novel *Streptococcus* species isolated from a patient with infective endocarditis. J. Clin. Microbiol. **40:**805–810.
28. **Woo, P. C. Y., E. Y. L. Cheung, K. W. Leung, and K. Y. Yuen.** 2001. Identification by 16S ribosomal RNA gene sequencing of an *Enterobacteriaceae* species with ambiguous biochemical profile from a renal transplant recipient. Diagn. Microbiol. Infect. Dis. **39:**85–93.
29. **Woo, P. C. Y., H. W. Tsoi, K. W. Leung, P. N. L. Lum, A. S. P. Leung, C. H. Ma, K. M. Kam, and K. Y. Yuen.** 2000. Identification of *Mycobacterium neoaurum* isolated from a neutropenic patient with catheter-related bacteremia by 16S rRNA sequencing. J. Clin. Microbiol. **38:**3515–3517.
30. **Woo, P. C. Y., J. H. C. Li, W. M. Tang, and K. Y. Yuen.** 2001. Acupuncture mycobacteriosis. N. Engl. J. Med. **345:**842–843.
31. **Woo, P. C. Y., K. T. K. Chong, K. W. Leung, T. L. Que, and K. Y. Yuen.** 2001. Identification of *Arcobacter cryaerophilus* isolated from a traffic accident victim with bacteraemia by 16S ribosomal RNA gene sequencing. Diagn. Microbiol. Infect. Dis. **40:**125–127.
32. **Woo, P. C. Y., K. W. Leung, H. W. Tsoi, S. S. Y. Wong, J. L. L. Teng, and K. Y. Yuen.** 2002. Thermo-tolerant *Campylobacter fetus* bacteraemia identified by 16S ribosomal RNA gene sequencing: an emerging pathogen in immunocompromised patients. J. Med. Microbiol. **51:**740–746.
33. **Woo, P. C. Y., K. W. Leung, S. S. Y. Wong, K. T. K. Chong, E. Y. L. Cheung, and K. Y. Yuen.** 2002. Relative alcohol-resistant mycobacteria are emerging pathogens in patients receiving acupuncture treatment. J. Clin. Microbiol. **40:**1219–1224.
34. **Woo, P. C. Y., P. K. L. Leung, K. W. Leung, and K. Y. Yuen.** 2000. Identification by 16S ribosomal RNA gene sequencing of an *Enterobacteriaceae* species from a bone marrow transplant recipient. Mol. Pathol. **53:**211–215.
35. **Yuen, K. Y., P. C. Y. Woo, J. L. L. Teng, K. W. Leung, M. K. M. Wong, and S. K. P. Lau.** 2001. *Laribacter hongkongensis* gen. nov., sp. nov., a novel gram-negative bacterium isolated from a cirrhotic patient with bacteremia and empyema. J. Clin. Microbiol. **39:**4227–4232.