JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2005, p. 6205–6208 Vol. 43, No. 12
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.12.6205–6208.2005
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# Life-Threatening Invasive *Helcococcus kunzii* Infections in Intravenous-Drug Users and *ermA*-Mediated Erythromycin Resistance

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Received 5 July 2005/Returned for modification 6 September 2005/Accepted 26 September 2005

**We report the first two cases of life-threatening invasive *Helcococcus kunzii* infection, with primary bacteremia and empyema thoracis, respectively. Gram smears of both *H. kunzii* isolates showed a mixture of gram-positive and gram-negative cocci. The isolate from the first patient, resistant to erythromycin and clindamycin, possessed an *ermA* gene.**

## CASE REPORTS

**Case 1.** A 41-year-old Chinese man was hospitalized in October 1999 because of general malaise for 1 week. He was an intravenous-drug user. On admission, the patient was in septic shock but afebrile. There was jaundice, and multiple scars were present over both thighs. In addition, a 10-by-5-cm suppurative skin ulcer and multiple injection sites were seen on the left thigh. The total white cell count was $17.0 \times 10^9$/liter (neutrophil count of $16.1 \times 10^9$/liter), the hemoglobin level was 11.4 g/dl, and the platelet count was $7 \times 10^9$/liter. The serum albumin level was 22 g/liter, the globulin level was 28 g/liter, the bilirubin level was 225 μmol/liter, the alkaline phosphatase level was 173 IU/liter, the aspartate aminotransferase level was 65 IU/liter, and the alanine aminotransferase level was 21 IU/liter. The urea level was elevated to 16.5 mmol/liter, but serum creatinine was within normal limits. Two sets of blood culture were performed, and intravenous cloxacillin and penicillin G were commenced. Fluid resuscitation was instituted. Ultrasonographic examination revealed no evidence of acute cholangitis. Transthoracic echocardiography revealed good left ventricular function with no vegetations.

On day 2 postincubation, both sets of blood cultures turned positive with a mixture of gram-positive and gram-negative cocci arranged in pairs, clusters, and tetrads. The fever gradually subsided, and his jaundice diminished. The white cell and platelet counts, liver function tests, and serum urea level also gradually returned to within normal limits. The patient was discharged after 3 weeks of antibiotics. There was no relapse of the bacteremia up to the time this report was written, 5 years after discharge.

**Case 2.** A 55-year-old Chinese man was hospitalized in October 2002 because of hemoptysis for 1 week. He had a productive cough; progressive shortness of breath; night sweating and a weight loss of 5 kg in the past 3 weeks; and fever, chills, and rigors for 3 days. He was a smoker, an alcoholic, and an intravenous-drug user. On admission, his body temperature was 38°C and he was in septic shock. There were percussion dullness and crackles in the lower areas of the chest. A chest radiograph showed left lower zone airspace shadows and pleural effusion. His total white cell count was $33.6 \times 10^9$/liter (neutrophil count of $30.7 \times 10^9$/liter), his hemoglobin level was 6.3 g/dl, and his platelet count was $259 \times 10^9$/liter. His clotting profile was mildly abnormal with a prolonged prothrombin time of 15.4 s and an activated partial thromboplastin time of 42.6 s. His serum albumin level was 20 g/liter, his globulin level was 55 g/liter, his bilirubin level was 19 μmol/liter, his alkaline phosphatase level was 126 IU/liter, his aspartate aminotransferase level was 21 IU/liter, and his alanine aminotransferase level was 8 IU/liter. Renal function test results were within normal limits. A blood culture was performed, and sputum samples were collected for Gram smear, bacterial culture, Ziehl-Neelsen staining, and culture for acid-fast bacilli. Empirical intravenous amoxicillin-clavulanate and inotropic support were commenced. He developed respiratory failure requiring ventilatory support.

Contrast computed tomography of the thorax revealed left lung consolidation associated with a large, septated empyema and perihilar lymphadenopathy. Computed-tomography-guided drainage of the collection was performed, and 1 liter of was pus obtained. A Gram smear of the pus showed numerous leukocytes and a mixture of gram-positive and gram-negative cocci arranged in pairs, clusters, and tetrads. Culture of the pus recovered pure heavy growth of a bacterium, the Gram smear of which also showed a mixture of gram-positive and gram-negative cocci arranged in pairs, clusters, and tetrads. Blood culture, Ziehl-Neelsen staining, and culture for acid-fast bacilli were negative. His condition was gradually stabilized, and he recovered with a total of 8 weeks of amoxicillin-clavulanate. There was no relapse of the illness up to the time of writing, 30 months after discharge.

**Microbiological data.** Clinical specimens were collected and handled according to standard protocols (12). The BACTEC 9240 blood culture system (Becton Dickinson) was used. Gram

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smears of both isolates showed a mixture of gram-positive and gram-negative cocci arranged in pairs, clusters, and tetrads with pleomorphic appearances. There were no differences in the appearances of the gram smears of both isolates after subculturing in Tween 80-enriched medium. Both grew on sheep blood agar as nonhemolytic, gray, pinpoint colonies after 24 h of incubation at 37°C in ambient air. At 48 h, the colonies of both isolates showed alpha hemolysis. Both were catalase negative, nonmotile, and positive for pyrrolidonyl arylamidase but negative for leucine aminopeptidase. Both isolates were identified by the API system (20 STREP; bioMérieux Vitek, Hazelwood, Mo.) as *Aerococcus viridans* of doubtful significance, with a numerical profile of 4100413. MICs of several antibiotics were determined by the broth macrodilution method (11). The isolate from the first patient was resistant to erythromycin (MIC, >256 μg/ml) and clindamycin (MIC, 0.5 μg/ml) but susceptible to penicillin (MIC, 0.064 μg/ml), cefotaxime (MIC, 0.25 μg/ml), and vancomycin (MIC, 0.5 μg/ml). The isolate from the second patient was susceptible to erythromycin (MIC, 0.25 μg/ml), clindamycin (MIC, 0.25 μg/ml), penicillin (MIC, 0.032 μg/ml), vancomycin (MIC, 0.5 μg/ml), and cefotaxime (MIC, 0.032 μg/ml).

**16S rRNA gene sequencing.** Bacterial DNA extraction, PCR amplification, and DNA sequencing of the 16S rRNA genes of the isolates were performed according to our previous publication, with LPW55 and LPW205 (Gibco BRL, Rockville, MD) as the PCR and sequencing primers (23). There were 1.9% and 2.1% difference between the 16S rRNA gene sequences of the two isolates and that of *Helcococcus kunzii* (GenBank accession number X69837) (Fig. 1).

***erm* and *mef* gene amplification and sequencing.** PCR amplification and DNA sequencing of the *ermT*, *ermA*, *ermB*, and *mef* genes were performed according to our previous publication (21). The *H. kunzii* isolate that was resistant to erythromycin and clindamycin possessed the *ermA* gene. Neither possessed the *mef*, *ermT*, or *ermB* gene. There was no difference between the nucleotide sequence of the *ermA* gene of the erythromycin-resistant isolate and that of a previously described *ermA* gene of *Streptococcus pyogenes* (GenBank accession number AF002716).

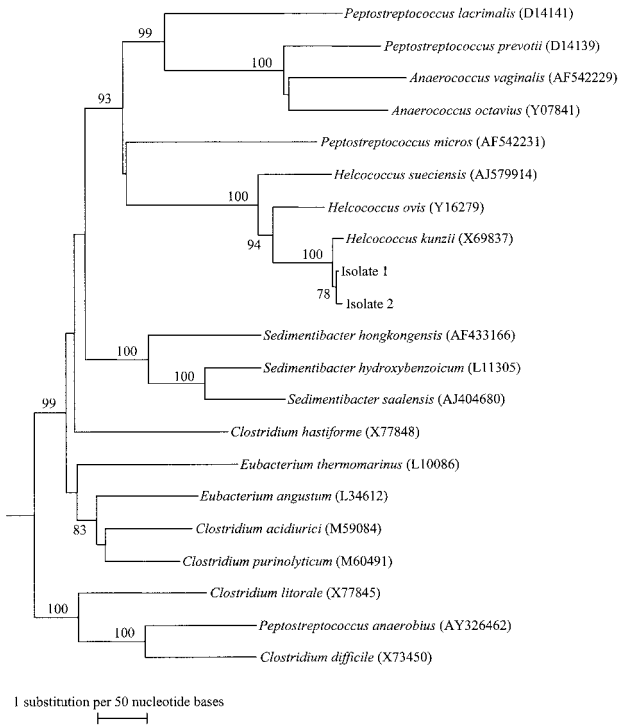


FIG. 1. Phylogenetic trees showing the relationship of the two isolates from our patients to closely related species. The tree was inferred from 16S rRNA gene sequence data (1,269 nucleotide positions) by the neighbor-joining method and was rooted by using *Bacillus subtilis* (accession no. AB065370). The scale bar indicates the estimated number of substitutions per 50 bases by using the Jukes-Cantor correction. Values (percentages) at nodes indicate levels of bootstrap support calculated from 1,000 trees. All names and accession numbers are given as cited in the GenBank database.

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*Helcococcus* is a genus of catalase-negative, facultatively anaerobic, nonsporulating, gram-positive cocci first described in 1993, with *H. kunzii* as the first discovered member (3). Since then, three more species, *Helcococcus ovis*, *Helcococcus pyogenes*, and *Helcococcus sueciensis*, have been described, of which *H. pyogenes* and *H. sueciensis* are associated with human infections (4, 5, 13, 18). The present report represents the first two cases of invasive *H. kunzii* infection in intravenous-drug users. Since the discovery of *H. kunzii* 12 years ago, 13 cases of infection associated with recovery of *H. kunzii* have been reported in the literature with clinical details (1, 2, 14, 16). Of these 13 cases, only 5 were due to *H. kunzii* infection (Table 1), whereas in the other 8 cases, the *H. kunzii* isolates recovered were probably just colonizers. No *H. kunzii* isolates have been recovered from blood cultures. The present two cases probably represent the two most serious cases of *H. kunzii* infection reported in the literature. Both cases were associated with life-threatening invasive infection with septic shock, and in both patients the *H. kunzii* isolates were recovered in pure cultures, with one from blood and the other from empyema pus. The seriousness of these two cases demonstrated that the bacterium probably possesses greater pathogenic potential than previously believed.

The skin is probably the portal of entry in cases of *H. kunzii* infection. Haas et al. have shown that *H. kunzii* was a member of the skin flora and suggested that it might be a component of the polymicrobial flora of lower-extremity ulcers (9). This is in line with the observation that most of the infections were superficial skin and soft-tissue infections and 9 out of the 13 previously reported cases were localized infections or colonization of the lower limbs (1, 2, 14, 16). In the present two cases, both patients were intravenous-drug users, with repeated needle injections providing breaks in the skin barrier for the bacteria to enter the bloodstream. In the first patient, although transthoracic echocardiography did not show any vegetation, the recovery of *H. kunzii* from two separate sets of blood cultures, the patient's underlying condition and a lack of other infective foci still make infective endocarditis of the tricuspid valve a distinct possibility. In the second patient, the seeding of the bacterium in the pleural cavity was probably further predisposed by the patient's immunocompromised state as a chronic alcoholic.

Identification of *H. kunzii* must be performed cautiously, as pitfalls are present in both phenotypic and genotypic identifi-

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TABLE 1. Summary of reported cases of *H. kunzii* infection

| Case | Reference | Sex[a]/age (yr) | Underlying condition(s) | Other bacterium isolated | Diagnosis | Outcome |
|---|---|---|---|---|---|---|
| 1 | 1 | F/96 | Hypertension | Coagulase-negative staphylococcus | Left lower limb cellulitis | Recovered |
| 2 | 1 | M/59 | Coronary artery disease | Coagulase-negative staphylococcus | Right lower limb ulcer with cellulitis | Recovered |
| 3 | 14 | M/36 | Hypertension, obesity, hypercholesterolemia | None | Infected sebaceous cyst over right shoulder | Recovered |
| 4 | 2 | F/57 | None | None | Infected sebaceous cyst of left breast | Recovered |
| 5 | 16 | F/36 | None | *Staphylococcus simulans* | Postsurgical foot abscess | Recovered |
| 6 | Present study: (case 1) | M/41 | Intravenous-drug abuse | None | Primary bacteremia | Recovered |
| 7 | Present study: (case 2) | M/55 | Intravenous-drug abuse, alcoholism | None | Empyema thoracis | Recovered |

[a] F, female; M, male.

cation systems. When *H. kunzii* was discovered, the authors described it as an *Aerococcus*-like organism due to its phenotypic resemblance to *A. viridans*, especially as both species are positive for pyrrolidonyl arylamidase but negative for leucine aminopeptidase. Although sometimes commercial systems can provide discriminating information, they often fail to identify *H. kunzii* by name. For example, as with the present two strains, biochemical testing of *H. kunzii* by the API system (20 STREP) often resulted in a numerical profile of 4100413, but it would be mislabeled as "doubtful *Aerococcus viridans*." Genotypically, we speculate that the 16S rRNA gene sequence of the *H. kunzii* isolate deposited in GenBank (accession no. X69837) may be atypical. When a multiple-sequence alignment of the 16S rRNA gene sequences of the two *H. kunzii* isolates in the present study, that of the *H. kunzii* isolate from GenBank (accession no. X69837), and those of other, closely related, species was performed, it was observed that there was a series of nine extra bases in the *H. kunzii* accession no. X69837 sequence compared to the others, including the sequences of the present two *H. kunzii* isolates. Furthermore, when the MicroSeq 500 16S rRNA gene-based bacterial identification system, the most popular commercially available database for 16S rRNA gene sequence analysis, was used, *H. kunzii* was misidentified as *Clostridium hastiforme* (22). Although the MicroSeq 500 16S rRNA gene-based bacterial identification system database was expanded recently to include more bacterial species, *H. kunzii* was still not included (7). Interestingly, the present two isolates showed a distinct Gram smear appearance of a mixture of gram-positive and gram-negative cocci, resembling those of cell wall-deficient forms that we recovered from our bone marrow transplant recipients and those of *Granulicatella adiacens* and *Abiotrophia defectiva* (20, 24), that has never been described before in *H. kunzii*. Similar to *G. adiacens* and *A. defectiva*, the Gram smear appearance of cell wall deficiency in *H. kunzii* remained the same after repeated subculturing. We speculate that the cell wall deficiency state of *H. kunzii*, similar to those of *G. adiacens* and *A. defectiva*, also occurs naturally. Although further electron microscopic studies are required for elucidating the cell wall structure of *H. kunzii*, the characteristic heteromorphic Gram smear appearance and positivity for pyrrolidonyl arylamidase but negativity for leucine aminopeptidase should raise the suspicion of *H. kunzii*.

Erythromycin resistance in the *H. kunzii* isolate in the present study was mediated through an *ermA* gene. The *ermTR* gene (renamed *ermA* in 1999 [17]) was first described in *S. pyogenes* in 1998 (19). Since then, the presence of this group of genes has been described in many other streptococci, such as Lancefield group B, C, and G streptococci, and other gram-positive cocci such as *Peptostreptococcus* spp. (6, 10, 15), although only a few "*ermTR*" gene sequences are available in GenBank. In fact, the *ermA* gene found in our *H. kunzii* isolate shared 100% nucleotide sequence identity with that of *S. pyogenes*, suggesting that horizontal transfer of this gene could be a common event among streptococci and other gram-positive cocci. This is in line with results of previous studies, which showed that the *ermA* gene could be transferred from *S. pyogenes* to a variety of other species, including *Enterococcus faecalis*, *Listeria innocua*, and beta-hemolytic group G streptococci (8). Other researchers have also highlighted the transferability of the *ermA* gene by obtaining *S. pyogenes* transconjugants from conjugation with *Peptostreptococcus* species harboring the *ermA* gene (15).

**Nucleotide sequence accession numbers.** The 16S rRNA and *ermA* gene sequences of the *H. kunzii* isolates have been lodged in the GenBank sequence database under accession numbers DQ082898, DQ082899, and DQ082900.

This work was partly supported by the University Development Fund and a Committee for Research and Conference grant from The University of Hong Kong.

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