

Helcococcus kunzii as Sole Isolate from an Infected Sebaceous Cyst

MARGARET M. PEEL,^{1*} JENNIFER M. DAVIS,¹ KEVIN J. GRIFFIN,² AND DAVID L. FREEDMAN³

Microbiological Diagnostic Unit, Department of Microbiology, The University of Melbourne, Parkville,¹ and Department of Pathology² and Department of Surgery,³ Swan Hill District Hospital, Swan Hill, Victoria, Australia

Received 14 June 1996/Returned for modification 28 August 1996/Accepted 8 October 1996

***Helcococcus kunzii* was isolated in pure culture from pus drained from an infected sebaceous cyst associated with marked cellulitis. The cyst was excised one month later after the inflammation had subsided with flucloxacillin treatment. This is the first report of the isolation of *H. kunzii* as the sole pathogen from an infected site.**

Helcococcus kunzii was first described in 1993 as a new genus and species of catalase-negative, facultatively anaerobic, gram-positive cocci (2). *H. kunzii* shares many phenotypic characteristics with *Aerococcus viridans* but differs in its growth rate and requirements and colonial morphology. *H. kunzii* produces pinpoint, nonhemolytic or slightly α -hemolytic colonies on sheep blood agar after incubation for 24 h (1). The amount of growth and size of colonies are the same on blood agar plates incubated aerobically, in 5% CO₂, or anaerobically. In contrast, *A. viridans* grows poorly or not at all under anaerobic conditions and gives strong α -hemolysis around colonies of 0.5 to 1 mm in diameter on blood agar plates incubated aerobically for 24 h (2, 3). Apart from the phenotypic differences between these two taxa, comparison of the G+C content of their DNA and comparative analysis of their 16S rRNA sequence data indicate that they are phylogenetically different and distinct (2).

We record the isolation of *H. kunzii* in pure culture and heavy growth from pus from an infected sebaceous cyst. This is the first report of the isolation of *H. kunzii* in pure culture from an infected site, and it indicates that this newly described bacterium is capable of acting as the sole pathogen.

Case report. A 36-year-old man was admitted to the hospital with an infected sebaceous cyst on his right shoulder. Although the cyst had been present for 2 years, it first showed signs of inflammation 2 weeks before his admission. He had a history of hypertension, obesity, and hypercholesterolemia. The cyst measured 3 by 4 cm in size and was associated with a marked cellulitis. It was incised and drained, and a swab of the pus was submitted for microbiological examination. Culture of the pus yielded a gram-positive coccus, which was later identified as *H. kunzii*.

Therapy with flucloxacillin was instituted pending identification of the infective agent. The initial treatment schedule consisted of four 1-g doses given intravenously at 8-h intervals. The patient was then discharged on a regimen of 0.5 g of flucloxacillin orally every 8 h for 5 days. The inflammation subsided, and the cyst was excised 1 month later. Histopathologic examination of the cyst showed an epidermal cyst with chronic inflammation.

Microbiology. Examination of a gram-stained smear of the pus showed moderate numbers of polymorphonuclear cells and of gram-positive cocci. Culture of the pus on horse blood agar incubated aerobically and anaerobically at 35°C and on chocolate agar incubated in a 7.5% CO₂-enriched atmosphere yielded pinpoint colonies after 16 h, and small, grayish colonies with slight α -hemolysis after a further 24 h. An initial attempt at identification by the rapid ID 32 Strep identification system (bioMérieux, Marcy-l'Étoile, France) was unsuccessful, and the isolate was referred to the Microbiological Diagnostic Unit for identification.

At the reference laboratory, the isolate grew slowly on horse blood agar, producing pinpoint, grayish, nonhemolytic colonies after 24 h of incubation at 35°C, which showed slight α -hemolysis after 48 h. Equivalent growth was obtained on horse blood agar plates incubated aerobically, anaerobically, and in a 5% CO₂-enriched atmosphere. The isolate did not grow on MacConkey agar or at 45°C, and it required the addition of 5% serum to all basal media for satisfactory growth.

A gram-stained smear of the culture showed gram-positive cocci arranged mainly in pairs and clusters with some tetrads. The cocci were nonmotile, catalase negative, and susceptible to a 30- μ g vancomycin disk (3). The enzyme profile and biochemical characteristics of the isolate were determined by the API 20 Strep identification system (bioMérieux) and in conventional test media enriched by the addition of 5% inactivated newborn calf serum. Acid production from carbohydrates was tested in a serum-enriched 1% peptone base with 0.5% NaCl and bromocresol purple indicator.

The API 20 Strep numerical profile for the isolate was 4100413, which is identical to that previously reported for *H. kunzii* (2). This means that the isolate gave positive reactions in this system for esculin hydrolysis, pyrrolidonylamidase activity, and acid production from lactose, trehalose, starch, and glycogen. It gave negative reactions for acetoin production, hippurate hydrolysis, and the enzymes α -galactosidase, β -glucuronidase, β -galactosidase, alkaline phosphatase, leucine aminopeptidase, and arginine dihydrolase, and it did not produce acid from ribose, arabinose, mannitol, sorbitol, inulin, and raffinose. The results of testing in serum-supplemented conventional test media were the same for tests that repeated those of the API 20 Strep system. In addition, the isolate gave negative reactions on serum-enriched conventional media for gelatin liquefaction, nitrate reduction, and urease activity. It grew in the presence of 6.5% NaCl, and it produced pyrazinamidase and acid from cellobiose, glucose, and maltose but not

* Corresponding author. Mailing address: Microbiological Diagnostic Unit, Department of Microbiology, The University of Melbourne, Parkville, Victoria, 3052, Australia. Phone: 61 3 9344 7736. Fax: 61 3 9344 7833.

from sucrose. No gas was produced from glucose in MRS (de Man, Rogosa, Sharpe) broth. The morphological and biochemical characteristics indicate that the isolate is *H. kunzii* (1–3).

Antimicrobial susceptibility testing, as determined by the E-test method (1), showed that the isolate was susceptible to penicillin (MIC, ≤ 0.094 $\mu\text{g/ml}$), vancomycin (MIC, ≤ 0.75 $\mu\text{g/ml}$), and flucloxacillin (MIC, = 0.5 $\mu\text{g/ml}$).

Discussion. All 10 patients from whom the isolation of *H. kunzii* has been reported (1) have yielded this bacterium in mixed cultures that included such bacteria as *Staphylococcus aureus* and anaerobes. However, the only other bacterium isolated from two patients with cellulitis was a coagulase-negative *Staphylococcus* sp., which was considered unlikely to have contributed to the cellulitis. Nonetheless, isolation in mixed culture makes it difficult to assign a clear-cut pathogenic or potentially pathogenic role to this new genus.

Seven of the 10 patients were in an older age group (≥ 57 years of age). In seven of the cases, the site of isolation was the lower extremities. One isolate came from an infected sebaceous cyst on the right side of the face of a 69-year-old woman, but *S. aureus* was also isolated from her cyst. Our case resembles this case in that *H. kunzii* was isolated from an infected

sebaceous cyst on the right shoulder. The important difference between our case and all previously reported cases (1) is that *H. kunzii* was isolated in pure culture and heavy growth from the pus specimen collected by incision and drainage of an infected sebaceous cyst. This is evidence of a pathogenic role, probably an opportunistic pathogenic role, for *H. kunzii*.

We thank Janet Strachan and the Department of Microbiology and Infectious Diseases of the Royal Children's Hospital, Parkville, Victoria, for the antimicrobial susceptibility testing of the isolate by the E-test method.

REFERENCES

1. **Caliendo, A. M., C. D. Jordan, and K. L. Ruoff.** 1995. *Helcococcus*, a new genus of catalase-negative, gram-positive cocci isolated from clinical specimens. *J. Clin. Microbiol.* **33**:1638–1639.
2. **Collins, M. D., R. R. Facklam, U. M. Rodrigues, and K. L. Ruoff.** 1993. Phylogenetic analysis of some *Aerococcus*-like organisms from clinical sources: description of *Helcococcus kunzii* gen. nov., sp. nov. *Int. J. Syst. Bacteriol.* **43**:425–429.
3. **Facklam, R., and J. A. Elliott.** 1995. Identification, classification, and clinical relevance of catalase-negative, gram-positive cocci, excluding the streptococci and enterococci. *Clin. Microbiol. Rev.* **8**:479–495.