

Breast Abscess Associated with *Helcococcus kunzii*

ABDUL H. CHAGLA,^{1*} ALEXANDER A. BORCZYK,² RICHARD R. FACKLAM,³
AND MARGUERITE LOVGREN⁴

London Public Health Laboratory, London,¹ and Central Public Health Laboratory, Toronto,²
Ontario, and National Centre for Streptococcus, Edmonton, Alberta,⁴ Canada, and
Centers for Disease Control, Atlanta, Georgia³

Received 16 January 1998/Returned for modification 6 March 1998/Accepted 12 May 1998

***Helcococcus kunzii*, a nonvirulent member of the human skin flora, has recently been implicated in causing infections in immunosuppressed patients. We report a case of breast abscess associated with *H. kunzii* in an immunocompetent patient and discuss the criteria used in its identification and our observations of susceptibility testing for this species.**

Helcococcus is a newly described genus of catalase-negative, gram-positive cocci (2). *H. kunzii*, the only member of this genus, is thought to be an avirulent member of the human skin flora that is rarely associated with wound infections (1, 5). We report the isolation of *H. kunzii* from a case of breast abscess in an immunocompetent patient and our experience with the identification and susceptibility testing of this species.

Case report. A 57-year-old immunocompetent, nondiabetic female presented to her physician with an infected sebaceous cyst in the skin around the upper inner quadrant of her left breast. She was afebrile and had no systemic signs of infection. Two days prior to her presentation, she had developed local redness, swelling, and tenderness around the left breast area. The cyst was incised and drained under local anesthesia. She was treated postsurgically with 0.5 g of cephalexin orally every 8 h for 5 days.

A Gram stain of the cyst drainage showed numerous polymorphonuclear leukocytes and gram-positive cocci in pairs and clumps. The pus from the incision was inoculated onto Trypticase soy agar containing 5% sheep blood, onto chocolate agar, onto MacConkey agar, and into cooked meat medium. The 5% sheep blood agar and chocolate agar plates were incubated at 36°C in a CO₂-enriched (5%) atmosphere for 24 h. A separate 5% sheep blood agar plate was also inoculated with pus and incubated anaerobically for 72 h at 36°C. Cooked meat medium and MacConkey agar plates were incubated aerobically for 48 h at 36°C. After 24 h of incubation, a pure and heavy growth of pinpoint, greyish, nonhemolytic colonies appeared on the 5% sheep blood agar and chocolate agar plates incubated in CO₂-enriched (5%) atmosphere. Identical growth characteristics were observed for the 5% sheep blood agar plate that was incubated under anaerobic conditions. A subculture from the cooked meat medium also yielded similar growth on 5% sheep blood agar and chocolate agar and no growth on MacConkey agar. A Gram stain of the isolate showed gram-positive cocci in pairs and clusters. Initial spot biochemical tests were negative for catalase and positive for pyrrolidonyl arylamidase (PYR). A leucine aminopeptidase (LAP) test using specific aminopeptidase substrate (diagnostic) tablets (A/S Rosco, Taastrup, Denmark) was negative after 24 h of incubation at 36°C. Biochemical identification by use of

the API 20S Strep system (bioMerieux Vitek, Inc., Hazelwood, Mo.) resulted in profile no. 4100413 (esculin and PYR positive, LAP negative, and acid reaction from lactose, trehalose, starch, and glycogen), indicating a doubtful identification of *Aerococcus viridans*. The isolate was identified as *H. kunzii* by the National Centre for Streptococcus (NCS), Edmonton, Alberta, Canada, and confirmed by the Centers for Disease Control and Prevention, Atlanta, Ga. The biochemical characteristics of this strain are presented in Table 1 together with typical reactions for eight other *H. kunzii* clinical isolates that have been examined at the NCS. All strains, including the case isolate, were further confirmed as *H. kunzii* by 16S rRNA sequencing done at the AFRC Institute of Food Research, Reading, United Kingdom.

Antimicrobial susceptibility testing with various antibiotics was performed by the disk diffusion method using Mueller-Hinton agar supplemented with 5% sheep blood. The disk diffusion plates were incubated at 36°C in 5% CO₂ for 24 h. Based on the recommendations made by the National Committee for Clinical Laboratory Standards (6) for interpretive standards for *Streptococcus* species, the results of the disk diffusion assay suggested that the current isolate was sensitive to penicillin, ampicillin, and vancomycin but resistant to erythromycin and clindamycin. The MICs of drugs for the isolate were determined by use of the Etest system (AB Biodisk, Piscataway, N.J.) on Mueller-Hinton agar supplemented with 5% sheep blood. The Etest susceptibility plates were incubated at 36°C in 5% CO₂ for 24 h. The MICs of penicillin, ampicillin, and cephalothin for the isolate were 0.12, 0.25, and 0.5 mg/liter, respectively.

Examination of the patient 1 week posttreatment revealed some residual pus. The patient was asked to irrigate the area once or twice daily by allowing warm water to flow over her breast while showering. On reassessment after a month, she showed no sign of tenderness, erythema, or palpable induration. Since there was no lump, excision of the cyst was not done.

Weaknesses of commercial gram-positive identification systems require the use of conventional testing media for accurate classification of this species. Due to close phenotypic resemblance, *Helcococcus* may be misidentified as *A. viridans* (2, 3). However unlike *Aerococcus*, *Helcococcus* is lipophilic (1, 2), and this can be demonstrated by enhanced growth in Todd-Hewitt broth supplemented with 0.1% Tween 80. At the NCS, we have observed that *Helcococcus* strains grow best in heart infusion-based carbohydrate media as described by Facklam and Elliot (3). It is important to note, however, that even

* Corresponding author. Mailing address: London Public Health Laboratory, P.O. Box 5704, Terminal A, London, Ontario, Canada N6A 4L6. Phone: (519) 455-9310. Fax: (519) 455-3363. E-mail: chaglaa@gov.on.ca.

TABLE 1. Identification of *H. kunzii*

Test	Reaction ^a	
	Case isolate	Typical of strain collection ^b
PYR	+	+
LAP	-	-
Arginine	-	-
Esculin	+	+
Hippurate	-	-
Urease	-	-
Voges-Proskauer	-	-
6.5% NaCl ^c	+	+(del)
10°C	NG	NG
45°C	NG	NG
Fermentation ^d		
Arabinose	-	-
Glucose	+	+
Glycerol	-	-
Inulin	+	-
Lactose	+	+
Maltose	+	+
Mannitol	-	-
Melibiose	-	-
Raffinose	-	-
Salicin	+	V
Sorbitol	-	-
Sorbitol	-	-
Starch	+	+
Sucrose	-	-
Trehalose	-	+

^a Symbols and abbreviations: +, ≥88% positive; -, ≤12% positive; V, variable; del, delayed; NG, no growth.

^b Data from the NCS, based on eight clinical isolates.

^c Supplemented with 0.1% Tween 80.

^d Fermentation in 1% carbohydrate in heart infusion broth supplemented with 0.1% Tween 80; incubation at 35°C for up to 7 days.

though the *Helcococcus* strains that we examined grew well in this medium, fermentation, as indicated by color change, was not demonstrated unless this medium was supplemented with 0.1% Tween 80. We do not use bovine serum as a medium supplement for this genus since it may cause a color change in the base and make interpretation difficult. The CDC has not observed fermentation in the carbohydrate media for the case isolate or for other *Helcococcus* isolates that they have tested since they do not supplement the media with 0.1% Tween 80 or bovine serum. The fermentation of inulin and the failure to ferment trehalose in conventional media observed for the case isolate are atypical for this species both in our experience and in previous reports (2, 8). Laboratories using the API 20S Strep system (2, 4, 8) have reported this species as inulin negative and an acidifier of trehalose, but one investigator (4) also noted acidification of inulin for an isolate which they called "Helcococcus-like." Failure to ferment trehalose in unsupplemented heart infusion-based media has been reported previously (2). Acidification of inulin and failure to ferment trehalose in Tween 80-supplemented heart infusion-based media for our case isolate were reproducible in repeat testing, and these atypical reactions may reflect our limited knowledge of this newly described organism.

H. kunzii should be considered a possible identification for any isolate that grows on blood agar as tiny, nonhemolytic or very slightly alpha-hemolytic, grey, translucent colonies which are catalase negative and vancomycin susceptible and which stain as gram-positive cocci arranged in pairs or clusters. Demonstration of enhanced growth in broth supplemented with

0.1% Tween 80 offers further support for this identification. Like other reports (2, 4, 8), biochemical testing of our isolate resulted in API profile no. 4100413. Even though *H. kunzii* is not currently in the API database, laboratories using this system may find this profile number useful for preliminary identification.

Susceptibility testing of *Helcococcus* is complicated by its lipophilic nature and growth requirement for hemin, as demonstrated by the inability to synthesize porphyrin (2). At the NCS, we have observed that this genus grows very poorly, or not at all, in Mueller-Hinton broth, even when the broth is supplemented with 3% lysed blood. The difficulties encountered when broth-based susceptibility testing systems are used have been noted by others (1). Susceptibility testing by Etest has been used by some laboratories (1, 8). At the NCS we have applied National Committee for Clinical Laboratory Standards testing criteria for *Streptococcus* to *Helcococcus* species (7). Our experience suggests that agar dilution on Mueller-Hinton agar supplemented with 5% sheep blood provides the most reliable method for MIC testing of drugs for this species. By this method, the MICs of penicillin and ampicillin for our case isolate were both 0.25 mg/liter, which is similar to those produced by the Etest. However, the MIC of cephalothin was 2.0 mg/liter, fourfold higher than the Etest MIC that was originally reported. Resistance to erythromycin (MIC > 2.0 mg/liter) was confirmed by agar dilution for the case isolate, but the result for clindamycin was susceptible (MIC = 0.12 mg/liter). It should be noted that of nine *Helcococcus* isolates currently in the NCS collection, eight are resistant to erythromycin (MIC ≥ 1.0 mg/liter) and all are susceptible to clindamycin at MICs of ≤0.25 mg/liter.

Aerococcus and *Helcococcus* are considered to be part of the normal flora of the human skin; however, unlike *Aerococcus*, which is present in the environment, the natural habitat of *Helcococcus* is not well characterized (9). In humans, these organisms can cause opportunistic infections such as endocarditis, bacteremia, meningitis, and wound infections (1). *H. kunzii* can cause wound infections in immunosuppressed patients; however, in the present case, the patient was immunocompetent. Peel and coworkers (8) have reported the isolation of *H. kunzii* in pure culture and heavy growth from an infected sebaceous cyst on the shoulder of a male patient. These investigators have suggested that *H. kunzii* can act as an opportunistic pathogen. Recently, Haas and coworkers, in a study of skin colonization by *H. kunzii* in diabetic and nondiabetic subjects, have suggested that this species, in addition to being a colonizer of human skin, may also be a previously unrecognized member of the polymicrobial flora of lower-extremity ulcers (4). Accurate identification of *H. kunzii* is important when investigating the role *H. kunzii* as a skin colonizer and a potential pathogen. Furthermore, our observations suggest that resistance to erythromycin may be expected for this species. Our finding provides further support for the opportunistic role of *H. kunzii* in causing infection in both immunosuppressed and immunocompetent patients.

We thank M. D. Collins at ARFC UK for the 16S rRNA sequence testing and P. Rawte for his technical assistance.

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. R., and J. A. Elliot. 1995. Identification, classification, and clinical

- relevance of catalase-negative, gram-positive cocci, excluding the streptococci and enterococci. *Clin. Microbiol. Rev.* **8**:479–495.
4. **Haas, J., S. L. Jernick, R. J. Scardina, J. Teruya, A. M. Caliendo, and K. L. Ruoff.** 1997. Colonization of skin by *Helcococcus kunzii*. *J. Clin. Microbiol.* **35**:2759–2761.
 5. **Janda, W. M.** 1994. *Streptococci* and “Streptococcus-like” bacteria: old friends and new species. *Clin. Microbiol. Newsl.* **16**:161–170.
 6. **National Committee for Clinical Laboratory Standards.** 1997. Performance standards for antimicrobial disk susceptibility tests, 6th ed. Approved standard M2-A6. National Committee for Clinical Laboratory Standards, Villanova, Pa.
 7. **National Committee for Clinical Laboratory Standards.** 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 4th ed. Approved standard. NCCLS document M7-A4. National Committee for Clinical Laboratory Standards, Villanova, Pa.
 8. **Peel, M. M., J. M. Davis, K. J. Griffin, and D. L. Freedman.** 1997. *Helcococcus kunzii* as sole isolate from an infected sebaceous cyst. *J. Clin. Microbiol.* **35**:328–329.
 9. **Ruoff, K.** 1997. *Leuconostoc, Pediococcus, Stomatococcus*, and miscellaneous gram-positive cocci that grow aerobically, p. 315–323. *In* P. R. Murray, E. J. Baron, M. A. Tenover and R. H. Tenover (ed.), *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.