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Colonization of Skin by *Helcococcus kunzii*

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In order to investigate the role of *Helcococcus kunzii* as a colonizer of skin and as a possible participant in diabetic foot ulcers, we used a selective medium to culture both lower- and upper-extremity skin from a study group of podiatry patients (60 diabetics and 60 nondiabetics) and a control group of 50 healthy volunteers. Although differences in colonization were not statistically significant, a trend toward higher colonization rates in the group of podiatry patients was noted. *H. kunzii* appears to preferentially colonize the skin of the feet, and while its pathogenic role in diabetic foot ulcers is difficult to establish, it may be a previously unrecognized component of the polymicrobial flora characteristically isolated from patients with these infections.

Helcococcus kunzii, a recently described gram-positive coccoid bacterium, has been isolated in mixed cultures from clinical microbiology specimens at the Massachusetts General Hospital (MGH) (2, 3). A recent report has also described the recovery of *H. kunzii* as the sole isolate from a culture of an infected sebaceous cyst (7). These bacteria are catalase-negative, facultative anaerobes with some phenotypic resemblance to *Aerococcus viridans* (5). Both helcococci and aerococci are pyrrolidonyl arylamidase (PYR) positive and leucine aminopeptidase (LAP) negative and produce acid but not gas from glucose. Helcococci differ from aerococci by demonstrating variable growth in 6.5% NaCl and by having a lipophilic nature. Isolates grow as pinpoint gray colonies on horse blood agar, and Gram-stained smears show pairs, small chains, and irregular clusters of gram-positive cocci. The 16S rRNA sequencing data showed that helcococci are only distantly related to previously described gram-positive cocci (3).

Previously published information suggested a possible link between colonization of skin by *H. kunzii* and the presence of diabetes. Caliendo and coworkers (2) found that diabetes was an underlying condition in 4 of 10 patients from whom clinical specimens yielded *H. kunzii* in culture. Three of these patients, along with five of six nondiabetic patients, had infected lower-extremity wounds. These data led the investigators to hypothesize that *H. kunzii* might be a previously unrecognized member of the polymicrobial flora of diabetic foot ulcers and that diabetics are predisposed to colonization by this bacterium. In view of these observations we attempted to gather more information on the incidence of skin colonization by *H. kunzii* in both diabetic and nondiabetic hosts by culturing samples on a medium selective for *H. kunzii*. We also sampled both lower- and upper-extremity sites to determine the presence of *H. kunzii* in these body areas.

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MATERIALS AND METHODS

Specimen collection. Specimens were obtained from a study group of 120 podiatry patients and a control group of 50 healthy volunteers. The podiatry group consisted of 60 diabetics (either insulin- or non-insulin-dependent diabetics) and 60 nondiabetics, all of whom were being seen on a regular basis by a podiatrist. None of the subjects in the control group had a known history of diabetes or regular podiatry care. Specimens were obtained by vigorously rubbing a dry swab over the skin of the area being sampled (an area of approximately 1 to 2 cm^2), specifically, the skin between the toes and/or the sole of the foot and the skin of the hand or forearm for the upper-extremity sample. Any wounds present in the group of podiatry patients were also sampled with dry swabs. Oral consent was obtained from all individuals prior to sampling.

Specimens were planted immediately onto the selective agar medium described below. The inoculated plates were held at room temperature until they were transported to the laboratory for incubation at 35°C in the presence of 5% CO_2. The time between inoculation and the beginning of the incubation period varied from a few hours to as long as 24 h.

Bacteriology. Material from the swabs was plated onto a medium prepared by autoclaving a solution of 37 g of brain heart infusion broth (Difco, Detroit, Mich.), 15 g of agar (Difco), and 1 ml of Tween 80 (Fisher, Fairlawn, N.J.) in 990 ml of distilled water. After tempering the autoclaved medium at 50°C, 10 ml of a filter-sterilized (pore size, 0.45 μm; Millipore Products Division, Bedford, Mass.) aqueous solution of novobiocin (1 mg/ml; Sigma, St. Louis, Mo.) and colistin (750 μg/ml; Sigma) was added. After gentle mixing, the medium was poured into sterile petri dishes.

Thirteen strains of *H. kunzii* that had previously been isolated at the MGH Microbiology Laboratories and one strain each of *Escherichia coli*, *Edwardsiella tarda*, *Proteus rettgeri*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Morganella morganii*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus warneri*, *Staphylococcus capitis*, *Staphylococcus saprophyticus*, *Staphylococcus cohnii*, and *Staphylococcus hominis* from our culture collection were used to test the selectivity of the medium. After routine culture of these organisms on brucella agar with 5% horse blood (Becton Dickinson Microbiology Systems, Cockeysville, Md.), 10 μl of a saline suspension of each organism at approximately 10^4 CFU was spotted onto the selective agar medium, and the medium was observed for growth after 24 and 48 h of incubation at 35°C in an aerobic atmosphere containing 5% CO_2. Mixtures of *S. aureus* (10^6 CFU), *E. coli* (10^6 CFU), and *H. kunzii* (10^4 CFU) were also tested on the selective medium. Duplicate aliquots from all bacterial suspensions were also plated onto blood agar to check viability. Although horse blood agar was used in this study, *H. kunzii* grows equally well on tryptic soy agar with sheep blood (8).

Plates containing selective medium inoculated with patient specimens were examined after 24, 48, and 72 h of incubation at 35°C in the presence of 5% CO_2. All pinpoint colonies were subcultured onto horse blood agar, Gram stained, and tested for catalase activity. All isolates forming pinpoint, gray colonies on blood agar that appeared as gram-positive cocci in clusters when they were Gram stained and that produced a negative reaction in the catalase test were further identified with the API20 Strep system (bioMérieux Vitek, Inc., Hazelwood, Mo.).

Statistical analysis. The data collected were analyzed for statistical significance by the two-tailed Fisher exact test. Comparisons were made between the control and study groups, the diabetic and nondiabetic podiatry patients in the

study group, and patients with and without wounds. *P* values were calculated for all the comparison groups.

RESULTS

H. kunzii strains from our culture collection exhibited good growth on the selective medium, with colonies apparent after 24 h of incubation. The *P. rettgeri*, *S. saprophyticus*, and *S. cohnii* strains tested grew on the selective medium, but the growth of all other non-*Helcococcus* strains was inhibited. Each of the 13 *H. kunzii* strains tested was recovered on streak plates of the selective medium when the strains were coinoculated with 100-fold greater (10^6 versus 10^4 CFU) numbers of both *E. coli* and *S. aureus* cells. When these 13 mixtures were streaked onto blood agar, colonies of *H. kunzii* were detected in only one case; the *E. coli* and *S. aureus* growth obscured any *H. kunzii* colonies present in the remaining 12 cultures.

The podiatry study group was enlisted from both the MGH podiatry clinic and a private practice in Rhode Island and was composed of 60 (50%) diabetics (either non-insulin- or insulin-dependent diabetics) and 60 (50%) nondiabetics. The age of this group ranged from 24 to 86 years (mean age, 55 years). Ten percent (12 of 120) of the podiatry patients yielded helcococci from cultures of foot swab specimens only. Four of these 12 isolates (33%) were from the nondiabetic group. The remaining 8 (67%) strains were from diabetic patients. Only 6 of the 120 patients (5%) had wounds at the time of examination, all of which were located on the foot. Five of these patients were diabetic; only one wound (from a diabetic) yielded a strain of *Helcococcus*, which was also recovered from this patient's intact foot skin. Helcococci were not recovered from the intact skin of the other five patients with foot wounds. In addition, one *Helcococcus*-like isolate was obtained from the diabetic group.

The control group of 50 healthy subjects was composed of volunteers from the MGH Microbiology Laboratories and pathology residents. The ages ranged from approximately 25 to 60 years, similar to the age range of the subjects in the study group. As mentioned previously, none of the control subjects had a known history of diabetes or were being seen by a podiatrist. Cultures of specimens from only 1 of the 50 (2%) control subjects grew helcococci. This individual had positive cultures for specimens from the foot and hand.

The two-tailed Fisher exact test was used to determine any statistically significant difference in the isolation of helcococci from the groups studied. The first analysis compared the isolation of helcococci from the study group to that from the control group. The calculated *P* value was 0.1113. The second comparison, diabetics versus nondiabetics in the study group, yielded a *P* value of 0.3621. The *P* value for the isolation of helcococci from wounds versus nonwound sites was 0.4761. None of the calculated *P* values were statistically significant, although the comparison of the frequency of isolation from the study group to that from the control group approached statistical significance.

All *Helcococcus* isolates had an API 20 Strep profile of 4100413, corresponding to "doubtful *A. viridans*," which was typical of previously reported *Helcococcus* strains (3). The "doubtful *A. viridans*" identification occurs because helcococci display positive PYR and negative LAP reactions, a profile typical of *A. viridans*. Additional reactions displayed by the helcococci on the API 20 Strep strip were positive reactions for hydrolysis of esculin and acidification of lactose, trehalose, starch, and glycogen. Negative reactions were observed in tests for acetoin production; hydrolysis of hippurate; production of α-galactosidase, β-glucuronidase, β-galactosidase, alkaline phosphatase, and arginine dehydrolase; and acidification of ribose, L-arabinose, mannitol, sorbitol, inulin, and raffinose. The isolate referred to as *Helcococcus*-like acidified more carbohydrates than typical *H. kunzii* strains; acidification of inulin and raffinose was observed, as was a weakly positive reaction with mannitol (API 20 Strep profile, 4100573). If conventional media instead of a commercially available kit are used for characterization of these organisms, positive reactions in the PYR and esculin hydrolysis tests, variable results for growth in the presence of 6.5% NaCl, absence of the LAP enzyme, susceptibility to vancomycin, and stimulation of growth by Tween 80 are to be expected for isolates of helcococci.

DISCUSSION

The selective medium developed in this study appeared to be effective for culture of helcococci from samples from sites containing other microbial flora. Experiments in which mixtures of helcococci (10^4 CFU), *S. aureus* (10^6 CFU), and *E. coli* (10^6 CFU) yielded growth of only helcococci on the selective medium (although in 12 of 13 trials growth of only *S. aureus* and *E. coli* on blood agar was observable), attest to the suitability of the medium. From our observations it seems likely that slowly growing helcococcal colonies could be obscured by more vigorous normal flora or pathogenic microorganisms when clinical specimens are cultured on nonselective media. Difficulty in isolating helcococci when they are present with other organisms may have contributed to their belated recognition in cultures of clinical specimens. Once isolated, the helcococci recovered in our study were readily identified by the API 20 Strep kit, with only one *Helcococcus*-like isolate producing aberrant results.

Cultures of specimens from both the foot and hand of only one individual in the control group yielded helcococci; the remaining 12 isolates recovered in the study were cultured only from foot specimens. These observations suggest that helcococci preferentially inhabit the lower extremities or that they are more readily cultured from these sites. The higher rate of recovery of helcococci from cultures of foot specimens could be a reflection of the higher bacterial counts present on moist versus dry regions of the skin (9). Assuming that our sampling and culture techniques were optimal, our results suggest that helcococci are not as common (2% in the control group and 10% in the podiatric patients) as other skin-colonizing microorganisms such as staphylococci.

Although differences in the rates of *Helcococcus* colonization in the groups that we studied fell short of statistical significance, our data suggest that patients with podiatric problems may be more likely than the general population to be colonized with helcococci. Since many podiatry patients, both diabetic and nondiabetic, have vascular problems, it is possible that vascular insufficiencies may be linked to colonization with *H. kunzii*. Within this patient population it is well known that diabetics are predisposed to a number of infections; those involving foot ulcers are mostly polymicrobial and include both aerobes and anaerobes (1). The most common microorganisms isolated include *S. aureus*, enterococci, coagulase-negative staphylococci, *E. coli*, peptostreptococci, and *Bacteroides fragilis* (1, 4, 6). Our data demonstrating the unreliability of isolation of helcococci from mixtures of bacteria plated on nonselective media and the currently and previously (2) reported isolation of these organisms from foot ulcers suggest that helcococci may represent a formerly unrecognized additional

member of the polymicrobial flora of infected lower-extremity ulcers as well as colonizers of the skin.

The isolation of helcococci along with other bacteria from cultures of wound specimens (2) casts doubt on the pathogenic role of these organisms. Only one of the six cultures of wound specimens examined in our study was *Helcococcus* positive. This culture was matched with a positive culture of a foot skin specimen from the same patient, while the five patients with *Helcococcus*-negative wound specimens also had negative skin specimen cultures, suggesting that helcococci in wound specimen cultures could be present as colonizers. Peel and coworkers (7), however, reported the isolation of *H. kunzii* in pure culture from an infected sebaceous cyst. Thus, this bacterium may also be capable of causing an opportunistic infection. Increased awareness of this organism will no doubt lead to a clearer understanding of its role as an isolate in cultures of clinical specimens.

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REFERENCES

1. **Borrero, E., and M. Rossini.** 1992. Bacteriology of 100 consecutive diabetic foot infections and in vitro susceptibility to ampicillin/sulbactam versus cefoxitin. Angiology **43:**357–361.
2. **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.
3. **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.
4. **Eckman, M. H., S. Greenfield, W. C. Mackey, J. B. Wong, S. Kaplan, L. Sullivan, K. Dukes, and S. G. Pauker.** 1995. Foot infections in diabetic patients. JAMA **273:**712–720.
5. **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.
6. **Gentry, L. O.** 1993. Diagnosis and management of the diabetic foot ulcer. J. Antimicrob. Chemother. **32**(Suppl. A)**:**77–89.
7. **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.
8. **Ruoff, K. L.** Unpublished data.
9. **Tally, F. P.** 1993. Skin and soft tissue, p. 748–759. *In* M. Schaecter, G. Medoff, and B. I. Einstein (ed.), Mechanisms of microbial disease. The Williams & Wilkins Co., Baltimore, Md.