JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2005, p. 1448–1450 Vol. 43, No. 3
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.3.1448–1450.2005


# Erroneous Reporting of Coagulase-Negative Staphylococci as *Kocuria* spp. by the Vitek 2 System

R. Ben-Ami,[1] S. Navon-Venezia,[2] D. Schwartz,[3] Y. Schlezinger,[2] Y. Mekuzas,[2] and Y. Carmeli[1,2,4]*

*Infectious Diseases Unit,[1] Laboratories of Molecular Epidemiology[2] and Microbiology,[3] and Department of Epidemiology,[4] Tel Aviv Sourasky Medical Center, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel*

Received 19 February 2004/Returned for modification 4 June 2004/Accepted 18 October 2004

**Misidentification of coagulase-negative staphylococci (CoNS) may delay appropriate treatment. We investigated 20 clinical isolates identified as *Kocuria* spp. by the Vitek 2 system. All were identified as CoNS by 16S rRNA gene sequencing (18 *Staphylococcus epidermidis*, 1 *Staphylococcus haemolyticus*). Four *Kocuria* isolates were shown to be identical to CoNS from the same patient by pulsed-field gel electrophoresis. Isolates identified by Vitek 2 as *Kocuria* most likely represent misidentified CoNS, and if clinically indicated, should be investigated further by genomic methods.**

Coagulase-negative staphylococci (CoNS) are increasingly important human pathogens, often involved in infections related to implanted medical devices, such as intravenous catheters, prosthetic heart valves, and orthopedic implants (9). Correct identification of CoNS by microbiology laboratories facilitates early recognition and management of these infections, often necessitating removal of the infected device. The Vitek 2 system (Vitek Systems, bioMerieux, St. Louis, Mo.) is an automated bacterial identification and susceptibility testing system that uses fluorescence-based technology. This system was previously reported to correctly identify ~90% of CoNS to the species level (8). With the introduction of the Vitek 2 system into our microbiology laboratory, we noticed a dramatic increase in the number of clinical isolates identified as *Kocuria* spp. Our investigation of one case of continuous bacteremia with *Kocuria* spp. revealed all isolates to be identical by pulsed-field gel electrophoresis (PFGE); isolates were subsequently identified as *Staphylococcus epidermidis* by 16S RNA gene sequencing (3). We hypothesized that phenotypic variation, a feature of pathogenic *S. epidermidis* (10), may cause misidentification by standard laboratory procedures. These findings prompted us to review the clinical and microbiological features of isolates identified as *Kocuria* spp. by our microbiology laboratory. In order to concentrate on clinically significant isolates, only isolates from blood or implanted medical devices were studied.

Sequential clinical isolates, identified as *Kocuria* spp. by Vitek 2 using the ID-GPC card were collected. Antimicrobial susceptibility profiles were determined by Vitek 2. An investigation was conducted in each case, with the aim of determining the clinical significance of the index isolate.

Growth on Congo red agar (CRA) has previously been noted to enable morphological characterization of phenotypically variable staphylococci (4, 5). Colonial morphology was studied on Trypticase soy agar plates containing 5% sheep blood (Hy-Labs, Rehovot, Israel) and on homemade CRA plates containing 0.01% Congo red (4, 5). Plates were incubated at 35°C with $CO_2$ (5%) for 48 h and at room temperature for another 48 h.

PFGE analysis was performed on all available clinical isolates of *Kocuria* spp., and on any CoNS isolated from the same patient, to determine genetic relatedness. DNA preparation and cleavage were performed as described previously (2). Chromosomal restriction fragments were separated in a 1.0% SeaKem LE agarose gel (FMC BioProducts, Rockland, Maine) with the CHEF-DR III apparatus (Bio-Rad, Hercules, Calif.). Electrophoresis conditions were 6 V/cm for 22 h at 14°C, with pulse times ranging from 5 to 40 s. DNA was stained with ethidium bromide, photographed (Bio-Rad Gel Doc 2000), and analyzed using diversity-fingerprinting software (Bio-Rad).

For 16S rRNA gene amplification, DNA samples were prepared using a Wizard genomic DNA purification kit according to the manufacturer's instructions (Promega, Madison, Wis.). The nearly complete sequence of the 16S rRNA gene was amplified by PCR with the conserved primers 8F (5′-AG AGTTTGATYMTGGCTCAG-3′) and 1942R (5′-ACCTTGT TACGACTT-3′). Amplification was carried out using Vent DNA polymerase (New England BioLabs) and genomic DNA template. PCR conditions were as follows: denaturation at 95°C for 5 min, followed by 30 cycles of 60 s at 95, 56, and 72°C and a final elongation step of 10 min at 72°C. PCR products were purified using a Promega Wizard PCR Preps kit (Promega) and sequenced using an Applied Biosystems (Foster City, Calif.) model 373A sequencer. Sequences were analyzed and compared using BLASTN software (http://www.ncbi.nlm.nih.gov/BLAST/) (1).

Twenty clinical isolates from seven patients were identified as *Kocuria* spp. by Vitek 2 (19 *K. varians* and 1 *K. rosea*); of these, 19 were available for further study (Table 1). Sources of *Kocuria* isolates were as follows: blood culture (12 isolates), pacemaker electrode (3 isolates), pacemaker pocket (2 isolates), and one isolate each from a pleural empyema, a central venous catheter, and an intraosseous nail of an Ilizarov external fixator. Five of the seven patients were judged to have a clinically significant infection involving an implanted medical device.

Eleven of 19 *Kocuria* isolates (58%) exhibited variability in colony morphology on CRA (Table 1). In addition, three pa-

* Corresponding author. Mailing address: Infectious Diseases Unit, Tel-Aviv Sourasky Medical Center, Tel-Aviv 64239, Israel. Phone and fax: 972-3-697-4996. E-mail: ycarmeli@bidmc.harvard.edu.

TABLE 1. Characteristics of 18 clinical isolates identified as *Kocuria* spp. by Vitek 2

| Patient | Age/sex[a] | Clinical diagnosis | Vitek 2 ID (no. of isolates) | Source | Variability on CRA | PFGE clone | ID by 16S rRNA gene sequencing |
|---|---|---|---|---|---|---|---|
| 1 | 70 yr/M | Infected pacemaker electrode | *K. rosea* (1) | Blood | − | A | *S. epidermidis* |
| | | | *S. epidermidis* (8) | Blood | + | A | |
| | | | CoNS (3) | Blood | − | A | |
| | | | *K. varians* (1) | Pacemaker pocket | − | A | |
| 2 | 85 yr/M | Infected pacemaker electrode | *K. varians* (2) | Electrode | − | B | *S. epidermidis* |
| | | | *K. varians* (1) | Pacemaker pocket | − | B | |
| | | | *K. varians* (1) | Electrode | + | D | |
| | | | CoNS (1) | Blood | − | C | |
| | | | CoNS (1) | Blood | − | B | |
| 3[b] | 47 yr/F | Infected ventriculoatrial shunt | *K. varians* (9) | Blood | + | F | *S. epidermidis* |
| | | | *S. hyicus* (5) | Blood | + | F | |
| | | | *S. epidermidis* (1) | Blood | + | F | |
| | | | CoNS (4) | Blood | + | F | |
| 4 | 15 yr/M | Infected Ilizarov external fixator | *K. varians* (1) | Intraosseous nail | − | G | *S. hemolyticus* |
| 5 | 61 yr/M | Postsurgical pleural empyema | *K. varians* (1) | Blood | + | E | *S. epidermidis* |
| | | | CoNS (1) | Blood | − | E | |
| | | | CoNS (1) | Blood | + | B | |
| | | | CoNS (2) | Pleural empyema | NA[d] | | |
| | | | *K. varians* (1) | Pleural empyema | NA | | |
| 6 | 34 yr/F | Routinely extracted Port-o-Cath | *K. varians* (1) | CVC[c] | − | H | *S. epidermidis* |
| 7 | 3 wk/F | Pseudobacteremia | *K. varians* (1) | Blood | − | I | *S. epidermidis* |

[a] M, male; F, female.
[b] Patient has been previously reported (3).
[c] CVC, central venous catheter.
[d] NA, not available.

tients also had CoNS blood isolates exhibiting colonial variability. Colonial variability manifested as colonies of different sizes (typically pinpoint and large colonies), various elevations above the agar (dome-shaped and flat colonies), and variable uptake of Congo red resulting in dark red or light pink colonies.

For all four patients who had CoNS isolated from blood (patients 1 to 4), PFGE patterns for the *Kocuria* isolates were identical to at least one of the CoNS. Of 19 available clinical isolates identified as *Kocuria* by Vitek 2, 18 were identified as *S. epidermidis* by 16S rRNA gene sequencing, and one was identified as *Staphylococcus haemolyticus*. Isolates from different patients were shown to be unrelated by PFGE (Table 1; Fig. 1).

The Vitek 2 system is not optimized for reporting *Kocuria* spp. The biochemical reactions used in the ID-GPC card are either negative or variably positive with the quality control strain of *K. rosea* (ATCC 186); none of the reactions included in this protocol yields a positive result in 95% or more of *K. rosea* strains (Vitek 2 ID-GPC quality control table, bioMerieux, 1999). All of the clinical isolates identified by Vitek 2 as *Kocuria* that we examined were in fact CoNS, 58% were phenotypically variable and most represented clinically significant infections associated with implanted medical devices. Although these results are uncontrolled, they suggest that identification of *Kocuria* by Vitek 2 may be a marker for invasive infection caused by CoNS. Phenotypic variation, manifesting as variability in colony morphology, is characteristic of virulent strains of *S. epidermidis* (10). It is possible that phenotypic variation is also responsible for misidentification of CoNS by biochemical methods, leading to erroneous reporting of CoNS as *Kocuria* by Vitek 2.

Clinical isolates reported by Vitek 2 as *Kocuria* spp. are not rare. Our laboratory, which serves a 1,200-bed hospital and processes about 47,000 blood cultures a year, identified 117

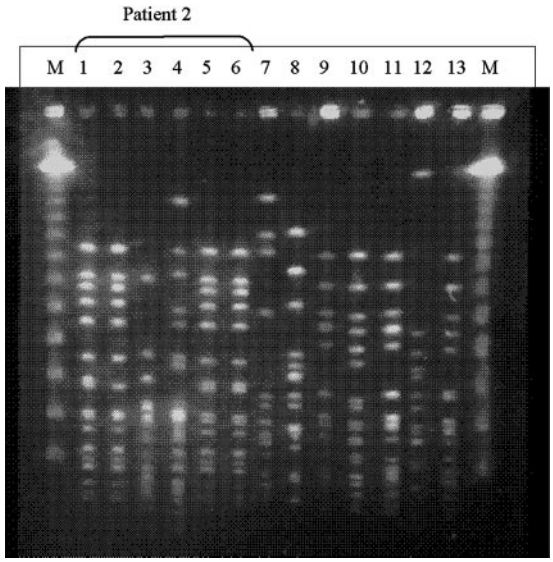


FIG. 1. PFGE patterns produced by SmaI macrorestriction from clinical isolates reported as CoNS and *Kocuria* spp. by Vitek 2. Lanes 1 to 6 contain isolates from patient 2, reported as *Kocuria* (lanes 1, 2, and 4) and CoNS (lanes 3, 5, and 6). The isolates identified as *K. varians* from the pacemaker pocket (lane 1) and an electrode (lane 2) were identical to each other, as well as to CoNS isolates from another electrode and blood (lanes 5 and 6, respectively). Another CoNS cultured from blood (lane 3) and an isolate identified as *K. varians* that was cultured from an electrode (lane 4) were of different clones. Lanes 7 to 13 each contain an isolate identified as *Kocuria* spp. from a different patient, shown by PFGE to belong to different clones. Lanes M, lambda standard markers. Isolate 7 was identified by 16S rRNA gene sequencing as *S. haemolyticus*; all other isolates were identified as *S. epidermidis.*

clinical isolates as *Kocuria* spp. between 1 December 2002 and 1 December 2003, among them 23 blood isolates and 21 vascular catheter isolates. If, as our data suggests, most of these isolates are in fact misidentified CoNS, then the problem presented here may be of significant magnitude.

Incorrect identification of CoNS by the microbiology laboratory increases the likelihood that a significant infection will be erroneously ascribed to contamination (6, 7). This problem may be addressed in several ways. Clearly, CoNS are best identified by genomic methods, such as 16S rRNA gene sequencing, thus eliminating confusion arising from phenotypic variation. However, high cost and the intensive labor required limit the use of such methods in clinical practice. Laboratories using the Vitek 2 system may wish to investigate the significance of isolates identified as *Kocura* spp., as we have done. Recognizing that *Kocuria* isolates frequently represent misidentified CoNS, it is reasonable to suppress reports of *Kocuria* by the laboratory and exchange them with "unidentified gram-positive cocci." In any case, a clinical specimen growing *Kocuria* should raise suspicion of CoNS infection, especially if the patient has an indwelling medical device and additional cultures are positive for CoNS. In these cases PFGE may be useful in demonstrating the genetic identity of different isolates, which strongly suggests true infection rather than contamination (6, 7). Finally, since phenotypic variation is known to be affected by the culture medium and growth conditions (4), the performance of the Vitek 2 system may improve when CoNS are cultured in conditions that minimize phenotypic variation.

## REFERENCES


1. **Altschul, S. F., A. A. Madden, J. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman.** 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25:**3389–3402.
2. **Bannerman, T. L., G. A. Hancock, F. C. Tenover, and J. M. Miller.** 1995. Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus.* J. Clin. Microbiol. **33:**551–555.
3. **Ben-Ami, R., S. Navon-Venezia, D. Schwartz, and Y. Carmeli.** 2003. Infection of a ventriculoatrial shunt with phenotypically variable *Staphylococcus epidermidis* masquerading as polymicrobial bacteremia due to various coagulase-negative staphylococci and *Kocuria varians.* J. Clin. Microbiol. **41:**2444–2447.
4. **Deighton, M., J. Capstick, and R. Borland.** 1992. A study of phenotypic variation of *Staphylococcus epidermidis* using Congo red agar. Epidemiol. Infect. **109:**423–432.
5. **Deighton, M., S. Pearson, J. Capstick, D. Spelman, and R. Borland.** 1992. Phenotypic variation of *Staphylococcus epidermidis* isolated from a patient with native valve endocarditis. J. Clin. Microbiol. **30:**2385–2390.
6. **Khatib, R., K. M. Riederer, J. A. Clark, S. Khatib, L. E. Briski, and F. M. Wilson.** 1995. Coagulase-negative staphylococci in multiple blood cultures: strain relatedness and determinants of same-strain bacteremia. J. Clin. Microbiol. **33:**816–820.
7. **Kim, S. D., L. C. McDonald, W. R. Jarvis, S. K. McAllister, R. Jerris, L. A. Carson, and J. M. Miller.** 2000. Determining the significance of coagulase-negative staphylococci isolated from blood cultures at a community hospital: a role for species and strain identification. Infect. Control Hosp. Epidemiol. **21:**213–217.
8. **Ligozzi, M., C. Bernini, M. G. Bonora, M. de Fatima, J. Zuliani, and R. Fontana.** 2002. Evaluation of the VITEK 2 system for identification and antimicrobial susceptibility testing of medically relevant gram-positive cocci. J. Clin. Microbiol. **40:**1681–1686.
9. **Rupp, M. E., and G. L. Archer.** 1994. Coagulase-negative staphylococci: pathogens associated with medical progress. Clin. Infect. Dis. **19:**231–243.
10. **Ziebuhr, W., C. Heilmann, F. Gotz, P. Meyer, K. Wilms, E. Straube, and J. Hacker.** 1997. Detection of the intercellular adhesion gene cluster (*ica*) and phase variation in *Staphylococcus epidermidis* blood culture strains and mucosal isolates. Infect. Immun. **65:**890–896.