## Acinetobacter baumannii-Infected Vascular Catheters Collected from Horses in an Equine Clinic

## MARIO VANEECHOUTTE,<sup>1\*</sup> LUC A. DEVRIESE,<sup>2</sup> LENIE DIJKSHOORN,<sup>3</sup> BENEDICTE LAMOTE,<sup>2</sup> PIET DEPREZ,<sup>2</sup> GERDA VERSCHRAEGEN,<sup>1</sup> AND FREDDY HAESEBROUCK<sup>2</sup>

Department of Clinical Chemistry, Microbiology and Immunology, University Hospital Ghent,<sup>1</sup> and Faculty of Veterinary Medicine, University of Ghent,<sup>2</sup> Ghent, Belgium, and Department of Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands<sup>3</sup>

Received 6 March 2000/Returned for modification 2 June 2000/Accepted 27 August 2000

Acinetobacter baumannii was isolated from tips clipped from seven intravenous jugular catheters collected from horses in the Ghent University equine clinic. They originated from seven different horses. Three of the seven showed evidence of local infection.

Acinetobacter baumannii is a well-known cause of a wide spectrum of nosocomial infections in hospitals. Multiresistant strains of this species are particularly important as pneumonia agents in intensive care units (8). Pneumonia and catheterrelated infection are the common sources of *A. baumannii* bacteremia. Unlike other *Acinetobacter* species, *A. baumannii* is found only rarely on human skin in nonepidemic situations (9), and its natural habitat remains unknown.

The ecology of the different (genomic) species of the genus Acinetobacter is scarcely known. Two reports have attested to the presence of unspecified Acinetobacter strains in samples from lower respiratory tract infections in horses without evidence of any involvement in the pathologic conditions (5, 12). One paper described the frequent isolation of Acinetobacter strains from infectious keratitis in horses, again without presenting evidence of a pathologic role for the bacteria (7). Several publications from the 1960s and 1970s reported on the occurrence of acinetobacters (at the time designated by several names that are no longer valid: Herellea vaginicola, Moraxella lwoffi, or Bacterium anitratum) in animals (4). In a Polish study (6), a high proportion (32.3%) of workers on a horse farm were found to have precipitins to Acinetobacter calcoaceticus sensu lato, which may indicate an immunological response to acinetobacters in their environment. Since the authors used the name A. calcoaceticus, which in the past was used for all members of the genus, it is not clear against which of the presently known (genomic) species antibodies were directed.

Here we report the isolation of *A. baumannii* from jugular catheter tips collected from horses suffering from a variety of conditions and hospitalized in the Ghent University equine clinic. Four of the horses were hospitalized for colic surgery, and three were hospitalized for treatment of enteritis. All animals survived and were discharged. Seven *Acinetobacter* isolates were obtained from seven catheter tips originating from seven horses. The *Acinetobacter* cases were part of an investigational series comprising 32 catheter tips derived from 23 horses with or without evidence of catheter infection. The other organisms belonged to *Enterobacteriaceae* (n = 7), *Staphylococcus* spp. (n = 3), and gram-negative nonfermenters (n = 3). One of the *Acinetobacter* cases had a mixed infection with *Pseudomo*-

nas aeruginosa, and another had an infection with Enterococcus faecalis. Pus formation was noticed in the latter case and in one horse from which A. baumannii was obtained in pure culture. The organism was isolated in pure culture from a case of thrombophlebitis as well. The tips and subcutaneous inner and/or outer segments of the catheters were cultured by the semiquantitative roll plate method on Columbia agar plates enriched with 5% sheep blood. Catheter colonization was defined when at least 15 CFU of a similar morphology was obtained. Isolates were identified to the genus Acinetobacter if theywere nonmotile, catalase-positive, oxidase-negative, nonfermenting, gram-negative coccobacilli. Further phenotypic characterization below the genus level was based on the oxidation-fermentation test for oxidative-fermentative acidification of glucose, the determination of hemolysis of the blood agar plate, growth at 44°C using the method described by Bouvet and Grimont (2), and the tests included in the commercial API 20NE system (BioMérieux, Marcy l'Étoile, France). None of the strains was hemolytic. By use of the test results of the API 20NE system with Database version 6.0 and with measurement of growth at 44°C as a complementary test, the organisms were identified as A. baumannii. However, during a previous study validating the API 20NE system with a large set of strains that had been identified to (genomic) species by DNA-DNA hybridization (1), it was found that one strain with API code 0001473 was not identifiable and that some of the isolates had to be identified as belonging to the A. calcoaceticus-A. baumannii complex. This complex also contains A. cal*coaceticus*, DNA group 3, and DNA group 13 sensu Tjernberg and Ursing, in addition to A. baumannii (10). Since growth at 44°C has been found to be positive both in A. baumannii and in DNA group 13 sensu Tjernberg and Ursing, the strains were phenotypically allocated to one of the latter (genomic) species. In this study, identification by means of amplified ribosomal DNA restriction analysis (3, 11) unambiguously allocated the seven strains to A. baumannii. Restriction digestion with the enzymes CfoI, MboI, and AluI of the amplified 16S rRNA gene vielded restriction profile 1,1,1, which is specific for A. baumannii (3, 11; http://allserv.rug.ac.be/~mvaneech/ARDRA /Acinetobacter.html).

All strains were resistant to the following antibiotics used in equine practice: amoxicillin, amoxicillin and clavulanic acid, ceftiofur (a cephalosporin), tetracyclines, and potentiated sulfonamides. They were intermediately susceptible or resistant to gentamicin and susceptible to neomycin. Two strains had

<sup>\*</sup> Corresponding author. Mailing address: Laboratory Bacteriology & Virology, Blok A, University Hospital Ghent, De Pintelaan 185, 9000 Ghent, Belgium. Phone: 32 9 2403692. Fax: 32 9 2403659. E-mail: Mario.Vaneechoutte@rug.ac.be.

Strain designation	Resistance of isolates to drugs:								API 20NE
	A	A + C	С	Т	S + T	G	Ν	Е	code
4982	R	R	R	R	R	Х	S	S	0001073
5007	R	R	R	R	R	Х	S	S	0041473
5014	R	R	R	R	R	R	S	R	0041473
5017	R	R	R	R	R	Х	S	S	0041473
5022	R	R	R	R	R	R	S	R	0041473
5412	R	R	R	R	R	Х	S	S	0041073
5440	R	R	R	R	R	R	S	S	0001473

<sup>*a*</sup> A, amoxicillin; A + C, amoxicillin plus clavulanic acid; C, ceftiofur; T, tetracyclines; S + T, sulfonamides plus trimethoprim; G, gentamicin; N, neomycin; E, enrofloxacin; R, resistant; X, intermediate; S, susceptible.

acquired resistance against the fluoroquinolone antibiotics flumequine and enrofloxacin (Table 1).

The pathogenic significance for the horses of the bacteria studied was probably low. Intensive care of debilitated animals is less often carried out in veterinary medicine, which curbs the possibility of serious pneumonia and bacteremia in these animals. The present study indicated the occurrence of *A. baumannii* in horses, but this was not always associated with disease. In light of the poor knowledge of the ecology of these bacteria, the present findings (including the resistance to multiple antibiotics) and the old and nearly forgotten literature are a stimulus to search for these organisms in animals. Whether or not horses or animals in general are a potential source of *A. baumannii* in humans remains to be determined.

## ADDENDUM IN PROOF

After the manuscript was submitted, comparable findings were published (T. Francey, F. Gaschen, J. Nicolet, and A. P. Burnens, J. Vet. Intern. Med. **14**:177–183, 2000).

## REFERENCES

- Bernards, A. T., J. van der Toorn, C. P. A. van Boven, and L. Dijkshoorn. 1996. Evaluation of the ability of a commercial system to identify *Acinetobacter* genomic species. Eur. J. Clin. Microbiol. Infect. Dis. 15:303– 308.
- Bouvet, P. J. M., and P. A. D. Grimont. 1986. Taxonomy of the genus Acinetobacter with the recognition of Acinetobacter baumannii sp. nov., Acinetobacter haemolyticus sp. nov., Acinetobacter johnsonii sp. nov., and Acinetobacter junii sp. nov. and emended descriptions of Acinetobacter calcoaceticus and Acinetobacter lwoffii. Int. J. Syst. Bacteriol. 36:228–240.
- Dijkshoorn, L., B. van Harsselaar, I. Tjernberg, P. J. M. Bouvet, and M. Vaneechoutte. 1998. Evaluation of amplified ribosomal DNA restriction analysis for identification of *Acinetobacter* genomic species. Syst. Appl. Microbiol. 21:33–39.
- Henriksen, S. D. 1973. Moraxella, Acinetobacter, and the Mimae. Bacteriol. Rev. 37:522–561.
- Kester, R. M., S. Lesser, and L. L. Dowd. 1993. Bacteria isolated from equine respiratory cultures. Equine Pract. 15:33–36.
- Mackiewicz, B., Z. Prazmo, J. Milanowski, J. Dutkiewicz, and B. Fafrowicz. 1996. Exposure to organic dust and microorganisms as a factor affecting respiratory function of workers of purebred horse farms. Pneumonol. Alergol. Pol. 64(Suppl. 1):19–24. (In Polish.)
- Moore, C. P., B. K. Collins, and W. H. Fales. 1995. Antimicrobial susceptibility patterns for microbial isolates associated with infectious keratitis in horses: 63 cases (1986–1994). J. Am. Vet. Med. Assoc. 207:928–933.
- Rodriguez-Bano, J. 1999. Nosocomial bacteraemia due to Acinetobacter baumannii. Rev. Med. Microbiol. 10:67–77.
- Seifert, H., L. Dijkshoorn, P. Gerner-Smidt, N. Pelzer, I. Tjernberg, and M. Vaneechoutte. 1997. Distribution of *Acinetobacter* species on human skin: comparison of phenotypic and genotypic identification methods. J. Clin. Microbiol. 35:2819–2825.
- Tjernberg, I., and J. Ursing. 1989. Clinical strains of *Acinetobacter* classified by DNA-DNA hybridization. Acta Pathol. Microbiol. Immunol. Scand. 97: 595–605.
- Vaneechoutte, M., L. Dijkshoorn, I. Tjernberg, A. Elaichouni, P. De Vos, G. Claeys, and G. Verschraegen. 1995. Identification of *Acinetobacter* genomic species by amplified ribosomal DNA restriction analysis. J. Clin. Microbiol. 33:11–15.
- Wood, J. L., M. H. Burrell, C. A. Roberts, N. Chanter, and Y. Shaw. 1993. Streptococci and *Pasteurella* spp. associated with disease of the equine lower respiratory tract. Equine Vet. J. 25:314–318.