JCM Journals.ASM.org

CASE REPORT

A Taxonomically Unique *Acinetobacter* Strain with Proteolytic and Hemolytic Activities Recovered from a Patient with a Soft Tissue Injury

Marisa Almuzara,[b,c] German Matías Traglia,[a] Lenka Krizova,[d] Claudia Barberis,[b] Sabrina Montaña,[a] Romina Bakai,[c] Alicia Tuduri,[c] Carlos Vay,[b] Alexandr Nemec,[d] María Soledad Ramírez[a,e]

Instituto de Microbiología y Parasitología Médica (IMPaM, UBA-CONICET),[a] Laboratorio de Bacteriología Clínica, Departamento de Bioquímica Clínica, Hospital de Clínicas José de San Martín, Facultad de Farmacia y Bioquímica,[b] and Laboratorio de Bacteriología, Hospital Interzonal de Agudos Eva Perón, San Martín,[c] Buenos Aires, Argentina; Laboratory of Bacterial Genetics, National Institute of Public Health, Šrobárova, Prague, Czech Republic[d]; Center for Applied Biotechnology Studies, Department of Biological Science, California State University, Fullerton, Fullerton, California, USA[e]

A taxonomically unique bacterial strain, *Acinetobacter* sp. A47, has been recovered from several soft tissue samples from a patient undergoing reconstructive surgery owing to a traumatic amputation. The results of 16S rRNA, *rpoB*, and *gyrB* gene comparative sequence analyses showed that A47 does not belong to any of the hitherto-known taxa and may represent an as-yet-unknown *Acinetobacter* species. The recognition of this novel organism contributes to our knowledge of the taxonomic complexity underlying infections caused by *Acinetobacter*.

CASE REPORT

A 59-year-old female patient was admitted to an emergency room owing to severe left forearm trauma and traumatic amputation of her left foot secondary to a road accident. On the day of admission, she underwent a surgical toilette of the forearm and infrapatellar leg amputation. At that moment, samples of skin, soft tissue, and bone were sent for culture with negative results. Six days after the surgical debridement, wound dehiscence with purulent discharge of the amputation stump with exposed bone was observed. A new surgical debridement was performed, and a wound secretion sample and two soft tissue samples were sent for bacteriological examination. In the Gram stain, abundant Gram-negative (GN) bacilli together with Gram-positive cocci arranged in chains were seen. After 24 h of incubation at 35°C, colonies formed by both a Gram-negative organism and *Enterococcus faecalis* were observed on sheep blood agar. The colonies of the Gram-negative bacterium were about 2 mm in diameter, with an irregular spreading edge surrounded by wide zones of beta-hemolysis. Both organisms were considered the possible causative agent of the infection because of their association with clinical signs in the infected tissue (purulent secretion and wound dehiscence). As these microorganisms were isolated 6 days after the patient's admission to hospital, the infection was considered hospital acquired.

The Gram-negative strain (designated A47) was first investigated by conventional biochemical tests. Positive results were obtained for D-glucose oxidation and gelatin hydrolysis whereas negative results were recorded for oxidase, swimming motility, dissimilative nitrate reduction, urease, or acetamide hydrolysis. Identification by the use of an automated Vitek 2 compact system (bioMérieux) using a GN Colorimetric Identification Card yielded a biocode of 0001012100500300 which corresponds to *Acinetobacter baumannii* with a probability of 94%. Species identification was also carried out by using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonik) and the current Bruker Daltonics database (MBT-BDAL-5627 MSP library). Using this approach, the organisms was identified only at the genus level (maximum score of <2.0) although the first identification hit was *Acinetobacter parvus* with a score of 1.93. However, the appearance of its colonies (see above) was not congruent with the description of *A. parvus*, which is a small-colony-forming, nonhemolytic species (1). Furthermore, we determined the 16S rRNA gene sequence (1,332 bp) of A47 (NCBI accession no. KM386667). A BLAST-based search using NCBI databases for this sequence revealed its highest similarity (95%) to be with that of *A. parvus* X32 (KC477206). Altogether, these results indicated that A47 belonged to the genus *Acinetobacter* but its identity at the species level remained obscure.

Antibiotic susceptibility testing was performed using a Vitek 2 system and an AST-079 panel (Gram-negative-susceptibility [GNS] card). MIC results were interpreted using the CLSI breakpoints for *Acinetobacter* except for ampicillin, cephalothin, and cefoxitin, for which the breakpoints for *Enterobacteriaceae* were used (2). Strain A47 was susceptible to ampicillin-sulbactam, piperacillin-tazobactam, ceftazidime, cefepime, imipenem, meropenem, amikacin, gentamicin, ciprofloxacin, colistin, and trimethoprim-sulfamethoxazole, whereas it appeared resistant to

Received 10 September 2014 **Returned for modification** 2 October 2014
Accepted 29 October 2014

Accepted manuscript posted online 12 November 2014

Citation Almuzara M, Traglia GM, Krizova L, Barberis C, Montaña S, Bakai R, Tuduri A, Vay C, Nemec A, Ramírez MS. 2015. A taxonomically unique *Acinetobacter* strain with proteolytic and hemolytic activities recovered from a patient with a soft tissue injury. J Clin Microbiol 53:349–351. doi:10.1128/JCM.02625-14.

Editor: G. A. Land

Address correspondence to Alexandr Nemec, anemec@szu.cz, or María Soledad Ramírez, msramirez@fullerton.edu.

Supplemental material for this article may be found at http://dx.doi.org/10.1128/JCM.02625-14.

doi:10.1128/JCM.02625-14

ampicillin, cefalotin, cefoxitin, and cefotaxime. Based on these results, treatment with imipenem at 500 mg/6 h of intravenous (i.v.) administration and with ampicillin at 2 g/6 h (owing to the presence of ampicillin-susceptible *Enterococcus faecalis*) for 30 days was conducted. After completing treatment and because of the good clinical course, the patient was discharged to her home in stable condition.

Given the conflicting results of the species identification based on routine and commercial diagnostic systems, we decided to assess precisely the taxonomic position of strain A47 using a set of taxonomic methods relevant for the genus *Acinetobacter*. First, we performed the comparative sequence analysis of the *rpoB* gene, which is which is the single most frequently used gene taxonomic marker for the genus (3–7). The similarity calculations and cluster analysis were performed for a variable 861-bp region that included nucleotide positions 2915 to 3775 of the *rpoB* coding region of *A. baumannii* CIP 70.34^T (NCBI accession no. APRG00000000) as described previously (3, 6). The *rpoB*-based dendrogram for A47 and strains representing all known validly or provisionally named species of the genus is shown in Fig. S1A in the supplemental material. The intraspecies similarity values between A47 and the other members of the genus were in the range of 77.1% (*Acinetobacter qingfengensis*) to 90.9% (genomic species 17), which indicates the uniqueness of A47 at the species level. To verify the taxonomic distinctness of A47 inferred from *rpoB* sequences, we carried out comparative analysis of the partial sequences of the *gyrB* gene using the primers described by Yamamoto et al. (8). The similarity calculations and cluster analysis were performed for an 804-bp region corresponding to positions 421 to 1224 of the *gyrB* gene of *A. baumannii* CIP 70.34^T (see Fig. S1B). The identity values between A47 and the representatives of known *Acinetobacter* spp. ranged from 74.9% (*Acinetobacter bohemicus*) to 86.6% (*Acinetobacter gyllenbergii*), which further supports the taxonomic uniqueness of A47.

To define the phenotype of A47, we performed the in-house nutritional and physiological tests targeted to the *Acinetobacter* genus (3, 5). A47 lysed both sheep erythrocytes and gelatin, produced acid from D-glucose, and grew at 41°C but not at 44°C. It utilized acetate, *trans*-aconitate, 4-aminobutyrate, adipate, L-arginine, azelate, β-alanine, citrate, glutarate, L-histidine, 4-hydroxybenzoate, DL-lactate, L-leucine, L-ornithine, phenylacetate, phenylalanine, putrescine, D-ribose, and tricarballylate as sole sources of carbon but did not grow on L-arabinose, L-aspartate, benzoate, butanediol, histamine, levulinate, citraconate, L-tartrate, gentisate, D-glucose, D-gluconate, trigonelline, or tryptamine. The combination of these properties is unique among those of nearly 800 phenotypically well-characterized strains which are deposited in the *Acinetobacter* collection of the Laboratory of Bacterial Genetics and which represent all validly named species and a number of novel tentative species (A. Nemec and L. Krizova, unpublished data).

The genotypic results described above clearly indicate that A47 is distinct from the hitherto known *Acinetobacter* taxa at the species level. Based on both the *rpoB* and *gyrB* sequences (see Fig. S1 in the supplemental material), this strain appears to be most closely related to the *Acinetobacter* spp. which typically include hemolytic and/or proteolytic strains, such as *A. gyllenbergii*, *Acinetobacter venetianus*, and genomic species described by Bouvet and Jeanjean et al. (9, 10). A47 clustered with these proteolytic/hemolytic species also using MALDI-TOF MS whole-cell profiling based on our in-house database (5) which encompasses the representatives of all validly named species and a number of provisionally termed genomic species of the genus *Acinetobacter* (Krizova and Nemec, unpublished). The hemolytic-proteolytic phenotype and MALDI-TOF MS protein spectrum of A47 are thus consistent with its taxonomic position based on genotypic markers.

In conclusion, the present report provides another example that illustrates the difficulties of the identification of *Acinetobacter* isolates in routine diagnostic laboratories. Such laboratories mostly depend on commercial systems which generally suffer from a small number of diagnostically relevant characteristics and/or the insufficient quality of reference data (11). This report also demonstrates the usefulness of *Acinetobacter*-targeted taxonomic methods such as *rpoB*- and *gyrB*-based comparative analysis, which may help in the elucidation of the taxonomic position of unusual strains and to uncover novel, clinically relevant species.

Nucleotide sequence accession numbers. The 16S rRNA, *gyrB*, and *rpoB* genes of *Acinetobacter* sp. A47 were deposited in GenBank under accession numbers KM386667, KM386668, and KM386669, respectively.

ACKNOWLEDGMENTS

M.S.R. is a career investigator of CONICET, Argentina. G.M.T. has doctoral fellowships from CONICET. This study was supported by grant PIP 11420100100152 to M.S.R. and grant PICT 2012-00120 to M.S.R. and by grants from the "Secretaría de Ciencia y Técnica de la Universidad de Buenos Aires" (UBACyT) to C.V. A.N. and L.K. were supported by grant 13-26693S from the Czech Science Foundation.

REFERENCES

1. **Nemec A, Dijkshoorn L, Cleenwerck I, De Baere T, Janssens D, Van Der Reijden TJ, Jezek P, Vaneechoutte M.** 2003. *Acinetobacter parvus* sp. nov., a small-colony-forming species isolated from human clinical specimens. Int J Syst Evol Microbiol **53:**1563–1567. http://dx.doi.org/10.1099/ijs.0.02631-0.
2. **CLSI.** 2013. Performance standards for antimicrobial susceptibility testing; M100-S23, 23th informational supplement. Clinical and Laboratory Standards Institute, Wayne, PA.
3. **Nemec A, Musílek M, Maixnerová M, De Baere T, van der Reijden TJ, Vaneechoutte M, Dijkshoorn L.** 2009. *Acinetobacter beijerinckii* sp. nov. and *Acinetobacter gyllenbergii* sp. nov., haemolytic organisms isolated from humans. Int J Syst Evol Microbiol **59**(Pt 1):118–124. http://dx.doi.org/10.1099/ijs.0.001230-0.
4. **Karah N, Haldorsen B, Hegstad K, Simonsen GS, Sundsfjord A, Samuelsen Ø; Norwegian Study Group of Acinetobacter.** 2011. Species identification and molecular characterization of *Acinetobacter* spp. blood culture isolates from Norway. J Antimicrob Chemother **66:**738–744. http://dx.doi.org/10.1093/jac/dkq521.
5. **Krizova L, Maixnerova M, Sedo O, Nemec A.** 2014. *Acinetobacter bohemicus* sp. nov. widespread in natural soil and water ecosystems in the Czech Republic. Syst Appl Microbiol **37:**467–473. http://dx.doi.org/10.1016/j.syapm.2014.07.001.
6. **Nemec A, Krizova L, Maixnerova M, van der Reijden TJ, Deschaght P, Passet V, Vaneechoutte M, Brisse S, Dijkshoorn L.** 2011. Genotypic and phenotypic characterization of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex with the proposal of *Acinetobacter pittii* sp. nov. (formerly *Acinetobacter* genomic species 3) and *Acinetobacter nosocomialis* sp. nov. (formerly *Acinetobacter* genomic species 13TU). Res Microbiol **162:**393–404. http://dx.doi.org/10.1016/j.resmic.2011.02.006.
7. **Turton JF, Shah J, Ozongwu C, Pike R.** 2010. Incidence of *Acinetobacter* species other than *A. baumannii* among clinical isolates of *Acinetobacter*: evidence for emerging species. J Clin Microbiol **48:**1445–1449. http://dx.doi.org/10.1128/JCM.02467-09.
8. **Yamamoto S, Bouvet PJ, Harayama S.** 1999. Phylogenetic structures of

the genus *Acinetobacter* based on *gyrB* sequences: comparison with the grouping by DNA-DNA hybridization. Int J Syst Bacteriol **49**(Pt 1)**:**87–95. http://dx.doi.org/10.1099/00207713-49-1-87.

9. **Bouvet PJ, Jeanjean S.** 1989. Delineation of new proteolytic genomic species in the genus *Acinetobacter*. Res Microbiol **140:**291–299. http://dx.doi.org/10.1016/0923-2508(89)90021-1.
10. **Touchon M, Cury J, Yoon E-J, Krizova L, Cerqueira G, Murphy C, Feldgarden M, Wortman J, Clermont D, Lambert T, Grillot-Courvalin C, Nemec A, Courvalin P, Rocha EPC.** 2014. The genomic diversification of the whole *Acinetobacter* genus: origins, mechanisms, and consequences. Genome Biol Evol **6:**2866–2882. http://dx.doi.org/10.1093/gbe/evu225.
11. **Dijkshoorn L, Nemec A, Seifert H.** 2007. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. Nat Rev Microbiol **5:**939–951. http://dx.doi.org/10.1038/nrmicro1789.