Genome-based taxonomic reclassification of *Acinetobacter* species using type and reference strains

Masato Suzuki (✉ suzuki-m@nih.go.jp)

National Institute of Infectious Diseases <https://orcid.org/0000-0001-8975-2193>

Shotaro Maehana

Kitasato University

Hidero Kitasato

Kitasato University

Short Report

Keywords: Acinetobacter, genome-based taxonomic classification, core genome phylogeny, in silico DNA–DNA hybridization, average nucleotide identity

Posted Date: July 6th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-677553/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Abstract

Acinetobacter species are widely distributed in the environment and clinical settings worldwide and serve as natural reservoirs of antimicrobial resistance genes and occasional human pathogens responsible for nosocomial infections. In this study, we performed genomic analysis of *Acinetobacter seohaensis* DSM 16313, a type strain of the proposed *Acinetobacter* species. This species was estimated to be evolutionary close to *Acinetobacter towneri* but the genome sequence of *A. seohaensis* was not publicly available. Pangenome analysis of the genome sequence of *A. seohaensis* along with those of genome-available type and reference strains of 82 *Acinetobacter* species including *A. towneri* suggested that three groups of *Acinetobacter* species, *A. seohaensis* and *A. towneri*; *Acinetobacter pullorum* and *Acinetobacter portensis*; and *Acinetobacter idrijaensis*, *Acinetobacter mesopotamicus*, and *Acinetobacter lwoffii*, were phylogenetically very similar to each other. Genome comparisons based on *in silico* DNA-DNA hybridization and the average nucleotide identity confirmed that these three groups of *Acinetobacter* species are conspecific. Based on the rules of priority, *A. seohaensis*, *A. pullorum*, and *A. idrijaensis*/ *A. mesopotamicus* should be reclassified as later heterotypic synonyms of *A. towneri*, *A. portensis*, and *A. lwoffii*, respectively.

Main Text

Acinetobacter towneri, belonging to the genus *Acinetobacter* whose members are gram-negative aerobic coccobacilli, is often isolated from water environments worldwide (1). This species has become increasingly important in recent years as a natural reservoir of antimicrobial resistance (AMR) genes (2–6). *Acinetobacter baumannii*, a cause of opportunistic infections in humans, has acquired resistance mechanisms to various antimicrobials, including clinically important carbapenems, making antimicrobial therapy difficult (7). AMR genes, such as those for carbapenem-hydrolyzing enzymes (carbapenemases), have spread among environmental and clinical *Acinetobacter* species via mobile gene elements such as plasmids (2–4). Tigecycline is a last-resort antimicrobial with promising activity against carbapenemase-producing gram-negative bacteria, including *Acinetobacter* species; however, mobile genes for tigecycline-inactivating enzymes, *tet(X)*, have also emerged in *A. towneri* (5, 6) and *A. baumannii* (8–10). Accumulation of such clinically relevant AMR genes in environmental bacteria such as *A. towneri* and their transmission to human pathogenic bacteria such as *A. baumannii* poses a global public health threat.

As of July 1, 2021, the List of Prokaryotic names with Standing in Nomenclature (LSPN) listed 92 species of the genus *Acinetobacter* (<https://lpsn.dsmz.de/genus/acinetobacter>). Of these, *Acinetobacter venetianus* and *Acinetobacter refrigeratoris* (formerly *Acinetobacter refrigeratorensis*) were listed in duplicate. Additionally, *Acinetobacter grimontii*, *Acinetobacter guangdongensis*, *Acinetobacter pakistanensis*, and *Acinetobacter dijkshoorniae* were later identified as different species (*Acinetobacter junii*, *Acinetobacter indicus*, *Acinetobacter bohemicus*, and *Acinetobacter lactucae*, respectively) (11–14); therefore, the LSPN lists 86 unique species of *Acinetobacter* (Table S1). Of these, 68 species were validly published in the International Journal of Systematic and Evolutionary Microbiology (IJSEM), whereas the

remaining 18 species were not validly published (Table S1). To date, the genome sequences of type and reference strains of 79 species are available in the NCBI database, with all 68 species published in the IJSEM (68/68, 100%) and 11 species not validly published (11/18, 61.1%) (Table S1). Although not listed in the LSPN, one novel species of *Acinetobacter*, *Acinetobacter kanungonis*, has been validly published in the IJSEM (15), and two novel species of *Acinetobacter*, *Acinetobacter rongchengensis* and *Acinetobacter tianfuensis*, have been proposed from large-scale reanalysis on 3,956 genomes of *Acinetobacter* species in public databases and published in another journal (16) (Table S1).

During molecular epidemiological analysis of carbapenem-resistant *A. towneri* isolates from hospital sewage in Japan, we performed genomic analysis of *Acinetobacter seohaensis* DSM 16313, a proposed type strain whose genome sequence was estimated to be similar to that of *A. towneri* but the genome sequence of *A. seohaensis* was not publicly available (17). The 16S rRNA gene sequence of *A. seohaensis* DSM 16313 (accession no. AY633608) (17) was shown to be nearly identical (99.8%) to that of *A. towneri* DSM 14962^T (type strain, accession no. EF611416). The Illumina sequencing library (paired-end, insert size 500–900 bp) was prepared using the Nextera XT DNA Library Prep Kit (Illumina). Whole-genome sequencing using the HiSeq X system (Illumina) was performed, followed by *de novo* assembly of Illumina reads using Shovill v1.1.0 (<https://github.com/tseemann/shovill>) with default parameters. The resulting draft genome sequence of *A. seohaensis* DSM 16313 (accession no. BPEQ00000000) consisted of 298 contigs with a genome size of 2.99 Mbp and GC content of 41.3%.

We further performed pangenome analysis of the draft genome sequence of *A. seohaensis* DSM 16313 along with those of genome-available type and reference strains of the aforementioned 82 species of *Acinetobacter*, including *A. towneri* DSM 14962^T, using Roary v3.13.0 (<https://github.com/sanger-pathogens/Roary>) with the parameter of minimum percentage identity for blastp = 60%. Phylogenetic analysis with their core genome using RAxML v8.2.4 (<https://github.com/stamatak/standard-RAxML>) with default parameters suggested three groups of *Acinetobacter* species, *A. seohaensis* and *A. towneri*; *Acinetobacter pullorum* and *Acinetobacter portensis*; and *Acinetobacter idrijaensis*, *Acinetobacter mesopotamicus* and *Acinetobacter lwoffii*, to be phylogenetically very similar to each other (Fig. 1). Of these, two pairs of *Acinetobacter* species, *A. pullorum* and *A. portensis* as well as *A. mesopotamicus* and *A. lwoffii*, have been suggested to be phylogenetically identical to each other in journals other than the IJSEM published in May 2021 and January 2021, respectively (16, 18). We confirmed that the 16S rRNA gene sequences of *A. pullorum* B301 (proposed type strain, accession no. MN909715) and *A. portensis* 877^T (type strain, accession no. KX870877) are nearly identical (99.7%), and that those of *A. idrijaensis* MII (proposed type strain, GS19_03400 in accession no. JQCU01000127, *A. mesopotamicus* GC2 (proposed type strain, accession no. KJ867435), and *A. lwoffii* NCTC 5866^T (type strain, accession no. AB626125) are nearly identical (99.7% for *A. idrijaensis* compared with *A. lwoffii* and 99.7% for *A. mesopotamicus* compared with *A. lwoffii*), respectively.

The results of *in silico* DNA–DNA hybridization (DDH) analysis using the Type Strain Genome Server (<https://tygs.dsmz.de/>) and average nucleotide identity (ANI) analysis using FastANI v1.3 (<https://github.com/ParBLiSS/FastANI>) confirmed that *A. seohaensis* DSM 16313 (accession no.

BPEQ000000000) and *A. towneri* DSM 14962^T (accession no. JHZH000000000) (17) are conspecific with 77.5% of DDH and 97.7% of ANI according to their proposed minimal standards ($\geq 70\%$ of DDH or $\geq 95\%$ of ANI) (19) (Fig. 1). Moreover, our results confirmed that *A. pullorum* B301 (accession no. JAAARQ000000000) (20) and *A. portensis* AC 877^T (accession no. LWRV000000000) (21) are conspecific with 82.6% of DDH and 98.6% of ANI, and that *A. idrijaensis* MII (accession no. JQCU000000000) (22), *A. mesopotamicus* GC2 (accession no. JAALFF000000000) (23), and *A. lwoffii* NCTC 5866^T (accession no. CAADHN000000000) (24) are conspecific with 76.8% of DDH and 96.1% of ANI (*A. idrijaensis* compared with *A. lwoffii*) and 68.5% of DDH and 96.0% of ANI (*A. mesopotamicus* compared with *A. lwoffii*), respectively (Fig. 1). Thus, comparative genomic analysis demonstrated that the aforementioned three groups of *Acinetobacter* species are conspecific and suggested that genome-level comparisons are essential for proposing novel bacterial species among highly similar species.

The priority of prokaryotic names is governed by the International Code of Nomenclature of Prokaryotes (25). Rule 23a of the code states that, “In a given position, a species can bear only one correct epithet, that is, the earliest that is in accordance with the Rules of this Code”. Rules 23b, 24a, and 24b establish the priority of names based on their dates of valid publication in the IJSEM. In our case, *A. towneri* was validly published in the IJSEM in July 2003 (1) and *A. seohaensis* was published in another journal in November 2007 (17); *A. portensis* was validly published in the IJSEM in August 2020 (21) and *A. pullorum* was published in another journal in April 2020 (20); *A. lwoffii* was validly published in the International Journal of Systematic and Evolutionary Bacteriology (predecessor journal of the IJSEM) in April 1986 (24), and *A. idrijaensis* and *A. mesopotamicus* were published in other journals in November 2014 and October 2020, respectively (22, 23). Based on the rules of priority, *A. seohaensis*, *A. pullorum*, and *A. idrijaensis*/*A. mesopotamicus* are later heterotypic synonyms of *A. towneri*, *A. portensis*, and *A. lwoffii*, respectively.

References

1. Carr EL, Kämpfer P, Patel BKC, Gürtler V, Seviour RJ. Seven novel species of *Acinetobacter* isolated from activated sludge. **Int J Syst Evol Microbiol.** 2003 53(Pt 4):953-963.
2. Zou D, Huang Y, Liu W, Yang Z, Dong D, Huang S, He X, Ao D, Liu N, Wang S, Wang Y, Tong Y, Yuan J, Huang L. Complete sequences of two novel *bla*_{NDM-1}-harbouring plasmids from two *Acinetobacter towneri* isolates in China associated with the acquisition of Tn 125. **Sci Rep.** 2017 7(1):9405.
3. Jiang N, Zhang X, Zhou Y, Zhang Z, Zheng X. Whole-genome sequencing of an NDM-1- and OXA-58-producing *Acinetobacter towneri* isolate from hospital sewage in Sichuan Province, China. **J Glob Antimicrob Resist.** 2019 16:4-5.
4. Wang K, Li P, Li J, Hu X, Lin Y, Yang L, Qiu S, Ma H, Li P, Song H. An NDM-1-Producing *Acinetobacter towneri* Isolate from Hospital Sewage in China. **Infect Drug Resist.** 2020 13:1105-1110.

5. Ma J, Wang J, Feng J, Liu Y, Yang B, Li R, Bai L, He T, Wang X, Yang Z. Characterization of Three Porcine *Acinetobacter towneri* Strains Co-Harboring *tet*(X3) and *bla*_{OXA-58}. **Front Cell Infect Microbiol.** 2020 10:586507.
6. Cheng Y, Chen Y, Liu Y, Song J, Chen Y, Shan T, Xiao Y, Zhou K. Detection of a new *tet*(X6)-encoding plasmid in *Acinetobacter towneri*. **J Glob Antimicrob Resist.** 2021 25:132-136.
7. Piperaki ET, Tzouveleakis LS, Miriagou V, Daikos GL. Carbapenem-resistant *Acinetobacter baumannii*: in pursuit of an effective treatment. **Clin Microbiol Infect.** 2019 25(8):951-957.
8. He T, Wang R, Liu D, Walsh TR, Zhang R, Lv Y, Ke Y, Ji Q, Wei R, Liu Z, Shen Y, Wang G, Sun L, Lei L, Lv Z, Li Y, Pang M, Wang L, Sun Q, Fu Y, Song H, Hao Y, Shen Z, Wang S, Chen G, Wu C, Shen J, Wang Y. Emergence of plasmid-mediated high-level tigecycline resistance genes in animals and humans. **Nat Microbiol.** 2019 4(9):1450-1456.
9. Wang L, Liu D, Lv Y, Cui L, Li Y, Li T, Song H, Hao Y, Shen J, Wang Y, Walsh TR. Novel Plasmid-Mediated *tet*(X5) Gene Conferring Resistance to Tigecycline, Eravacycline, and Omadacycline in a Clinical *Acinetobacter baumannii* Isolate. **Antimicrob Agents Chemother.** 2019 64(1):e01326-19.
10. Liu D, Zhai W, Song H, Fu Y, Schwarz S, He T, Bai L, Wang Y, Walsh TR, Shen J. Identification of the novel tigecycline resistance gene *tet*(X6) and its variants in *Myroides*, *Acinetobacter* and *Proteus* of food animal origin. **J Antimicrob Chemother.** 2020 75(6):1428-1431.
11. Vaneechoutte M, De Baere T, Nemec A, Musílek M, van der Reijden TJ, Dijkshoorn L. Reclassification of *Acinetobacter grimontii* Carr et al. 2003 as a later synonym of *Acinetobacter junii* Bouvet and Grimont 1986. **Int J Syst Evol Microbiol.** 2008 58(Pt 4):937-40.
12. Nemec A, Radolfova-Krizova L. *Acinetobacter guangdongensis* Feng et al. 2014 is a junior heterotypic synonym of *Acinetobacter indicus* Malhotra et al. 2012. **Int J Syst Evol Microbiol.** 2017 67(10):4080-4082.
13. Nemec A, Radolfova-Krizova L. *Acinetobacter pakistanensis* Abbas et al. 2014 is a later heterotypic synonym of *Acinetobacter bohemicus* Krizova et al. 2014. **Int J Syst Evol Microbiol.** 2016 66(12):5614-5617.
14. Dunlap CA, Rooney AP. *Acinetobacter dijkshoorniae* is a later heterotypic synonym of *Acinetobacter lactucae*. **Int J Syst Evol Microbiol.** 2018 68(1):131-132.
15. Das L, Deb S, Das SK. Description of *Acinetobacter kanungonis* sp. nov., based on phylogenomic analysis. **Int J Syst Evol Microbiol.** 2021 71(6).
16. Qin J, Feng Y, Lü X, Zong Z. Precise Species Identification for *Acinetobacter*: a Genome-Based Study with Description of Two Novel *Acinetobacter* Species. **mSystems.** 2021 e0023721.

17. Yoon JH, Kim IG, Oh TK. *Acinetobacter marinus* sp. nov. and *Acinetobacter seohaensis* sp. nov., isolated from sea water of the Yellow Sea in Korea. **J Microbiol Biotechnol.** 2007 17(11):1743-50.
18. Nemec A. Strain "*Acinetobacter mesopotamicus*" GC2 Does Not Represent a Novel Species, but Belongs to the Species *Acinetobacter lwoffii* as Revealed by Whole-Genome Sequence-Based Analysis. **Curr Microbiol.** 2021 78(1):369-370.
19. Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, da Costa MS, Rooney AP, Yi H, Xu XW, De Meyer S, Trujillo ME. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. **Int J Syst Evol Microbiol.** 2018 68(1):461-466.
20. Elnar AG, Kim MG, Lee JE, Han RH, Yoon SH, Lee GY, Yang SJ, Kim GB. *Acinetobacter pullorum* sp. nov., Isolated from Chicken Meat. **J Microbiol Biotechnol.** 2020 30(4):526-532.
21. Carvalho A, Gonzales-Siles L, Salvà-Serra F, Lindgren Å, Svensson-Stadler L, Thorell K, Piñeiro-Iglesias B, Karlsson R, Silva J, Teixeira P, Moore ERB. *Acinetobacter portensis* sp. nov. and *Acinetobacter guerra* sp. nov., isolated from raw meat. **Int J Syst Evol Microbiol.** 2020 70(8):4544-4554.
22. Campos-Guillén J, Caballero Pérez J, Cruz Medina JA, Molina Vera C, Salas Rosas LM, Limpens Gutiérrez C, García Salinas I, Hernández Ramírez MR, Soto Alonso G, Cruz Hernández A, Saldaña Gutiérrez C, Romero Gómez S, Pastrana Martínez X, Alvarez Hidalgo E, Gosar M, Dizdarevič T. Draft Genome Sequence of the Mercury-Resistant Bacterium *Acinetobacter idrijaensis* Strain MII, Isolated from a Mine-Impacted Area, Idrija, Slovenia. **Genome Announc.** 2014 2(6):e01177-14.
23. Acer Ö, Güven K, Poli A, Di Donato P, Leone L, Buono L, Güven RG, Nicolaus B, Finore I. *Acinetobacter mesopotamicus* sp. nov., Petroleum-degrading Bacterium, Isolated from Petroleum-Contaminated Soil in Diyarbakir, in the Southeast of Turkey. **Curr Microbiol.** 2020 77(10):3192-3200.
24. Bouvet PJM, Grimont PAD. 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 Evol Bacteriol.** 1986 36(2): 228-240
25. Parker CT, Tindall BJ, Garrity GM. International Code of Nomenclature of Prokaryotes Prokaryotic Code (2008 Revision). **Int J Syst Evol Microbiol.** 2019 69(1A):S1–S111.

Competing Interests

The authors declare no competing interests.

Figures

Fig. 1

Figure 1

Core genome phylogeny of publicly available genomes of type and reference strains of 83 species of *Acinetobacter*. Bar lengths represent the number of substitutions per site in the core genome. Names of type and reference strains of *Acinetobacter* species, accession nos. of the genome sequences, years when each strain was validly published in the International Journal of Systematic and Evolutionary Microbiology (or years when each strain was published in another journal), and average nucleotide

identity (ANI) values to reference genomes of *A. seohaensis* DSM 16313, *A. pullorum* B301, *A. idrijaensis* MII, and *A. mesopotamicus* GC2, respectively, are shown. Three groups of *Acinetobacter* species, *A. seohaensis* and *A. towneri*; *Acinetobacter pullorum* and *Acinetobacter portensis*; and *Acinetobacter idrijaensis*, *Acinetobacter mesopotamicus*, and *Acinetobacter lwoffii* are highlighted in red, blue, and green, respectively.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [210626TableS1.pdf](#)