Systematic and Applied Microbiology 42 (2019) 319–325

Contents lists available at ScienceDirect

Systematic and Applied Microbiology

journal homepage: http://www.elsevier.com/locate/syapm

Acinetobacter cumulans sp. nov., isolated from hospital sewage and capable of acquisition of multiple antibiotic resistance genes☆

Jiayuan Qin[a,b,c], Martina Maixnerová[d], Matěj Nemec[d], Yu Feng[a,b,c], Xinzhuo Zhang[e,f], Alexandr Nemec[d,g,*], Zhiyong Zong[a,b,c,**]

[a] Center of Infectious Diseases, West China Hospital, Sichuan University, Guoxuexiang 37, Chengdu 610041, Sichuan, China
[b] Division of Infectious Diseases, State Key Laboratory of Biotherapy, Guoxuexiang 37, Chengdu 610041, Sichuan, China
[c] Center for Pathogen Research, West China Hospital, Sichuan University, Guoxuexiang 37, Chengdu 610041, Sichuan, China
[d] Laboratory of Bacterial Genetics, Centre for Epidemiology and Microbiology, National Institute of Public Health, Šrobárova 48, 100 42 Prague 10, Czech Republic
[e] School of International Education, Southwest Medical University, No. 1 Xianglin Road, Luzhou 646000, Sichuan, China
[f] Department of Pathogenic Biology, School of Basic Medicine, Southwest Medical University, No. 1 Xianglin Road, Luzhou 646000, Sichuan, China
[g] Department of Laboratory Medicine, Third Faculty of Medicine, Charles University, Šrobárova 50, 100 34 Prague 10, Czech Republic

ARTICLE INFO

Article history:
Received 21 November 2018
Received in revised form 3 February 2019
Accepted 8 February 2019

Keywords:
Carbon source assimilation
Core genome phylogeny
MALDI-TOF MS
Whole genome sequence

ABSTRACT

We studied the taxonomic position of six phenetically related strains of the genus *Acinetobacter*, which were recovered from hospital sewage in China and showed different patterns of resistance to clinically important antibiotics. Whole-genome sequencing of these strains and genus-wide phylogeny reconstruction based on a set of 107 *Acinetobacter* core genes indicated that they formed a separate and internally cohesive clade within the genus. The average nucleotide identity based on BLAST and digital DNA–DNA hybridization values between the six new genomes were 97.25–98.67% and 79.2–89.3%, respectively, whereas those between them and the genomes of the known species were ≤78.57% and ≤28.5%, respectively. The distinctness of the strains at the species level was also supported by the results of the cluster analysis of the whole-cell protein fingerprints generated by MALDI-TOF MS. Moreover, the strains displayed a catabolically unique profile and could be differentiated from the phylogenetically closest species at least by their inability to grow on D,L-lactate. A total of 18 different genes were found in the six genome sequences which encode resistance to seven classes of antimicrobial agents, including clinically important carbapenems, oxyimino-cephalosporins, or aminoglycosides. These genes occurred in five different combinations, with three to 10 different genes per strain. We conclude that the six strains represent a novel *Acinetobacter* species, for which we propose the name *Acinetobacter cumulans* sp. nov. to reflect its ability to acquire and cumulate diverse resistance determinants. The type strain is WCHAc060092^T (ANC 5797^T = CCTCC AB 2018119^T = GDMCC 1.1380^T = KCTC 62576^T).

Introduction

Acinetobacter is a taxonomically highly diverse genus of Gammaproteobacteria, which is widely distributed in diverse natural ecosystems, including soil or water, and is commonly found in association with eukaryotic organisms [1,8,10,13,16,17]. Certain *Acinetobacter* species can reside on the skin or mucous membranes of mammals and/or are opportunistic human pathogens, especially in hospitalized patients in critical health condition [5]. These organisms can become resistant to multiple antibiotics via activation of inherent cellular mechanisms or horizontal acquisition of resistance genes, which complicates treatment options [19]. At the time of writing, the genus contains 60 validly published species names including four pairs of synonyms (www.szu.cz/anemec/Classification.pdf). Moreover, there is one recently pub-

Abbreviations: MALDI-TOF MS, Matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry; ANIb, Average nucleotide identity based on BLAST; dDDH, Digital DNA–DNA hybridization.

☆ The whole genome shotgun projects for *Acinetobacter cumulans* WCHAc060092T, WCHAc060001, WCHAc060002, WCHAc060003, WCHAc060004, and WCHAc060008 have been deposited at DDBJ/ENA/GenBank under accession numbers PYIW00000000, RCHE00000000, RAXZ00000000, RCHD00000000, RAXY00000000, and RAXW00000000, respectively. The versions described in this paper are PYIW01000000, RCHE01000000, RAXZ01000000, RCHD01000000, RAXY01000000, and RAXW01000000.

* Corresponding author at: Laboratory of Bacterial Genetics, Centre for Epidemiology and Microbiology, National Institute of Public Health, Šrobárova 48, 100 42 Prague 10, Czech Republic.

** Corresponding author at: Center of Infectious Diseases, West China Hospital, Sichuan University, Guoxuexiang 37, Chengdu 610041, Sichuan, China.

E-mail addresses: alexandr.nemec@szu.cz (A. Nemec), zongzhiy@scu.edu.cn (Z. Zong).

https://doi.org/10.1016/j.syapm.2019.02.001
0723-2020/

Table 1
Metabolic and physiological properties of *Acinetobacter cumulans* sp. nov. and phylogenetically related species.

Characteristic	*A. cumulans* (*n* = 6)	*A. bouvetii* (*n* = 1)	*A. celticus* (*n* = 6)	*A. gandensis* (*n* = 6)	*A. pragensis* (*n* = 7)
Growth at 37 °C	17 (D)	D	–	+	–
Growth at 35 °C	+	+	–	+	D
Growth at 32 °C	+	+	–	+	+
Utilization of					
L-Aspartate	–	–	+	–	–
2,3-Butanediol	–	–	–	33 (–)	29 (+)
Citrate (Simmons)	+	+	–	50 (+)	+
Ethanol	83 (+)	+	+	+	+
Gentisate	-	–	–	-	43 (+)
L-Glutamate	50 (D)	+	+	33 (+)	86 (D)
Glutarate	33 (–)	+	–	83 (+)	+
L-Histidine	–	+	–	–	–
4-Hydroxybenzoate	–	–	–	–	+
DL-Lactate	–	+	+	+	+
Levulinate	–	–	50 (–)	–	–
D-Malate	17 (–)	–	–	–	–
Malonate	–	–	+	17 (–)	+
Phenylacetate	–	–	67 (+)	–	71 (+)
L-Tartrate	–	–	–	–	+
Tricarballylate	–	–	–	–	71 (–)
Tryptamine	–	–	–	–	57 (–)

The results were obtained either in this study or have been published previously [10,16,17]. The culture temperature was 30 °C except for that used for *Acinetobacter celticus* (25 °C) and temperature growth tests. All strains grew on acetate and benzoate. None of the strains acidified D-glucose, liquefied gelatin, produced haemolysis on sheep blood agar, grew at ≥41 °C, or utilized *trans*-aconitate, adipate, β-alanine, 4-aminobutyrate, L-arabinose, L-arginine, azelate, citraconate, D-gluconate, D-glucose, histamine, L-leucine, L-ornithine, L-phenylalanine, putrescine, D-ribose, or trigonelline. +, All strains positive; –, all strains negative; D, (mostly) doubtful or irreproducible reactions. Numbers are percentages of strains with clearly positive reactions. For strain-dependent reactions, results for type strains are given in parentheses.

lished species name awaiting valid publication [13] and a number of putative novel *Acinetobacter* species with provisional designations (www.szu.cz/anemec/Classification.pdf). Here, we present the results of the analysis of a taxonomically novel group of six *Acinetobacter* strains recovered from hospital sewage. As evidenced further, this group is taxonomically both internally coherent and distinct from all hitherto known species at the species level of resolution. In addition, all these strains showed a remarkable capacity to acquire multiple genes encoding resistance to different classes of medically important antimicrobial agents.

Material and methods

Bacteria

The six novel *Acinetobacter* isolates (WCHAc060001, WCHAc060002, WCHAc060003, WCHAc060004, WCHAc060008, and WCHAc060092^T) were recovered from hospital sewage. One-ml samples collected from the influx mainstream of the wastewater treatment plant at West China Hospital in November 2017 (isolate WCHAc060092^T) and June 2018 (the other five isolates) were added to 10 ml nutrient broth (Oxoid, Basingstoke, UK) and was incubated with shaking at 30 °C overnight. The culture suspension was diluted to 0.5 McFarland standard and then further diluted to 1:100 using saline. A 100-μl aliquot was streaked onto an *Acinetobacter* chromogenic agar plate (CHROMagar, Paris, France). This plate was then incubated at 30 °C overnight. The six isolates were unique at the strain level as revealed by the diversity of their genomic fingerprints obtained by macrorestriction analysis of genomic DNA (Fig. S1) and differences in their whole genome sequences (Table S1). Representatives of all known *Acinetobacter* species with validly published names and several provisional taxa of the genus were included in the following analyses.

MALDI-TOF MS

Whole cell profiling by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was performed as described previously [17], using a standard matrix based on alpha-cyano-4-hydroxycinnamic acid solution. All measurements and data processing were carried out using the Microflex LT instrument (Bruker Daltonics; Billerica, MA, USA) and BioTyper software version 3.1 (Bruker Daltonics).

Whole-genome sequence analysis

Genomic DNA from an overnight culture of each of the novel strains was prepared using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and was then subjected to whole genome sequencing using the HiSeq X10 Sequencing platform (Illumina; San Deigo, CA, USA). Reads were de novo assembled into contigs using the program SPAdes [3]. The whole genome sequences of the type or reference strain of the known *Acinetobacter* species (https://apps.szu.cz/anemec/Genomes.pdf) were retrieved from the GenBank database. Genome sequences were compared using the average nucleotide identity based on BLAST (ANIb) and digital DNA–DNA hybridization (dDDH) parameters. ANIb and dDDH values were calculated, respectively, using the JSpecies (http://www.imedea.uib.es/jspecies) [18] and GGDC 2.1 (formula 2; http://ggdc.dsmz.de) [11] programs with the recommended parameters and/or default settings. For phylogenomic analysis, Prokka [20] was used to annotate genome sequences, followed by the application of an approach integrating the analysis of core genes as described previously [2]. A maximum-likelihood phylogenomic tree was inferred based on the 107 core gene sequences using bcgTree with default settings [2].

Phenotypic analysis

Metabolic and physiological features were assessed using a genus-targeted set of in-house, strictly standardized tests (Table 1) as described previously [10,12]. Assimilation tests were performed in fluid mineral medium supplemented with 0.1% (w/v) carbon source. Temperature growth tests were carried out in brain-heart infusion broth (Oxoid) using a thermostatically controlled water bath. The assimilation tests were interpreted after six days of culture, and the other tests after three (haemolytic and gelatinase activities) or two (D-glucose acidification, temperature-dependent

growth tests) days. Gram staining and tests for oxidase, catalase, nitrate reduction, motility, and anaerobic growth were performed as described by Radolfova-Krizova et al. [17].

Analysis of antimicrobial resistance phenotype and genotype

The minimal inhibitory concentrations (MICs) of amikacin, ceftazidime, ciprofloxacin, colistin, gentamicin, imipenem, meropenem, piperacillin/tazobactam, tobramycin, trimethoprim/sulfamethoxazole, and tigecycline were determined using the broth microdilution method of the Clinical and Laboratory Standards Institute (CLSI) [4]. Antimicrobial resistance genes were identified from genome sequences using the ABRicate program (https://github.com/tseemann/abricate) to query the ResFinder database (http://genomicepidemiology.org/).

Results and discussion

Genus allocation

All six strains had the basic features of the genus *Acinetobacter*, i.e. they were oxidase-negative, catalase-positive, and strictly aerobic Gram-stain-negative coccobacilli, incapable of dissimilative denitrification and swimming motility and positive in the transformation assay of Juni [8]. In addition, the affiliation of the strains to the genus was evidenced by the results of the core genome and MALDI-TOF MS-based analyses (Figs. 1 and 2).

MALDI-TOF MS-based analysis

The result of the genus-wide cluster analysis MALDI-TOF MS is shown in Fig. 1. The spectra of the six novel strains formed a coherent cluster at a high similarity level (distance level of <100%), which was well-separated from the other species. This picture agrees with those seen for other recently described *Acinetobacter* species [7,10,16,17]. The potential of MALDI-TOF MS for the identification of a novel taxonomic group represented by the six strains was assessed by matching the spectra of these strains to those of the current Bruker Daltonics database (version 8.0.0.0) supplemented with in-house entries for WCHAc060092^T and the type strains included in Fig. 1. Each of the novel strains matched with WCHAc060092^T with BioTyper log (scores) of >2.3 (A), which indicates highly probable species identification as well as species consistency.

Whole genome sequence-based analysis

The basic properties of the six novel genome sequences are summarized in Table S2. Whole genome sequencing generated 1.35–1.56 Gb clean bases for these sequences. The reads were assembled into 107 to 204 contigs (N_{50}, 58,841–154,547 bp). The draft genomes were 3.44–3.90 Mb with a G+C content of 40.1 or 40.2 mol% and the sequencing coverage was about 200×. The assembled genomes of the six strains are available in the GenBank database under accession nos. PYIW00000000.1 (WCHAc060092^T), RCHE00000000.1 (WCHAc060001), RAXZ00000000.1 (WCHAc060002), RCHD00000000.1 (WCHAc060003), RAXY00000000.1 (WCHAc060004), and RAXW00000000.1 (WCHAc060008). The values of the overall genome relatedness indexes calculated for the six genome sequences against each other and versus all known *Acinetobacter* species are summarized in Table S1. The pairwise ANIb and dDDH values between the six new genomes were 97.25–98.67% and 79.2–89.3%, respectively, whereas those between the six genomes and the genomes of the known *Acinetobacter* species were ≤78.57% and ≤28.5%, respectively. In light of the recommended threshold

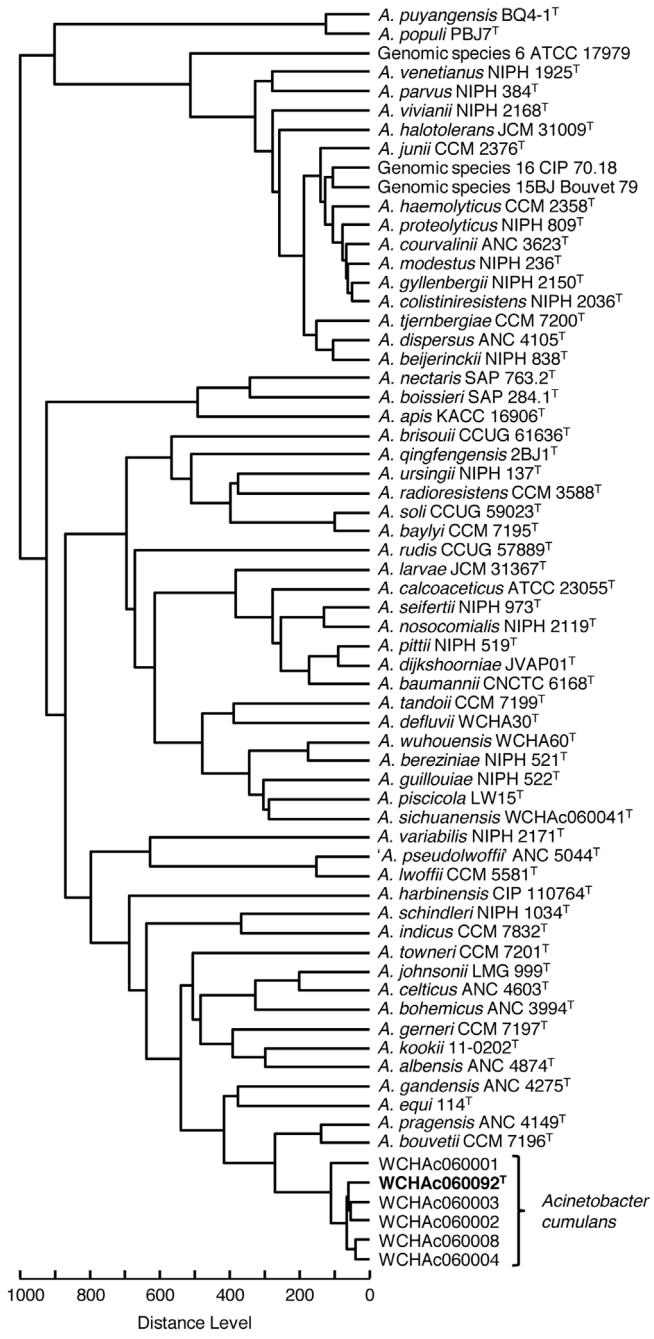

Fig. 1. The result of cluster analysis of the MALDI-TOF mass spectra of the six strains of *Acinetobacter cumulans* sp. nov. and those of the type strains of all known *Acinetobacter* species with validly published names and reference strains of three provisional taxa. The analysis was performed using the Biotyper MSP Dendrogram Creation Standard Method (Bruker Daltonics), with the correlation distance measure and average linkage algorithm.

values of ANIb (95–96% [18]) and dDDH (70% [11]) for species circumscription, these values indicate that the six new strains represent a novel *Acinetobacter* species, which is clearly separated from all known taxa. Even though the genome sequence of *Acinetobacter halotolerans* was not available at the time of our analyses, the six strains are clearly distinct from the only known strain of this species based on MALDI-TOF MS (Fig. 1) and phenotypic data (Table S3) as well as on of the comparative sequence analysis of the RNA polymerase β-subunit gene (nucleotide identity of 83.6–83.9%).

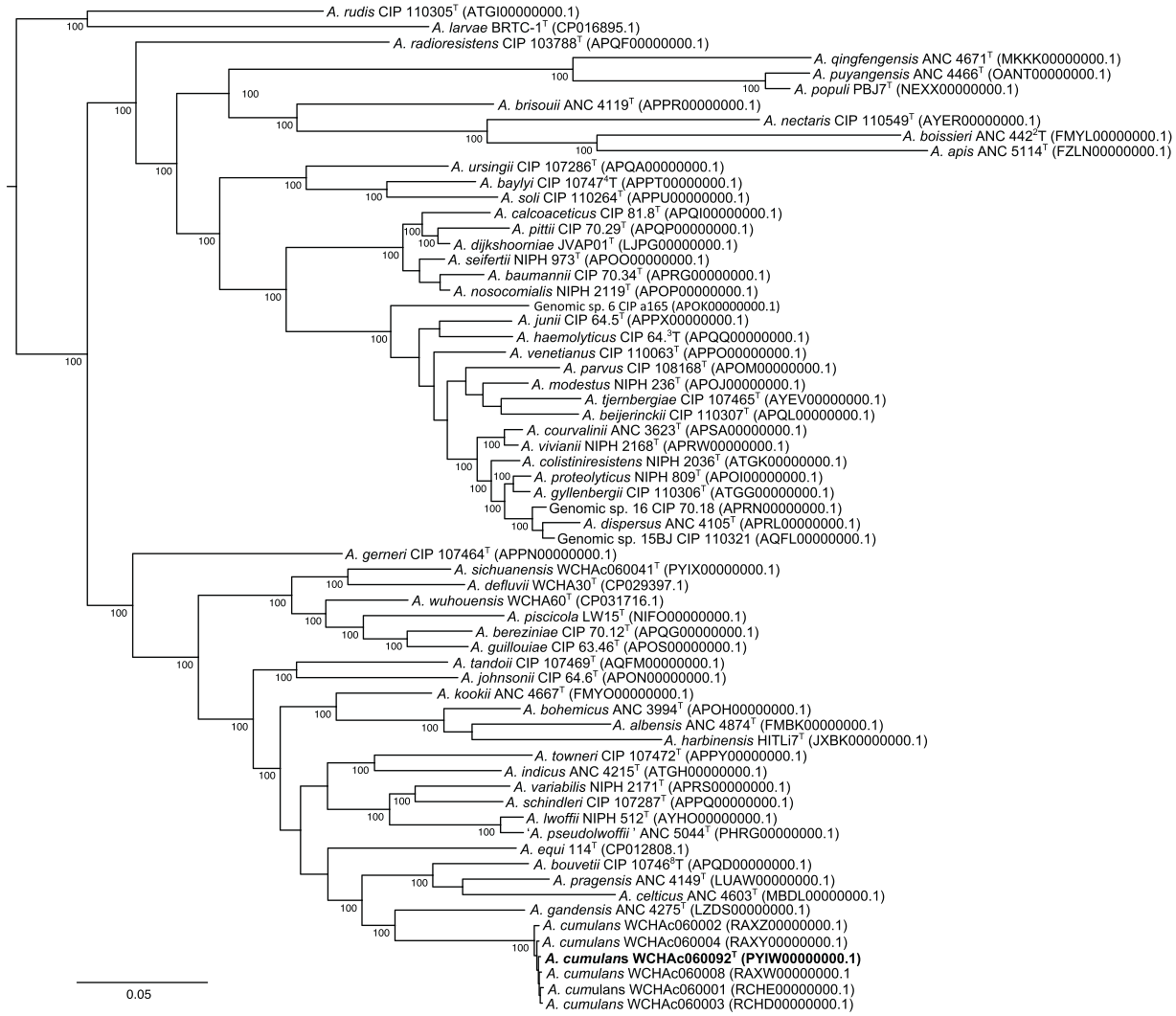

Fig. 2. Core genome ML phylogenetic tree for the genus *Acinetobacter* showing the phylogenetic placement of the six strains of *Acinetobacter cumulans* sp. nov. Included are all known *Acinetobacter* species with validly published names except for *A. halotolerans*. Numbers along branches indicate percentage bootstrap support; only values 100% are shown. Shown in parentheses are the DDBJ/ENA/GenBank accession numbers for the whole genome sequences. Bar, 0.05 changes per nucleotide position.

Phylogeny

Core genome-based phylogeny revealed that the six strains are only distantly related to any hitherto known *Acinetobacter* species (Fig. 2). *Acinetobacter gandensis* appeared the most related species to them although still being distant in terms of both phylogenetic distance and overall genotypic similarity (ANIb 77.41–77.64%; dDDH 22.4% or 22.5%).

Physiological and metabolic features

The phenotypic characteristics tested using the genus-targeted set of physiological and metabolic tests (Table 1) are presented in the standard way used in previous nomenclatural proposals [10,14]. Table S3 summarizes the phenotypes for the six new strains, together with those for all known *Acinetobacter* species with validly published names, while Table 1 presents a subset of these data, comparing the features of the six strains with those of the species closest to them in terms of core genome relatedness, i.e. *Acinetobacter bouvetii*, *Acinetobacter celticus*, *A. gandensis*, and *Acinetobacter pragensis* (Fig. 1). The six strains showed a generally low metabolic activity, with only acetate, benzoate, and citrate being assimilated by all of them. The only single feature that differentiated them from the four phylogenetically closest species was their inability to grow on D,L-lactate (Table 1). The numbers of phenotypic differences between the six strains and each of the known species with validly published names are indicated in Table S3. The combination of features which unambiguously differentiates the six strains from any of these species include their ability to assimilate benzoate and citrate along with their inability to haemolyse blood agar, to grow at 41 °C, and to assimilate D,L-lactate.

Antimicrobial susceptibilities and resistance genes

The susceptibility phenotypes of the six strains are shown in Table 2, while Table 3 summarizes the acquired resistance genes found in the whole genome sequences of these strains. The strains were all non-susceptible to ciprofloxacin (MIC, 2–4 mg/l), whereas they were susceptible to colistin (MIC, 1–2 mg/l) and tobramycin (≤0.5–2 mg/l) and their their MICs to tigecycline were ≤0.5 mg/l (there are no CLSI breakpoints available to define susceptibility categories). Otherwise, the strains exhibited varied susceptibility profiles. A total of 18 different genes were found in the six genome sequences, which encode resistance to seven classes of antimicrobial agents, including clinically relevant groups such as carbapenems or aminoglycosides. These genes occurred

Table 2
Minimal inhibitory concentrations (mg/l) of antimicrobial agents for *Acinetobacter cumulans* sp. nov.

Strain	Amikacin	Gentamicin	Tobramycin	Ceftazidime	Piperacillin/tazobactam	Meropenem	Imipenem	Ciprofloxacin	Colistin	Tigecycline	Trimethoprim/sulfamethoxazole
WCHAc060092^T	16 (S)	≤0.5 (S)	≤0.5 (S)	≥256 (R)	256/4 (R)	≥128 (R)	≥64 (R)	4 (R)	2 (S)	≤0.5	8/152 (R)
WCHAc060001	16 (S)	2 (S)	2 (S)	≥256 (R)	32/4 (I)	16 (R)	16 (R)	2 (I)	2 (S)	≤0.5	1/19 (S)
WCHAc060002	≤1 (S)	≤0.5 (S)	≤0.5 (S)	4 (S)	≤2/4 (S)	≤0.5 (S)	2 (S)	4 (R)	2 (S)	≤0.5	≤0.25/4.75 (S)
WCHAc060003	16 (S)	2 (S)	2 (S)	≥256 (R)	32/4 (I)	8 (R)	8 (R)	4 (R)	1 (S)	≤0.5	0.5/9.5 (S)
WCHAc060004	≤1 (S)	≤0.5 (S)	≤0.5 (S)	4 (S)	≤2/4 (S)	≤0.5 (S)	≤0.25 (S)	4 (R)	1 (S)	≤0.5	4/76 (R)
WCHAc060008	32 (I)	≥128 (R)	2 (S)	≥256 (R)	256/4 (R)	≥128 (R)	≥64 (R)	4 (R)	2 (S)	≤0.5	4/76 (R)

Susceptibility categories (S, susceptible; I, intermediate; R, resistant) according to the Clinical and Laboratory Standards Institute [4] are shown (no CLSI breakpoints for tigecycline available).

Table 3
Antimicrobial resistance genes in *Acinetobacter cumulans* sp. nov.

Strain	Genes conferring resistance to							
	Carbapenems	Other β-lactams	Aminoglycosides	Bleomycin	Macrolides	Rifampin	Sulfonamides	Tetracyclines
WCHAc060092^T	*bla*$_{NDM-1}$		*aph(3″)-Ib, aph(6)-Id, aph(3′)-VI*	*ble*	*mph(E), msr(E)*		*sul2*	*tet(39)*
WCHAc060001	*bla*$_{OXA-23}$, *bla*$_{OXA-58}$	*bla*$_{PER-1}$	*aac(6′)-Ib, aph(3′)-VIa*		*mph(E), msr(E)*	*arr-3*	*sul1*	*tet(39)*
WCHAc060002	*bla*$_{OXA-58}$				*mph(E), msr(E)*			
WCHAc060003	*bla*$_{OXA-23}$, *bla*$_{OXA-58}$	*bla*$_{PER-1}$	*aac(6′)-Ib, aph(3′)-VIa*		*mph(E), msr(E)*	*arr-3*	*sul1*	*tet(39)*
WCHAc060004	*bla*$_{OXA-58}$		*aph(3″)-Ib, aph(6)-Id*		*mph(E), msr(E)*		*sul2*	*tet(39), tet(Y)*
WCHAc060008	*bla*$_{NDM-1}$, *bla*$_{OXA-58}$		*aac(3)-IId, aph(6)-Id, aph(3″)-Ib, aph(3′)-Ia, aph(3′)-VIa*	*ble*			*sul2*	*tet(Y)*

Table 4
Description of *Acinetobacter cumulans* sp. nov according to Digital Protologue TA00727, assigned by the www.imedea.uib.es/dprotologue website.

Taxonumber	TA00727
Type of description	New description
Species name	*Acinetobacter cumulans*
Genus name	*Acinetobacter*
Specific epithet	*cumulans*
Species status	sp. nov.
Species etymology	cu'mu.lans, L. part. adj. *cumulans* cumulating, pertaining to the ability of the species to cumulate different genes encoding antibiotic resistance
Authors	Jiayuan Qin, Martina Maixnerová, Matěj Nemec, Yu Feng, Xinzhuo Zhang, Alexandr Nemec, Zhiyong Zong
Title	*Acinetobacter cumulans* sp. nov., isolated from hospital sewage and capable of acquisition of multiple antibiotic resistance genes
Journal	Systematic and Applied Microbiology
Volume & pages	Submitted
Corresponding author	Alexandr Nemec
E-mail of the corresponding author	alexandr.nemec@szu.cz
Submitter	Alexandr Nemec
E-mail of the submitter	alexandr.nemec@szu.cz
Designation of the type strain	WCHAc060092
Strain collection numbers	ANC 5797 = CCTCC AB 2018119 = GDMCC 1.1380 = KCTC 62576
16S rRNA gene accession number	PYIW01000063.1
Genome accession number [EMBL]	PYIW00000000.1
Genome status	Draft
Genome size	3439.554
GC mol%	40.2
Country of origin	China
Region of origin	Chengdu, Sichuan
Source of isolation	Hospital sewage
Sampling date	2017-11-01
Latitude	30°40′0.01″N
Longitude	104°4′0.01″E
Number of strains in study	6
Source of isolation of non-type strains	Hospital sewage
Growth medium, incubation conditions [temperature, pH, and further information] used for standard cultivation	Tryptic soy agar, nutrient agar or other ordinary agar media; culturable at ≈30 °C and pH of ≈7.0
Is a defined medium available	Int. J. Syst. Evol. Microbiol. 2009; 59, 118–124
Conditions of preservation	Lyophilisation or storage in broth with glycerol (50% by volume) at temperatures from −80 °C to −10 °C
Gram stain	Negative
Motility	Nonmotile
Colony morphology	Colonies on tryptic soy agar (Oxoid) after incubation at 30 °C for 24 h are 1.5–2.5 mm in diameter, grey-white, slightly opaque, circular, convex and smooth, with entire margins; neither haemolysis nor greenish discoloration is observed on agar media supplemented with sheep erythrocytes
Temperature range	15–35 °C
pH category	Neutrophile
Relationship to O_2	Aerobe
O_2 conditions for strain testing	Aerobiosis
Carbon source used [specific compounds]	Acetate, benzoate, citrate (Simmons)
Carbon source not used [specific compounds]	*trans*-Aconitate, adipate, β-alanine, 4-aminobutyrate, L-arabinose, L-arginine, L-aspartate, azelate, 2,3-butanediol, citraconate, gentisate, D-gluconate, D-glucose, histamine, L-histidine, 4-hydroxybenzoate, DL-lactate, L-leucine, levulinate, malonate, L-ornithine, phenylacetate, L-phenylalanine, putrescine, D-ribose, L-tartrate, tricarballylate, trigonelline, tryptamine
Carbon source variable [specific compounds]	Ethanol, L-glutamate, glutarate, D-malate
Acid formation for carbohydrates (all negative)	D-Glucose
Nitrogen source	Ammonium
Terminal electron acceptor	Oxygen
Energy metabolism	Chemoorganotroph
Oxidase	Negative
Catalase	Positive
Negative tests	Gelatin hydrolysis
Biotic relationship	Unknown
Miscellaneous, extraordinary features relevant for the description	Free-living
	Positive in a transformation assay [J. Bacteriol. 1972; 112, 917-931]; the draft genome sequence (PYIW01000063.1) of the type strain contains one complete copy of the 16S rRNA gene (PYIW01000063.1, locus_tag: C9E88_16080)

in five different combinations, with three to 10 distinct genes present per genome. Notably, the genomes of WCHAc060001 and WCHAc060003 carried the same combination of 10 genes even though these organisms are different at the strain level of resolution (Table S1, Fig. S1). The fact that none of the genes was found in all six strains indicates their independent acquisition via horizontal gene transfer. In addition, amino acid sustitutions in GyrA and ParC that have been reported to confer quinolone resistance are present in four of the six strains, while the remaining two strains have amino acid sustitutions at locations in GyrA and ParC that are known to confer resistance to quinolones in the presence of other amino acid substitutions (Table S4).

Overall, the resistance genotypes agreed with the resistance phenotypes found in the six strains (Tables 2 and 3). Two strains, WCHAc060092^T and WCHAc060008, were highly resistant to meropenem and imipenem (MIC, ≥64 mg/l) and to ceftazidime (MIC, ≥256 mg/l), which could be due to the presence of the carbapenemase gene bla_{NDM-1}. Two other strains, WCHAc060001 and WCHAc060003, were also resistant to carbapenems, although at a lower level (MIC, 8–16 mg/l), and both carried genes encoding two OXA-type carbapenemases, OXA-23 and OXA-58. Even though OXA-23 and OXA-58 have only weak activities against broad-spectrum cephalosporins [9,15], the two strains were high-level resistant to ceftazidime (MIC, ≥256 mg/l), which could be explained by the presence of the extended-spectrum β-lactamase-encoding gene bla_{PER-1}. By contrast, the remaining two strains, WCHAc060002 and WCHAc060004, carried the bla_{OXA-58} gene only and were susceptible to carbapenems (MIC, ≤2 mg/l). As OXA-58 has been shown to have only weak carbapenemase activity [15], its presence does not necessarily lead to carbapenem resistance in *Acinetobacter* [6]. Regarding aminoglycosides, decreased susceptibility to amikacin correlated with the presence of the gene encoding phosphotransphefase APH(3′)-VI, which is a common cause of amikacin resistance in clinical *Acinetobacter* isolates.

The inspection of the NCBI Nucleotide database (https://www.ncbi.nlm.nih.gov/nuccore) revealed a genome sequence (accession no. QUWR00000000.1) similar to those of our novel group. The pairwise ANIb values between this sequence and the genomes of our six strains were 97.46–98.61%, which indicates its conspecifity with our taxon but also its uniqueness at the strain level. Moreover, this additional sequence was found to contain *aac(3)-IId*, *aph(3″)-Ib*, *aph(3′)-VIa*, *aph(6)-Id*, bla_{NDM-1}, *mph(E)*, *msr(E)*, and *tet(Y)*, which are genes found in our genomes (Table 3) but arranged in a unique combination. The sequence was obtained from a strain isolated from hospital sewage in China in July 2017, i.e. before our strains

had been collected. These data further support the capacity of the members of the novel taxon for the independent acquisiton of mutiple resistence determinants.

Conclusions

The results presented here indicate that the six strains represent a new species within the genus *Acinetobacter*, which is clearly distinct from all known species with validly published names. We propose the name *Acinetobacter cumulans* sp. nov. for this species, which reflects its ability to accumulate and integrate diverse resistance genes via horizontal gene transfer, and its formal description, which is given in Table 4 with Taxonumber TA00727.

Funding

The work was partially supported by grants from the National Natural Science Foundation of China (project no. 81772233, 81661130159, and 81572030), the Newton Advanced Fellowship, Royal Society, UK (NA150363) and MH CZDRO (National Institute of Public Health – NIPH, 75010330).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.syapm.2019.02.001.

References

[1] Alvarez-Perez, S., Lievens, B., Jacquemyn, H., Herrera, C.M. (2013) *Acinetobacter nectaris* sp nov. and *Acinetobacter boissieri* sp. nov., isolated from floral nectar of wild Mediterranean insect-pollinated plants. Int. J. Syst. Evol. Microbiol. 63, 1532–1539.
[2] Ankenbrand, M.J., Keller, A. (2016) bcgTree: automatized phylogenetic tree building from bacterial core genomes. Genome 59, 783–791.
[3] Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V.M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A. (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19, 455–477.
[4] CLSI, 2017 Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Seventh Informational Supplement. M100-S27, Clinical and Laboratory Standards Institute, Wayne, PA, USA.
[5] Dijkshoorn, L., Nemec, A., Seifert, H. (2007) An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. Nat. Rev. Microbiol. 5, 939–951.
[6] Heritier, C., Poirel, L., Lambert, T., Nordmann, P. (2005) Contribution of acquired carbapenem-hydrolyzing oxacillinases to carbapenem resistance in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. 49, 3198–3202.
[7] Hu, Y., Feng, Y., Qin, J., Radolfova-Krizova, L., Maixnerova, M., Zhang, X., Nemec, A., Zong, Z. (2018) *Acinetobacter wuhouensis* sp. nov., isolated from hospital sewage. Int. J. Syst. Evol. Microbiol. 68, 3212–3216.
[8] Juni, E. (1972) Interspecies transformation of *Acinetobacter:* genetic evidence for a ubiquitous genus. J. Bacteriol. 112, 917–931.
[9] Kaitany, K.C., Klinger, N.V., June, C.M., Ramey, M.E., Bonomo, R.A., Powers, R.A., Leonard, D.A. (2013) Structures of the class D Carbapenemases OXA-23 and OXA-146: mechanistic basis of activity against carbapenems, extended-spectrum cephalosporins, and aztreonam. Antimicrob. Agents Chemother. 57, 4848–4855.
[10] Krizova, L., Maixnerova, M., Sedo, O., Nemec, A. (2015) *Acinetobacter albensis* sp nov., isolated from natural soil and water ecosystems. Int. J. Syst. Evol. Microbiol. 65, 3905–3912.
[11] Meier-Kolthoff, J.P., Auch, A.F., Klenk, H.P., Goker, M. (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatic 14, 60.
[12] Nemec, A., Musilek, M., Maixnerova, M., De Baere, T., van der Reijden, T.J., 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, 118–124.
[13] Nemec, A., Radolfova-Krizova, L., Maixnerova, M., Nemec, M., Clermont, D., Bzdil, J., Jezek, P., Spanelova, P. (2018) Revising the taxonomy of the *Acinetobacter lwoffii* group: The description of *Acinetobacter pseudolwoffii* sp. nov. and emended description of Acinetobacter lwoffii. Syst. Appl. Microbiol., http://dx.doi.org/10.1016/j.syapm.2018.1010.1004, in press.
[14] Nemec, A., Radolfova-Krizova, L., Maixnerova, M., Sedo, O. (2017) *Acinetobacter colistiniresistens* sp nov. (formerly genomic species 13 sensu Bouvet and Jeanjean and genomic species 14 sensu Tjernberg and Ursing), isolated from human infections and characterized by intrinsic resistance to polymyxins. Int. J. Syst. Evol. Microbiol. 67, 2134–2141.
[15] Poirel, L., Marque, S., Heritier, C., Segonds, C., Chabanon, G., Nordmann, P. (2005) OXA-58, a novel class D β-lactamase involved in resistance to carbapenems in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. 49, 202–208.
[16] Radolfova-Krizova, L., Maixnerova, M., Nemec, A. (2016) *Acinetobacter celticus* sp nov., a psychrotolerant species widespread in natural soil and water ecosystems. Int. J. Syst. Evol. Microbiol. 66, 5392–5398.
[17] Radolfova-Krizova, L., Maixnerova, M., Nemec, A. (2016) *Acinetobacter pragensis* sp nov., found in soil and water ecosystems. Int. J. Syst. Evol. Microbiol. 66, 3897–3903.
[18] Richter, M., Rossello-Mora, R. (2009) Shifting the genomic gold standard for the prokaryotic species definition. Proc. Natl. Acad. Sci. U.S.A. 106, 19126–19131.
[19] Roca, I., Espinal, P., Vila-Farres, X., Vila, J. (2012) The *Acinetobacter baumannii* oxymoron: commensal hospital dweller turned pan-drug-resistant menace. Front. Microbiol. 3, 148.
[20] Seemann, T. (2014) Prokka: rapid prokaryotic genome annotation. Bioinformatics 30, 2068–2069.