Introduction

Acinetobacter is a genus of Gram-negative bacteria that are important soil organisms. Over 30 species have been identified with validly published names [1,2]. During recent decades, Acinetobacter spp., particularly A. baumannii, have been suggested as an important public-health concern because of multiple drug resistance. The emergence of carbapenem-resistant A. baumannii (CRAB) has become a major international public health concern (CRAB) has become a major international public health concern [3]. Different Acinetobacter spp. might possess distinct capability in invasion and virulence [4], thus ensuring the urgency of developing an accurate identification method for Acinetobacter spp.

The most common phenotypic and genotypic identification methods utilized in the species determination of Acinetobacter spp. are biochemical systems and 16S rRNA gene sequencing. Biochemical systems such as VITEK 2 are prone to being influenced by culture conditions [5]. The major limitation of 16S rRNA gene sequencing is that it is too conserved to distinguish all species [6]. A more effective identification method is required for clinical or laboratory application. In this case, rpoB sequencing and Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS) were evaluated as two alternative methods.

The rpoB gene has a housekeeping role, and its size differs between species, ranging from 3411 bp (Staphylococcus aureus) to 4185 bp (Neisseria meningitidis) [7]. The variability of the rpoB gene sequence ensures that it is impossible to design universal primers to amplify this gene for all bacteria. As a result, rpoB is more suitable for typing subspecies and is frequently used as a multiplex locus sequence typing (MLST) locus for many bacterial species [8]. Species is a taxonomic unit between genus and subspecies, and whether rpoB sequencing is feasible for species identification is unknown. Recently, many scientists have attempted to use the rpoB gene to identify clinical isolates of Acinetobacter spp [9,10]; however, few systematic application of the rpoB gene have been applied to identify clinical Acinetobacter spp. As a result of the incompleteness of the rpoB database, many reference strains have not received an accurate name, which adds confusion regarding the nomenclature in the clinical field.

Mass spectrometry is a recently developed method for clinical bacterial identification that is primarily based on ribosomal proteins [11,12]. This method has high sensitivity, high accuracy and high resolution, leading to its wide research application in life sciences and other fields. For bacterial identification, mass
spectrometry has the advantage of low cost, rapidity and ease of use. Several misidentification cases in nonfermentative Gram-negative bacilli have been reported [6].

In this study, we compared the pros and cons of the four identification methods to find a convenient approach for Acinetobacter spp. identification at the species level.

**Materials and Methods**

**Bacterial isolates**

A total of 2582 non-duplicate clinical Acinetobacter spp. isolates were collected from 27 provinces in China from January 2009 to September 2010 [13]. Among them, 385 isolates of blaOXA-51-like-negative Acinetobacter spp. were selected for further identification. A total of 24 A. baumannii isolates, which were confirmed by blaOXA-51-like-positive and MLST, were selected as the reference, i.e., one isolate per province and ATCC17978 [13]. The primers used for amplifying the blaOXA-51-like gene are listed in Table 1. The isolates were preliminarily identified by the VITEK 2 system (Sysmex-bioMérieux, Marcyl’Etoile, France).

**PCR amplification and gene sequencing**

The rpoB sequences of 32 Acinetobacter spp. reference strains were obtained from the NCBI GenBank database or the Acinetobacter spp. genomes (Table S1). The PCR degenerate primers were designed online at the region between positions 2300 and 3300 (http://blocks.fhcrc.org/codehop.html) [7]. The primers used to amplify and sequence the rpoB gene are listed in Table 1. The primers of the 16S rRNA gene were designed based on 32 sequences of standard strains downloaded from the website for prokaryotic nomenclature (http://www.bacterio.net/a/acinetobacter.html) (Table S2).

The interspecies diversity was evaluated using the similarities of the rpoB gene sequences between A. baumannii ATCC 17978 and reference strains of different Acinetobacter species. The similarities of its trimmed sequences where our in-house designed primers were located were calculated using the identical method. To accurately evaluate the highest similarity between the closest relationship groups, the genomes of 16 A. baumannii strains and nine A. nosocomialis strains were retrieved, and the complete rpoB gene sequences were trimmed with the identical standard to calculate their intraspecies similarity.

The sequences of the rpoB and 16S rRNA genes were aligned using the multiple alignment program for amino acid or nucleotide sequences (MAFFT version 7) [14]. The Neighbor-Joining (NJ) tree was constructed with the 32 reference strains and the representative isolates, which were selected from each lineages using the MEGA 5.2 program [15–17].

**MALDI-TOF MS**

The isolates were identified on the VITEK MS system (bioMérieux, Marcyl’Etoile, France) in accordance with the manufacturer’s instructions. E. coli ATCC 8739 was used as a quality control.

**Susceptibility test**

Susceptibility testing of imipenem and meropenem was performed by disk diffusion. A. baumannii ATCC 17978 and E. coli ATCC 25922 were used for quality control. For the disk diffusion testing, a single clone was cultured at 37°C overnight in a Mueller-Hinton (MH) agar plate, recovered and diluted to 0.5 McFarland turbidity with 0.9% NaCl (w/v) solution. The diluted suspension was distributed on an MH plate with a cotton swab. After the antimicrobial susceptibility test discs were used, the plate was incubated at 37°C for 24 h. The data interpretation was performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) 2012 guideline [18]. The Zone Diameter Interpretive Criteria for imipenem and meropenem were as follows: susceptible, inhibition zone ≤14 mm; intermediate susceptible inhibition zone = 15 to 17 mm; resistant, inhibition zone ≥18 mm.

**Results**

**rpoB sequencing**

The rpoB gene similarities between A. baumannii ATCC 17978 and the reference strains of different Acinetobacter species were distributed as 84.8–95.6%, and the highest similarities of these trimmed rpoB gene sequences were up to 96.5% (Table 2). Because the two species of A. baumannii and A. nosocomialis showed the highest interspecies similarity, the complete sequence similarity of the rpoB gene between them was 95.6±0.1%, whereas the similarity of their conservative region amplified by our

---

**Table 1. PCR primers used for species identification.**

| Target gene | Primer name | Nucleotide sequence 5′→3′ | Product size (bp) |
|-------------|-------------|--------------------------|-------------------|
| rpoB        | rpoB-F1     | CCTCATGACCTGGAAYGGNTA     | 940               |
|             | rpoB-R1     | TCCAGGATCTGONCNACRITCAT   | 940               |
|             | rpoB-F2     | CATGACCTGGAACCGTAAYAYGA   | 1210              |
|             | rpoB-R2     | TGTTACGCTCAGCATRACATRTA   | 1210              |
| 16S rRNA    | 16S-F1      | GAGTAATGCTTAAGGAATCTGC    | 130               |
|             | 16S-R1      | GGGAAACGCCCCTCTTGG        | 130               |
|             | 16S-F2      | GGGAAACGCGTGGATATG        | 1030              |
|             | 16S-R2      | GCTGGGAAATAGGAAAA         | 1030              |
| blaOXA-51-like | blaOXA-51-like-F1 | TAATGTTCTTGACGCCCTTGG | 353               |
|             | blaOXA-51-like-R1 | TGGATTGCACTCATCTTGG    | 353               |
|             | blaOXA-51-like-F2 | TAGTACTGCTAATCCAAAT     | 670               |
|             | blaOXA-51-like-R2 | AAGGGAGAACCGCTCAAT     | 670               |

Note: R = A/G,Y = C/T,N = A/C/G/T,
doi:10.1371/journal.pone.0104882.0001
in-house designed PCR primers was 96.6±0.2% (Table 3). The intraspecies similarity of the *A. baumannii* and *A. nosocomialis* reference strains were all above 99%, which indicated that it was an intraspecies conservative gene and, therefore, not appropriate for strain typing. Because the *rpoB* gene has the characteristics of interspecies polymorphisms and is intraspecies conservative, it could offer high discriminative power for *Acinetobacter* spp. identification. Setting a pairwise identity of 97% as the appropriate criterion for delineating this species was thus a reasonable hypothesis.

According to such criteria, a total of 409 clinical isolates, including 385 isolates of *bla*<sup>OXA-51</sup>-negative *Acinetobacter* spp. and 24 *A. baumannii* isolates, could be clearly divided into 11 lineages (Table 4, Figure 1(a)). Our study indicated that a pairwise identity of 98.5% could be set as the criterion for delineating the clinical species.

In addition, *A. ptii* and *A. calcoaceticus* were not differentiated from each other, and their 189 isolates constituted the largest branch. The second largest branch was *A. nosocomialis*, which was composed of 134 isolates. The branch of *A. junii*/*A. grimontii* contained 25 isolates. *A. lwofii* SH145 and *A. lwofii* WJ10621 were located on different branches, the latter being clustered with *A. johnsonii* SH046. Similarly, *A. calcoaceticus* RUH2202 was separated from other *A. calcoaceticus* reference strains and were clustered with *A. oleivorans* isolates. Initially, *A. lwofii* WJ10621 and *A. calcoaceticus* RUH2202 were identified incorrectly.

*A. haemolyticus, A. soli, A. bereziniae* and *A. ursingii* formed their own branch, although with a small number of isolates. One branch contained 11 isolates and did not contain any reference strains. This branch might represent a novel species, which was entitled *genomic species 33YU* (GenBank accession numbers: KP982810-KP982820).

### 16S RNA gene sequencing

A pairwise identity of 99% was set as the criterion for delineating the species of *Acinetobacter* spp. The 409 isolates were grouped into seven branches. The Neighbor-Joining (NJ) tree, which was constructed by the 32 reference strains and the representative isolates, is shown in Figure 1(a). The nucleotide similarity values of each lineage with the reference strains are list in Table 4.

*A. calcoaceticus* and *A. nosocomialis* were not differentiated by 16S rRNA gene sequencing, and these 340 isolates were combined into one large branch that was labeled *A. calcoaceticus-A. baumannii* complex (*Acb*) complex, including 11 isolates of the *genomic species 33YU* branch that were revealed by *rpoB* gene sequencing. The *A. baumannii* branch contained 24 isolates and was located near the *A. junii* branch, which was consistent with the phylogenetic relationships in other studies [19]. *A. junii* and *A.

---

### Table 2. The *rpoB* gene similarity between *A. baumannii* ATCC 17978 and reference strains of different species.

| reference strains | A (%) | B (%) | reference strains | C (%) | D (%) |
|-------------------|-------|-------|-------------------|-------|-------|
| *A. genomosporic* | 95.6  | 96.5  | *Acinetobacter* sp. _HA | 87.3  | 89.3  |
| *A. nosocomialis* | 95.4  | 96.3  | *A. johnsonii* SH046 | 87.3  | 87.1  |
| *A. ptii* | 93.7  | 94.0  | *A. bereziniae* LMG_1003 | 87.3  | 85.6  |
| *A. calcoaceticus* SH024 | 93.3  | 93.6  | *A. lwofii* WJ10621 | 87.3  | 87.0  |
| *A. oleivorans* DR1 | 92.5  | 94.1  | *Acinetobacter* sp. _P8-3-8 | 87.1  | 85.8  |
| *A. calcoaceticus* RUH2202 | 92.0  | 93.2  | *A. schindleri* TG19614 | 86.8  | 86.3  |
| *A. venetianus* RAG_1 | 90.0  | 90.8  | *Acinetobacter* sp. _WC-743 | 86.7  | 85.6  |
| *Acinetobacter* NBR100985 | 89.7  | 93.6  | *A. tandoi* DSM_14970 | 86.7  | 85.8  |
| *A. tjiernbergia* DSM_14971 | 89.3  | 89.3  | *A. lwofii* SH145 | 86.6  | 85.4  |
| *A. calcoaceticus* NCTC7422 | 89.2  | 91.2  | *A. baylyi* ADP1 | 86.1  | 84.4  |
| *A. haemolyticus* ATCC191914 | 88.9  | 89.2  | *A. ursingii* DSM_16037 | 85.5  | 83.5  |
| *A. parvus* DSM16617 | 88.8  | 88.3  | *A. bouvetii* DSM_14964 | 85.5  | 84.5  |
| *A. junii* SH025 | 88.6  | 89.6  | *A. townesi* DSM_14962 | 85.4  | 84.3  |
| *A. grimontii* CIP07470 | 88.5  | 89.4  | *A. soli* CIP_110264 | 85.3  | 84.1  |
| *A. genomosa* | 87.5  | 87.7  | *A. radioresistens* DSM_6976 | 84.8  | 83.4  |
| *A. genomosporic* | 87.3  | 85.4  | *(GenBank accession numbers: KP982810-KP982820)*

Note: The column A and C indicated the complete *rpoB* gene sequences. The column B and D indicated the trimmed partial *rpoB* gene sequences where our designed primers located.

doi:10.1371/journal.pone.0104882.t002

### Table 3. The *rpoB* gene variation of *A. baumannii* and *A. nosocomialis* reference strains.

|                           | Complete CDS (%) | Trimmed sequences (%) |
|---------------------------|------------------|-----------------------|
| The intraspecies similarity of *A. baumannii* (%) | 99.5±0.1         | 99.3±0.2              |
| The intraspecies similarity of *A. nosocomialis* (%) | 99.6±0.3         | 99.5±0.5              |
| The interspecies similarity (%) | 95.6±0.1         | 96.6±0.2              |

Note: The column A and C indicated the complete CDS (%) Trimmed sequences (%).

doi:10.1371/journal.pone.0104882.t003
Comparison of different methods

Because the 16S rRNA gene was more conservative than the rpoB gene, the former resolution was even worse than the latter. As a result, in this study, their criteria for delineating the species of Acinetobacter were quite different. If the pairwise identity 98.5% was set as the identical criteria for the 16S rRNA gene, most of the strains could not be differentiated. If the pairwise identity of 99% were set as the criteria for the rpoB gene, however, the strains would be divided into numerous clusters. According to the current standards, the consistency between these two methods was most satisfactory.

The VITEK 2 system showed unsatisfactory performance in species identification, and the result of VITEK MS was a little better. The latter method was able to identify A. junii, A. haemolyticus and A. johnsonii accurately; however, it failed to discriminate the Acb complex. These results could be expected because this clinical identification system relies on the database that is built on the knowledge of the understanding of the genus Acinetobacter by engineers and scientists. The taxonomy of Acinetobacter is unclear, even in the lab, and the related findings are rarely transferred to these identification systems in a timely manner.

Susceptibility data

The resistance rate of the non-A. baumannii isolates to imipenem and/or meropenem was 2.6%. Among the 10 resistant isolates, three isolates belong to the A. calcoaceticus/A. pittii branch, and five isolates were A. nosocomialis, one isolate was A. soli, and one isolate was genomic species 33YU.

False-positive rate and false negative rate of blaOXA-51-like

None of the 385 non-A. baumannii isolates carried the blaOXA-51-like gene. Of the 2197 blaOXA-51-like-positive Acinetobacter spp. isolates, one isolate was inserted by ISAba19.

Discussion

This study provided a combined genotypic and phenotypic assessment of identification methods for clinical Acinetobacter spp. isolates collected from 23 provinces in China. As a classic identification method, 16S rRNA gene sequencing was highly reliable at the genus level; however, it showed poor discriminatory ability on the species level [20]. The full lengths of the 16S rRNA gene sequence of A. pittii, A. nosocomialis, A. calcoaceticus and A. baumannii were nearly identical to each other. Distinguishing these species merely by 16S rRNA gene sequencing is impossible.

The high variability of the rpoB gene among Acinetobacter spp. ensured that it is appropriate for species typing; however, designing universal primers is difficult. A conserved region within the rpoB gene provided a unique target for primer designing [7]. Except for the different resolution, 16S rRNA and rpoB gene sequencing showed a consistent typing result, which indicated that both 16S rRNA and rpoB were rarely involved in recombination between Acinetobacter spp. The evolutionary tree based on the two genes could reflect their original phylogenetic relationship.

As early as 2006, rpoB gene sequencing was proposed for identifying species of Acinetobacter [21]. In 2009, Vijay A. K. B. Gundl et al. identified 99 Acinetobacter clinical isolates by rpoB gene sequencing and confirmed that an unnamed Acinetobacter genomic species (gen. sp.) 3 was the second dominant species after A. baumannii and A. nosocomialis.
A. baumannii in patients [9]. Some studies reported that the Acb complex could be further classified into the following four species: A. pittii, A. nosocomialis, A. calcoaceticus and A. baumannii by rpoB gene sequences. [22,23]. The fragment lengths of the partial rpoB gene amplified by their in-house designed PCR primers were only 350 and 450 bp, and the locations of the two fragments were completely different. Some of these so-called reference sequences of the rpoB gene have not been verified by whole genome sequencing. The similarity of the rpoB reference sequences in some species were even greater than 99%, e.g., A. calcoaceticus SH024 and A. pittii D499, whereas A. lwoffii WJ10621 and A. calcoaceticus RUH2202 were not located on their expected phylogenetic tree branches, which was consistent with their whole genome sequencing data [24]. These phenomena reiterated the current confusion of the nomenclature of Acinetobacter spp., the severity of which has ensured that sequences retrieved from public databases are unreliable. Excessively short fragments of the rpoB gene sequence might encounter marked difficulties, particularly in large-scale clinical applications, e.g., some closely related strains remain difficult to differentiate. Most researchers were continuing to follow these primers for Acinetobacter species identification, whereas others were attempting to replace rpoB gene sequencing by other housekeeping genes, e.g., gyrB [22]. In our study, the fragment lengths of the partial rpoB gene amplified using our newly re-designed PCR primers were 940 and 1210 bp, and the location of these fragments were nearly consistent. The criteria for delineating the species of Acinetobacter were proposed in this study, and the classification results were relatively ideal. Additionally, our study confirmed that A. pittii/A. calcoaceticus was the second dominant species, after A. baumannii, that is isolated from patients, which was consistent with a study in European countries, whereas A. nosocomialis was the second dominant species in South Korea [5,9,22]. Additionally, our study confirmed that genomic species 33YU was a clinically significant species in patients and should be monitored with care.
Table 5. The difference identification results of 409 Acinetobacter isolates by three different methods.

| Isolates No. | rpoB (n) | VITEK2 (n) | MALDI-TOF MS (n) |
|--------------|----------|------------|-----------------|
| A1-A189      | A.pittii/A.calcoaceticus (189) | Acb complex (180), A.haemolyticus (1)*, A.Jwolffii (8)* | Acb complex (188), E.coli (1)* |
| A190-A195    | A.oelovenar/A.calcoaceticus_RUH2202 (6) | Acb complex (6) | Acb complex (6) |
| A196-A329    | A.nosocomialis (134) | Acb complex (131), A.Jwolffii (3)* | Acb complex (134) |
| A354-A364    | A. genic species 33YU (11) | Acb complex (11)* | Acb complex (11) |
| A330-A353    | A.baumannii (24) | Acb complex (24) | Acb complex (23), Non-Acinetobacter spp. (1)* |
| A365-A366    | A.haemolyticus (2) | Acb complex (2)* | A.haemolyticus (2) |
| A367-A370    | A.johnsonii/A.Jwolffii_WJ10621 (4) | A.Jwolffii (2)*, Acb complex (2)* | A.johnsonii (4) |
| A371-A395    | A.junii/A.grimontii (25) | A.junii (16), A.haemolyticus (2)*, A.Jwolffii (3)*, Acb complex (4)* | A.junii (25) |
| A396-A404    | A.soli (9) | A.Jwolffii (1)*, Acb complex (8)* | Non-Acinetobacter spp. (6), Acb complex (3)* |
| A405-A408    | A.berezinaiae (4) | A.Jwolffii (4)* | Acb complex (4)* |
| A409         | A.ursingii (1) | Acb complex (1)* | Acb complex (1)* |

Note: * indicates mis-identification.
doi:10.1371/journal.pone.0104882.t005

Additionally, Toidi Ade’ kambi et al. compared the rpoB gene sequence similarity of 230 bacterial species representative of 45 genera and revealed that the interspecific diversity based on the rpoB gene sequence was 98.2–100%, which could be considered a suitable supplement to DNA-DNA hybridization [25]. For Acinetobacter species, the interspecies similarities were 84.8–95.6%, whereas the intraspecies similarity of the complete rpoB gene sequences in A. baumannii and A. nosocomialis were above 99%. We hypothesize that, considering the irreplaceable advantages of rpoB gene sequencing, it is a promising tool for species identification because of its appropriate interspecies polymorphisms.

Vaneechoutte M et al. proposed that A. grimontii is a heterotypic synonym of A. junii by DNA-DNA hybridization and amplified fragment length polymorphism (AFLP) [26]. However, on the website of prokaryotic nomenclature, A. grimontii and A. junii are considered two different species. We found that the identity of their rpoB sequences was greater than 99% and did not reach the threshold of 98.5%, and we suggest combining them into one species.

The blaOXA-51-like gene has been proposed as a marker for A. baumannii [27]. Previous studies proposed that detecting the blaOXA-51-like gene using multiplex PCR was not reliable for the identification of A. baumannii because this gene could be disrupted by ISAba15 or ISAba19, or that the blaOXA-51-like gene could be acquired by non-A. baumannii by horizontal gene transfer [28,29]. We hypothesize that the above evidence was not sufficient to negate the value of the blaOXA-51-like gene as a genetic marker of A. baumannii in that such events were remarkably rare. Of our 2197 A. baumannii isolates, one isolate was inserted by ISAba19, with the band of PCR product continuing, with its length increasing from 670 bp to 2000 bp. Our previous study confirmed that 875 carbapenem-resistant blaOXA-51-like-positive Acinetobacter isolates could be investigated using a MLST scheme, which demonstrated that they belong to A. baumannii [13]. None of the 85 non-A. baumannii isolates carried the blaOXA-51-like gene. The false positive rate and the false negative rate were low using the blaOXA-51-like gene as a genetic marker of A. baumannii.

The large number of our collected isolates guarantees the inclusion of most of the nosocomial Acinetobacter spp. One novel species, i.e., genomic species 33YU, was newly identified, indicating that most nosocomial Acinetobacter spp. have been discovered.

We suggest that rpoB sequencing is the best reference method for identifying Acinetobacter species. We designed primers for the rpoB gene that applied to most non-A. baumannii isolates successfully. A database of rpoB sequences was constructed, which can be used by scientists and physicians worldwide for identifying Acinetobacter spp. Other than A. baumannii, the most commonly isolated Acinetobacter spp. in a nosocomial environment were A. calcoaceticus, A. pittii and A. nosocomialis. Few non-A. baumannii showed high resistance to carbapenem, and they could be used as a control for studies to determine the reasons that A. baumannii evolved to be a multi-drug resistant superbug.

Supporting Information

Table S1 The rpoB gene reference strains of Acinetobacter used in this study.

(DOCX)

Table S2 The 16S rRNA reference strains of Acinetobacter used in this study.

(DOCX)

Acknowledgment

We would like to express our gratitude to Prof. Xinzhen Zhang and her colleagues for providing VITEK MS system and identification assistance.

Ethical approval: Not required.

Author Contributions

Conceived and designed the experiments: YSY Y. Feng. Performed the experiments: JFW Y. Fu HPW. Analyzed the data: ZR YJ. Contributed reagents/materials/analysis tools: ZR YJ HPW. Wrote the paper: JFW ZR Y. Feng.
References

1. Sieniawski K, Kaczkó K, Racinska M, Gagis L, Pomorski Ł. (2013) Acinetobacter baumannii nosocomial infections. Pol Przegl Chir 83: 483–490.

2. Li Y, Piao CG, Mu YC, He W, Wang HM, et al. (2013) Acinetobacter puyangensis sp. nov., isolated from the healthy and diseased part of Populus xuramericana canker bark. Int J Syst Evol Microbiol 63: 2963–2969.

3. Kaase M, Szabados F, Pfenninger I, Anders A, Gen G, et al. (2013) Description of the metallo-beta-lactamase GIM-1 in Acinetobacter pittii. J Antimicrob Chemother.

4. Bitrián M, Solari CM, Gonzalez RH, Nudel CB (2012) Identification of virulence markers in clinically relevant strains of Acinetobacter genospecies. Int Microbiol 15: 79–88.

5. Karah N, Haldorsen B, Hegstad K, Simonsen GS, Sundsfjord A, et al. (2011) Identification of Acinetobacter species: is Bruker biotyper MALDI-TOF mass spectrometry a good alternative to molecular techniques? Infect Genet Evol 12: 345–349.

6. Alvarez-Boylla A, Colebras E, Picaeo JF (2012) Identification of Acinetobacter pittii species: is Bruker biomarker MALDI-TOF mass spectrometry a good alternative to molecular techniques? Infect Genet Evol 12: 345–349.

7. Adekambi T, Drancourt M, Raoult D (2009) Validation of Acinetobacter baumannii complex. J Clin Microbiol 47: 4302–4309.

8. Bartual SG, Seffert H, Hippler C, Luzon MA, Wüiblinghoff H, et al. (2005) Development of a multilocus sequence typing scheme for characterization of clinical isolates of Acinetobacter baumannii. J Clin Microbiol 43: 4302–4309.

9. Gundi VA, Dijkshoorn L, Burignat S, Raoult D, La Scola B (2009) Validation of partial rpoB gene sequence analysis for the identification of clinically important and emerging Acinetobacter species. Microbiology 155: 233–2341.

10. Lee MJ, Jiang SJ, Li XM, Park G, Kook JK, et al. (2013) Comparison of rpoB gene sequencing, 16S rRNA gene sequencing, gyrB multiplex PCR, and the VITEK2 system for identification of Acinetobacter clinical isolates. Diagn Microbiol Infect Dis.

11. Sodó O, Nemec A, Krizova I, Kacakova M, Zdrahal Z (2013) Improvement of MALDI-TOF MS profiling for the differentiation of species within the Acinetobacter calcoaceticus-Acinetobacter baumannii complex. Syst Appl Microbiol.

12. Patel R (2013) Matrix-assisted laser desorption ionization-time of flight mass spectrometry in clinical microbiology. Clin Infect Dis 57: 564–572.

13. Ruan Z, Chen Y, Jiang Y, Zhou H, Zhou Z, et al. (2013) Wide distribution of CC92 carbapenem-resistant and OXA-23-producing Acinetobacter baumannii in multiple provinces of China. Int J Antimicrob Agents 42: 322–328.

14. Katoh K, Standley DM (2014) MAFFT: Iterative Refinement and Additional Methods. Methods Mol Biol 1079: 131–146.

15. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406–425.

16. Kiyota N, Kusahashi I, Kobaichi M, Tsukagushi H, Kyo A, et al. (2013) Genetic analysis of the VP4/VP2 coding region in human rhinovirus species C in patients with acute respiratory infection in Japan. J Med Microbiol 62: 610–617.

17. Hall BG (2013) Building phylogenetic trees from molecular data with MEGA. Mol Biol Evol 30: 1289–1295.

18. Cockerill F (2012) Performance Standards for Antimicrobial Susceptibility Testing: Twenty-second Informational Supplement. Clinical and Laboratory Standards Institute.

19. Gerischer U (2008) Acinetobacter molecular biology: Horizon Scientific Press.

20. Janda JM, Abbott SL (2007) 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: phases, perils, and pitfalls. J Clin Microbiol 45: 2761–2764.

21. La Scola B, Gundi VA, Khamis A, Raoult D (2006) Sequencing of the rpoB gene and flanking spacers for molecular identification of Acinetobacter species. J Clin Microbiol 44: 4327–4332.

22. Lee MJ, Jiang SJ, Li XM, Park G, Kook JK, et al. (2014) Comparison of rpoB gene sequencing, 16S rRNA gene sequencing, gyrB multiplex PCR, and the VITEK2 system for identification of Acinetobacter clinical isolates. Diagn Microbiol Infect Dis 78: 29–34.

23. Nemec A, Krizova I, Maixnerova M, van der Reijden TJ, Deschagt P, et al. (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 3TU). Res Microbiol 162: 393–404.

24. Sahl JW, Gillice JD, Schupp JM, Waddell VG, Driebe EM, et al. (2013) Evolution of a pathogen: a comparative genomics analysis identifies a genetic pathway to pathogenesis in acinetobacter. PLoS One 8: e54307.

25. Adekambi T, Shinmuck TM, Raoult D, Drancourt M (2008) Complete rpoB gene sequencing as a suitable supplement to DNA-DNA hybridization for bacterial species and genus delineation. Int J Syst Evol Microbiol 58: 1807–1814.

26. Vanezourette M, De Baere T, Nemec A, Musilek M, van der Reijden TJ, et al. (2008) Reclassification of Acinetobacter grimontii Carr, et al. 2003 as a later synonym of Acinetobacter junii Bouvet and Grimont 1986. Int J Syst Evol Microbiol 58: 937–940.

27. Turton JF, Woodford N, Glover J, Yardie S, Kaufmann ME, et al. (2006) Identification of Acinetobacter baumannii by detection of the blaOXA-51-like carbapenemase gene intrinsic to this species. J Clin Microbiol 44: 2974–2976.

28. Zander E, Higgins PG, Fernandez-Gonzalez A, Seifert H (2013) Detection of intrinsic blaOXA-51-like by multiplex PCR on its own is not reliable for the identification of Acinetobacter baumannii. Int J Med Microbiol 303: 88–90.

29. Lee YT, Kuo SC, Chiang MC, Yang SP, Chen CP, et al. (2012) Emergence of rpoB gene and flanking spacers for the differentiation of clinical isolates of Acinetobacter baumannii. Int J Med Microbiol 303: 88–90.

30. Janda JM, Abbott SL (2007) 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: phases, perils, and pitfalls. J Clin Microbiol 45: 2761–2764.

31. Adekambi T, Drancourt M, Raoult D (2009) Validation of Acinetobacter baumannii complex. J Clin Microbiol 47: 4302–4309.