Clinical Microbiology and Infection 27 (2021) 126.e1–126.e5

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Original article

Development of a database for the rapid and accurate routine identification of *Achromobacter* species by matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS)

T. Garrigos [1], C. Neuwirth [1, 2], A. Chapuis [1], J. Bador [1, 2], L. Amoureux [1, 2, *], Collaborators

[1)] *Department of Bacteriology, University Hospital of Dijon, BP 37013, 21070, Dijon Cedex, France*
[2)] *UMR/CNRS 6249 Chrono-environnement, University of Bourgogne- Franche-Comté, Besançon, France*

ARTICLE INFO

Article history:
Received 20 September 2019
Received in revised form
17 February 2020
Accepted 26 March 2020
Available online 10 April 2020

Editor: P. T. Tassios

Keywords:
Achromobacter
MALDI-TOF
mass spectrometry
nrdA
species identification

ABSTRACT

Objectives: *Achromobacter* spp. are emerging pathogens in respiratory samples from cystic fibrosis patients. The current reference methods (*nrdA*-sequencing or multilocus sequence typing) can identify 18 species which are often misidentified by conventional techniques as *A. xylosoxidans*. A few studies have suggested that matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF/MS) provides accurate identification of the genus but not of species. The aims of this study were (a) to generate a database for MALDI-TOF/MS Bruker including the 18 species, (b) to evaluate the suitability of the database for routine laboratory identification, and (c) to compare its performance with that of the currently available Bruker default database.

Methods: A total of 205 isolates belonging to the 18 species identified by *nrdA* sequencing were used to build a local database. Main spectra profiles (MSPs) were created according to Bruker's recommendations for each isolate with the Biotyper software. Performance of the default Bruker database and ours for routine use were compared by testing 167 strains (including 38 isolates used from MSP creation) belonging to the 18 species identified by *nrdA* sequencing directly from colonies cultivated on various media.

Results: Our new database accurately identified 99.4% (166/167) of the isolates from the 18 species (score $\geq$2.0) versus only 50.9% (85/167) with the Bruker database. In the Bruker database 17.3% of the isolates (29/167) were incorrectly identified as another species despite a score of $\geq$2.0.

List of collaborators: Emmanuel Andre (Laboratory of Clinical Bacteriology and Mycology, Department of Microbiology and Immunology, KU Leuven, Leuven, Belgium), Elodie Barbier (INRA, UMR 1347 Agroécologie, Dijon Cedex, France; Université de Bourgogne Franche Comté, Dijon Cedex, France), Jocelyne Caillon (Centre Hospitalier Universitaire de Nantes, service de Bactériologie-Hygiene, Nantes, France), Emilie Cardot-Martin (Department of Microbiology, Foch Hospital, Suresnes, France), Vincent Cattoir (Service de Bactériologie-Hygiène hospitalière, Centre Hospitalier Universitaire de Rennes, Rennes, France), Anne Doléans-Jordheim (Institut des agents infectieux, Hospices Civils de Lyon, Lyon, France), Fedoua Echahidi (Department of Microbiology, National Reference Center for Burkholderia Cepacia Complex, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel, Brussels, Belgium), Agnès Ferroni (Assistance Publique-Hôpitaux de Paris, Laboratoire de Microbiologie, Hôpital Necker-Enfants Malades, Paris, France; Université Paris Descartes, Faculté de médecine, Paris, France), Hélène Guet-Revillet (Service de Bactériologie-Hygiène, Centre Hospitalier Universitaire de Toulouse, Toulouse, France), Geneviève Héry-Arnaud (Unité de Bactériologie, Pôle de Biologie-Pathologie, Centre Hospitalier Régional et Universitaire de Brest, Hôpital de la Cavale Blanche, Boulevard Tanguy Prigent, Brest, France), John Lipuma (Department of Pediatrics, University of Michigan, Ann Arbor, MI 48109 USA), Niels Nørskov-Lauritsen (Department of clinical microbiology, Aarhus University Hospital, Aarhus, Denmark), Charlotte Peeters (Laboratory of Microbiology, National Reference Center for Burkholderia Cepacia Complex, Ghent University, Ghent, Belgium), Denis Pierard (Department of Microbiology, National Reference Center for Burkholderia Cepacia Complex, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel, Brussels, Belgium), Christine Segonds (Service de Bactériologie-Hygiène, Centre Hospitalier Universitaire de Toulouse, Toulouse, France), Elise Thomas (Centre Hospitalier Universitaire de Nantes, service de Bactériologie-Hygiene, Nantes, France), Patrick Plésiat (Laboratoire de Bactériologie, Centre Hospitalier Universitaire de Besançon, France), Peter Vandamme (Laboratory of Microbiology, National Reference Center for Burkholderia Cepacia Complex, Ghent University, Ghent, Belgium), Alexia Verroken (Department of Microbiology, Cliniques Universitaires Saint-Luc, Université catholique de Louvain, Brussels, Belgium), Hoang Vu-Thien (Institute for Integrative Biology of the Cell, CNRS, Université Paris Sud, Université Paris-Saclay, Orsay, France)

* Corresponding author. L. Amoureux, Laboratoire de Bactériologie, Hôpital Universitaire, Plateau Technique de Biologie, BP 37013, 21070, Dijon CEDEX, France.
E-mail address: lucie.amoureux@chu-dijon.fr (L. Amoureux).

https://doi.org/10.1016/j.cmi.2020.03.031

Conclusions: The use of MALDI-TOF/MS in combination with a database developed with samples from 18 *Achromobacter* species provides rapid and accurate identification. This tool could be used to help future clinical studies. **T. Garrigos, Clin Microbiol Infect 2021;27:126.e1–126.e5**

Introduction

Achromobacter spp. are increasingly being reported in various infections in both immunocompromised and immunocompetent patients [1–3]. They are emerging pathogens in cystic fibrosis (CF) patients [4–6] but their role in the decline of respiratory function is not clear [6,7].

Over the last decade, the taxonomy of the genus *Achromobacter* has been revised. Two current reference methods can be used to identify *Achromobacter* spp. to the species level: *nrdA* sequencing (765 bp fragment) or multilocus sequence typing (MLST/MLSA) (five- or seven-gene sequencing) [8–10]. The only available database, developed by Spilker and Lipuma (https://pubmlst.org/achromobacter/), provides online identification for 18 of the 21 described species: *A. xylosoxidans* [11], *A. denitrificans* [12], *A. ruhlandii, A. piechaudii* [13], *A. insolitus, A. spanius* [14], *A. marplatensis* [15], *A. insuavis, A. dolens, A. aegrifaciens, A. anxifer* [16], *A. animicus, A. mucicolens, A. pulmonis* [17], *A. agilis, A. pestifer, A. kerstersii* and *A. deleyi* [18]. These time-consuming techniques are not used for routine identification. Consequently *Achromobacter* spp. are mostly referred to as *A. xylosoxidans* in studies because conventional methods—biochemical tests, *rrs* sequencing, mass spectrometry (MS)—are not powerful enough to differentiate between species [10,19–21]. The few studies performed with correct identification of the isolates have revealed that *A. xylosoxidans* was the most frequently encountered species and that the distribution of the other species was variable: from six to 14 species among CF patients, and up to 12 species among non-CF patients [10,22–25].

Given that the intrinsic antimicrobial resistance and probably also virulence properties differ from one species to another, a rapid and accurate method of identification is urgently needed [26]. For this purpose, mass spectrometry is a promising technique. However, the IVD-CE-certified databases currently available in France (VITEK® MS bioMerieux and microflex LT Bruker) harbour only two (*A. xylosoxidans* and *A. denitrificans* for VITEK® MS V3.0) or six species (*A. xylosoxidans*, *A. denitrificans, A. ruhlandii, A. piechaudii, A. insolitus* and *A. spanius* for Bruker MS MBT-IVD-DB-7712).

To date, no study has evaluated the performance of species identification by MS on isolates identified by a reference method. Therefore, we aimed to generate a local database by matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF/MS) Bruker for routine identification of the 18 species of *Achromobacter* included in the pubMLST database (*nrdA* analysis) when the study started and to compare its performance with that of the currently available Bruker default database.

Methods

Bacterial isolates (Supplementary Material Tables S1, S2 and S3)

In total 334 isolates belonging to 18 *Achromobacter* species were included. All had been previously identified to the species level by a reference method: sequencing 765 bp of the housekeeping gene *nrdA* [10] using the pubMLST database (https://pubmlst.org/) which contained 18 species. For each *nrdA*-765 allele not matching an allele listed in the database, we contacted T. Spilker to ensure a correct identification [4,23]. The number of strains and alleles by species are listed in the Supplementary Material Table S3 (total of 92 alleles). Of 334 isolates, 192 were collected from the sputum of CF patients (96 from our CF centre, Dijon, 48 from nine other French centres, 25 from Belgium, nine from Denmark, and 14 from the USA), 71 from various samples from non-CF patients, and 53 from environmental samples (domestic, hospital, natural environments). Isolates of the various sources were included for both MSP (main spectra profile) creation and database verification.

Type strains from the 18 *Achromobacter* species were also included.

Generation of a local database (MSP creation) (Supplementary Material Table S1)

A total of 205 *Achromobacter* isolates, including the 18 type strains, were used to generate the local database (MSP creation): *A. aegrifaciens* ($n = 9$), *A. agilis* ($n = 1$), *A. animicus* ($n = 5$), *A. anxifer* ($n = 1$), *A. deleyi* ($n = 3$), *A. denitrificans* ($n = 3$), *A. dolens* ($n = 5$), *A. insolitus* ($n = 8$), *A. insuavis* ($n = 17$), *A. kerstersii* ($n = 1$), *A. marplatensis* ($n = 10$), *A. mucicolens* ($n = 12$), *A. pestifer* ($n = 1$), *A. piechaudii* ($n = 1$), *A. pulmonis* ($n = 4$), *A. ruhlandii* ($n = 8$), *A. spanius* ($n = 9$), *A. xylosoxidans* ($n = 101$), and six isolates belonging to a new *Achromobacter* species not characterized to date (novel species according to MLST analysis and confirmed by Dr P. Vandamme, personal communication) [4,23].

All isolates were cultivated on Mueller–Hinton (MH) agar (Bio-Rad®) overnight at 37°C. MH is adapted to the culture of non-fastidious bacteria like *Achromobacter*. Extraction was performed according to Bruker instructions (MSP creation protocol V1.1). Briefly, a 1-μL loop of bacteria was suspended in 300 μL ultrapure water (HPLC grade) then mixed with 900 μL of 90% ethanol and centrifuged (12 000 *g* for 2 min). The pellet was dried, resuspended in 50 μL of 70% formic acid (Optima™, Fisher chemical)/acetonitrile (Carlo Erba Reagents) (V/V) and centrifuged again. The supernatant was used for further analysis.

Data acquisition was performed according to Bruker instructions (Maldi Biotyper protocol V.2.4). For each extract, the supernatant was spotted eight times (1 μL) on a disposable target, coated with 1 μL HCCA matrix solution (α-cyano-4-hydroxy-cinnamic acid, Bruker Daltonics) and air-dried. The target was inserted in MALDI-TOF/MS Microflex LT (Bruker Daltonics), and FlexControl software (version 3.3, Bruker Daltonics) was used. Each spot was measured three times, resulting in 24 spectra by extract.

Each spectrum was generated with 240 laser shots (six series of 40 laser shots) using linear positive mode with mass range m/z 2000 to 20 000, an acceleration voltage of 20kV in FlexControl software. Calibration was performed with the bacterial test standard calibrant (BTS) (Bruker Daltonics, Germany).

The resulting 24 spectra were carefully inspected using flexAnalysis software (version 3.4 Bruker Daltonics), according to Bruker's recommendations. At least 20 spectra were selected to generate a single MSP onto the MALDI Biotyper 4.1 software (Bruker Daltonics) using the Biotyper MSP Creation Standard

Method with default parameters (25% desired peak frequency minimum and 70 maximum desired peak number for the MSP). The MSPs were used to generate a new local database. After constitution of the novel *Achromobacter* database, each individually created MSP was analysed against the database itself to check whether the best match was obtained with one MSP belonging to the same species.

Evaluation for routine identification by direct colony transfer (Supplementary Material Table S1)

Fifty isolates used for MSP creation (the 18 type strains and representative strains of all species) were selected to test the direct colony transfer method and to assess the potential influence of culture media widely used for clinical sample analyses (sheep-blood agar and Drigalski agar, BioMérieux). Isolates were tested in duplicate by direct colony transfer: colony fragments from overnight cultures at 37°C were spotted onto the target and overlaid with 1 μL of HCCA-matrix. MS analysis was performed as described above but with a single measure of each spot. The spectra obtained were compared to the newly generated database. The algorithm generated a log score value ranging from 0 to 3.0. We used the criteria provided by Bruker to interpret the score: a log score ≥2.0 indicated 'high confidence identification' at the species level, a score value between 1.7 and 2.0 indicated 'low confidence identification' and a score value <1.7 'no organism identification possible'.

Accuracy of the newly generated database and comparison with default Bruker database (Supplementary Material Table S2)

A total of 167 *Achromobacter* isolates (clinical and environmental) belonging to the 18 species were tested for database verification and comparison with the Bruker default database: *A. aegrifaciens* ($n = 7$), *A. agilis* ($n = 1$), *A. animicus* ($n = 2$), *A. anxifer* ($n = 1$), *A. deleyi* ($n = 3$), *A. denitrificans* ($n = 3$), *A. dolens* ($n = 6$), *A. insolitus* ($n = 8$), *A. insuavis* ($n = 18$), *A. kerstersii* ($n = 1$), *A. marplatensis* ($n = 5$), *A. mucicolens* ($n = 14$), *A. pestifer* ($n = 1$), *A. piechaudii* ($n = 1$), *A. pulmonis* ($n = 3$), *A. ruhlandii* ($n = 13$), *A. spanius* ($n = 6$) and *A. xylosoxidans* ($n = 74$). Among these 167 isolates, 38 had already been used for the creation of the MSPs (Supplementary Material Table S2 and Fig. S1). All isolates were cultivated (overnight at 37°C) on sheep blood agar, and 48 of them also on MH and Drigalski agar (although MH and Drigalski are not approved media for the use of IVD-CE Bruker database).

Each isolate was tested in duplicate by direct colony transfer. Each spectrum was compared by the MALDI Biotyper software both to the default Bruker database (DB-7712 MSP) and to our database, and the best matches and scores were gathered.

Results

Verification of the newly generated database

When analysing each individual MSP against the newly generated database, the best match corresponded to an MSP belonging to the same species and a score ≥2.0 in 94.6% of cases (194/205) including the six MSPs of the novel species 1. The remaining cases involved the five species represented only once in the database (2.4%, 5/205), two isolates not matching with a score ≥2.0, and four isolates matching with another species (score ≥2.0), including one *A. ruhlandii* isolate that matched with *A. xylosoxidans.*

Identification performed by direct colony transfer of the 50 isolates led to 100% matching with their own corresponding MSP or another MSP from the same species with an excellent degree (scores ≥2.0), indicating that the direct transfer method is suitable. This identification occurred with colonies picked from sheep-blood agar. Colonies picked from Drigalski agar yielded scores ≥2.0 for 78.0% of the isolates (39/50).

Accuracy of species identification by default Bruker database (Table 1)

Briefly, the default Bruker database resulted in a correct identification (i.e. score ≥2.0 and concordance with *nrdA* sequencing) in only 50.9% of the isolates (85/167). Surprisingly, the species was misidentified in 17.3% of the isolates (29/167) despite high confidence scores (≥2.0). All of these erroneously matched with *A. xylosoxidans* except for one strain of *A. piechaudii* that was identified as *A. spanius*. These errors mostly involved species not included in the database, with the exception of *A. ruhlandii* and *A. piechaudii.*

The other isolates led to scores <2.0 (25.2% (42/167) low confidence, and 6.6% (11/167) no match). The isolates not identified (score <1.7) belonged to species not found in the Bruker database.

Accuracy of species identification with the local database (Table 1)

Overall, 99.4% (166/167) of the isolates were correctly identified with our local database (i.e. score ≥2.0 and concordance with *nrdA* sequencing). The only discrepancy observed involved a single isolate out of 167 (0.6%) corresponding to one *A. ruhlandii* isolate for which the best match was obtained once with *A. xylosoxidans* (with *A. ruhlandii* as the second-best match with a score ≥2.0). We therefore reiterated spots for all of the 13 *A. ruhlandii* isolates and observed that *A. xylosoxidans* erratically matched with high confidence scores as best or second-best match for four strains. For these 13 isolates, we also tested the extraction method (used for database creation), which did not improve the results obtained. We did not detect any species-specific peaks in the spectra. In particular, none could be used to distinguish *A. xylosoxidans* from *A. ruhlandii*. The '10% difference rule' proposed by several authors states that "*any species scoring >10% below the top-scoring match may be excluded*" [27–29]. We observed that 11 out of 167 isolates failed this rule: two *A. mucicolens*, one *A. insolitus*, two *A. dolens*, and interestingly six *A. ruhlandii* isolates.

Concerning the culture media used, high confidence scores were obtained for all isolates whether picked on MH or sheep-blood agar. The scores were lower on Drigalski agar, and no score ≥2.0 was obtained for species other than *A. xylosoxidans.*

Discussion

In this study we constructed the first MALDI-TOF database to include 18 species currently described within the genus *Achromobacter* using a wide collection of isolates identified with a reference method (*nrdA* sequencing). Because the pathogenicity of these bacteria remains controversial in CF patients, there is a strong need for developing rapid and accurate *Achromobacter* species identification methods that are not currently available for routine laboratory analysis. Concerning MALDI-TOF MS, the commercially available libraries are incomplete: MALDI Biotyper® (Bruker) and VITEK® MS databases include respectively only six and two *Achromobacter* species.

Here we underscore the large number of erroneous identifications obtained with the Bruker database, despite high confidence scores, leading mostly to the misidentification of certain isolates as *A. xylosoxidans,* as previously described [24]. One might assume that the spectra from isolates belonging to species not included in the database would not find a match, but this was not the case for

Table 1
Comparison of the results of species identification of 167 *Achromobacter* isolates by Bruker default database and the newly generated database

Species (*nrdA*)	No. of strains	Bruker default database						New database					
		log score ≥2.0		1.7 ≤ log score <2.0		log score <1.7		log score ≥2.0		1.7 ≤ log score <2.0		log score <1.7	
		C	NC	C	NC	C	NC	C	NC	C	NC	C	NC
A. aegrifaciens	7	0	0	0	6 (85.7%)	0	1 (14.3%)	7 (100%)	0	0	0	0	0
A. agilis	1	0	0	0	1 (100%)	0	0	1 (100%)	0	0	0	0	0
A. animicus	2	0	0	0	0	0	2 (100%)	2 (100%)	0	0	0	0	0
A. anxifer	1	0	0	0	0	0	1 (100%)	1 (100%)	0	0	0	0	0
A. deleyi	3	0	0	0	1 (33.3%)	0	2 (66.7%)	3 (100%)	0	0	0	0	0
A. denitrificans[a]	3	2 (66.7%)	0	1 (33.3%)	0	0	0	3 (100%)	0	0	0	0	0
A. dolens	6	0	3 (50.0%)	0	3 (50.0%)	0	0	6 (100%)	0	0	0	0	0
A. insolitus[a]	8	5 (62.5%)	0	3 (37,5%)	0	0	0	8 (100%)	0	0	0	0	0
A. insuavis	18	0	17 (94.4%)	0	1 (5.6%)	0	0	18 (100%)	0	0	0	0	0
A. kerstersii	1	0	0	0	1 (100%)	0	0	1 (100%)	0	0	0	0	0
A. marplatensis	5	0	0	0	3 (60.0%)	0	2 (40.0%)	5 (100%)	0	0	0	0	0
A. mucicolens	14	0	0	0	12 (85.7%)	0	2 (14.3%)	14 (100%)	0	0	0	0	0
A. pestifer	1	0	1 (100%)	0	0	0	0	1 (100%)	0	0	0	0	0
A. piechaudii[a]	1	0	1 (100%)	0	0	0	0	1 (100%)	0	0	0	0	0
A. pulmonis	3	0	0	0	2 (66.7%)	0	1 (33.3%)	3 (100%)	0	0	0	0	0
A. ruhlandii[a]	13	0	7 (53.8%)	3 (23.1%)	3 (23.1%)	0	0	12 (92.3%)	1 (7.7%)	0	0	0	0
A. spanius[a]	6	4 (66.7%)	0	2 (33.3%)	0	0	0	6 (100%)	0	0	0	0	0
A. xylosoxidans[a]	74	74 (100%)	0	0	0	0	0	74 (100%)	0	0	0	0	0
Total	167	85 (50.9%)	29 (17.3%)	9 (5.4%)	33 (19.8%)	0	11 (6.6%)	166 (99.4%)	1 (0.6%)	0	0	0	0

C, result of identification concordant with *nrdA* sequencing; NC, result of identification non-concordant with *nrdA* sequencing.
[a] Species included in Bruker default database.

isolates belonging to *A. insuavis, A. dolens* and *A. pestifer*. In these cases it was not possible to determine a score cut-off for distinguishing erroneous and correct '*A. xylosoxidans*' identifications. Users should be aware of the risk of misidentification.

Moreover, the isolates belonging to species present in the Bruker database were not always correctly identified. Surprisingly, none of the 13 *A. ruhlandii* isolates was correctly identified. This issue has already been pointed out by Rodrigues et al. in a study including nine isolates of *A. ruhlandii* [21].

Finally, 6.6% of the isolates (11/167) led to scores <1.7, preventing genus identification. There may be several reasons for the issues with the Bruker database. First, the number of isolates included in the database is limited and some species are absent, as already highlighted by Papalia et al. [24]. Secondly, with the exception of the type strains, the method of species identification of the isolates is not provided by the manufacturer, making it difficult to ascertain reliability. This also applies to the VITEK® MS system.

To overcome the weaknesses found in the existing databases, first we included only isolates identified by reference methods using the pubMLST database [10] and representatives of 18 species (corresponding to all the species that could be identified with this database at the beginning of the study). Second, the database was built up with a large number of MSPs (205) created with a wide variety of clinical and environmental isolates, either from our collection or from other collections in Denmark, Belgium, France, and the USA.

We built MSP dendrograms, but we noticed that the results depended on the parameters and the set of strains included for their construction (data not shown) despite the excellent results of species identification obtained with our database. This might be explained by the fact that Bruker algorithms for dendrogram construction and bacterial identification are not based on the same parameters.

With the use of our database, the rate of correct identifications jumped from 50.9% (85/167) (Bruker default database) to 99.4% (166/167) with high confidence scores (score ≥2.0) for all 167 isolates tested by direct colony transfer from sheep-blood agar. The use of Drigalski agar, which is not validated by Bruker, yielded lower scores, which could be explained by the drier texture of the colonies.

We recognize that our database has certain limitations. First, some species are rarely found in clinical samples [10,22–25] and therefore are not easily available from laboratory collections. As a result, few isolates were included in the database for some species: only one representative for *A. agilis, A. anxifer, A. kerstersii, A. pestifer*, and *A. piechaudii*. In addition, accuracy was verified with fewer than three isolates for the five species listed above as well as for *A. animicus*. In the future, additional strains belonging to these species or to novel species should be included when available to expand the database.

Second, the results were not satisfactory for some *A. ruhlandii* isolates. Indeed, the only incorrect species identification with our database (0.6%, 1/167) involved a single isolate of *A. ruhlandii* erroneously identified as *A. xylosoxidans*, and we observed this possible misidentification in the MSP evaluation step. Gomila et al. also noticed that "*A. xylosoxidans* and *A. ruhlandii* showed a close relationship by MALDI-TOF MS analysis" in a study including ten *A. ruhlandii* isolates [30]. The inclusion of more strains of *A. ruhlandii* in our database might help to rectify this problem. On the contrary, for the 74 *A. xylosoxidans* isolates identified with our database, *A. ruhlandii* was never proposed with a high confidence score. Therefore, another method (*nrdA* analysis or MLST) should be used when both *A. ruhlandii* and *A. xylosoxidans* are presented as the best and second-best match with high confidence scores.

In conclusion, we built a reliable database for the identification of clinical or environmental *Achromobacter*. This database has several valuable potential uses, including the enrichment of epidemiological data and the improvement of current understanding of the clinical impact of the various species of *Achromobacter*, which is particularly important for patients with CF.

Transparency declaration

The authors declare no conflict of interest. No external funding was received for this publication.

Acknowledgements

We would like to thank Suzanne Rankin for her help in reviewing the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2020.03.031.

References

[1] Aisenberg G, Rolston KV, Safdar A. Bacteremia caused by *Achromobacter* and *Alcaligenes* species in 46 patients with cancer (1989–2003). Cancer 2004;101:2134–40.

[2] Molina-Cabrillana J, Santana-Reyes C, González-García A, Bordes-Benítez A, Horcajada I. Outbreak of *Achromobacter xylosoxidans* pseudobacteremia in a neonatal care unit related to contaminated chlorhexidine solution. Eur J Clin Microbiol Infect Dis 2007;26:435–7.

[3] Swenson CE, Sadikot RT. *Achromobacter* respiratory infections. Ann Am Thorac Soc 2015;12:252–8.

[4] Amoureux L, Bador J, Bounoua Zouak F, Chapuis A, de Curraize C, Neuwirth C. Distribution of the species of *Achromobacter* in a French Cystic Fibrosis Centre and multilocus sequence typing analysis reveal the predominance of *A. xylosoxidans* and clonal relationships between some clinical and environmental isolates. J Cyst Fibros 2016;15:486–94.

[5] Cools P, Ho E, Vranckx K, Schelstraete P, Wurth B, Franckx H, et al. Epidemic *Achromobacter xylosoxidans* strain among Belgian cystic fibrosis patients and review of literature. BMC Microbiol 2016;16:122.

[6] Edwards BD, Greysson-Wong J, Somayaji R, Waddell B, Whelan FJ, Storey DG, et al. Prevalence and outcomes of *Achromobacter* species infections in adults with cystic fibrosis: a North American cohort study. J Clin Microbiol 2017;55:2074–85.

[7] Recio R, Brañas P, Martínez MT, Chaves F, Orellana MA. Effect of respiratory *Achromobacter* spp. infection on pulmonary function in patients with cystic fibrosis. J Med Microbiol 2018;67:952–6.

[8] Ridderberg W, Wang M, Nørskov-Lauritsen N. Multilocus sequence analysis of isolates of *Achromobacter* from patients with cystic fibrosis reveals infecting species other than *Achromobacter xylosoxidans*. J Clin Microbiol 2012;50:2688–94.

[9] Spilker T, Vandamme P, Lipuma JJ. A multilocus sequence typing scheme implies population structure and reveals several putative novel *Achromobacter* species. J Clin Microbiol 2012;50:3010–5.

[10] Spilker T, Vandamme P, Lipuma JJ. Identification and distribution of *Achromobacter* species in cystic fibrosis. J Cyst Fibros 2013;12:298–301.

[11] Yabuuchi E, Yano I. *Achromobacter* gen. nov. and *Achromobacter xylosoxidans* (ex Yabuuchi and Ohyama 1971) nom. rev. Int J Syst Evol Microbiol 1981;31:477–8.

[12] Coenye T, Vancanneyt M, Cnockaert MC, Falsen E, Swings J, Vandamme P. *Kerstersia gyiorum* gen. nov., sp. nov., a novel *Alcaligenes faecalis*-like organism isolated from human clinical samples, and reclassification of *Alcaligenes denitrificans* Rüger and Tan 1983 as *Achromobacter denitrificans* comb. nov. Int J Syst Evol Microbiol 2003;53:1825–31.

[13] Yabuuchi E, Kawamura Y, Kosako Y, Ezaki T. Emendation of genus *Achromobacter* and *Achromobacter xylosoxidans* (Yabuuchi and Yano) and proposal of *Achromobacter ruhlandii* (Packer and Vishniac) comb. nov., *Achromobacter piechaudii* (Kiredjian *et al.*) comb. nov., and *Achromobacter xylosoxidans* subsp. *denitrificans* (Rüger and Tan) comb. nov. Microbiol Immunol 1998;42:429–38.

[14] Coenye T, Vancanneyt M, Falsen E, Swings J, Vandamme P. *Achromobacter insolitus* sp. nov. and *Achromobacter spanius* sp. nov., from human clinical samples. Int J Syst Evol Microbiol 2003;53:1819–24.

[15] Gomila M, Tvrzová L, Teshim A, Sedlácek I, González-Escalona N, Zdráhal Z, et al. *Achromobacter marplatensis* sp. nov., isolated from a pentachlorophenol-contaminated soil. Int J Syst Evol Microbiol 2011;61:2231–7.

[16] Vandamme P, Moore ERB, Cnockaert M, Peeters C, Svensson-Stadler L, Houf K, et al. Classification of *Achromobacter* genogroups 2, 5, 7 and 14 as *Achromobacter insuavis* sp. nov., *Achromobacter aegrifaciens* sp. nov., *Achromobacter anxifer* sp. nov. and *Achromobacter dolens* sp. nov., respectively. Syst Appl Microbiol 2013;36:474–82.

[17] Vandamme P, Moore ERB, Cnockaert M, De Brandt E, Svensson-Stadler L, Houf K, et al. *Achromobacter animicus* sp. nov., *Achromobacter mucicolens* sp. nov., *Achromobacter pulmonis* sp. nov. and *Achromobacter spiritinus* sp. nov., from human clinical samples. Syst Appl Microbiol 2013;36:1–10.

[18] Vandamme PA, Peeters C, Inganäs E, Cnockaert M, Houf K, Spilker T, et al. Taxonomic dissection of *Achromobacter denitrificans* Coenye *et al.* 2003 and proposal of *Achromobacter agilis* sp. nov., nom. rev., *Achromobacter pestifer* sp. nov., nom. rev., *Achromobacter kerstersii* sp. nov. and *Achromobacter deleyi* sp. nov. Int J Syst Evol Microbiol 2016;66:3708–17.

[19] Traglia G, Papalia M, Almuzara M, Gutkind G, Centrón D, Vay C, et al. Presence of OXA-type enzymes in *Achromobacter insuavis* and *A. dolens*. Curr Microbiol 2014;69:501–6.

[20] Dupont C, Michon A-L, Jumas-Bilak E, Nørskov-Lauritsen N, Chiron R, Marchandin H. Intrapatient diversity of *Achromobacter* spp. involved in chronic colonization of cystic fibrosis airways. Infect Genet Evol 2015;32:214–23.

[21] Rodrigues ERA, Ferreira AG, Leão RS, Leite CCF, Carvalho-Assef AP, Albano RM, et al. Characterization of *Achromobacter* species in cystic fibrosis patients: comparison of bla OXA-114 PCR amplification, multilocus sequence typing, and matrix-assisted laser desorption ionization–time of Flight mass spectrometry. J Clin Microbiol 2015;53:3894–6.

[22] Barrado L, Brañas P, Orellana MÁ, Martínez MT, García G, Otero JR, et al. Molecular characterization of *Achromobacter* isolates from cystic fibrosis and non-cystic fibrosis patients in Madrid, Spain. J Clin Microbiol 2013;51:1927–30.

[23] Amoureux L, Bador J, Verrier T, Mjahed H, DE Curraize C, Neuwirth C. *Achromobacter xylosoxidans* is the predominant *Achromobacter* species isolated from diverse non-respiratory samples. Epidemiol Infect 2016:1–4.

[24] Papalia M, Steffanowski C, Traglia G, Almuzara M, Martina P, Galanternik L, et al. Diversity of *Achromobacter* species recovered from patients with cystic fibrosis, in Argentina. Rev Argent Microbiol 2020;52:13–8.

[25] Coward A, Kenna DTD, Perry C, Martin K, Doumith M, Turton JF. Use of *nrdA* gene sequence clustering to estimate the prevalence of different *Achromobacter* species among cystic fibrosis patients in the UK. J Cyst Fibros 2016;15:479–85.

[26] Bador J, Neuwirth C, Liszczynski P, Mézier M-C, Chrétiennot M, Grenot E, et al. Distribution of innate efflux-mediated aminoglycoside resistance among different *Achromobacter* species. New Microbes New Infect 2016;10:1–5.

[27] Degand N, Carbonnelle E, Dauphin B, Beretti J-L, Le Bourgeois M, Sermet-Gaudelus I, et al. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of nonfermenting gram-negative bacilli isolated from cystic fibrosis patients. J Clin Microbiol 2008;46:3361–7.

[28] Khot PD, Couturier MR, Wilson A, Croft A, Fisher MA. Optimization of matrix-assisted laser desorption ionization-time of flight mass spectrometry analysis for bacterial identification. J Clin Microbiol 2012;50:3845–52.

[29] Saffert RT, Cunningham SA, Ihde SM, Jobe KEM, Mandrekar J, Patel R. Comparison of Bruker Biotyper matrix-assisted laser desorption ionization-time of flight mass spectrometer to BD Phoenix automated microbiology system for identification of gram-negative bacilli. J Clin Microbiol 2011;49:887–92.

[30] Gomila M, Prince-Manzano C, Svensson-Stadler L, Busquets A, Erhard M, Martínez DL, et al. Genotypic and phenotypic applications for the differentiation and species-level identification of *Achromobacter* for clinical diagnoses. PLOS ONE 2014;9:e114356.