Species distribution, sequence types and antimicrobial resistance of *Acinetobacter* spp. from cystic fibrosis patients

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Abstract

Acinetobacter spp. are important healthcare pathogens, being closely linked to antibiotic resistance and outbreaks worldwide. Although such species are rarely observed in patients with cystic fibrosis (CF), we describe the characteristics of 53 strains of *Acinetobacter* spp. isolated from the sputum of 39 Brazilian patients with CF. The species distribution was *A. baumannii* ($n = 29$), *A. pittii* ($n = 13$), *A. nosocomialis* ($n = 8$), *A. seifertii* ($n = 1$), *A. soli* ($n = 1$) and *A. variabilis* ($n = 1$) determined by partial *rpoB* gene sequencing. Sixteen strains (10 *A. baumannii*, 3 *A. pittii* and 3 *A. nosocomialis*) were multidrug-resistant (MDR) by disk diffusion test (30%) and eight MDR carbapenem-resistant *A. baumannii* strains harboured the $bla_{\text{OXA-23-like}}$ oxacillinase gene. Thirty-three sequence types (STs) were identified by multilocus sequence typing of which eight were novel (*A. baumannii*: 843, 844, 845, 847, 848; *A. pitti*: 643; *A. nosocomialis*: 862 and *A. seifertii*: 846); six STs (2 *A. baumannii*, 3 *A. pittii* and 1 *A. nosocomialis*) were found in more than one patient. Four strains of *A. baumannii* were assigned to two common clonal complexes (CCs), namely, CC1 (ST1, ST20 and ST160), and CC79 (ST79). This study underlines the extensive species diversity of *Acinetobacter* spp. strains in CF lung infections which may present difficulties for therapy due to significant antimicrobial resistance.

Introduction

Some members of the genus *Acinetobacter* are important healthcare-associated pathogens, being closely linked to outbreaks worldwide [1]. The most common species isolated from clinical samples is *A. baumannii*, however, with improved laboratory identification methods, an increasing number of other *Acinetobacter* species are now recognised as being clinically significant particularly due to their association with nosocomial outbreaks and antibiotic resistance [2]. *A. calcoaceticus*, *A. baumannii*, *A. pittii* and *A. nosocomialis* are closely related species that are phenotypically indistinguishable by routine laboratory technologies, hence the term '*A. calcoaceticus–A. baumannii* (Acb) complex'. Recently, *A. seifertii* and *A. dijkshoorniae* have been included in this complex [3, 4].

Acinetobacter spp. are widespread in the hospital environment, especially *A. baumannii* strains belonging to worldwide distributed clones such as European clones I, II and III. These are defined by multilocus sequence typing and assigned according to the Pasteur Institute scheme (MLST-IP) to clonal complexes (CCs) 1, 2 and 3, respectively [1]. More recently, the isolation of multidrug-resistant (MDR) strains of *A. pittii* and *A. nosocomialis* of various sequence types (STs) in healthcare facilities has been described around the world [5]. Carbapenems are the drug of choice against infections caused by MDR *Acinetobacter* and the production of oxacillinase (OXA)-type carbapenemases is the main resistance mechanism to these antimicrobials. These comprise four main groups, namely, OXA-23-like; OXA-24/40-like; OXA-58-like; OXA-51-like (intrinsic in *A. baumannii*); OXA-143-like and OXA-235-like are quite rare [6].

Patients with cystic fibrosis (CF) are frequently admitted to hospitals for the treatment of acute pulmonary exacerbations and severe complications due to a limited range of bacterial pathogens, primarily *Pseudomonas aeruginosa*, *Staphylococcus aureus*, among others, but *Acinetobacter* spp. are seldom detected in these patients [7]. We report here the characterisation of a series of isolates of *Acinetobacter* spp. recovered from the sputum of Brazilian patients with CF with regard to species distribution, CC and antimicrobial resistance mechanisms.

Materials and methods

We conducted a retrospective study of *Acinetobacter* spp. isolated from CF patients from June 2005 to February 2014 attending the Instituto Nacional da Saúde da Mulher, da Criança e do Adolescente Fernandes Figueira (IFF-FIOCRUZ), and Hospital Universitário Pedro Ernesto (HUPE-UERJ), two reference centres for paediatric and adult patients with CF, respectively, in Rio de Janeiro, Brazil. Respiratory samples were cultured in accordance with standard bacteriological protocols for CF samples [8] and presumptive *Acinetobacter* spp. isolates were confirmed by phenotypic tests and assigned to species level by PCR amplification and partial sequencing of the *rpoB* gene as described previously [9].

Sequence typing

All isolates classified as members of the Acb complex were subjected to MLST, by PCR amplification and sequence analysis of seven housekeeping genes (*cpn60*, *fusA*, *gltA*, *pyrG*, *recA*, *rplB* and *rpoB*) in accordance with the MLST-IP scheme [10]. CCs were identified by the eBURST algorithm (http://eburst.mlst.net/), and defined as a cluster formed by the founder ST linked to other STs sharing six identical (single locus variants – SLVs) allele profiles at each of the seven MLST loci [11].

Antimicrobial susceptibility

Isolates were tested for antimicrobial susceptibility by the disk diffusion method according to the Clinical and Laboratory Standards Institute guidelines [12] with the following agents: amikacin, ampicillin/sulbactam, cefepime, cefotaxime, ceftazidime, ciprofloxacin, gentamicin, imipenem, meropenem, piperacillin/tazobactam and tobramycin (Oxoid Ltd Basingstoke, UK). *P. aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as quality controls. Strains that were resistant to at least one agent in three classes of antimicrobials were defined as MDR. Multiplex PCR for the detection of genes encoding OXA $bla_{OXA23-like}$, $bla_{OXA24-like}$, $bla_{OXA51-like}$, $bla_{OXA58-like}$ and $bla_{OXA143-like}$ was performed as previously described [13, 14].

Results

Fifty-three *Acinetobacter* spp. strains were isolated from 32 paediatric and seven adults (Table 1). In seven paediatric patients, more than one *Acinetobacter* strain defined by species, ST and/or resistance markers was isolated. The mean age of first isolation of *Acinetobacter* spp. was 6 and 26 years for paediatric and adults, respectively, and in four patients (one child and three adults) colonisation was first detected on hospitalisation. Twenty of the 39 patients were also colonised with pathogens commonly found in CF, with *P. aeruginosa* being the most frequent.

Partial *rpoB* gene sequencing identified the species distribution as *A. baumannii* (*n* = 29), *A. pittii* (*n* = 13), *A. nosocomialis* (*n* = 8), and one each of *A. seifertii*, *A. soli* and *A. variabilis*.

Although most strains showed susceptibility to the majority of the antimicrobials tested, with the exception of cefotaxime (Table 1), 16 (30%) were classified as MDR (10 *A. baumannii*, three *A. pittii* and three *A. nosocomialis*). Eight of the MDR *A. baumannii* were resistant to carbapenems (meropenem and imipenem) and these were the only isolates harbouring the $bla_{OXA23-like}$ gene (Table 1). None of the other OXA genes tested was present in any of the 53 strains.

MLST analysis identified 33 unique STs among the 51 Acb complex strains: 21 STs (five novel) for *A. baumannii*, five (one novel) for *A. pittii*, six (one novel) for *A. nosocomialis* and one novel ST for *A. seifertii* (Table 1). By reference to the MLST-IP scheme database, 11 STs (two *A. nosocomialis* and nine *A. baumannii*) had previously been identified in two states in Southeastern Brazil (Rio de Janeiro and São Paulo). Among the eight novel STs identified, five (*A. pittii* ST643, *A. nosocomialis* ST862, ST843, ST844 and ST848 of *A. baumannii*) had a hitherto unrecognised allelic arrangement; three novel allele profiles (*A. seifertii* ST846, ST845 and ST847 of *A. baumannii*) were added to the MLST-IP database (Table 1).

Seven paediatric patients (#6, 8, 9, 10, 18, 32 and 34) had more than one positive culture for *Acinetobacter* species. Two of them had persistent colonisation with the same *A. baumannii* ST: patient 6 by ST 411 and patient 9 by ST 228. On one occasion, patient 6 was co-colonised with *A. nosocomialis* (ST410). Three different *Acinetobacter* species were recovered from patient 10 from 2008 to 2010: *A. baumannii* (ST216 and ST285), *A. nosocomialis* (ST71) and *A. variabilis* (ST not determined). Moreover, two patients were sporadically colonised by different *A. nosocomialis* STs (patient 18: ST842 and ST410; patient 32: ST410 and ST161). Likewise, patient 34 harboured *A. soli* (ST not determined) and *A. pittii* (ST 643), and patient 8 yielded *A. baumannii* of two different STs, one of which was MDR corresponding to European clone I (ST1) (Table 1). Two *A. baumannii*, three *A. pittii* and one *A. nosocomialis* STs were also found in different patients but often separated in time and/or hospital (Table 2).

eBURST analysis identified STs belonging to three important *A. baumannii* CCs: CC1 (ST1, ST20 and ST160), CC25 (ST228) and CC79 (ST79) (Fig. 1). Moreover, comparison of the profiles of the four *A. nosocomialis* STs found in this study with the MLST-IP database showed that ST71, ST161 and ST410 (group founder) are SLVs of the latter (Fig. 2).

Discussion

In the present study all, but two, of the strains of *Acinetobacter* spp. identified from CF patients were assigned to the Acb complex (96%) with *A. baumannii* being the most common. These results corroborate the distribution of these species found in hospitalised patients but the proportion of species other than *A. baumannii* normally found in the latter group of patients is usually considerably lower than the 45% of strains identified here [15]. Most isolates (70%) were susceptible to antimicrobials which suggests that these strains, in common with other opportunist Gram-negative bacteria, may have been acquired by CF patients from environmental sources, given their ubiquity in nature [1, 5].

To our knowledge, this is the first description of *A. nosocomialis*, *A. seifertii*, *A. soli* and *A. variabilis* (previously group of 15 sensu DNA Tjernberg & Ursing) [16] isolated from CF patients. Although initially reported as environmental microorganisms, *A. seifertii* and *A. soli* have both been recently described from bacteraemia in hospitalised patients [17, 18].

There was marked genetic diversity among the strains investigated here which suggests that they were acquired by patients from different sources. Nevertheless, three STs accounted for 11 of 13 patients who grew *A. pittii*; ST 643 was identified in six patients, five of which were sampled in May 2009. This could conceivably be due to contamination of specimens, but if not, it is strongly indicative of cross-infection between these patients, or alternatively, acquisition of the strain from a single common

Table 1. General features of *Acinetobacter* spp. strains isolated from Brazilian patients with CF

Patient	Hospital	Isolation date	Strain	Species	ST	Resistance profile	Other microorganisms identified in the same culture
1	IFF	02/06/2005	4799	*A. baumannii*	32[a]	CIP-CTX	–
2	IFF	08/11/2005	5177	*A. pittii*	220	CTX	–
3	IFF	07/03/2007	6044	*A. nosocomialis*	785	CTX	–
4	IFF	28/03/2007	6085	*A. baumannii*	845	–	*Burkholderia cepacia complex P. aeruginosa*
5	IFF	22/03/2007	6117	*A. baumannii*	739	CTX	–
6	IFF	27/08/2007	6564	*A. baumannii*	411	CTX-FEP	–
	IFF	03/09/2007	6576	*A. baumannii*	411	CTX	–
	IFF	25/10/2007	6776	*A. baumannii*	411	CN	–
	IFF	25/10/2007	6775	*A. nosocomialis*	410	CN	–
7	IFF	26/09/2007	6635	*A. seifertii*	846	CTX	*P. aeruginosa*
8	IFF	21/02/2008	7135	*A. baumannii*	303[a]	CN	*Stenotrophomonas maltophilia*
	IFF	03/01/2013	15 832	*A. baumannii*	1[a,b]	AK-CAZ-CIP-CN-CTX-FEP-IMP-MEM-SAM-TOB-TZP	*Achromobacter xylosoxidans*
9	IFF	11/06/2008	7493	*A. baumannii*	228[a]	CTX	*P. aeruginosa*
	IFF	17/09/2008	7935	*A. baumannii*	228[a]	CTX	*P. aeruginosa*
	IFF	11/02/2009	8494	*A. baumannii*	228[a]	CIP	*P. aeruginosa*
	IFF	11/03/2009	8617	*A. baumannii*	228[a]	CIP	*P. aeruginosa*
	IFF	17/06/2009	8999	*A. baumannii*	228[a]	–	*P. aeruginosa*
10	IFF	30/07/2008	7686	*A. baumannii*	216	CTX-TZP	Methicillin-resistant *Staphylococcus aureus*
	IFF	22/05/2009	8896	*A. nosocomialis*	71[a]	CTX	Methicillin-resistant *S. aureus*
	IFF	22/01/2010	9881	*A. baumannii*	285	CTX	*B. cepacia complex*
	IFF	08/08/2011	13 458	*A. variabilis*	ND	–	*B. cepacia complex*
11	IFF	26/11/2008	8179	*A. pittii*	563	CTX	*P. aeruginosa*
12	IFF	17/12/2008	8257	*A. pittii*	220[b]	CAZ-CN-CTX-TZP	–
13	IFF	01/12/2008	8278	*A. pittii*	457	CTX	*B. cepacia complex*
14	IFF	18/12/2008	8279	*A. pittii*	457	CTX-TZP	–
15	IFF	05/03/2009	8598	*A. pittii*	220	–	–
16	HUPE	22/04/2009	8760	*A. baumannii*	79[a,b]	AK-CAZ-CIP-CN-CTX-FEP-IMP-MEM-SAM-TOB-TZP	*B. cepacia complex P. aeruginosa*
17	IFF	11/05/2009	8863	*A. pittii*	643	CTX	*P. aeruginosa*
18	IFF	07/05/2009	8874	*A. nosocomialis*	862	CN-CTX	–
	IFF	05/05/2011	12 627	*A. nosocomialis*	410	TZP-CTX-FEP	–
19	IFF	14/05/2009	8876	*A. pittii*	643[b]	CIP-CTX-TZP	–
20	IFF	20/05/2009	8893	*A. pittii*	643	CN-CTX	*P. aeruginosa*

21	IFF	20/05/2009	8895	A. pittii	643	CTX	P. aeruginosa
22	IFF	27/05/2009	8941	A. pittii	643	CTX	P. aeruginosa
23	IFF	18/03/2010	10 046	A. nosocomialis	68[b]	AK-CAZ-CN-CTX-FEP-SAM-TOB-TZP	S. maltophilia Pseudomonas aeruginosa
24	HUPE	14/05/2010	10 324	A. baumannii	188[a,b]	CIP-CN-CTX-FEP-IMP-MEM-TOB-TZP	B. cepacia complex
25	IFF	19/05/2010	10 376	A. baumannii	843	CTX	–
26	IFF	09/05/2011	12 770	A. baumannii	847[b]	AK-CN-CTX-TZP	–
27	HUPE	15/06/2011	13 083	A. baumannii	847	AK-CTX	P. aeruginosa
28	HUPE	01/07/2011	13 145	A. baumannii	20[b,c]	CAZ-CIP-CN-CTX-FEP-IMP-MEM-TOB-TZP	B. cepacia complex S. maltophilia
29	HUPE	04/07/2011	13 195	A. baumannii	844[b]	AK-CAZ-CIP-CN-CTX-FEP-IMP-MEM-SAM-TOB-TZP	Serratia marcescens P. aeruginosa
30	IFF	10/11/2011	13 999	A. baumannii	188[a,b]	CIP-CTX-TZP-MEM-IMP-FEP-CN-TOB	P. aeruginosa
31	IFF	22/12/2011	14 104	A. baumannii	162[a,b]	AK-CAZ-CIP-CN-CTX-FEP-IMP-MEM-SAM-TOB-TZP	–
32	IFF	19/01/2012	14 141	A. nosocomialis	410[b]	CTX-TOB-TZP	–
	IFF	19/07/2012	14 851	A. nosocomialis	161[a,b]	AK-CAZ-CTX-TOB-TZP	–
33	IFF	09/02/2012	14 268	A. baumannii	10	CAZ-CTX	–
34	IFF	03/05/2012	14 455	A. soli	ND	CAZ-CTX	–
	IFF	18/12/2013	17 303	A. pittii	643[b]	AK-CIP-CTX-TZP	–
35	HUPE	09/08/2012	15 009	A. pittii	795	CAZ-CTX	Elizabethkingia meningoseptica
36	IFF	06/09/2012	15 216	A. baumannii	137	CTX	–
37	HUPE	25/09/2012	15 331	A. baumannii	160[a,b]	AK-CAZ-CIP-CN-CTX-FEP-IMP-MEM-SAM-TOB-TZP	–
38	IFF	12/11/2013	17 149	A. baumannii	848	AK-CTX	–
39	IFF	05/02/2014	17 461	A. baumannii	490[b]	AK-CTX-TZP	B. cepacia complex P. aeruginosa

ND, MLST not determined.
Novel ST deposited in PubMLST from this study.
[a]Also isolated from non-CF patients in Rio de Janeiro state [25].
[b]MDR strain.
[c]Also isolated from non-CF patients in São Paulo state [26].
AK, amikacin; CAZ, ceftazidime; CIP, ciprofloxacin; CN, gentamicin; CTX, cefotaxime; FEP, cefepime; IMP, imipenem; MEM, meropenem; SAM, ampicillin/sulbactam; TOB, tobramycin; TZP, piperacillin/tazobactam.

Table 2. *Acinetobacter baumannii* complex STs shared by different patients with CF

Species	ST	Patient	Date	Hospital
A. baumannii	188	24	14/05/2010	HUPE
		30	10/11/2011	IFF
	847	26	09/05/2011	IFF
		27	15/06/2011	HUPE
A. pittii	220	02	08/11/2005	IFF
		12	17/12/2008	IFF
		15	05/03/2009	IFF
	457	13	01/12/2008	IFF
		14	18/12/2008	IFF
	643	17	11/05/2009	IFF
		19	14/05/2009	IFF
		20	20/05/2009	IFF
		21	20/05/2009	IFF
		22	27/05/2009	IFF
		34	18/12/2013	IFF
A. nosocomialis	410	06	25/10/2007	IFF
		18	05/05/2011	IFF
		32	19/01/2012	IFF

source. Considerably less sharing of strains of the same STs between patients was evident for *A. nosocomialis* and *A. baumannii*.

Although *A. baumannii* is the species most commonly associated with antimicrobial resistance in hospital outbreaks [10], there was no evidence of clonal spread of resistance among the patient cohort for this species or for *A. pittii* and *A. nosocomialis*.

Indeed, only two strains of the same ST (188) of *A. baumannii* proved to be MDR and these were recovered from two patients attending different hospitals and sampled over a year apart. Likewise, the two MDR strains of *A. pittii* ST 643 were separated by an interval of over 4 years (Tables 1 and 2). It is noteworthy that overall only 16 of the 53 strains were found to be MDR, and 21 strains were susceptible to all, but one, of the antimicrobial agents tested. This finding suggests that antimicrobial resistance alone does not necessarily confer a selective advantage for the establishment or dissemination of *Acinetobacter* spp. in respiratory infections in CF patients. There is, however, some evidence that *A. pittii* was relatively more successful in adhering to human bronchial epithelial cells and in inducing IL-8 expression than *A. baumannii* or *A. nosocomialis* [19]. In addition, recent whole-genome sequencing data of an *A. pittii* ST643 MDR strain revealed the presence of genes associated with virulence, biofilm formation and antimicrobial resistance [20].

Three patients grew two or three different species of *Acinetobacter*, and four individuals yielded strains of the same species distinguishable by ST. Studies on the lung microbiome in CF show the presence of widely diverse bacterial populations which with increasing age of the host become well adapted to the CF lung environment and compete with other resident species for dominance [21]. It is likely that *Acinetobacter* spp. are not well adapted to this relatively unique environment and this might explain their low frequency of isolation from these patients.

One patient (#32, Table 1) was colonised with two distinguishable but phylogenetically related STs of *A. nosocomialis* (ST161 and its SLV ST410). *A. nosocomialis* ST161 has been found in clinical (MDR profile) and environmental samples (non-MDR) in Rio de Janeiro [22]. In this study, the ST161 clone was MDR, which might indicate that it was acquired by the patient through cross-transmission in the hospital setting. This scenario might also explain the acquisition of the European clone I *A. baumannii* ST1 by patient #8. Although there are no reports of cross-transmission of *Acinetobacter* spp. between patients with CF, it is generally accepted that cross-transmission in hospitals occurs
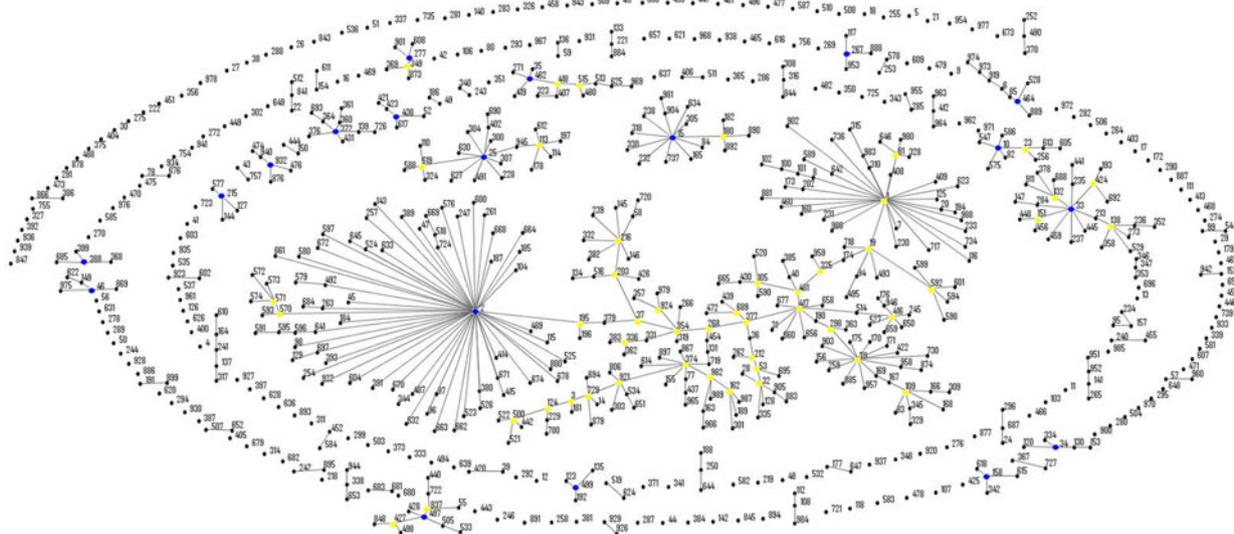

Fig. 1. Population snapshot of *A. baumannii* isolates in this study and existing isolates in the Institut Pasteur's MLST database by eBURST algorithm (http://pubmlst.org/abaumannii/; 24 April 2017, date last accessed). Each ST is represented by a black dot. Blue and yellow dots correspond to group and subgroup founders, respectively. SLVs are linked by lines, and clonal complexes (CC) correspond to the group of connected STs.
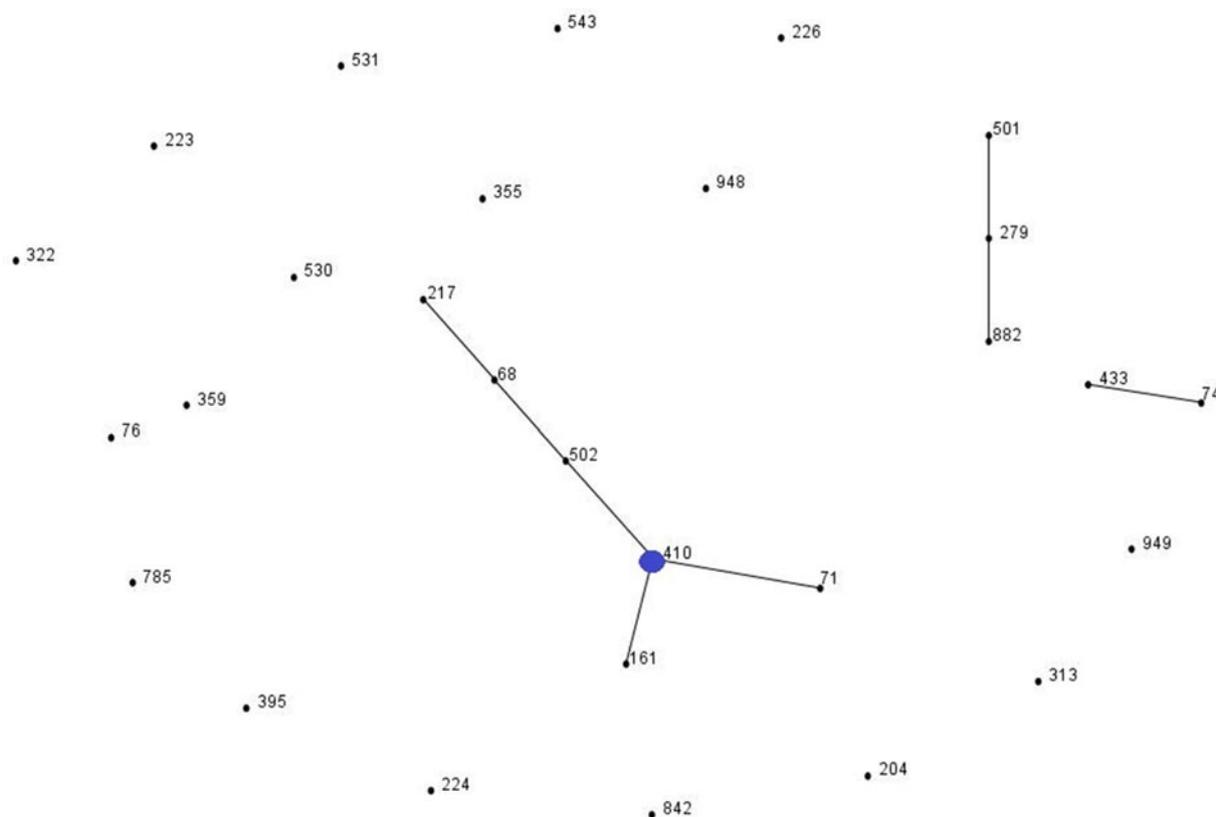
Fig. 2. Population snapshot of *A. nosocomialis* isolates in this study and existing isolates in the Institut Pasteur's MLST database by eBURST algorithm (http://pubmlst.org/abaumannii/; 24 April 2017, date last accessed). Each ST is represented by a black dot. Blue dot correspond to group founder. SLVs are linked by lines, and clonal complexes (CC) correspond to the group of connected STs.

through transient colonisation of the hands of healthcare workers and the survival of the organism on inanimate surfaces [23]. Patient #9 was colonised for 2 years by the same *A. baumannii* ST (ST228), which is a SLV of ST25 (CC25). ST25 is an emerging genotype responsible for recent epidemics worldwide that has been associated with elevated resistance to desiccation, high biofilm-forming capacity on abiotic surfaces, and adherence to pneumocytes [24].

Eight strains of MDR *A. baumannii* were also resistant to imipenem and meropenem, and carried the $bla_{OXA-23-like}$ gene. Around the world, including Brazil, this resistance determinant is widely associated with the spread of carbapenem resistance [15], and to the best of our knowledge, this study is the first to report its occurrence in this species from CF patients. Further, four of the MDR strains belonged to two globally distributed CCs in *A. baumannii*, CC1 (ST1, ST20 and ST160) and CC79 (ST79), which have been associated with antimicrobial resistance and dispersion of the $bla_{OXA-23-like}$ gene in Rio de Janeiro and wider Brazilian hospitals [25, 26].

A major limitation of this retrospective study is the lack of clinical and epidemiological data to provide a context of the significance of the findings and impact on patient care. However, in our paediatric and adult CF centres, *Acinetobacter* spp. are rare in the patients and we have been able to document successfully the species distribution and genetic relatedness of the different species recovered from patients over a long time period. Unlike other prevalent Gram-negative species isolated from CF, we found little evidence of widespread clonal transmission which suggests that

acquisition of *Acinetobacter* spp. in our hospitals was most often due to sporadic and widely diverse strain populations more associated with environmental rather than hospital sources. Nevertheless, our data do raise concerns regarding the risk of gene dissemination, since the MDR *A. baumannii* CC79 and CC1 lineages carrying the $bla_{OXA23-like}$ gene may be a silent source of resistance genes which could spread to other pathogens and compromise treatment and management of patients. Further prospective studies are therefore warranted to establish the clinical significance of *Acinetobacter* spp. in CF and distinguish between benign colonisation and increase in infectious risk.

References

1. **Peleg AY, Seifert H and Paterson DL** (2008) *Acinetobacter baumannii*: emergence of a successful pathogen. *Clinical Microbiology Reviews* **21**, 538–582.
2. **Yamamoto M, et al.** (2013) Regional dissemination of *Acinetobacter* species harbouring metallo-β-lactamase genes in Japan. *Clinical Microbiology and Infection* **19**, 729–736.

3. **Nemec A, *et al.*** (2015) *Acinetobacter seifertii* sp. nov., a member of the *Acinetobacter calcoaceticus–Acinetobacter baumannii* complex isolated from human clinical specimens. *International Journal of Systematic and Evolutionary Microbiology* **65**, 934–942.

4. **Cosgaya C, *et al.*** (2016) *Acinetobacter dijkshoorniae* sp. nov., a member of the *Acinetobacter calcoaceticus–Acinetobacter baumannii* complex mainly recovered from clinical samples in different countries. *International Journal of Systematic and Evolutionary Microbiology* **66**, 4105–4111.

5. **Al Atrouni A, *et al.*** (2016) Reservoirs of non-*baumannii Acinetobacter* species. *Frontiers in Microbiology* **7**, 49.

6. **Higgins PG, *et al.*** (2013) OXA-235, a novel class D β-lactamase involved in resistance to carbapenems in *Acinetobacter baumannii*. *Antimicrobial Agents and Chemotherapy* **57**, 2121–2126.

7. **Coenye T, *et al.*** (2002) Characterization of unusual bacteria isolated from respiratory secretions of cystic fibrosis patients and description of *Inquilinus limosus* gen. nov., sp. nov. *Journal of Clinical Microbiology* **40**, 2062–2069.

8. **Gilligan PH, Kiska DL and Appleman MD** (2006) *Cumitech 43: Cystic Fibrosis Microbiology*. Washington, DC: ASM Press, p. 36.

9. **Gundi VA, *et al.*** (2009) Validation of partial rpoB gene sequence analysis for the identification of clinically important and emerging *Acinetobacter* species. *Microbiology* **155**, 2333–2341.

10. **Diancourt L, *et al.*** (2010) The population structure of *Acinetobacter baumannii*: expanding multiresistant clones from an ancestral susceptible genetic pool. *PLoS ONE* **5**, e10034.

11. **Feil EJ, *et al.*** (2004) eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. *Journal of Bacteriology* **186**, 1518–1530.

12. **Clinical and Laboratory Standards Institute** (2017) Performance Standards for Antimicrobial Susceptibility Testing. Document M100-S27. Wayne, PA: Clinical and Laboratory Standards Institute, p. 224.

13. **Woodford N, *et al.*** (2006) Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. *Journal of Antimicrobial Agents* **27**, 351–353.

14. **Higgins PG, Lehmann M and Seifert H** (2010) Inclusion of OXA-143 primers in a multiplex polymerase chain reaction (PCR) for genes encoding OXA carbapenemases in *Acinetobacter* spp. *International Journal of Antimicrobial Agents* **35**, 305.

15. **Vasconcelos AT, *et al.*** (2015) The changing epidemiology of *Acinetobacter* spp. producing OXA carbapenemases causing bloodstream infections in Brazil: a BrasNet report. *Diagnostic Microbiology and Infectious Disease* **83**, 382–385.

16. **Krizova L, *et al.*** (2015) *Acinetobacter variabilis* sp. nov. (formerly DNA group 15 sensu Tjernberg & Ursing), isolated from humans and animals. *International Journal of Systematic and Evolutionary Microbiology* **65**, 857–863.

17. **Kishii K, *et al.*** (2016) The first cases of human bacteremia caused by *Acinetobacter seifertii* in Japan. *Journal of Infection and Chemotherapy* **22**, 342–345.

18. **Endo S, *et al.*** (2014) High frequency of *Acinetobacter soli* among *Acinetobacter* isolates causing bacteremia at a tertiary hospital in Japan. *Journal of Clinical Microbiology* **52**, 911–915.

19. **Peleg AY, *et al.*** (2012) The success of acinetobacter species; genetic, metabolic and virulence attributes. *PLoS One* **7**, e46984.

20. **Rocha GA, *et al.*** (2016) Draft genome sequence of *Acinetobacter pittii* ST643 shared by cystic fibrosis patients. *Memórias do Instituto Oswaldo Cruz* **111**, 592–593.

21. **Cox MJ, *et al.*** (2010) Airway microbiota and pathogen abundance in age-stratified cystic fibrosis patients. *PLoS One* **5**, e11044.

22. **Girão VBC, *et al.*** (2013) Dissemination of *Acinetobacter nosocomialis* clone among critically ill patients and the environment. *Journal of Clinical Microbiology* **51**, 2707–2709.

23. **D'Agata EMC, Thayer V and Schaffner W** (2000) An outbreak of *Acinetobacter baumannii*: the importance of cross-transmission. *Infection Control and Hospital Epidemiology* **21**, 588–591.

24. **Giannouli M, *et al.*** (2013) Virulence-related traits of epidemic *Acinetobacter baumannii* strains belonging to the international clonal lineages I–III and to the emerging genotypes ST25 and ST78. *BMC Infectious Diseases* **13**, 282.

25. **Grosso F, *et al.*** (2011) OXA-23-producing *Acinetobacter baumannii*: a new hotspot of diversity in Rio de Janeiro? *Journal of Antimicrobial Chemotherapy* **66**, 62–65.

26. **Chagas TP, *et al.*** (2014) Characterization of carbapenem-resistant *Acinetobacter baumannii* in Brazil (2008–2011): countrywide spread of OXA-23 producing clones (CC15 and CC79). *Diagnostic Microbiology and Infectious Disease* **79**, 468–472.