International Journal of Infectious Diseases 52 (2016) 29–36

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Wide spread of OXA-23-producing carbapenem-resistant *Acinetobacter baumannii* belonging to clonal complex II in different hospitals in Lebanon

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ARTICLE INFO

Article history:
Received 16 August 2016
Accepted 15 September 2016

Corresponding Editor: Eskild Petersen, Aarhus, Denmark.

Keywords:
Acinetobacter baumannii
Carbapenem resistance
OXA-23
Clonal complex II
Lebanon

SUMMARY

Objectives: To investigate the molecular epidemiology of *Acinetobacter baumannii* strains isolated from different hospitals in Lebanon.
Methods: A total of 119 non-duplicate Acinetobacter strains were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and partial *rpoB* gene sequencing. Antibiotic susceptibility testing was performed by disc diffusion method and all identified carbapenem-resistant isolates were investigated by PCR assays for the presence of the carbapenemase-encoding genes. Multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) were used for molecular typing.
Results: Of the 119 *A. baumannii* isolates, 76.5% were resistant to carbapenems. The most common carbapenemase was the OXA-23-type, found in 82 isolates. The study of population structure using MLST revealed the presence of 30 sequence types (STs) including 18 new ones, with ST2 being the most commonly detected, accounting for 61% of the isolates typed. PFGE performed on all strains of ST2 identified a major cluster of 53 isolates, in addition to three other minor clusters and ten unique profiles.
Conclusions: This study highlights the wide dissemination of highly related OXA-23-producing carbapenem-resistant *A. baumannii* belonging to the international clone II in Lebanon. Thus, appropriate infection control measures are recommended in order to control the geographical spread of this clone in this country.

1. Introduction

Acinetobacter baumannii is one of the most common opportunistic Gram-negative bacteria associated with community-acquired infections, outbreaks, and nosocomial infections, particularly in critically ill patients. This bacterium is involved in respiratory tract infections, bloodstream infections, skin and soft tissue infections, urinary tract infections, and meningitis, leading to increased rates of mortality and morbidity.[1,2]

Infections caused by *A. baumannii* are an increasing threat in many countries. Indeed, this bacterium has a strong ability to resist desiccation and to survive on inanimate surfaces. Moreover, the prevalence of multidrug-resistant (MDR) isolates has increased worldwide in recent decades,[3,4] due to its ability to develop multidrug resistance through either the acquisition of genetic determinants such as plasmids, integrons, and transposons, or through the acquisition of mutations leading to the modification of antibiotic targets, efflux pump expression, and membrane permeability.[5] As a consequence, the treatment of infections caused by such isolates has become more and more difficult, even with most clinically available drugs, including tigecycline, colistin, and carbapenems.[1,4,6]

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http://dx.doi.org/10.1016/j.ijid.2016.09.017

A recent retrospective nationwide study conducted by the Lebanese Society of Infectious Diseases showed that the percentage of resistance to imipenem among *Acinetobacter spp* increased significantly from 57.6% in 2011 to 84.5% in 2013.[7] In *A. baumannii*, carbapenem resistance was mostly associated with the production of carbapenem-hydrolyzing class D β-lactamases (CHDLs), including OXA-23, OXA-24, OXA-58, OXA-143, and OXA-235, in addition to carbapenemases from classes B and A.[6]

Nowadays, understanding the molecular characteristics of epidemic strains is the key strategy to track outbreaks and to control the spread of *A. baumannii*, both in hospitals and in the community.[8,9] Several molecular typing methods have been developed for this purpose. Among them, pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) have been the most frequently used and have represented the gold standard methods to investigate strain clonality and the bacterial population structure, respectively.[10] Molecular epidemiological studies have revealed *A. baumannii* outbreaks to have been caused mainly by MDR strains belonging to the international clonal complexes CC1, CC2, and CC3.[8,11] In Lebanon, previous studies have reported the predominance of carbapenem-resistant *A. baumannii* strains belonging to the international clone II .[12,13]

The main objectives of this work were to investigate the prevalence of carbapenem-resistant *A. baumannii* isolates (CRAb) circulating in some Lebanese hospitals between 2013 and 2015, and to characterize the common resistance mechanisms. The population structure, as well as the genetic relatedness of CRAb isolates, was also studied in order to track the evolution and the clonality of these strains.

2. Materials and methods

2.1. Bacterial strain collection and identification

A total of 119 non-replicate *A. baumannii* clinical isolates were analyzed. These strains were collected between October 2013 and December 2015 from the following hospitals in Lebanon: Tripoli Governmental Hospital (TGH; 100 beds), Nini Hospital (NH; 120 beds), Makassed General Hospital (MGH; 150 beds), Saydet Zgharta Hospital (SZH; 149 beds), Hanane Hospital (HH; 45 beds), Al Youssef Medical Centre (YMC; 100 beds), Seblin Governmental Hospital (SGH; 70 beds), and Dar Ajaza Hospital (DAH; 580 beds). Isolates were sent to the Laboratory of Microbiology Health and Environment (LMSE), Tripoli, Lebanon, and were stored at −80 °C. Bacterial identification was performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) using the Vitek MS (bioMérieux, Marcy l'Etoile, France), and confirmed at the species level by partial RNA polymerase β-subunit (*rpoB*) gene sequencing, as described previously.[13] For each isolate, information on the sex and age of the patient, the clinical specimen, and the type of ward were provided.

2.2. Antibiotic susceptibility testing and investigation of carbapenem resistance mechanisms

Antibiotic susceptibility testing was performed by disc diffusion method according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (http://www.eucast.org). A panel of 14 antibiotics was tested, including ticarcillin, ticarcillin–clavulanic acid, piperacillin–tazobactam, ceftazidime, imipenem, ciprofloxacin, amikacin, gentamicin, tobramycin, trimethoprim–sulfamethoxazole, colistin, doxycycline, tigecycline, and rifampicin. Carbapenem resistance was confirmed by determining minimum inhibitory concentration (MICs) against imipenem and meropenem by Etest strips (bioMérieux).

Carbapenem-resistant isolates were investigated by PCR assays for the presence of the carbapenemase-encoding genes $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-58-like}}$, $bla_{\text{OXA-143}}$, bla_{NDM}, bla_{IMP}, and bla_{VIM}.[12] Sequencing of the entire gene was performed on all New Delhi metallo-β-lactamase (NDM)-positive isolates.

2.3. Epidemiological typing

2.3.1. Multilocus sequence typing (MLST)

MLST was performed according to the Pasteur scheme (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Abaumannii.html). The internal fragments of seven housekeeping genes (*fusA*, *pyrG*, *rpoB*, *rplB*, *cpn60*, *gltA*, and *recA*) were amplified, then purified and sequenced using an ABI 3130XL DNA sequencer (Applied Biosystems, Foster City, CA, USA). The allelic number and sequence types (STs) were analyzed using the *A. baumannii* MLST database. New STs were submitted and assigned by the Institut Pasteur MLST database. The clustering of related STs (defined as clonal complexes, CCs) was analyzed using eBURST software (http://eburst.mlst.net). A CC is defined as a set of similar ST(s) having six identical loci among seven, so a CC is formed by the founder ST and its single locus variants (SLV).

2.3.2. Pulsed field gel electrophoresis (PFGE)

A. baumannii DNA was digested with *Apa*I (TaKaRa, Dalian, Liaoning Province, China) as a restriction enzyme. DNA fragments were separated on 1% w/v agarose gels in 0.5× Tris/borate/ethylenediaminetetraacetic acid (TBE) buffer at 14 °C using a CHEF DRII apparatus (Bio-Rad) with 6 V/cm, pulsed from 3 to 20 s for 21 h; gels were stained with Gel Red. The Dice coefficient was used to calculate similarities, and the unweighted pair group method using average linkages (UPGMA) was used for cluster analysis with fingerprinting II software (Bio-Rad, Marne La Coquette, France). Isolates were considered to belong to the same PFGE clone if their Dice similarity index was ≥80%.

3. Results

3.1. Bacterial strain collection and identification

The 119 isolates analyzed in this study were collected from seven hospitals situated in different regions in Lebanon: North of Lebanon, Beirut, and Mount of Lebanon. Forty-eight isolates were from Tripoli Governmental Hospital (Tripoli, North of Lebanon), 40 from Nini Hospital (Tripoli, North of Lebanon), 21 from Makassed General Hospital (Beirut), four from Saydet Zgharta Hospital (Zgharta, North of Lebanon), two from each of Hanane Hospital (Tripoli, North of Lebanon) and Al Youssef Medical Centre (Akkar, North of Lebanon), and one from each of Dar Ajaza (Beirut) and Seblin Governmental Hospital (Seblin, Mount of Lebanon). The isolates were mostly recovered from the respiratory tract (n = 35; 29%) and skin and soft tissue (n = 33; 28%), followed by urine (n = 14; 12%), blood (n = 9; 7.6%), and vascular catheters (n = 6; 5%). Of note, 19 (13.4%) patients were asymptomatically colonized (Table 1).

3.2. Antibiotic susceptibility testing and investigation of carbapenem resistance mechanisms

Antibiotic susceptibility testing showed that the *A. baumannii* isolates analyzed were MDR. Indeed, most of them were resistant to β-lactams, aminoglycosides, ciprofloxacin, and doxycycline. However, only four isolates were resistant to rifampicin and one isolate to tigecycline, while colistin and tigecycline maintained their activity against all isolates. Among the isolates, 91 (76.5%) showed an imipenem-resistant phenotype. Etest results confirmed

Table 1
Characteristics of *Acinetobacter baumannii* isolates

Strain ID	Sex	Age, years	Origin	Year of study	City	Hospital	Department	Carbapenem phenotype[a]	Resistance genes	ST	CC[b]
1	M	NA	Tracheal aspirate	2013	Tripoli	TGH	ICU	S	-	698	S
2	F	65	Rectal swab	2013	Tripoli	NH	ICU	R	OXA-23	2	2
3	F	76	Tracheal aspirate	2013	Tripoli	NH	ICU	R	OXA-23	2	2
4	M	18	Wound	2013	Tripoli	TGH	IM	R	OXA-23	2	
5	F	NA	Broncho-tracheal lavage	2013	Tripoli	TGH	IM	R	OXA-23	2	2
6	M	NA	Wound	2013	Tripoli	TGH	IM	R	OXA-23	2	2
7	M	66	Tracheal aspirate	2013	Tripoli	TGH	IM	R	OXA-23	2	2
8	M	NA	Wound	2013	Tripoli	TGH	IM	R	OXA-23	2	2
9	M	45	Catheter	2013	Tripoli	HH	NA	R	OXA-23	2	
10	F	81	Rectal swab	2014	Tripoli	NH	ICU	R	OXA-23	2	2
11	F	39	Wound	2014	Tripoli	TGH	IM	R	OXA-23	2	2
12	M	NA	Broncho-tracheal lavage	2014	Tripoli	TGH	ICU	R	OXA-23	2	2
13	M	NA	Blood	2014	Tripoli	TGH	IM	R	NDM-1	85	85
14	M	82	Rectal swab	2014	Tripoli	NH	IM	S	-	600	2
15	F	NA	NA	2014	Tripoli	HH	NA	R	OXA-23	2	2
16	F	86	Tracheal aspirate	2014	Tripoli	NH	ICU	R	OXA-23	2	2
19	F	23	Urine	2014	Tripoli	NH	OP	S	-	701	S
20	F	84	Nasal swab	2014	Tripoli	NH	ICU	R	OXA-23	2	2
21	M	21	Wound	2014	Tripoli	TGH	IM	R	OXA-23	2	2
22	F	NA	Sputum	2014	Tripoli	TGH	ICU	R	OXA-23/OXA-24	2	2
23	M	60	NA	2014	Zgharta	SZH	NA	S	-	2	2
24	M	NA	Blood	2014	Tripoli	TGH	ICU	S	-	702	S
26	F	NA	Wound	2014	Tripoli	TGH	IM	R	OXA-23	2	2
27	M	83	Rectal swab	2014	Tripoli	NH	IM	S	-	2	2
28	M	NA	Sputum	2014	Tripoli	TGH	ICU	R	OXA-23	2	2
29	F	38	Urine	2014	Tripoli	NH	OP	S	-	704	S
30	F	NA	Catheter	2014	Tripoli	TGH	ICU	S	-	702	S
33	M	NA	Sputum	2014	Tripoli	TGH	ICU	S	-	702	S
34	F	78	Urine	2014	Tripoli	NH	NA	S	-	46	S
35	M	NA	NA	2014	Zgharta	SZH	NA	R	OXA-23	705	S
36	M	NA	Sputum	2014	Tripoli	TGH	NA	S	-	702	S
38	M	44	Tracheal aspirate	2014	Tripoli	NH	ICU	R	OXA-23	2	2
39	M	NA	Broncho-tracheal lavage	2014	Tripoli	TGH	IM	R	OXA-23	2	2
40	M	84	Urine	2014	Tripoli	NH	IM	S	-	2	2
43	M	27	Rectal swab	2014	Tripoli	NH	ICU	S	-	25	25
44	M	NA	Wound	2014	Tripoli	TGH	IM	R	OXA-143	2	2
45	F	60	Wound	2014	Tripoli	TGH	ICU	R	OXA-23	2	2
46	M	NA	Wound	2014	Tripoli	TGH	IM	R	OXA-23	2	2
47	M	NA	Wound	2014	Tripoli	TGH	IM	R	OXA-23	2	2
48	F	NA	Wound	2014	Tripoli	TGH	IM	R	OXA-23	2	2
50	M	NA	Sputum	2014	Tripoli	TGH	ICU	R	OXA-23	2	2
51	M	NA	Blood	2014	Tripoli	TGH	ICU	R	OXA-23	2	2
53	M	65	Rectal swab	2014	Tripoli	TGH	ICU	S	-	52	S
54	F	NA	Wound	2014	Tripoli	TGH	OP	S	-	690	S
55	M	81	Bed swab	2014	Seblin	SGH	ICU	R	OXA-23	706	S
56	M	77	Wound	2014	Tripoli	NH	ICU	S	-	25	25
58	M	NA	Wound	2014	Tripoli	TGH	IM	R	OXA-23	2	2
59	F	NA	Urine	2014	Tripoli	TGH	IM	R	OXA-23/OXA-24	2	2
60	M	47	Pus	2014	Tripoli	NH	OP	S	-	1	1
61	M	82	Sputum	2014	Zgharta	SZH	NA	R	OXA-23	2	2
62	M	23	Wound	2014	Tripoli	TGH	IM	R	OXA-23	2	2
63	M	64	LCR	2014	Tripoli	NH	ICU	S	-	25	25
64	F	NA	Wound	2014	Tripoli	TGH	IM	R	OXA-23	2	2
65	F	69	Tracheal aspirate	2015	Tripoli	NH	ICU	R	OXA-23	2	2
66	F	NA	Wound	2015	Tripoli	TGH	ICU	R	NDM-1	85	85
67	M	75	Pus	2015	Tripoli	NH	ICU	R	OXA-23	707	S
68	M	NA	Pus	2015	Tripoli	TGH	IM	R	NDM-1	708	S
69	M	75	Nasal swab	2015	Tripoli	TGH	ICU	R	OXA-23/OXA-58	2	2
70	M	75	Rectal swab	2015	Tripoli	NH	ICU	R	OXA-23	2	2
71	NA	NA	Pus	2015	Zgharta	SZH	NA	R	OXA-23	2	2
72	F	18	Catheter	2015	Tripoli	NH	ICU	R	OXA-23	2	2
73	M	84	Rectal swab	2015	Tripoli	NH	ICU	R	OXA-23	2	2
74	M	75	Nasal swab	2015	Tripoli	NH	ICU	R	OXA-23	2	2
75	F	18	Tracheal aspirate	2015	Tripoli	NH	ICU	R	OXA-23	2	2
76	M	82	Rectal swab	2015	Tripoli	NH	ICU	S	-	2	2
77	F	69	Axillary swab	2015	Tripoli	NH	ICU	R	OXA-23	2	2
78	F	63	Urine	2015	Tripoli	NH	ICU	S	-	807	S
79	M	84	Urine	2015	Tripoli	NH	ICU	S	-	25	25
80	M	NA	Catheter	2015	Tripoli	NH	ICU	S	-	25	25
81	F	78	Urine	2015	Beirut	DAH	IM	R	OXA-23/OXA-24	2	2
82	M	NA	Blood	2015	Tripoli	TGH	ICU	S	-	424	33
83	M	NA	Tracheal aspirate	2015	Tripoli	TGH	ICU	R	NDM-1	708	S
84	M	70	Blood	2014	Beirut	MGH	IM	R	OXA-23	2	2
85	M	64	Tracheal aspirate	2014	Beirut	MGH	IM	R	OXA-23	2	2
86	M	66	Tracheal aspirate	2015	Beirut	MGH	IM	R	OXA-23	2	2

Table 1 (*Continued*)

Strain ID	Sex	Age, years	Origin	Year of study	City	Hospital	Department	Carbapenem phenotype[a]	Resistance genes	ST	CC[b]
87	M	82	Tracheal aspirate	2014	Beirut	MGH	ICU	R	OXA-23/OXA-24	711	S
88	F	81	Tracheal aspirate	2015	Beirut	MGH	IM	R	OXA-23	2	2
89	M	83	Tracheal aspirate	2015	Beirut	MGH	IM	R	OXA-23	2	2
90	F	83	Pus	2015	Beirut	MGH	IM	R	OXA-23	570	2
91	F	87	Blood	2015	Beirut	MGH	IM	R	OXA-23/OXA-24	715	S
92	M	19	Tracheal aspirate	2015	Beirut	MGH	ICU	R	OXA-23	570	2
93	F	67	Blood	2015	Beirut	MGH	CCU	R	OXA-23	570	2
94	M	58	Tracheal aspirate	2015	Beirut	MGH	CCU	R	OXA-23	600	2
95	M	26	Tracheal aspirate	2014	Beirut	MGH	IM	R	OXA-23	2	2
96	F	71	Tracheal aspirate	2015	Beirut	MGH	SURG	R	OXA-23	570	2
97	M	63	Tracheal aspirate	2015	Beirut	MGH	IM	R	OXA-23	2	2
98	M	31	Pus	2015	Beirut	MGH	IM	R	OXA-23	2	2
99	M	79	Sputum	2015	Beirut	MGH	CCU	R	OXA-23	2	2
100	F	47	Sputum	2015	Beirut	MGH	IM	R	OXA-23	713	2
101	F	74	Tracheal aspirate	2015	Beirut	MGH	IM	R	OXA-23	2	2
102	F	84	Blood	2015	Beirut	MGH	CCU	R	OXA-23/OXA-24	714	S
103	F	84	Tracheal aspirate	2015	Beirut	MGH	ICU	R	OXA-23	2	2
104	F	93	Tracheal aspirate	2015	Beirut	MGH	ICU	R	OXA-23	600	2
105	F	50	Urine	2015	Tripoli	NH	IM	S	-	808	S
106	M	42	Pus	2015	Tripoli	NH	IM	R	OXA-23	1	1
107	M	NA	Wound	2015	Tripoli	TGH	IM	R	OXA-23	85	85
108	M	NA	Wound	2015	Tripoli	TGH	IM	R	OXA-24	636	S
109	M	74	Rectal swab	2015	Tripoli	NH	ICU	R	OXA-23	2	2
110	M	65	Wound	2015	Akkar	YMC	NA	R	OXA-23	2	2
111	M	55	Urine	2015	Tripoli	NH	IM	R	OXA-23	2	2
112	F	NA	Catheter	2015	Tripoli	TGH	IM	S	-	193	S
113	F	38	Wound	2015	Tripoli	TGH	IM	R	OXA-23	2	2
114	F	NA	Pus	2015	Tripoli	TGH	ICU	R	OXA-23	2	2
115	F	NA	Catheter	2015	Tripoli	TGH	ICU	R	OXA-23	2	2
116	M	5	Urine	2015	Tripoli	NH	OP	S	-	809	S
117	F	19	Urine	2015	Tripoli	NH	OP	S	-	810	S
118	M	NA	Urine	2015	Akkar	YMC	NA	S	-	811	S
119	M	80	Wound	2015	Tripoli	NH	OP	R	OXA-23	2	2
120	F	NA	Broncho-tracheal lavage	2015	Tripoli	TGH	IM	R	OXA-23	2	2
121	F	71	Rectal swab	2015	Tripoli	NH	CCU	R	OXA-23	2	2
122	M	NA	Wound	2015	Tripoli	TGH	IM	R	OXA-23	2	2
123	F	NA	Broncho-tracheal lavage	2015	Tripoli	TGH	ICU	R	OXA-24	2	2
124	F	32	Blood	2015	Tripoli	NH	ICU	R	OXA-23	2	2
125	M	NA	Broncho-tracheal lavage	2015	Tripoli	TGH	ICU	R	OXA-23	2	2
126	F	75	Pus	2015	Tripoli	NH	P	R	OXA-23	2	2
127	M	46	Catheter	2015	Tripoli	NH	ICU	R	OXA-23	2	2
128	M	46	Nasal swab	2015	Tripoli	NH	ICU	R	OXA-23	25	25
129	F	NA	Wound	2015	Tripoli	TGH	NA	R	OXA-23	2	2
130	M	NA	Urine	2015	Tripoli	TGH	IM	R	OXA-24	812	S

CC, clonal complex; CCU, cardiac care unit; DAH, Dar Ajar Hospital; F, female; HH, Hanane Hospital; ICU, intensive care unit; IM, internal medicine; CSF, cerebrospinal fluid; M, male; MGH, Makassed General Hospital; NA, not assigned; NH, Nini Hospital; OP, outpatient; P, paediatric; SGH, Seblin Governmental Hospital; ST, sequence type; SURG, surgery; SZH, Sayedt Zgharta Hospital; TGH, Tripoli Governmental Hospital; YMC, Al Youssef Medical Centre.

[a] Phenotype: 'R' resistant or 'S' susceptible to carbapenems.

[b] S: singleton.

carbapenem resistance since the MICs for imipenem and meropenem were >32 mg/l. Carbapenemase determinants were detected in all carbapenem-resistant isolates. $bla_{\text{OXA-23-like}}$ was the most common gene found ($n = 76$; 83.5%). Furthermore, three $bla_{\text{OXA-24-like}}$ and four $bla_{\text{NDM-1}}$ were detected. Of note, six isolates carried both $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24-like}}$ genes and one isolate carried both $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-58-like}}$ genes. Finally, $bla_{\text{OXA-143-like}}$ was found in one isolate.

3.3. Epidemiological typing

3.3.1. Multilocus sequence typing

MLST was performed on all isolates. As a result, 30 STs including 18 new ones were identified in this study. ST2 was the most commonly observed (73/119 isolates), accounting for 61% of the isolates typed. Other less common STs were ST25 (six isolates) and ST570 (four isolates), followed by ST85, ST600 (both with three isolates) and ST1 (two isolates). The remaining STs were found sporadically in this collection (Table 1). Based on eBURST analysis, ST2 as well as ST570, ST600, and ST713 (which are SLVs of ST2) belonged to CC2, while ST706 was a double locus variant (DLV) of ST2. Furthermore, ST1, ST25, ST85, and ST424 (a SLV of ST33) belonged to CC1, CC25, CC85, and CC33, respectively. ST690 was a DLV of CC25, and ST193 and ST702 were DLVs of CC33. Finally, ST46 was a SLV of ST622, and ST715 was a SLV of ST636. The remaining STs were singletons, since they did not share any homology with a known ST in the database (Fig. 1).

3.3.2. PFGE analysis

Based on the above MLST results, all strains of ST2 were selected for PFGE typing in order to investigate their clonality. Overall, 73 strains were analyzed. As shown in Fig. 2, PFGE revealed the presence of four clusters with two or more isolates and 10 unique profiles. The major cluster (cluster C) comprised 52 carbapenem-resistant isolates harbouring the $bla_{\text{OXA-23-like}}$ gene with or without either the $bla_{\text{OXA-24-like}}$ gene or the $bla_{\text{OXA-58-like}}$ gene, as well as one

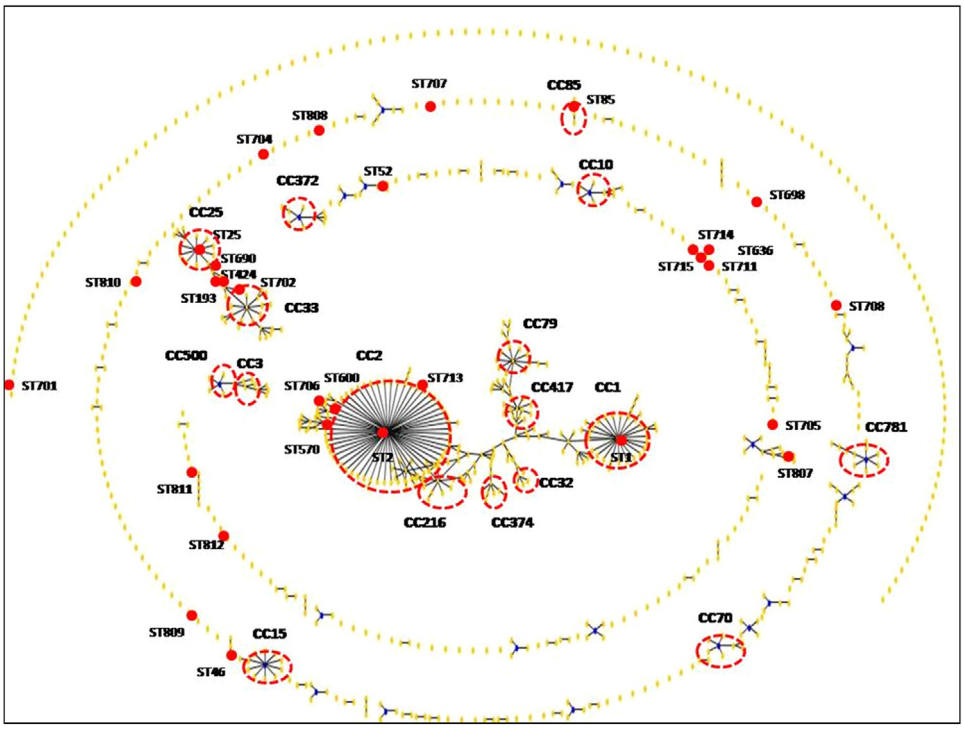

Fig. 1. Population snapshot analysed by eBURST on 836 sequences present in the MLST Pasteur database (05/05/2016). Red points and circles show sequence types identified in this study and the most important clonal complexes, respectively.

carbapenem-susceptible strain (strain 23). Interestingly, some of these strains displayed identical PFGE patterns, although they were isolated from different hospitals in different locations, mainly in Tripoli (40 isolates), Beirut (nine isolates), Zgharta (three isolates), and Akkar (one isolate). The second cluster (cluster A) contained five isolates producing the $bla_{\text{OXA-23-like}}$ gene that were recovered from two distinct hospitals in Tripoli (Table 1). Furthermore, the OXA-23-producing isolates (numbers 5, 85, and 103) that were isolated in Tripoli (TGH) and Beirut (MGH) belonged to cluster B. Finally, cluster D contained two carbapenem-resistant strains harbouring the $bla_{\text{OXA-23-like}}$ gene isolated from the same hospital (NH).

4. Discussion

A. baumannii is the most common opportunistic pathogen causing nosocomial infections, particularly respiratory tract infections in intensive care units among critically ill patients.[14] Nowadays, the excessive use of antimicrobial agents in and outside hospitals may be an important cause of the isolation of MDR *A. baumannii* strains around the world.[15–18] The major problem is that infections caused by such isolates are associated with greater morbidity and greater healthcare costs.[19,20]

The current survey was conducted in different hospitals distributed across the country, with isolates mostly being recovered from two main hospitals in Tripoli, North of Lebanon and one main hospital in Beirut. A total of 119 *A. baumannii* clinical strains were included in this study to evaluate antibiotic resistance phenotypes and molecular epidemiological features. These strains were isolated from nosocomial infections, but also from infections present at the time of admission to the hospitals, and a number of patients were only colonized.

Antibiotic susceptibility testing showed a very serious situation. Of all *A. baumannii* isolates, 76.5% were carbapenem-resistant. This rate is higher than those reported in other countries such as China, Saudi Arabia, Greece, Italy, and the USA, where reported rates were 29.4%, 69%, 57.4%, 45.7% and 34% respectively.[9,14,21–23] Interestingly, in previous studies in Lebanon, the rate of CRAb isolates was significantly different.[13,24] Moreover, 60.5% of the isolates presented here were resistant to aminoglycosides and 87% were resistant to fluoroquinolone, while tigecycline and colistin maintained their activity against all isolates.

The $bla_{\text{OXA-23}}$ gene is considered a significant cause of carbapenem resistance in *A. baumannii* worldwide.[25] This study revealed that carbapenem resistance in *A. baumannii* was also mainly associated with this gene. The present findings are similar to those reported previously from Lebanon and other countries.[13,22,26–28] Additionally, the co-occurrence of two OXA-type genes has also been reported in *A. baumannii*.[22,29] In the present study, the coexistence of either $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-24}}$ or $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-58}}$ was found in seven isolates. Moreover, the first description of a $bla_{\text{OXA-143-like}}$ strain is reported here, which was isolated in 2014 from a wound sample of a female patient admitted to TGH. Alarmingly, since the first description in Lebanon of a $bla_{\text{NDM-1}}$-positive *A. baumannii* [30] and *Acinetobacter pittii*,[31] four more isolates were found in this study.

Epidemiological tools are important to track outbreaks and to investigate strain relatedness in order to control the spread of MDR isolates. The evolutionary history and the dynamic spread of *A. baumannii* in this study were investigated by MLST. The data revealed that CC2 (ST2, ST570, ST600, ST713) was the main clonal complex found in Lebanon over recent years. Interestingly, almost all of these isolates were resistant to carbapenems, which is in agreement with previous studies that have used MLST for epidemiological characterization of *A. baumannii*.[8,12,21,32,33] In Lebanon, even if different clones and STs have been described, CC2 and particularly ST2 appear to be widely distributed. The capacity for biofilm formation, adherence to abiotic surfaces, and acquisition of antimicrobial resistance determinants could be the main factors that enhance the spread and the persistence of this CC in the hospital setting, as has been described previously.[34] Moreover, CC1 and CC25 have also been responsible for several

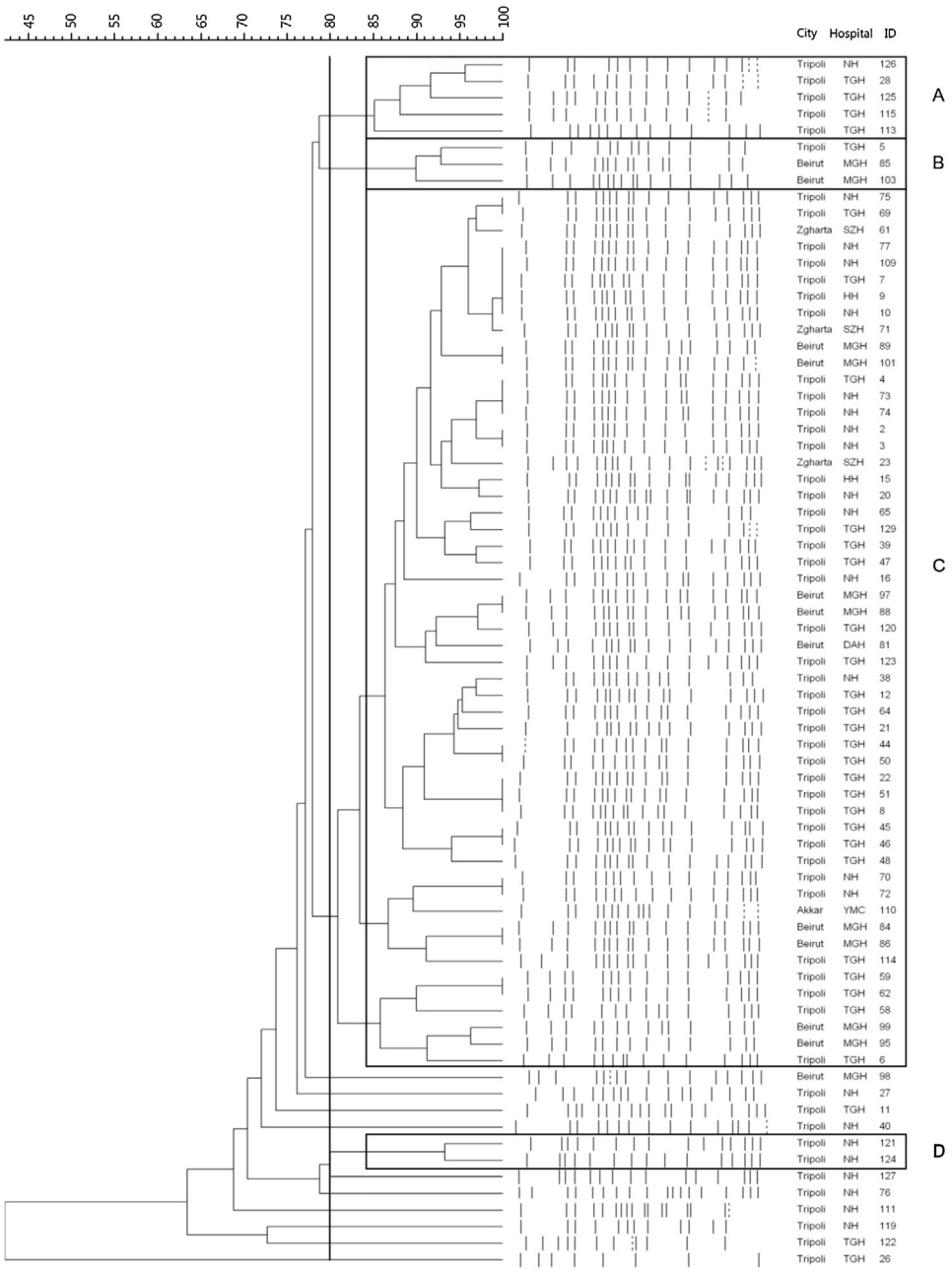

Fig. 2. Dendrogram illustrating the PFGE patterns of *Acinetobacter baumannii* isolates.

outbreaks of *A. baumannii* worldwide and have been described as emerging clones.[8,32,35] Strains belonging to these clones were detected in the present study. However, these strains, with the exception of one isolate (ST25), were all susceptible to carbapenems, which is not consistent with the results of Rafei et al., who found only carbapenem-resistant strains belonging to CC1 and CC25 in Lebanon.[13] These findings may highlight the potential role of hospitals as reservoirs for carbapenem resistance genes. Another new emerging clone, ST85 (CC85), which has long been associated with NDM-1,[13,36,37] was also detected in three isolates. Among them, two were NDM-1-producers and the remaining isolate was an OXA-23-producer. Of note, the detection of several new STs may support and highlight the continuous clonal evolution of *A. baumannii*. This diversity may be due to several mechanisms, such as mutations or the transfer and mobilization of genetic elements including insertion sequences.[38]

As shown in Table 1, 73 strains belonging to ST2 were further selected for PFGE typing. Around 72.6% (n = 53) of them belonged to the same PFGE cluster, which appeared to be a predominant clone in Lebanon. Noteworthy, among this clone, several strains sharing identical PFGE patterns were detected, although they were not isolated in the same hospital or in the same location, such as strains 69, 71, and 75 (Fig. 2). This finding may suggest a possible inter-hospital transmission of these carbapenem-resistant strains. On the other hand, the presence of identical strains in the same hospital (Fig. 2) may also highlight the existence of pseudo outbreaks. Thus, early surveillance is warranted and the development of appropriate infection control measures (environmental and equipment cleaning and disinfection, implementation of a hand hygiene programme for healthcare workers, isolation of patients with MDR *A. baumannii*, early screening of the hospitalized patients, genotyping of the isolated strains, etc.) is recommended in order to eradicate the geographical spread of this clone.

MLST approaches have been used widely for the genotyping of *A. baumannii* due to their reproducibility and portability, facilitating comparisons between laboratories. Although MLST is an expensive typing method, it provides good information on the molecular epidemiology across different hospitals and locations.[10,39] Even if it is not suitable for inter-laboratory comparison, PFGE allows the investigation of outbreaks due to its high discriminatory power. PFGE analysis showed that the majority of the isolates belonging to ST2 were highly related, which is in agreement with the findings of previous studies.[12,39] However, it is recommended that both techniques are used when testing a high number of strains isolated from different locations at different time points.

Finally, although the clinical isolates collected may be limited, these data provide a global view on the molecular epidemiology of CRAb circulating in Beirut and the North of Lebanon during recent years. Of note, *A. baumannii* has evolved as a global pathogen causing community-acquired infections and war and natural disaster-related infections.[40] Thus, there is no doubt that the overall epidemiology of *A. baumannii* in Lebanon could be highly influenced by the Syrian War, since several wounded Syrian refugees have been hosted in different Lebanese cities and admitted to different Lebanese hospitals.[13] Therefore, further research is needed including more strains from other hospitals situated in different governorates in order to ensure the continuous surveillance of these MDR organisms in Lebanon.

In conclusion, this study provides evidence of the wide dissemination of OXA-23-producing carbapenem-resistant *A. baumannii* belonging to international clone II in Lebanon. Although PFGE results did not always correlate with MLST, both techniques remain nevertheless useful for the investigation of the molecular epidemiology and clonal spread of *A. baumannii*.

Acknowledgements

We thank the team of the Institute Pasteur MLST system (Paris, France) for importing novel alleles, profiles, and isolates at http://www.pasteur.fr/mlst. We are very grateful to the clinical laboratory departments at all hospitals for their collaboration in this study. The authors gratefully thank Catherine Quinqueneau, Catherine Ramont, Maryam Yehya, Taha Abdo, Majd Mouzawak, Marianne Ekko, Aicha Borghol, and Rola Hajjar for their excellent technical assistance.

Funding: This work was supported in part by the Lebanese University and the National Council for Scientific Research, Lebanon.

Conflict of interest: We have no conflicts of interest.

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