

Extrahuman Epidemiology of *Acinetobacter baumannii* **in Lebanon**

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The presence of *Acinetobacter baumannii* **outside hospitals is still a controversial issue. The objective of our study was to explore the extrahospital epidemiology of** *A. baumannii* **in Lebanon. From February 2012 to October 2013, a total of 73 water samples, 51 soil samples, 37 raw cow milk samples, 50 cow meat samples, 7 raw cheese samples, and 379 animal samples were analyzed by cultural methods for the presence of** *A. baumannii***. Species identification was performed by** *rpoB* **gene sequencing. Antibiotic susceptibility was investigated, and the** *A. baumannii* **population was studied by two genotyping approaches: multilo**cus sequence typing (MLST) and *bla*_{OXA-51} sequence-based typing (SBT). A. *baumannii* was detected in 6.9% of water samples, **2.7% of milk samples, 8.0% of meat samples, 14.3% of cheese samples, and 7.7% of animal samples. All isolates showed a susceptible phenotype against most of the antibiotics tested and lacked carbapenemase-encoding genes, except one that harbored a** *bla***OXA-143 gene. MLST analysis revealed the presence of 36 sequence types (STs), among which 24 were novel STs reported for the** first time in this study. $bla_{\text{OXA-51}}$ SBT showed the presence of 34 variants, among which 21 were novel and all were isolated from **animal origins. Finally, 30 isolates had new partial** *rpoB* **sequences and were considered putative new** *Acinetobacter* **species. In conclusion, animals can be a potential reservoir for** *A. baumannii* **and the dissemination of new emerging carbapenemases. The roles of the novel animal clones identified in community-acquired infections should be investigated.**

A*cinetobacter baumannii* is an opportunistic pathogen involved in a large number of hospital-acquired infections and associated with increased mortality and morbidity [\(1\)](#page-6-0). One of the main reasons for the current increased interest in *A. baumannii* is its remarkable ability to acquire mechanisms of resistance to almost all available antimicrobial agents, including carbapenems $(1-3)$ $(1-3)$ $(1-3)$. Genotyping approaches have attributed its global spread to a limited number of successful clones responsible for the majority of the worldwide nosocomial outbreaks [\(4](#page-6-3)[–](#page-6-4)[6\)](#page-6-5). Among them, international clones 1 and 2 have been extensively disseminated in more than 30 countries [\(2\)](#page-6-1).

Despite the fact that the hospital ecology of the bacterium has been intensively studied, its ecology outside hospitals remains unclear and is the subject of great debate [\(7](#page-6-6)[–](#page-6-7)[9\)](#page-7-0). Difficulties regarding *A. baumannii* identification methods enhance this ambiguity [\(9\)](#page-7-0). *A. baumannii* can cause severe community-acquired pneumonia occurring mainly during the warm and humid months in tropical and subtropical zones [\(10,](#page-7-1) [11\)](#page-7-2). In addition, *A. baumannii* isolates have been recovered from wounds of survivors of natural disasters [\(12,](#page-7-3) [13\)](#page-7-4), as well as from soldiers [\(14\)](#page-7-5) and civilians [\(15\)](#page-7-6) during warfare. Reports studying *A. baumannii* human carriage in the community are rare, and the prevalence has varied according to the countries and the identification methods used, from 0.5% to 3% in Europe [\(16](#page-7-7)[–](#page-7-8)[18\)](#page-7-9), 4% in Hong Kong [\(19\)](#page-7-10), and 5.4% in Senegal [\(20\)](#page-7-11) to 10.4% in the United States [\(21\)](#page-7-12). *A. baumannii*was also found in environments such as soil [\(22,](#page-7-13) [23\)](#page-7-14) and water [\(24,](#page-7-15) [25\)](#page-7-16) and in food products, such as vegetables [\(22,](#page-7-13) [26\)](#page-7-17), fish, meat [\(22\)](#page-7-13), and raw bulk tank milk [\(27\)](#page-7-18). Finally, in animals, the bacterium has been described as an emerging pathogen in veterinary clinics in Germany [\(28\)](#page-7-19) and Switzerland [\(29\)](#page-7-20). It was also involved in asymptomatic carriage in some animals [\(20,](#page-7-11) [30,](#page-7-21) [31\)](#page-7-22). Moreover, its presence was reported worldwide in human body and head lice [\(20,](#page-7-11) [32](#page-7-23)[–](#page-7-24)[34\)](#page-7-25), as well as in arthropods [\(35\)](#page-7-26).

Unlike clinical strains, there are limited reports using genotyping methods to explore the extrahospital epidemiology of *A. baumannii* [\(20](#page-7-11)[–](#page-7-12)[22,](#page-7-13) [30,](#page-7-21) [31,](#page-7-22) [33,](#page-7-24) [36,](#page-7-27) [37\)](#page-7-28). In Lebanon, only strains be-longing to a clinical context have been studied [\(15,](#page-7-6) [38](#page-7-29)-[40\)](#page-7-31), and no data concerning the occurrence of *A. baumannii* outside Lebanese hospitals are available. The aim of this study was to look for the presence of *A. baumannii* in different environments, to study its susceptibility to antibiotics, and to characterize the predominant community genotypes by using two genotyping approaches: multilocus sequence typing (MLST) and *bla*_{OXA-51} sequence-based typing (SBT).

MATERIALS AND METHODS

Sample collection and cultivation. Sampling was performed from February 2012 to October 2013 from soil, animals, and food products in different regions of Lebanon [\(Fig. 1\)](#page-1-0). The majority of samples were taken from two regions in North Lebanon: Tripoli, the second-largest city of the country after Beirut, and Akkar, which is a rural district.

Fifty-one soil samples were collected in urban and agricultural zones. They were suspended in water at 10%, vortexed for 15 min to fully homogenize the suspension, and then decanted for 30 min. Fifty

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FIG 1 Map of Lebanon showing the distribution of epidemiological samples between districts. E, environmental samples (soil and water); A, animal samples; F, food samples (meat, milk, and cheese).

minced meat and seven raw cheese samples, purchased from butchers and shops, were cut aseptically into very small pieces and homogenized by using a stomacher bag (Interscience, Saint Nom, France) and then suspended at 10% in sterile water. Seventy-three water samples and 37 raw cow milk samples were also collected. Finally, 379 samples from different animals recovered from fecal specimens or from rectum and/or mouth swabbing were collected. Feces were collected directly after defecation or from the cow anus to limit contamination with soil or any other sources and suspended in water at 10%. Consent was given orally by the farmers.

For all the samples except the rectal swabs, 5 ml was added to 20 ml of Baumann medium [\(41\)](#page-7-32), a minimum enrichment medium with acetate as the sole carbon source. The swabs were directly discharged in 20 ml of Baumann medium. Samples were then mixed on a rotor shaker at 200 rpm for 48 h at 37°C, and cultures were streaked on MacConkey agar with cephradine (40 mg/liter), amoxicillin (10 mg/liter), fosfomycin (30 mg/liter), and cycloheximide (400 g/liter) and incubated at 37°C for 48 h. In addition, 12 isolates identified as *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex and stored in the collection of the AZM Center for Research in Biotechnology and Its Application were incorporated in this study. They were recovered from raw cheese and lettuce.

Bacterial identification. Uncolored colonies grown on MacConkey agar were selected for further identification. Genus *Acinetobacter* identification was presumptively performed on the basis of Gram staining, a negative oxidase test, and a Vitek MS (bioMérieux, Marcy l'Étoile, France) test. Identification was further confirmed by partial sequencing of the *rpoB* gene [\(42\)](#page-7-33).

Susceptibility testing and Investigation of carbapenemase-encoding genes. Antibiotic susceptibility testing was performed by the disc diffusion method according to the guidelines of the French Comité de l'Antibiogramme de la Société Française de Microbiologie [\(http://www](http://www.sfm-microbiologie.org/UserFiles/files/casfm/CASFM_EUCAST_V1_0_2014(1).pdf) [.sfm-microbiologie.org/UserFiles/files/casfm/CASFM_EUCAST_V1_0_](http://www.sfm-microbiologie.org/UserFiles/files/casfm/CASFM_EUCAST_V1_0_2014(1).pdf) [2014\(1\).pdf\)](http://www.sfm-microbiologie.org/UserFiles/files/casfm/CASFM_EUCAST_V1_0_2014(1).pdf). The antibiotics tested were ticarcillin, piperacillin plus tazobactam, ceftazidime, imipenem, ciprofloxacin, amikacin, gentamicin, tobramycin, co-trimoxazole, colistin, netilmicin, doxycycline, and rifampin. Resistance to carbapenem was confirmed by determining the MICs of imipenem, meropenem, and doripenem with Etest strips (bioMérieux, Marcy l'Étoile, France). All identified *A. baumannii* isolates were investigated by PCR assays for the presence of the carbapenemase-encoding genes *bla*_{OXA-23} [\(43\)](#page-7-34), *bla*_{OXA-24} (43), *bla*_{OXA-58} (43), and *bla*_{OXA-143} [\(44\)](#page-8-0) and the insertion sequence IS*Aba1* [\(45\)](#page-8-1).

Genotyping. Genotyping by MLST and $bla_{\rm OXA-51}$ SBT was performed on all identified *A. baumannii* isolates. MLST was done according to the Pasteur scheme [\(http://www.pasteur.fr/recherche/genopole/PF8/mlst](http://www.pasteur.fr/recherche/genopole/PF8/mlst/Abaumannii.html) [/Abaumannii.html\)](http://www.pasteur.fr/recherche/genopole/PF8/mlst/Abaumannii.html). The bacterial population was analyzed with eBURST [\(46\)](#page-8-2) (on data available as of 8 July 2014), and when possible, isolates were assigned to their clonal complexes (CC), which are defined as the founder sequence type (ST) and its single-locus variants (SLV) [\(5\)](#page-6-4). The full length of the $bla_{\text{OXA-51}}$ gene (825 bp) was amplified and sequenced with the external primers OXA-69A/OXA-69B as described by Hamouda et al. [\(47\)](#page-8-3). The sequences were compared to those of all variants present in BLAST. New variants were submitted to GenBank and the Lahey database for beta-lactamase classification [\(http://www.lahey.org/studies/webt.asp\)](http://www.lahey.org/studies/webt.asp). Moreover, 24 new STs have been assigned by MLST Pasteur: ST286 to ST296 and ST464 to ST476.

Nucleotide sequence accession numbers. Twenty-one nucleotide sequences of bla_{OXA-51} genes were submitted to GenBank with the following accession numbers: [KF048909](http://www.ncbi.nlm.nih.gov/nuccore?term=KF048909) to [KF048919](http://www.ncbi.nlm.nih.gov/nuccore?term=KF048919) and [KJ584916](http://www.ncbi.nlm.nih.gov/nuccore?term=KJ584916) to [KJ584925.](http://www.ncbi.nlm.nih.gov/nuccore?term=KJ584925)

RESULTS

Bacterial identification. A total of 597 samples were analyzed. The 12 *Acinetobacter* strains stored in the collection of the AZM Center were also incorporated. Overall, 161 *Acinetobacter* species isolates were isolated, and among them, 42 were identified as *A. baumannii* by *rpoB* gene sequencing. [Table 1](#page-2-0) shows the distribution of the identified *Acinetobacter* species according to the sources of the samples. Briefly, no *A. baumannii* isolate was identified in soil samples. Most of the isolates were isolated from animals. Moreover, 30 isolates had new partial *rpoB* sequences and were considered putative new *Acinetobacter* species.

Antimicrobial susceptibility testing results for *A. baumannii***.** The 42 *A. baumannii* isolates identified showed a susceptible phenotype in response to most of the antibiotics tested. Two isolates were intermediate to rifampin, and one isolate was resistant to ciprofloxacin and doxycycline. Only one isolate showed resistance to carbapenems. It was isolated from a horse's mouth and was susceptible to imipenem ($MIC = 2$ mg/liter), intermediate to meropenem ($MIC = 4$ mg/liter), and resistant to doripenem $(MIC = 4 mg/liter)$. The $bla_{OXA-143}$ gene was detected by PCR and confirmed by sequencing. The insertion sequence IS*Aba1* was present in only two isolates from cats.

As for the other *Acinetobacter* sp. isolates, most were wild type, and few isolates exhibited resistance to co-trimoxazole, ciprofloxacin, doxycycline, and rifampin. Only one showed carbapenem resistance. It was identified as *Acinetobacter pittii* and was isolated from a rabbit's mouth. It showed high MICs of imipenem (16 mg/liter), meropenem ($>$ 32 mg/liter), and doripenem ($>$ 32 mg/liter) and was an OXA-24-producing isolate.

MLST analysis. MLST was performed on all 42 identified *A. baumannii* isolates [\(Table 2\)](#page-3-0). The isolates were grouped into 36 different STs, and among them, 30 were found in a single isolate. Twenty-four STs were new and reported for the first time in this study: 9 had new allelic combinations of previously known alleles (ST286 to ST288, ST293, ST464, and ST469 to ST472), and 15 had new alleles, leading to a new allelic profile (ST289 to ST292, ST294 to ST296, ST465 to ST468, and ST473 to ST476). The relationship between the STs in this study and the existing STs was studied by eBURST [\(Fig. 2\)](#page-4-0). Some detected STs belonged to clonal complexes, such as ST1 (the founder of CC1), ST216 (the founder of CC216), ST10 (the founder of CC10), ST138 and ST193 (belonging to CC33), and ST464 (belonging to CC85). Other STs shared

Acinetobacter species isolated	Total no. of isolates	Environmental isolates		Food isolates		Animal isolates	
		Source	No. of isolates found	Source	No. of isolates found	Source	No. of isolates found
A. baumannii	42	Water	$\mathbf 5$	Cheese	$\sqrt{2}$	Cow	$17\,$
				Meat	$\overline{4}$	Cat	$\mathbf{2}$
				Milk	$\mathbf{1}$	Horse	$\mathbf 1$
						Goat	3
						Dog	3
						Rabbit	$\,1$
						Donkey	$\mathbf{1}$
						Mule	$\mathbf{1}$
						Chicken	$\mathbf{1}$
A. pittii	61	Water	$\,$ 8 $\,$	Cheese	6	Cow	14
		Soil	$\boldsymbol{7}$	Lettuce	$\,4$	Horse	$\overline{4}$
				Meat	6	Goat	\overline{c}
						Dog	6
						Sheep	$\mathbf{2}$
						Rabbit	$\mathbf{1}$
						Chicken	$\mathbf{1}$
A. calcoaceticus	$\overline{4}$	Soil	$\,1$	Lettuce	$\,1$	Goat	\overline{c}
A. bereziniae	10			Meat	\mathfrak{Z}	Cow	$\overline{4}$
						Horse	$\,1$
						Dog	$\mathbf{1}$
						Pigeon	$\mathbf{1}$
A. johnsonii	$\mathbf{1}$					Rabbit	$\mathbf 1$
A. lwoffii	$\mathbf{1}$					Cat	$\mathbf{1}$
A. schindleri	\mathfrak{Z}					Cat	3
A. radioresistens	$\mathbf{1}$					Cat	$\mathbf{1}$
A. beijerinckii	$\mathbf{1}$					Cow	$\,1$
A. junii	$\mathbf{1}$					Cat	$\,1$
A. soli	$\mathbf{1}$			Lettuce	$\,1$		
A. gerneri	$\mathbf{1}$					Goat	$\mathbf{1}$
Gen. sp. 15 TU ^a	$\,4\,$					Cow	$\sqrt{2}$
						Cat	$\,1$
						Dog	$\mathbf{1}$
Putative novel Acinetobacter	30	Soil	$\,1$	Meat	$\,1$	Cow	20
species						Horse	\overline{c}
						Dog	$\mathbf{1}$
						Sheep	3
						Goose	$\mathbf{1}$
						Pig	$\mathbf 1$
Total	161		22		29		110

TABLE 1 Distribution of the identified *Acinetobacter* species according to the sources of samples

^a Gen. sp., genomic species.

similarities with known STs: ST46 with ST149, ST108 with ST112, ST221 with ST133, ST250 with ST188, and ST472 with ST439. The remaining identified STs (ST13, ST286 to ST296, ST465 to ST471, and ST473 to ST476) were singletons, and no ST similar to them has been characterized yet.

 $bla_{\text{OXA-51}}$ **SBT analysis.** The full-length sequence analysis of the *bla*_{OXA-51} genes of the 42 *A. baumannii* isolates revealed the presence of 34 gene variants, and among them, 26 were singletons and 21 had not been described before (GenBank accession numbers [KF048909](http://www.ncbi.nlm.nih.gov/nuccore?term=KF048909) to [KF048919](http://www.ncbi.nlm.nih.gov/nuccore?term=KF048919) and [KJ584916](http://www.ncbi.nlm.nih.gov/nuccore?term=KJ584916) to [KJ584925;](http://www.ncbi.nlm.nih.gov/nuccore?term=KJ584925) Lahey numbers OXA-338 to OXA-346 and OXA-407 to OXA-413) [\(Ta](#page-3-0)[ble 2](#page-3-0) and [Fig. 3\)](#page-5-0). These 34 $bla_{\text{OXA-51}}$ gene variants coded for 31 OXA protein variants, with 16 being new ones [\(Fig. 3\)](#page-5-0). [Figure 4](#page-6-8) shows an alignment of the full amino acid sequences of these new enzymes. It should be noted that the OXA-410 protein [\(KJ584919\)](http://www.ncbi.nlm.nih.gov/nuccore?term=KJ584919) had an adenine insertion at bp 820, leading to the

modification of the last amino acid (L274Y) and the addition of 4 supplementary amino acids. The OXA-409 protein [\(KJ584918\)](http://www.ncbi.nlm.nih.gov/nuccore?term=KJ584918) had an insertion of a cysteine at amino acid position 19. The new enzyme OXA-338 is encoded by two different nucleotide sequences (GenBank accession numbers [KF048909](http://www.ncbi.nlm.nih.gov/nuccore?term=KF048909) and [KJ584925\)](http://www.ncbi.nlm.nih.gov/nuccore?term=KJ584925). Finally, the [KJ584925](http://www.ncbi.nlm.nih.gov/nuccore?term=KJ584925) gene sequence differed from that of [KF048909](http://www.ncbi.nlm.nih.gov/nuccore?term=KF048909) by 6 synonymous mutations.

Comparison between MLST and $bla_{\text{OXA-51}}$ **typing.** Most of the STs described in this study had a specific *bla*_{OXA-51}-like gene variant [\(Table 2\)](#page-3-0), with the exception of ST216 and ST470, both of which had the *bla*_{OXA-51} variant, and ST295 and ST468, both of which had the $bla_{\rm OXA\text{-}344}$ variant. Overall, each ST led to a specific OXA protein variant, except ST469 and ST475, both of which had the OXA-71 protein variant; ST1 and ST292, which had the OXA-69 protein variant; and ST118 and ST286, which had the OXA-338 protein variant [\(Table 2](#page-3-0) and [Fig. 3\)](#page-5-0).

^a The novel STs found in this study are in boldface.

b OXA-51 protein variants found for the first time in this study. GenBank accession numbers are provided in parentheses for new *bla*_{OXA-51} gene variants described for the first time in this study.

DISCUSSION

Although the ubiquitous existence of *A. baumannii* in nature has been considered a common misconception by some authors [\(7\)](#page-6-6), several recent studies have undeniably highlighted the presence of extrahospital reservoirs [\(9,](#page-7-0) [22,](#page-7-13) [30,](#page-7-21) [31,](#page-7-22) [33\)](#page-7-24). These observations have mainly been made through recent implementation of molecular methods, such as *bla*_{OXA-51}-like PCR or *rpoB* gene sequencing, improving detection and identification of *A. baumannii* and other species of the genus *Acinetobacter* [\(42,](#page-7-33) [48,](#page-8-4) [49\)](#page-8-5). In our study, we evidenced the extrahospital presence of *A. baumannii* in Lebanon. We showed that 8% of the animals studied carried *A. baumannii* in their flora. These animals lived on farms or were wild animals and had never been in contact with a hospital environment, such as veterinary clinics. *A. baumannii* has previously been documented as an animal colonizer with different prevalences in different countries: in Senegal, 5.1% [\(20\)](#page-7-11); in Scotland, 1.2% [\(30\)](#page-7-21); and on La Reunion Island, 6.5% [\(31\)](#page-7-22). We have also found the

bacterium in food produced from animals, such as cow meat, raw cheese, and raw milk, reinforcing the idea that animals could be a potential reservoir of *A. baumannii*. Additionally, we have isolated the bacterium from water samples, while all the soil samples tested were negative. Two hypotheses can arise from the detection of *A. baumannii* in water: that water is a normal habitat of *A. baumannii* or that the presence of the bacterium results from human or animal contamination. We cannot exclude either of these hypotheses, but the detection of a novel ST (ST294) in a horse's mouth, as well as in an artesian well, could support the second hypothesis.

We have studied the *A. baumannii* population structure by MLST typing, which is considered a gold standard and is intensively applied in the characterization of genotypes circulating in hospitals. The current MLST-based global population structure is formed by 26 clones divided into 18 international clones and 8 European- or Asian-restricted clones [\(2\)](#page-6-1). However, it is evident that there are no sufficient data regarding genotypes of isolates

FIG 2 Population snapshot determined by eBURST analysis of 587 sequences present in the MLST Pasteur database (last update, 8 July 2014). The dots represent STs. The STs identified in this study are shown next to their corresponding dots. Boldface indicates a new ST described in this study. The large circles indicate that our identified ST belonged to a clonal complex, whose name is shown next to the circle.

isolated outside hospitals. Improving the MLST database with extrahospital *A. baumannii* genotypes could be important, since it may improve our understanding of the potential reservoirs, the origins of human infections, and the acquisition of resistance mechanisms in the species. In our study, we identified 36 STs of *A. baumannii*; some were identical to those isolated in human infections, and 24 were new genotypes never reported previously. They were all found in animals or in animal-derived food and showed huge diversity in population structures. This diversity was not in accordance with the findings of Hamouda et al. [\(30\)](#page-7-21), who found only 4 different genotypes in the isolates recovered from cattle and pigs in their study, and the results of Belmonte et al. [\(31\)](#page-7-22), who revealed the presence of a single pulsed-field gel electrophoresis (PFGE) genotype/ST25 in pets recovered from geographically distant veterinary clinics on La Reunion Island.

The $bla_{\text{OXA-51}}$ gene is an intrinsic carbapenemase gene specific to *A. baumannii* and is regarded as a tool for *A. baumannii* identification [\(50\)](#page-8-6). Analysis of our 42 *A. baumannii* isolates allowed the identification of 34 *bla*_{OXA-51} gene variants. Among them, 21 were new. These 21 sequences were all associated with animal origins, showing a potentially huge diversity in the *A. baumannii* population. These observations have previously been made by Hamouda et al., who reported three new *bla*_{OXA-148}, *bla*_{OXA-149}, and *bla*_{OXA-150} variants in cattle in Scotland [\(30\)](#page-7-21). In addition, our results illustrate the usefulness of $bla_{\text{OXA-51}}$ as a single-locus-based typing method [\(51\)](#page-8-7). We observed good correlation between

MLST and $bla_{\text{OXA-51}}$ typing, since each $bla_{\text{OXA-51}}$ like gene variant had its specific ST, with two exceptions: ST216 and ST470, which had the same *bla*_{OXA-51} gene, and ST295 and ST468, which had the same *bla*_{OXA-344} gene. Our *bla*_{OXA-51} gene-sequencing results were concordant with the worldwide MLST results reported in other studies, such as $bla_{\text{OXA-69}}$ usually being associated with ST1 [\(51\)](#page-8-7), $bla_{\text{OXA-120}}$ with ST193 [\(52\)](#page-8-8), $bla_{\text{OXA-68}}$ with ST10 [\(3,](#page-6-2) [53\)](#page-8-9), and *bla*_{OXA-94} with ST85 [\(15\)](#page-7-6). Finally, some *bla*_{OXA-51} variants found in our new STs have previously been described in human STs. Thus, bla_{OXA-71} , detected in our ST469 *A. baumannii* isolate, has been described in the international clone 3 [\(51\)](#page-8-7). Similarly, *bla*_{OXA-64}, previously reported in ST25 [\(51\)](#page-8-7), has been found in ST138. It is interesting that ST138 is a trilocus variant of ST25.

Analysis of the antibiotic susceptibility results showed that a susceptible *A. baumannii* population prevailed outside hospitals in Lebanon. The majority of the isolates (40/42) lacked IS*Aba1*, an insertion sequence that is considered the first step in resistance evolution in *A. baumannii* [\(54\)](#page-8-10). However, we identified a *bla*_{OXA-143} gene in an *A. baumannii* isolate from a horse, as well as a *bla*_{OXA-24} gene in an *A. pittii* isolate from a rabbit. These results highlight the potential presence of reservoirs of resistance genes in the environment. The $bla_{\text{OX}_{A-143}}$ gene has recently been detected and reported only in Brazil and South Korea [\(50\)](#page-8-6). Until now, most of the *A. baumannii* populations detected outside hospitals were susceptible to antibiotics [\(21,](#page-7-12) [27,](#page-7-18) [30](#page-7-21)[–](#page-7-22)[32\)](#page-7-23). However, there have been growing concerns after the description of a *bla*_{OXA-23} gene from

FIG 3 Maximum-likelihood nucleotide tree of 35 *bla*OXA-51-like genes. These 35 genes correspond to 34 *bla*OXA-51 variants identified in this study and the *bla*_{OXA-66} gene (the blaOXA-51 representative of clonal complex 2). MEGA 6 was used to build the phylogenetic tree. Bootstrap values are shown at the nodes. One thousand replicates were used to calculate the bootstrap values.

human stool and lice in Senegal [\(20\)](#page-7-11); NDM-1-producing *A. baumannii* tk;4from a pig in China [\(55\)](#page-8-11); and other carbapenemase-producing *Acinetobacter* spp. from pets, food, and their environments [\(56\)](#page-8-12).

One other important finding in our study is the identifica-

tion of 30 isolates with low levels of nucleotide homology with all available described *Acinetobacter* species, which are assumed to be putative novel species. These observations show the species diversity of environmental isolates within the genus *Acinetobacter*. Several publications have described new isolates [\(22,](#page-7-13) [36,](#page-7-27) [57\)](#page-8-13),

FIG 4 Amino acid sequence alignment of the 16 new OXA-51-like proteins detected in this study and of OXA-51 (the founding member of OXA-51-like beta-lactamases), OXA-66 (an OXA-51 representative variant of CC2), OXA-69 (an OXA-51 representative variant of CC1), and OXA-71 (an OXA-51 representative variant of CC3) [\(52\)](#page-8-8). Protein accession numbers: OXA-51, [WP_002033109.1;](http://www.ncbi.nlm.nih.gov/nuccore?term=WP_002033109.1) OXA-66, [YP_001846219.1;](http://www.ncbi.nlm.nih.gov/nuccore?term=YP_001846219.1) OXA-69, [YP_001713983.1;](http://www.ncbi.nlm.nih.gov/nuccore?term=YP_001713983.1) and OXA-71, [WP_001021785.1.](http://www.ncbi.nlm.nih.gov/nuccore?term=WP_001021785.1)

which indicates that our knowledge of the genus *Acinetobacter* is still evolving.

In conclusion, our paper reports the occurrence of *A. baumannii* isolates outside Lebanese hospitals and is one of a limited number of worldwide studies exploring the population in the environment, food, and animals. Detection of successful human genotypes, such as international clones 1 and 10, in water and animals is a worrying issue for public health. Furthermore, the roles of newly identified animal clones and their involvement in human diseases, especially in community-acquired infections, should be investigated. Our findings suggest that animals could be a potential reservoir for *A. baumannii* and the spread of new, emerging carbapenemase genes, such as $bla_{\text{OXA-143}}$, to humans. Additional large epidemiological studies are required to confirm the significance of our primary results and to determine the real distribution of these clones in Lebanon and the possible interactions between the different environments.

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