Emergence and multi-lineages of carbapenemase-producing Acinetobacter baumannii-calcoaceticus complex from canine and feline origins

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ABSTRACT. The carbapenemase-producing Acinetobacter baumannii is an important opportunistic bacterium and frequently causes hospital-acquired infections in humans. It also has increasingly been reported in veterinary medicine. This study illustrates multiple clones of carbapenemase-producing A. baumannii disseminating and causing diseases in dogs and cats in Thailand. Between 2016 and 2020, 44 A. baumannii and two A. pittii isolates exhibiting imipenem resistance (MIC≥16 μg/mL) from diagnostic samples were characterized by Pasteur multilocus sequence typing (MLST), sequence grouping (SG), repetitive extragenic palindromic element (rep)-PCR fingerprint analysis and antimicrobial resistance (AMR) profiling. All isolates contained blaOXA-23 in the Tn2006 family, and A. baumannii showed the sequence type (ST) 16 (14/44), ST149 (12/44), ST25 (6/44), ST2 (4/44), ST1581 (3/44), ST23 (2/44), ST1575 (1/44) and ST1576 (1/44). DNA fingerprint analysis and SG illustrated clonal relationships in the STs and its single locus variants, and AMR gene profiles, including tetracycline and aminoglycoside resistance genes, showed minor variations in the clones. The findings suggest that blaOXA-23 has been spread in multiple clones of A. baumannii and A. pittii from canine and feline hosts. With the collection of multiple AMR genes and intrinsic resistance, antimicrobial options are limited for treatment, and pets can be a potential reservoir of extensively drug-resistant, carbapenemase-producing A. baumannii in the community. Epidemiological tracking by passive and active surveillance in animals, veterinary personnel and hospital environment and preventive measurements should be promoted to decrease the risk of infection and transmission to humans.

KEYWORDS: Acinetobacter baumannii, carbapenemase, cat, dog, multi-lineages

Acinetobacter baumannii is a major non-fermenting gram-negative opportunistic pathogen in humans and a member of the A. baumannii-calcoaceticus (Abc) complex, which includes A. calcoaceticus, A. baumannii, A. pittii, A. nosocomialis, A. seifertii and A. dijkshoorniae [8, 10, 27]. Intrinsic β-lactamases and efflux pumps in these species naturally mediate resistance to various antimicrobial classes, such as amino-penicillins, first- to third- generation cephalosporins, clavulanic acid, macrolides, lincosamides and nitrofurantoin [19]. Particular clones associated with hospital outbreaks develop additional resistance by acquiring resistance genes, making them extensively drug-resistant (XDR) strains, limiting therapeutic options for hospitalized patients and leading to life-threatening outcomes [12, 15]. Carbapenems are most critically last-resort antimicrobials for the treatment of multidrug-resistant (MDR) gram-negative bacterial infections in humans and small animals [3, 20]. However, A. baumannii can hydrolyze carbapenems by acquiring genes encoding carbapenemases. The blaOXA-23 is the most common variant identified in this species and widely associated with Tn2006, Tn2007 and Tn2008 families [25]. Molecular characterization has identified major nosocomial outbreak clones, including

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sequence type (ST) 1 and ST2 by the Pasteur multilocus sequence typing (MLST) scheme, which are resembled to international clone (IC) 1 and IC2, which spread globally [9].

In companion animals, A. baumannii infection is predisposed by prolonged hospitalization, intubation and anesthesia, mechanical ventilation, urinary catheterization, prior cephalosporin treatment, prior operation and immunosuppression [18]. Opportunistic infections in various body systems caused by Acinetobacter spp. have been reported, with increasing prevalence of antimicrobial resistance (AMR) to the drugs used in small animal practice [5, 24, 28, 31, 41]. A carbapenemase-producing strain of A. baumannii ST2 harboring bla\textsubscript{OXA-23} was first detected in a cat with urinary tract infection (UTI) in Portugal, and a closely identical strain of ST2 was isolated from a dog with UTI in Thailand [5, 31], indicating the emergence of the dissemination of pandemic nosocomial clones to animals in the community.

From our observation in routine diagnostic samples submitted between 2016 and 2020, carbapenem-resistant A. baumannii (CR-Ab) and A. pittii that displayed a variety of MDR patterns have been identified, indicating proliferation or clonal variation of the isolates from canine and feline origins. Information regarding CR-Ab in companion animals is relatively rare compared with that in humans and of other MDR bacterial pathogens, and little is known about its clonal spreading. Therefore, this study aims to illustrate a variety of genetic and AMR characteristics of the clinical CR-Ab isolates from clinical specimens from diseased dogs and cats.

**MATERIALS AND METHODS**

**Sources of Abc complex isolates**

The Abc isolates were obtained from routine clinical samples submitted from 10 small animal hospitals or clinics to the Veterinary Diagnostic Laboratory of the Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand, between April 2016 and June 2020. Acinetobacter spp. and antimicrobial susceptibilities were examined by Vitek 2 identification (ID-GN card) and antimicrobial susceptibility testing (AST) (AST-GN65 or AST-GN97 card), using an automated system (bioMerieux, Marcy L’Étoile, France). The bacteria were kept in 20% glycerol stock at −80°C. The CR-Ab isolates presenting an imipenem minimum inhibitory concentration (MIC) level ≥16 μg/mL were regrown on 5% sheep blood agar for further procedures.

**Species identification and AST**

Species of the Abc complex isolates were identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Microflex Biotyper) (Bruker Dalnontics GmbH, Bremen, Germany). The MIC to antimicrobials was determined by the broth microdilution assay, using Sensititre EUVSEC and COMPANIF plates (Thermo Scientific, East Grinstead, West Sussex, UK). Acquired resistance was interpreted according to the Clinical and Laboratory Standards Institute (CLSI) interpretive criteria, which included ticarcillin/clavulanic acid (≥128/2 μg/mL), ceftazidime (≥32 μg/mL), meropenem (≥8 μg/mL), imipenem (≥8 μg/mL), gentamicin (≥16 μg/mL), amikacin (≥64 μg/mL), tetracycline (≥16 μg/mL), doxycycline (≥16 μg/mL), ciprofloxacin (≥4 μg/mL), trimethoprim/sulfamethoxazole (≥24/76 μg/mL) and colistin (≥4 μg/mL) [7].

**Detection of AMR genes**

Genomic DNA was isolated using the Nucleospin Tissue DNA extraction kit (Machery Nagel, Düren, Germany). Acquired AMR genes, which encode mechanisms of resistance to carbapenems (bla\textsubscript{OXA-23-like}), tetracyclines [tet(B) and tet(39)] and aminoglycosides (str\textsubscript{A}, str\textsubscript{B}, aac(3)-Ia and aac(6′)-Im) in Acinetobacter spp., were detected by simplex PCRs (Supplementary Table 1). Additional aminoglycoside-modifying enzyme (AME)-encoding genes including aac(3)-Ia, aac(4)-Ib, aph(3′)-VI, ant(2′)-Ia, aph(3′)-Ia and aac(6′)-Ib were identified by multiplex PCRs [30]. A 25-μL PCR mixture was prepared using the 5X Firepol Master Mix (Solis BioDye, Tartu, Estonia) with 0.2 μM of each primer. Entire bla\textsubscript{OXA-23} were analyzed by Sanger’s capillary sequencing. Specific regions of IS\textsubscript{Abi} and IS\textsubscript{Abi4} at both extremities of the bla\textsubscript{OXA-23}-containing transposon were amplified by PCR to illustrate the presence of transposable elements [25].

**Molecular typing**

The IC of A. baumannii isolates was identified by tri-locus sequence-based typing (3LST), specifically amplifying omp\textsubscript{A}, csu\textsubscript{E} and bla\textsubscript{OXA-51-like} [38, 42]. The STs were identified by Pasteur’s MLST scheme, which analyzed internal sequence of cmn\textsubscript{600}, fus\textsubscript{A}, glt\textsubscript{A}, pyr\textsubscript{G}, rec\textsubscript{A}, rpl\textsubscript{B} and rpo\textsubscript{D} to differentiate clones of the isolates. New STs and alleles were assigned by submission to the curator (www.pubmlst.org). Repetitive extragenic palindromic element-PCR (rep-PCR) was performed in 50-μL PCR reaction with 1 μM of REP-1 and REP-2 primers [40]. The PCR products were run in 1% Tris-acetate EDTA agarose gel electrophoresis to illustrate DNA fingerprint patterns. To illustrate clonal relationships, a dendrogram was constructed by UPGMA with 1% position tolerance, using the BioNumeric Software (Applied Maths, Sint-Martens-Latem, Belgium).

**RESULTS**

**Prevalence of Acinetobacter spp. from canine and feline clinical samples**

Of the 8,457 samples, Acinetobacter spp. was isolated from 125 (1.48%) samples submitted during a 4-year period between April 2016 and June 2020. Of 125 isolates, the Abc complex was the predominant (103 isolates). The non-baumannii-calcoaceticus complex Acinetobacter spp. included A. lwoffi (14 isolates), A. radioresistens (3 isolates), A. junior (2 isolates), A. haemolyticus (2 isolates), A. ursingii (1 isolate) (Table 1). Of 103 Abc complex isolates, 53 and 49 isolates were recovered from canine and feline samples,
respectively, and one isolate was from an unknown host species. Four *A. baumannii* isolates, presenting different AST patterns, were isolated at four sample collection times from two wound lesions of the same cat within a 1-month period in 2019. Origins related to clinical manifestation of the Abc complex isolates are illustrated in Table 2. Forty-six Abc complex isolates were resistant to imipenem, presenting MIC ≥16 μg/mL by Vitek AST.

**bla**\textsubscript{OXA-23}-harboring Abc complex

The 46 carbapenem-resistant isolates were *A. baumannii* (44 isolates) and *A. pittii* (2 isolates) and contained **bla**\textsubscript{OXA-23} that were recovered from samples submitted from six small animal hospitals (A to G) in Bangkok (Table 3). Two *A. pittii* were isolated from hospital A in 2018 only. The proportions of the **bla**\textsubscript{OXA-23} carriage in the Abc complex isolated from clinical samples each year were 1/9 (2016), 3/14 (2017), 12/32 (2018), 25/36 (2019) and 5/12 (2020). Clinical manifestations of the animals infected with **bla**\textsubscript{OXA-23}-harboring Abc complex were UTI, followed by wounds and abscesses, body cavities or internal organs, as well as prostatic abscesses (Table 2).

**Genetic background and multiple clones of **bla**\textsubscript{OXA-23}-containing Abc complex**

All **bla**\textsubscript{OXA-23}-harboring Abc complexes consistently contained the composite transposon Tn2006-like, which is flanked by IS\textsubscript{Abal} at both extremities. Of 44 CR-Ab isolates, nine STs were identified, including ST2 (4 isolates), ST16 (14 isolates), ST23 (2 isolates), ST25 (6 isolates), ST149 (12 isolates), ST1093 (1 isolate), ST1575 (1 isolate), ST1576 (1 isolate) and ST1581 (3 isolates) (Table 3). Both ST1576 (allelic profile 3-3-2-4-224-2-4) and ST1581 (allelic profile 3-3-13-4-7-2-4) were new single locus variants (SLV) of ST25. Four ST25 isolates were from multiple wound samples of the same cat. The ST1576 had a new **recA** allelic number 224, presenting a single nucleotide polymorphism (T19A) in that of ST25. The ST1575 was a SLV of ST2 with a new **rpoB** allelic number 217 (C387T). Genes of SG2 in 3LST were negative in all isolates; **ompA**, **bla**\textsubscript{OXA-66} and **csuE** of SG1 were positive for ST2 and ST1575, which were IC2. Other STs had different positive genes of SG1, but all ST16 isolates were negative to all gene amplifications in 3LST (Table 3). Clonal relationships based on dendrogram construction from rep-PCR DNA fingerprint patterns presented six clusters (A to F) that were correlated with the sequence types (Fig. 1).

**Table 1.** Numbers of canine and feline clinical samples positive for *Acinetobacter* between 2016 and 2020

| Year | Total samples | No. of samples with *Acinetobacter* isolation | *A. baumannii* complex | *A. lwoffii* | *A. radioresistens* | *A. junii* | *A. haemolyticus* | *A. ursingii* |
|------|---------------|---------------------------------------------|------------------------|-------------|------------------|-------------|------------------|-------------|
| 2016 | 958           | 12                                          | 9                      | 2           | 0                | 0           | 1                | 0           |
| 2017 | 1,772         | 23                                          | 14                     | 6           | 1                | 0           | 1                | 1           |
| 2018 | 1,864         | 36                                          | 32                     | 3           | 1                | 0           | 0                | 0           |
| 2019 | 2,690         | 39                                          | 36                     | 1           | 1                | 1           | 0                | 0           |
| 2020 | 1,173         | 15                                          | 12                     | 2           | 0                | 1           | 0                | 0           |
| Total | 8,457         | 125                                         | 103                    | 14          | 3                | 2           | 2                | 1           |

**Table 2.** Number of *Acinetobacter* spp., *A. baumannii-calcoaceticus* complex (Abc) and carbapenem-resistant Abc complex isolates associated with clinical manifestation and sampling sites

| Sampling site or lesion | *Acinetobacter* spp. | *A. baumannii-calcoaceticus* complex | Carbapenem-resistant *A. baumannii-calcoaceticus* complex |
|-------------------------|-----------------------|--------------------------------------|--------------------------------------------------------|
| Wound and abscess       | 48                    | 40                                   | 15                                                     |
| Urinary tract           | 39                    | 33                                   | 25                                                     |
| Body cavity and internal organs | 14                  | 10                                   | 4                                                      |
| Skin                    | 4                     | 2                                    | 0                                                      |
| Oral cavity             | 4                     | 3                                    | 0                                                      |
| Bone fracture           | 3                     | 3                                    | 0                                                      |
| Ear canal               | 3                     | 2                                    | 0                                                      |
| Nasal discharge and mucosa | 3                     | 3                                    | 0                                                      |
| Prostatic abscess       | 2                     | 2                                    | 2                                                      |
| Lower respiratory tract | 2                     | 2                                    | 0                                                      |
| Subcutaneous            | 1                     | 1                                    | 0                                                      |
| Unknown                 | 2                     | 2                                    | 0                                                      |
| Total                   | 125                   | 103                                  | 46                                                     |
Table 3. Source, genetic characteristics and antimicrobial resistance gene of 44 carbapenemase-producing *Acinetobacter baumannii* isolated from canine and feline clinical samples (2016–2020)

| Sequence type (ST) | Year (No. of isolate) | Hospital (No. of isolate) | Host (No. of isolate) | Infection (No. of isolate) | Tn2006-like | Genes of sequence group I | Antimicrobial resistance genes |
|-------------------|-----------------------|---------------------------|----------------------|---------------------------|-------------|--------------------------|-------------------------------|
| ST2               | 2017 (3), 2018 (1)    | A (4)                     | Dog (3), Cat (1)     | UTI (3), Wound (1)        | +           | E + + +                  | blaoxa-23, tet(B), aac(6')-Im, aac(3)-Ia, strA, strB |
|                   |                       |                           |                      |                           |             |                         |                               |
| ST16              | 2018 (1)              | A (2)                     | Dog (1)              | UTI (1)                   | +           | C - -                    | blaoxa-23, tet(B), tet(39), aac(3)-Ia, strA, strB |
|                   |                       |                           |                      |                           |             |                         |                               |
| ST16              | 2019 (1), 2020 (1)    | A (1)                     | Dog (1), Cat (1)     | UTI (2)                   | +           | C - -                    | blaoxa-23, tet(B), strA, strB |
|                   |                       |                           |                      |                           |             |                         |                               |
| ST16              | 2018 (1), 2019 (10)   | A (8), F (2), D (1)       | Dog (5), Cat (6)     | UTI (5), Wound (5), Peritonitis (1) | +           | C - -                    | blaoxa-23, tet(B), aac(3)-Ia, strA, strB |
|                   |                       |                           |                      |                           |             |                         |                               |
| ST23              | 2016 (1)              | A (1)                     | Cat (1)              | UTI (1)                   | +           | F + -                    | blaoxa-23, tet(B), aac(6')-Ia, strA, strB |
|                   |                       |                           |                      |                           |             |                         |                               |
| ST23              | 2019 (1)              | A (1)                     | Dog (1)              | UTI (1)                   | +           | F + -                    | blaoxa-23, tet(B), aac(6')-Ia, strA, strB |
|                   |                       |                           |                      |                           |             |                         |                               |
| ST25              | 2018 (1)              | B (1)                     | Cat (1)              | UTI (1)                   | +           | A - -                    | blaoxa-23, tet(B), strA, strB, aac(3)-Ia |
|                   |                       |                           |                      |                           |             |                         |                               |
| ST25              | 2018 (1)              | A (1)                     | Cat (1)              | Wound (1)                 | +           | A - -                    | blaoxa-23, tet(B), strA, strB, aac(3)-Ia |
|                   |                       |                           |                      |                           |             |                         |                               |
| ST25              | 2019 (4)              | A (4)                     | Cat (4)*             | Wound (4)                 | +           | A - -                    | blaoxa-23, tet(B), strA, strB, aac(3)-Ia |
|                   |                       |                           |                      |                           |             |                         |                               |
| ST149             | 2018 (3), 2019 (3), 2020 (3) | A (7), C (1), G (1)     | Cat (3), Dog (6)    | UTI (6), Wound (1), Prostatic abscess (1) | +           | D + -                    | blaoxa-23, tet(B), strA, strB, aac(3)-Ia, strB, aac(6')-Ia, aph(3')-VI, aac(6')-Ib |
|                   |                       |                           |                      |                           |             |                         |                               |
| ST149             | 2019 (1)              | A (1)                     | Cat (1)              | Wound (1)                 | +           | D + -                    | blaoxa-23, tet(B), strA, strB, aac(3)-Ia, strB, aac(6')-Ia, aph(3')-VI, aac(6')-Ib |
|                   |                       |                           |                      |                           |             |                         |                               |
| ST149             | 2019 (1)              | A (1)                     | Dog (1)              | Wound (1)                 | +           | D + -                    | blaoxa-23, tet(B), strA, strB, aac(3)-Ia, strB, aac(6')-Ia, aph(3')-VI, aac(6')-Ib |
|                   |                       |                           |                      |                           |             |                         |                               |
| ST149             | 2019 (1)              | E (1)                     | Cat (1)              | UTI (1)                   | +           | D + -                    | blaoxa-23, tet(B), strA, strB, aac(3)-Ia, strB, aac(6')-Ia, aph(3')-VI, aac(6')-Ib |
|                   |                       |                           |                      |                           |             |                         |                               |
| ST1093            | 2018 (1)              | A (1)                     | Cat (1)              | UTI (1)                   | +           | A + -                    | blaoxa-23, tet(B), strA, strB, aac(3)-Ia, strB, aac(6')-Ia, aph(3')-VI, aac(6')-Ib |
|                   |                       |                           |                      |                           |             |                         |                               |
| ST1575            | 2018 (1)              | A (1)                     | Cat (1)              | Uroabdomen (1)            | +           | E + + +                  | blaoxa-23, tet(B), strA, strB, aac(3)-Ia, strB, aac(6')-Im |
|                   |                       |                           |                      |                           |             |                         |                               |
| ST1576            | 2018 (1)              | A (1)                     | Dog (1)              | Prostatic abscess (1)     | +           | A - +                    | blaoxa-23, tet(B), strA, strB |
|                   |                       |                           |                      |                           |             |                         |                               |
| ST1581            | 2019 (2), 2020 (1)    | A (3)                     | Dog (2), Cat (1)     | UTI (3)                   | +           | A - +                    | blaoxa-23, tet(B), strA, strB, aac(3)-Ia |

* Four isolates were from the same cat. UTI, urinary tract infection; Antimicrobial resistance genes: blaoxa-23, OXA-23 carbapenemase; strA, streptomycin resistance; strB, streptomycin resistance; tet(B), tetracycline efflux protein; tet(39), tetracycline efflux protein; aac(3)-Ia, aminoglycoside acetyltransferase (gentamicin resistance); aac(3)-Ia, amino-glycoside acetylation transferase (gentamicin resistance); aac(6')-Ia, aminoglycoside acetylation transferase (amikacin resistance); aac(6')-Im, aminoglycoside acetylation transferase (amikacin resistance); ant(2'')-Ia, aminoglycoside acetylation transferase (gentamicin resistance); aph(3')-VI, aminoglycoside phosphotransferase (amikacin resistance).

AMR phenotypes and genes

The AMR phenotype of the isolates is illustrated in Fig. 1. All blaoxa-23-containing *A. baumannii* and *A. pittii* isolates express resistance to imipenem, meropenem, ticarcillin/clavulanic acid and ciprofloxacin and showed high MIC levels for ticarcillin (>64 μg/mL), nalidixic acid (>128 μg/mL), enrofloxacin (>2 μg/mL) and marbofloxacin (>2 μg/mL). The rates of resistance to aminoglycosides, tetracyclines and potentiated sulfonamides varied, including those of gentamicin (44/46), amikacin (28/46), tetracycline (40/46), doxycycline (39/46) and sulfamethoxazole/trimethoprim (42/46). Non-susceptibility to cefazidime (MIC >8 μg/mL) was observed in 25 of 46 isolates; two CR-Ab isolates presented resistance to colistin.

The AMR gene profile illustrated in Table 3, AME-encoding genes were detected, including streptomycin resistance genes [strA and strB (42/46)], gentamicin resistance genes [ant(2'')-Ia (4/46); aac(3)-Ia (5/46); aac(3)-Ia (21/46)] and amikacin resistance genes [aph(3')-VI (12/46); aac(6')-Ib (12/46); aac(6')-Im (4/46)]. Each of the genes encoding gentamicin resistance was detected in 22 gentamicin-resistant isolates but not in 15 gentamicin-resistant isolates. In amikacin-resistant isolates, aph(3')-VI and aac(6')-Ib were detected in the common 12 isolates, and aac(6')-Im was found in five isolates. However, amikacin resistance genes were not observed in 11 of 28 isolates presenting amikacin resistance. Overall, 23 and 30 isolates contained tet(B) and tet(39), respectively, 11 of which carried both genes. Tetracycline resistance genes were not found in four tetracycline- and doxycycline-susceptible isolates. Of these, tet(39) and ant(2'')-Ia were identified in two *A. pittii* isolates that showed distinct AMR profiles (Fig. 1).

Clonal spread and antimicrobial resistance genotypes

Table 3 presents the antimicrobial resistance genes found in blaoxa-23-carrying *A. baumannii* STs. The ST149 carried the highest numbers of detected tet and AME-encoding genes; 9 of 12 isolates carried tetracycline resistance genes [tet(B); tet(39)], streptomycin
**Fig. 1.** Dendrogram illustrating clonal relationships by rep-PCR analysis of carbapenemase-producing *Acinetobacter baumannii-calcoaceticus* complex (Ab, *Acinetobacter baumannii*; Ap, *Acinetobacter pittii*; AMK, Amikacin; COL, Colistin; CTZ; Ceftazidime; DOX, Doxycycline; GEN, Gentamicin; SXT, Trimethoprim/sulfamethoxazole; TET, Tetracycline).
resistance genes (strA; strB) and amikacin resistance genes [aph(3’)-VI; aac(6’)-Ib]. The ST2 and ST1575 carried aac(6’)-Ib (amikacin acetyltransferase), but aac(3)-Ia (gentamicin acetyltransferase) was identified in ST2 only. The ant(2’)-Ia was specifically found in ST23, and aac(3)-Ia was carried by ST16, ST25 and ST1581.

The tet genes were more widely distributed in several STs; tet(B) was more common in ST149, clonal complex (CC) 2 (ST2 and ST1575) and CC25 (ST25, ST1576 and ST1581). Only two isolates of ST16 carried tet(B), but tet(39) was abundantly found in ST16, ST23 and ST149.

**DISCUSSION**

Since the first identification of canine carbapenemase-producing *A. baumannii* ST2 [5], multiple STs of carbapenemase-producing *Acinetobacter baumannii* and *A. pittii* have been isolated from canine and feline clinical samples. Similar to human samples, *A. baumannii* was the predominant species associated with infections in dogs and cats and mostly associated with blaOXA-23 carriage. However, carbapenem resistance has not frequently been presented [4]. As a nosocomial problem, infections caused by the MDR Abc complex lead to 70% mortality in an intensive care unit of a veterinary hospital, especially in the case of respiratory tract infection [18]. Respiratory tract infection is the most frequent complication caused by *A. baumannii* in critically ill animal patients [1, 18]. In this study, the highest number of Abc isolates originated from samples associated with wounds and abscesses as well as UTI, but isolates from the lower respiratory tract were not commonly found. Samples from bronchoalveolar lavage or endotracheal aspiration were unusually submitted because of the anesthesia-requiring invasive procedure, limiting the number of the bacterial isolates from this origin. Compared with human medicine, little is known regarding *A. baumannii* infections in veterinary medicine [26, 29, 39]. Extending the MALDI-TOF MS database allows rapid phenotypic differentiation of Abc complex isolates, which identified *A. baumannii* and *A. pittii* in this study [16, 36]. Effective diagnostic schemes by fast and accurate bacterial species identification and AST should be promoted and implemented to provide reliable information in veterinary practice.

The majority of the CR-Ab were ST16, ST149 and ST25, which have increasingly been found since 2018 in addition to ST2 [5], indicating multiclonal dissemination in small animals. The predominant ST16 and ST149 were previously isolated from medical settings in various countries, including Thailand [2, 6, 9, 23, 33–35]. In Thailand’s healthcare settings, CR-Ab ST16 has been reported as a minor lineage with approximately 2.96% [17]. Of the 44 CR-Ab isolates analyzed, 14 and 12 isolates belonged to ST16 and ST149, respectively, and were found in samples submitted from several veterinary settings, indicating that CR-Ab isolates belonging to these clones are widespread in dogs and cats as well. The CC25 presented the highest intraclonal variation by single locus variants, positive genes in 3LST and AMR genotypes and phenotypes. A heterogenous population and divergent evolution in this CC could be speculated based on mutation and gene loss. The ST25 isolated in 2018 contained tet(B), which was not found in the same ST isolated in 2019; however, tet(B) occurred in ST1581, which was first identified in 2019. French studies have reported blaOXA-23-containing *A. baumannii* ST25 in companion animals in the community [14, 22]. Combined with the findings of our study, animals visiting veterinary settings or discharged from hospitals could be a reservoir of XDR and carbapenemase-producing *A. baumannii* [32, 39]. Compared with other STs, CC2 was minor in 2017–2018 and has not been detected since 2019 in the current study. However, CC2 is one of the major nosocomial outbreak strains reported globally and represents dissemination of the significant human healthcare-associated *A. baumannii* clone in companion animals [9, 15, 37, 42]. These findings suggested that, in addition to ST2, pets could be an important reservoir of the emergent carbapenemase-producing *A. baumannii* clones in the community.

Clonal dissemination of carbapenemase-producing *A. baumannii* could be described by its genetic characteristics. In addition, resistance gene carriage was different within and among the clones, reflecting minor variations within the population. The Pasteur MLST scheme could differentiate the clones with adequate discriminatory power in this study and is more reliable in studies on *A. baumannii* during the temporal period toward core genome MLST to avoid an effect from the presence of the paralog Oxford gene gdhB (gdhB2) in some *A. baumannii* genomes [13]. Most of the isolates, except CC2, were not typeable into SG by 3LST; blaOXA-23-containing ST2 is a classical SG1 or IC2, causing nosocomial outbreaks worldwide [15]. The relevant results from rep-PCR fragment analysis and STs in this study were sufficient and optimal to differentiate the clones obtained from diagnostic samples and were suggested if further outbreaks in veterinary settings and animal-to-human transmission need to be investigated.

Both blaOXA-23 and Tn2006 were consistently found in all carbapenemase-producing Abc complex isolates in our study. Of these, Tn2006 is the most common blaOXA-23-containing transposon found in nosocomial OXA-23 carbapenemase-producing *A. baumannii* ST2 strains from in Asia [21, 25]. This element is also primarily detected in *A. baumannii* from pets [5, 31]. The detection of the same determinant in multiple *A. baumannii* clones and *A. pittii* strains suggests that the sharing of blaOXA-23 could be mediated by the horizontal spread and genome plasticity of the Abc complex. With no other blaOXA-23-related transposons detected in this study, Tn2006 was the key element for blaOXA-23 spread among clones persisting in these animal populations. The development of resistance by acquired mechanisms is crucial for the Abc complex. Genes encoding additional resistance mechanisms could not be identified at all isolates because of the limitation of the detection by PCR in this study. Genetic location and the resistome can be further explored to understand the accumulation of resistance genes and the variation in resistance gene carriage in the same or in different *A. baumannii* clones. High-resolution genome studies by whole-genome and mobilome analysis should be performed for tracing the spread of the resistance elements among *A. baumannii* clones in terms of genomic surveillance.

The increase in carbapenemase-producing *Acinetobacter* spp. is a new challenge for antimicrobial therapy in veterinary practice. Amikacin is an important therapeutic option for XDR bacterial infections. A high rate (60.9%) of amikacin resistance could limit the treatment of choice in infections caused by pan drug-resistant strains, especially systemic infections and UTI. The presence of multiple ST25 isolates from an individual cat confirms the challenging management of chronic infections and disease progression caused by
resistant bacteria. With the emergence of MDR bacteria in veterinary medicine, carbapenems might be more frequently used and could be a selective pressure of resistance strains in pets. The transmission of a carbapenemase-producing strain of *Pseudomonas aeruginosa* between a dog owner with a history of hospitalization and the dog presenting otitis externa has been approved by identical genetic and resistance characteristics [11]. Therefore, humans, including hospitalized patients, healthcare workers and veterinary staff, could be long-term carriers of *A. baumannii*. Tracing of the spread in veterinary settings, animal reservoirs and people associated with animals are necessary for the establishment of prevention and control policies.

CONFLICT OF INTEREST. The authors declare no conflicts of interest.

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