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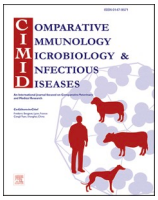

Evidence and antibiotic resistance profiles of clinical *Acinetobacter calcoaceticus-Acinetobacter baumannii* (ACB) and non-ACB complex members in companion animals: A 2020–2022 retrospective study

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ABSTRACT

To evaluate the frequency of *Acinetobacter* spp., belonging to both *Acinetobacter calcoaceticus-baumannii* (ACB) and non-ACB complex, and their antibiotic resistance profiles in veterinary medicine, a three-year (2020–2022) retrospective study was carried out on sick companion animals. Epidemiological data from different clinical canine, feline, and equine samples, were acquired. For each strain, MALDI-TOF MS identification and susceptibility to a panel of 11 antibiotics, by Kirby-Bauer and E-test methods, were performed. Out of 628 bacteriological examinations, 2.5% resulted positive for strains belonging to *Acinetobacter* genus. Frequencies of 2.3%, 1.9%, and 3% were obtained from both in-visiting and hospitalized dogs, cats, and horses, respectively. Members of ACB-complex accounted for 50% of isolates. Since all strains resulted susceptible to aminoglycosides and polymyxins, no pandrug-resistant (PDR) species were recorded. While 12.5% *A. baumannii* resulted extensively-drug resistant (XDR), a higher percentage of multidrug-resistant strains was recorded among non-ACB strains (35.5%) than ACB strains (25%). Susceptibility was observed in the same percentage in both groups (62.5%). All ACB strains confirmed their intrinsic resistances. Non-ACB species showed lower resistances against anti-pseudomonal penicillins plus beta-lactamase inhibitors (P=0.1306), III generation cephalosporins (P=0.0547), and tetracyclines (P=0.0209) than ACB species. Carbapenem-resistance was observed for XDR *A. baumannii* (12.5%) and, in particular for MDR non-ACB complex members (25%). To our knowledge, *A. lactucae* represents the first description in two sick dogs in Italy. Furthermore, our results emphasize the role of non-ACB-complex species as important zoonotic pathogens, which could be reservoirs of clinically relevant resistance profiles.

1. Introduction

Acinetobacter baumannii, known as one of the top-priority pathogens, has become the most clinically significant species with the extraordinary ability to survive in a hospital environment and acquire multidrug resistance. This earned *A. baumannii* a place among the most difficult 'ESKAPE' pathogens to treat by WHO [1]. Recently, members of *Acinetobacter calcoaceticus-Acinetobacter baumannii* (ACB) complex, *A. baumannii*, *A. calcoaceticus*, *A. lactucae* [2], *A. nosocomialis*, *A. pittii*, *A. seiffertii* [3], have become of particular concern in human medicine because they have emerged as health care-associated pathogens and have been involved in cases of co-infection with SARS-CoV-2 [4,5]. They are resilient bacteria with a diverse natural habitat, from moist environments to dry surfaces [6]. Outbreak investigations have demonstrated that environmental contamination with ACB species can be widespread and serve as sources of infection. Furthermore, *Acinetobacter baumannii* is becoming an opportunistic and emerging waterborne pathogen [7]. In addition, ACB microorganisms can colonize the human skin, bones, throat, eyes, heart, meninges, respiratory, gastrointestinal, and urinary tracts [8]. Human infections caused by members of non-ACB

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complex, such as *A. lwoffii*, *A. courvalinii*, *A. johnsonii*, and *A. bereziniae*, have been recently recognized also in veterinary medicine [3], due to the implementation of new technologies in clinical diagnostic laboratories and to the use of Matrix Assisted Laser Desorption Ionisation-Time Of Flight Mass Spectrometry (MALDI-TOF MS), which has emerged as a rapid and accurate identification method for bacterial species. In the last decades, their intrinsic antimicrobial resistance phenotypes were aggravated by the limited treatment options for these infections due to the ability of the *Acinetobacter* species to acquire broad antimicrobial resistance [4,8]. Even more concerning is the fact that the accumulation of resistance determinants in some ACB isolates has led to resistance to multiple categories of antimicrobial agents, sometimes referred to as "multidrug-resistance". Outbreaks of *Acinetobacter* infections, often caused by multi-, extensively- and pandrug-resistant phenotypes, have been widely reported, commonly in intensive care units (ICUs), such as burn wards, and spinal-cord-injury units [9]. From the most widespread β-lactamases to aminoglycosides and quinolones' resistances in *A. baumannii,* the dramatic rise in carbapenem and colistin resistance is of particular concern [9,10].

Despite the clinical relevance of members of the ACB complex, pathogenic *Acinetobacter* spp. have also been reported in drinking water and food, which could represent a neglected reservoir and source of bacterial pathogens to the human population [11,12]. In animals, while *A. baumannii* association with infections is increasingly reported [6, 13–16], there is a shortage of information regarding ACB and non-ACB complex members [14,17] and data on their antimicrobial resistance profiles are still scarce. In some cases, carbapenemase-producing isolates were described [15,18–21]. *Acinetobacter* spp. has been isolated from raw meat and from food animals in the UK [22,23], Lebanon [24], France [19] and China [20,21]. Some isolates have been associated with several types of infection in companion and large animals, such as canine pyoderma, feline necrotizing fasciitis, urinary tract infection, equine thrombophlebitis and lower respiratory tract infection, foal sepsis, pneumonia in mink, and cutaneous lesions in hybrid falcons [4, 11,15,25].

To fulfil the epidemiological gap on frequency and antibiotic susceptibility profiles of ACB and non-ACB complex members in hospitalized and in-visiting companion animals, a retrospective epidemiological study was carried out.

2. Materials and methods

2.1. Bacterial isolates

As we hypothesized that *Acinetobacter* species belonging both to ACB and non-ACB complex, isolated from canine, feline, and equine clinical specimens can harbour resistances against a broad spectrum of antimicrobials, a retrospective observational study on companion animals was carried out at the Laboratory of Medical Microbiology and Infectious Diseases – University of Camerino, Italy (Supplementary file). The veterinary bacteriological investigations performed from 2020 to 2022 were considered in the study. Only the biological samples collected from sick dogs (n=387), cats (n=107) and horses (n=134) were included. The sample distribution in relation to the animal species and anatomical sites, was described in Fig. 1.

Each specimen was collected from hospitalised and in-visiting companion animals, and delivered to the Laboratory of Medical Microbiology and Infectious Diseases for aetiological diagnosis. The clinical information present in the medical records (software ARGO 5.1.22.0804) and in animal cover sheets, was shown in Table 1.

Each sample was cultured according to standard protocols plating on Columbia Blood agar (Liofilchem®, Teramo, Italy), MacConkey agar (Liofilchem®, Teramo, Italy), Hektoen agar (Liofilchem®, Teramo, Italy), and incubated at 36±1 °C in aerobic atmosphere for 24–48 hours. All presumptive *Acinetobacter* isolates, which were Gram-negative short rods typically growing in pairs or in chains, non-lactose fermentative, and oxidase-negative, were identified by using the biochemical methods (RapID™ ONE System, Remel, Oxoid Milan), frozen and stored at –80 °C until species identification by MALDI-TOF MS (SOP Direct Transfer Procedure Revision.4; Bruker Microflex Lt®, Bruker Daltonics, Germany). *Acinetobacter* strains, isolated from sterile sites or from lower respiratory specimens collected with protected technique and cultured quantitatively, were considered as pathogens. The threshold determined to define clinically relevant bacterial growth was $> 1.7 \times 10^3$ colony-forming units (CFU) per millilitre of respiratory lavage [26].

For MALDI-TOF MS identification, stored *Acinetobacter* strains were cultured for 24 hours at 36±1 °C on Columbia Blood agar (Liofilchem®, Teramo, Italy), and then subcultured on MacConkey agar (Liofilchem®, Teramo, Italy). Following the manufacturer's instructions, Standard Operating Procedure - Direct Transfer Procedure was used for isolate identification. The protocol was the following: the bacterial colony was first inoculated in a MALDI-TOF MS target plate, and subsequently 1 μL of α-Cyano-4-hydroxycinnamic acid matrix solution (Bruker Matrix HCCA) was added to the sample and dried at room temperature for ten minutes. Afterward, the target plate was placed in the equipment for MALDI-TOF MS analysis. Mass spectra were processed using Flex Analysis (version 3.4; Bruker Daltonics, Germany) and BioTyper software (version 3.1; Bruker Daltonics, Germany). The identification was based on the score values released by the equipment's instructions. Specifically, score values below 1.7 indicated a non-reliable identification, between 1.70 and 1.99 a probable genus identification, and a score of ≥2.0 indicated a secure genus identification and a highly probable species-level identification. The row spectra obtained were compared with those present in the Biotyper database and log (score) ≥2.0 was considered. A bacterial test standard (BTS) (Bruker Daltonics, Germany) was used as a calibrator for quality control.

2.2. Antimicrobial susceptibility testing (AST)

Acinetobacter species were selected and tested against a panel of 11

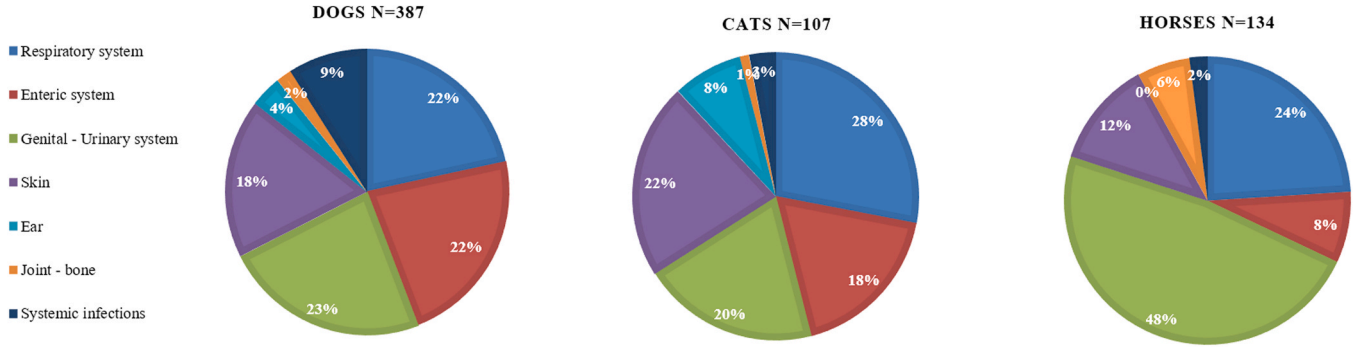

Fig. 1. Distribution (%) of anatomical sites sampled in relation to the animal species.

Table 1
- Clinical and anamnestic information collected from medical records (software ARGO 5.1.22.0804) and animal cover sheets.

Year	Season	Animals	Clinical history	Therapy (last 4 weeks)	Samples	Clinical settings
2020	Winter	Cat	Tracheitis, recurrent cough	Yes (Enrofloxacin)	Pharingeal swab	H
	Spring	Dog	Dyspnoea, recurrent cough	Yes (Amoxicillin and clavulanic acid)	Pharingeal swab	V
	Summer	Horse	Dyspnoic episodes with altered pulmonary parameters	Yes (Azithromycin)	Broncho-alveolar lavage	H
	Autumn	Dog	Rhinitis, muco-catarrhal secretion	No	Nasal swab	H
2021	Winter	Cat	Right forelimb fracture with purulent post-operative secretion	Yes (Cefadroxil)	Bone implant (intraoperative swab)	H
	Winter	Dog	Otitis media	Yes (Enrofloxacin)	Tympanic bubble wash	H
	Spring	Horse	Infertility and ultrasound collection of exudate in uterus	No	Uterine swab	V
	Summer	Dog	Lameness right hind limb	No	Synovial liquid	V
	Winter	Dog	Chronic otitis	Yes (Enrofloxacin)	Auricular swab	V
2022	Spring	Horse	Clinical mastitis	No	Mammal secretion	V
	Summer	Horse	Clinical mastitis with nervous symptoms at posterior train and altered liver and splenic parameters	No	Mammal secretion	V
	Autumn	Cat	Bite wound with purulent exudate	No	Swab of cutaneous purulent exudate	H
	Autumn	Dog	Post-traumatic lacerated hind limb wounds	Yes (Ceftriaxone)	Skin wound swab	H
	Autumn	Dog	Pyelonephritis	Yes (Enrofloxacin)	Cystocentesis urine sample	H
	Autumn	Dog	4-day-old puppy dog litter with high mortality with pulmonary abscess	No	Lung abscess	V
	Autumn	Dog	Tooth removal abscess with fistulization recurrent maxillary-ear proliferation	Yes (Amoxicillin and clavulanic acid)	Swab of dental fistula	H

Legend: H: hospitalized animals; V: in-visiting animals.

human and veterinary antibiotics (Liofilchem®, Teramo, Italy): ticarcillin and clavulanate acid (TTC 85 μg); imipenem (IMI-30μg); ceftriaxone (CRO-30μg, III gen. cephalosporin); cefquinome (CEQ-30μg, IV gen. cephalosporin); ciprofloxacin (CIP-5μg); sulfamethoxazole and trimethoprim (SXT-25μg); amikacin (AK-30μg); gentamicin (CN-30μg); tetracycline (TE-30μg); polymyxin B (PB-300IU), belonging to 8 categories: penicillins+beta-lactamase inhibitors, carbapenems, extended-spectrum cephalosporins (III and IV generation), fluoroquinolones, sulfonamides, aminoglycosides, tetracyclines, polymyxins. Kirby-Bauer and MIC (E-test Colistin CS 0.016–256, Liofilchem®, Teramo, Italy) methods were performed on Mueller-Hinton II agar plates (Liofilchem®, Teramo, Italy), and interpreted as recommended by EUCAST [27] and CLSI [28] for human and veterinary antibiotics, respectively.

Isolates that exhibited intermediate susceptibility were considered susceptible to the antibiotic as suggested by EUCAST guidelines [29]. Multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) *Acinetobacter* species were defined using the antimicrobial categories considered to categorize isolates, as suggested for *Acinetobacter* spp. by Magiorakos et al. [30]: aminoglycosides, antipseudomonal carbapenems, antipseudomonal penicillins+beta-lactamase inhibitors, antipseudomonal fluoroquinolones, extended-spectrum cephalosporins (III and IV generation), folate pathway inhibitors, polymyxins, tetracyclines. For *A. baumannii* and *A. pittii* belonging to ACB complex, the following categories: antipseudomonal penicillins+beta-lactamase inhibitors, III generation cephalosporins, and tetracyclines were not considered for MDR, XDR and PDR determination, because of their documented intrinsic resistance [27,28,31]

Precisely, ACB and non-ACB complex members were classified as S when non susceptible to <3 antimicrobial categories, MDR when non-susceptible to at least 1 agent in ≥3 antimicrobial categories, XDR when non-susceptible to at least 1 agent in all but 2 or fewer antimicrobial categories, and PDR when resistance to all agents in all antimicrobial categories was observed.

2.3. Statistical analysis

The univariate analysis, using the Fisher's exact tests for independent binomial variables as appropriate, was performed for categorical variables, such as frequency of *Acinetobacter* strains isolation and resistance rates compared between members of ACB and non-ACB-complex. The animal samples were stratified according to hospitalized or in-visiting animals, as local or systemic contaminated/infected animals, based on clinical signs and laboratory data recorded. Moreover, epidemiological data concerning the site of sampling, the season of *Acinetobacter* spp. isolation, and *Acinetobacter* spp. culture in purity or co-infection, were acquired to evaluate differences in the involvement of ACB or non-ACB complex members. To provide an estimate with 95% confidence intervals (CI_{95}) for the relationship between qualitative variables, association measures were reported as Odds Ratio (OR). The following formula was used for a 95 % confidence interval (CI):

Upper 95 % CI = e ^ [ln(OR) + 1.96 sqrt(1/a + 1/b + 1/c + 1/d)]

Lower 95 % CI = e ^ [ln(OR) - 1.96 sqrt(1/a + 1/b + 1/c + 1/d)]

where 'e' is the mathematical constant for the natural log, 'ln' is the natural log, 'OR' is the odds ratio calculated, 'sqrt' is the square root function and a, b, c and d are the values from the 2 ×2 table.

Data analysis was performed using Stata software, version 13.0 (©StataCorp LLC, College Station, TX, USA). The significance level threshold was a *P*-value < 0.05.

3. Results

3.1. Acinetobacter's clinical isolates in companion animals

From 2020–2022, out of 628 bacteriological cultures, 16 (2.5 %) resulted positive for strains belonging to the genus *Acinetobacter,* having a log (score) ≥2.0. Frequencies of 2.3% (n=9), 1.9% (n=3), and 3% (n=4) were observed in dogs (n=387), cats (n=107), and horses (n=134), respectively. The distribution by season showed that 18.8% (3/16) of the isolates were recorded in Spring, 18.7% (3/16) in Summer, 37.5% (6/16) in Autumn and 25% (4/16) in Winter. Although almost twice as many strains were isolated in Autumn/Winter (62.50%, 10/16), no difference emerged between ACB (75%, 6/8) and non-ACB complex members (50%, 4/8; χ^2=1.07, df=1, *P*=0.3017).

All strains were isolated from clinically diseased companion animals. In particular, 7/16 *Acinetobacter* spp. (43.75%) were cultured from in-visiting, and 9/16 (56.25%) from hospitalized animals (Table 2). Strains belonging to ACB complex were cultured from in-visiting companion animals (71.43%, 5/7), while 66.67% of the nosocomial strains (6/9) resulted non-ACB complex. In dogs, pure colonies of *Acinetobacter baumannii*, *A. lactucae*, *A. pittii*, belonging to ACB complex, were significantly isolated from 3 out of 4 in-visiting animals (75% χ^2=7.632, df=1, *P*=0.023). In particular, they were isolated from the pharynx (upper respiratory tract signs), the synovial fluid (arthritis), and a lung abscess (low respiratory tract signs).

Members of ACB complex represented 50% of total isolates: *A. baumannii* (n=4) was identified in dogs (22.2%, 2/9) and in horses (50%, 2/4), both in pure culture (50%) and in co-infection (50%); *A. pittii* strains (n=2) were isolated in pure culture from the respiratory system, and in co-infection from skin wound in dogs; *A. lactucae* (n=2) was recorded in dogs from infectious synovitis, in pure culture, and from respiratory system, in co-infection.

When non-ACB complex strains were identified, for *A. lwoffii* (n=3), *A. courvalinii* (n=2), *A. johnsonii* (n=2), and *A. bereziniae* (n=1) the nosocomial (75%, 6/8) *vs.* in-visiting (25%, 6/8) clinical setting was significantly represented (*P*=0.045), in particular when they were isolated in pure culture (37.5%, 3/8). The presence of *Acinetobacter* species belonging to the non-ACB complex was strongly associated with ear and dental infections (OR=4, CI_{95}: 1.2–28.8), with an occurrence of 50%, both in pure colony and in co-infection.

3.2. Antibiotic susceptibility testing

During the study period, all *Acinetobacter* spp. resulted susceptible to aminoglycosides and polymyxins no PDR *Acinetobacter* strains were recorded in clinically diseased companion animals (Table 3). One *A. baumannii* strain (1/16, 6.25%) resulted XDR, while 31.25% (5/16) and 62.5% (8/16) of isolates showed MDR and S patterns, respectively. Different percentages statistically significant were observed between S, MDR, and XDR (χ^2=11.44, df=2, *P*=0.0033) isolates. In particular, a higher significant percentage was recorded only for S *vs.* XDR (*P*=0.0021), not for S *vs.* MDR (*P*=0.1556), and for MDR *vs.* XDR (*P*=0.1719).

In detail, 25% (2 out of 8) of ACB complex strains *vs.* 35.5% (3 out of 8) of non-ACB complex strains) resulted MDR and the difference was not significant (χ^2=0.629, df=1, *P*=0.5896). The same percentage (62.5%, 5/8) of susceptibility was observed in both ACB and non-ACB complex members (Supplementary file).

All *A. baumannii*, *A. pittii*, and also *A. lactucae* strains confirmed the intrinsic resistance, showing significantly resistance rates twice as high compared to non-ACB complex to tetracyclines (100 % *vs.* 50%, χ^2=5.33, df=1, *P*=0.0209). Moreover, high resistances (100%, 8/8) were observed for ACB complex members against antipseudomonal penicillins plus beta-lactamase inhibitors and III generation cephalosporins, although the differences were not significant in comparison to what observed for non-ACB complex strains (75%, 6/8; χ^2=2.29, df=1, *P*=0.1306) and (62.5%, 3/5; χ^2=3.69, df=1, *P*=0.0547) (Fig. 2). On the other hand, resistance to IV generation cephalosporins (12.50%, 2/16), used in veterinary medicine only, was observed for strains belonging to ACB complex (*A. baumannii* and *A. lactucae*), which were found to be responsible for canine respiratory tract infections: one pure cultured XDR *A. baumannii*, isolated from a pharyngeal swab of an in-visiting dog, and one *A. lactucae* strain, cultured in co-infection from a nasal swab of hospitalized dog.

The same percentage of resistance was observed for fluoroquinolones (25%, 4/16) for both ACB (2 out of 8) and non-ACB (2 out of 8) complex members.

Resistance to sulfonamides (18.75%, 3/16) showed a twice as high for ACB strains (25%, 2/8) compared to non-ACB complex (12.5 %, 1/8; χ^2=0.41, df=1, *P*=0.5218) Overall, carbapenems resistance of 18.75% (3/16) was observed for XDR ACB complex (*A. baumannii*) and 2 (*A. courvalinii*) out of 5 MDR strains, both belonging to non-ACB complex (25 %, 2/8; χ^2=0.41, df=1, *P*=0.5218). The resistance to carbapenems proved to be associated with the XDR profile (OR=60, CI_{95}:

Table 2
- Results of 36 months bacteriological examinations by MALDI-TOF MS (Bruker Daltonics, Germany).

Animals	Total samples (N)	Frequency of *Acinetobacter* spp. (%)	MALDI-TOF MS *Acinetobacter* identification (%)	Anatomical sites	Bacterial Cultures	Clinical settings
Dogs	387	9 (2.3%)	*A. baumannii* (22.2%)	Pharynx	P	V
				UTI	Co-I	H
			A. lactucae (22.2%)	Nose	Co-I	H
				Synovia	P	V
			A. bereziniae (11.1%)	Ear	P	H
			A. lwoffii (11.1%)	Ear	Co-I	V
			A. pittii (22.2%)	Wound	Co-I	H
				Lung	P	V
			A. johnsonii (11.1%)	Dental fistula	Co-I	H
Cats	107	3 (1.9%)	*A. courvalinii* (66.7%)	Wound	Co-I	H
				Bone implant	P	H
			A. johnsonii (33.3%)	Pharynx	Co-I	H
Horses	134	4 (3.0%)	*A. baumannii* (50%)	Uterus	P	V
				Breast	Co-I	V
			A. lwoffii (50%)	BAL	Co-I	H
				Breast	Co-I	V
Total	628	16 (2.5%)				

Legend: MALDI-TOF MS: matrix-assisted laser desorption ionisation-time-of-flight mass spectrometry; UTI: Urinary Tract Infection; BAL: broncho-alveolar lavage; H: hospitalized animals; V: in-visiting animals; P: pure culture; co-I: bacterial co-infection.

Table 3
- Antimicrobial resistance profiles of clinical isolates of *Acinetobacter* spp. belonging to ACB and non-ACB complex, cultured from sick dogs, cats and horses.

Animals	Acinetobacter spp.	Belonging Complex	Infection sites	Resistance profiles	Patterns
Dogs	*A. baumannii*	ACB	Pharynx	IMI-CEQ-CIP-SXT	XDR
	A. baumannii	ACB	UTI	*Intrinsic resistances only*	S
	A. lactucae	ACB	Nose	TTC-CRO-CEQ-TE	MDR
	A. lactucae	ACB	Synovia	TTC-CRO-TE	MDR
	A. pittii	ACB	Wound	CIP-SXT	S
	A. pittii	ACB	Lung	*Intrinsic resistances only*	S
	A. lwoffii	non-ACB	Ear	TTC	S
	A. johnsonii	non-ACB	Dental fistula	TTC-CRO	S
	A. bereziniae	non-ACB	Ear	TTC- CRO-CIP-SXT-TE	MDR
Cats	*A. courvalinii*	non-ACB	Bone implant	TTC-IMI-CRO-CIP-TE	MDR
	A. courvalinii	non-ACB	Wound	TTC-IMI-CRO	MDR
	A. johnsonii	non-ACB	Pharynx	TE	S
Horses	*A. baumannii*	ACB	Uterus	*Intrinsic resistances only*	S
	A. baumannii	ACB	Breast	*Intrinsic resistances only*	S
	A. lwoffii	non-ACB	BAL	TTC-CRO	S
	A. lwoffii	non-ACB	Breast	TE	S

Legend: in *resistance profiles column*, TTC=ticarcillin and clavulanate; IMI-=imipenem; CRO=ceftriaxone (III gen. cephalosporin); CEQ=cefquinome (IV gen. cephalosporin); CIP=ciprofloxacin; SXT=sulfamethoxazole and trimethoprim; TE=tetracycline; in *patterns column*, XDR=extensively drug-resistant; S=susceptible to <3 other tested antimicrobial categories; MDR=multidrug-resistant.

2.25–3017.9).

Susceptibilities (62.5%, 10/16) were observed for either *Acinetobacter* groups (62.5%, 5/8) with no differences in relation to clinical settings: in-visiting (50%, 5/10), and hospitalized (50%, 5/10) companion animals. In particular, all susceptible strains showed a susceptibility to antipseudomonas carbapenems. They were three *A. baumannii*, exhibiting intrinsic resistances only, and two *A. pittii* isolates. About non-ACB complex, three were *A. lwoffii* strains, the first one isolated in Autumn/Winter in pure colonies from an in-visiting dog with chronic otitis, the other two isolated in Spring/Summer in co-infection from an in-visiting and hospitalized horses with clinical mastitis and pulmonary signs; lastly, two were *A. johnsonii* strains, isolated in co-infection during Autumn/Winter from hospitalized cat and dog, with tracheitis and dental fistula, respectively.

4. Discussion

The WHO has been using the term "silent pandemic" since the Fall of 2021 because, unlike COVID-19, antibiotic resistance is creeping into our society unnoticed. The issue is currently so serious that it is being treated with a degree of urgency, operating surveillance and studying new alternative molecules. In this context, there is growing concern that multidrug resistant *A. calcoaceticus-A. baumannii* (ACB) complex strains in hospitalized companion animals and horses may be emerging as a threat to veterinary and public health [25,32–35]. However, information on non-*A. baumannii* strains in veterinary medicine is still limited [11,18,36] and there is a lack of data comparable to strains isolated from humans [18–20,25,36].

The possibility of spread from humans to animals [32] or *vice versa* requires special attention as well as the correct identification of the strains that, luckily, today thanks to MALDI-TOF MS prove to be faster and accurate.

This study aimed at evaluating the presence of *Acinetobacter* spp., belonging to ACB and non-ACB complex, in companion animal clinical samples, comparing – between the two groups – the frequency of isolations obtained, as well as the detected phenotypic acquired resistance to human and veterinary antimicrobials. The main limitations of this study include its retrospective design and its restriction to a single University Veterinary Diagnostic Laboratory.

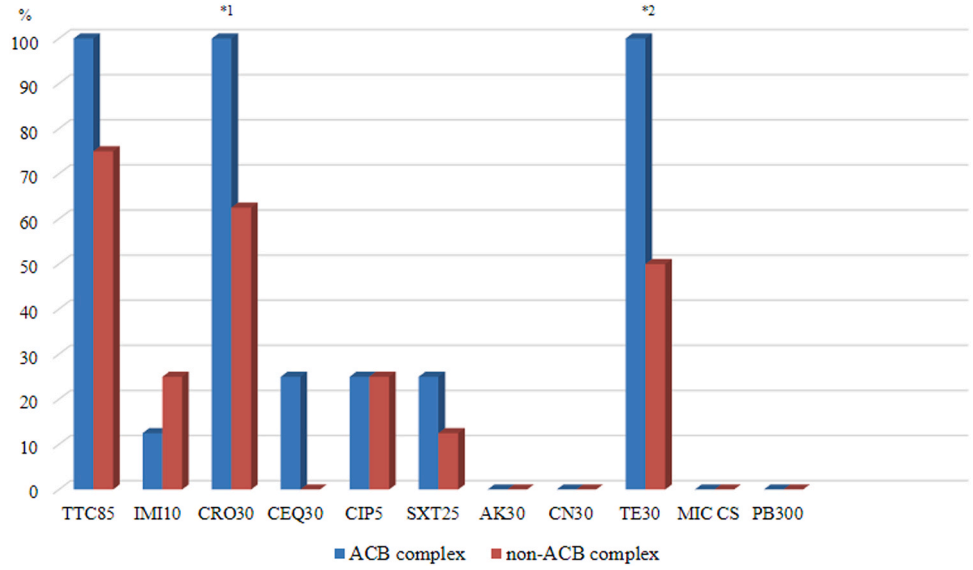

Fig. 2. Resistance (%) comparison between ACB and non-ACB complex members. AUG 30 μg = amoxicillin and clavulanate; IMI 30 μg = imipenem; CL 30 μg = cefalexin (I gen. cephalosporin); FOX 30 μg = cefoxitin (II gen. cephalosporin); CRO 30 μg = ceftriaxone (III gen. cephalosporin); CEQ 30 μg = cefquinome (IV gen. cephalosporin); CIP 5 μg = ciprofloxacin; SXT 25 μg = sulfamethoxazole and trimethoprim; AK 30 μg = amikacin; CN 30 μg = gentamicin; TE 30 μg = tetracycline; AZM 15 μg = azithromycin; ATM 30 μg = aztreonam; MIC CS = colistin; PB300 = polymyxin B. ^{*1} = 100 % *vs.* 62.5%, $\chi^2 = 3.69$, $P = 0.047$; ^{*2} = 100% *vs.* 50%, $\chi^2 = 5.33$, $P = 0.0209$.

The identification by MALDI-TOF MS yielded unambiguous protein spectra for all bacterial strains both at a genus and species level (log score ≥2.0). Although identification limits are documented [34], currently the software performances have improved and MALDI-TOF MS represents a valid tool also in terms of the speed of the identification procedure [3]. In this study, given the bioscore (>2.000) obtained, it was not necessary to resort to the addition of formic acid or extraction. The most frequently isolated species among ACB complex were *A. baumannii* (25%), *A. pittii* (12.5%), *A. lactucae* (12.5%). This work permitted the isolation, for the first time in Italy, of a strain of *A. lactucae* from synovial fluid in one in-visiting dog with arthritis. Furthermore, *A. lactucae* was isolated from the respiratory system of a hospitalized dog, in accordance with the literature where the authors, with the aim of assessing swallowing deficits in the canine species, performed tests on bronchoalveolar lavages, reporting the isolation of this bacterial species [35]. Among the isolates belonging to non-ACB complex, *A. lwoffii* (18.75%), *A. johnsonii* (12.25%), *A. courvalinii* (12.25%), *A. bereziniae* (6.25%), were herein recovered. Although these members of non-ACB complex were found distributed in different ecosystems: environment, animals, and humans, including food [11], they could represent opportunistic pathogens and determinants of animal infections. It was found that *A. lwoffii* is also the most frequently isolated species, in accordance with what was observed in some studies, where *A. lwoffii* was the most common strain isolated from wastewater treatment plants, in hydrocarbon-contaminated soil samples and on vegetables, as well as from horses and other domestic animals and humans [19,20,25].

As reported by Magiorakos et al. [30], the antibiotic panel selected for the study considered the various categories of human and veterinary antibiotics described for *Acinetobacter* spp. This study confirmed the intrinsic resistances for all ACB complex members, as documented by EUCAST [31], and also for two *A. lactucae* strains isolated in pure and co-infection from in-visiting and hospitalized sick dogs. On the contrary, a lower number of *Acinetobacter* strains belonging to non-ACB complex showed intrinsic resistances to antipseudomonal penicillins plus beta-lactamase inhibitors, III generation cephalosporins and tetracyclines, for which that difference resulted significant. Consequently, attention should be paid to the different antibiotic resistant profiles between the ACB complex and non-ACB complex strains. As already observed in other studies present in literature, 100% resistances concerning the ACB complex strains are recorded for penicillins and β-lactams, cephalosporins, and tetracyclines determined by an intrinsic resistance characteristic of *A. baumannii* and influenced by a use of these categories in human and veterinary medicine [6,8,15]. Nevertheless, non-ACB complex members harbored more phenotypic multidrug resistance. Percentages of MDR strains resulted to be highest, although not in a statistically significant way, for non-ACB compared to the ACB complex members.

Interestingly, in this study the resistance to carbapenems was observed for XDR and MDR strains, in both ACB (12.5%, 1/8) and non-ACB (25%, 2/8) complex members: demonstrating that non-ACB strains can also exhibit such phenotypic resistance. Another very interesting result was the different origin of the isolated XDR strain: the only one was *A. baumannii* belonging to the ACB complex group, recovered in pure culture from in-visiting dog, while all MDR strains belonging to the non-ACB complex group originated from hospitalized animals. In this retrospective study emerged that the risk of MDR *Acinetobacter* spp. exposition was related to the hospital setting, in particular for non-ACB complex members, in a 4/1 ratio. During the last decade, some studies have detected *A. baumannii* in dogs in the community [35,37]. These reports indicate that community-acquired *A. baumannii* infections among animals may be increasing and that animals outside clinical settings might represent a reservoir for *A. baumannii*, including strains resistant to carbapenems [25]. In this retrospective study, the resistance to carbapenems resulted to be associated with the XDR profile. No PDR *Acinetobacter* strains were detected in both groups. The only antibiotic categories for which all ACB and non-ACB complex strains were susceptible resulted polymyxins and aminoglycosides, as opposed to the other categories (carbapenems, fluoroquinolones, sulphonamides) where resistances ranged from 18.5% to 25%, without significant differences observed between ACB and non-ACB complex.

Considering our working experience and literature, it is difficult to delineate clear paths of diffusion of antimicrobial resistant bacteria. Certainly, the environment plays a key role and, above all, the hospital environment is the main place of dissemination of multi-resistant bacteria. In fact, many hospitals have introduced an antibiotic stewardship program in order to limit the selection of resistant bacteria by reducing overall the number of antibiotics prescribed and administered to patients.

Thus, monitoring frequency and resistance are the first steps to be taken to reduce the spread of resistant bacteria. Furthermore, this study underlines the importance of correctly identifying the *Acinetobacter* at species level, paying attention to all strains, members and non-members of ACB complex. Finally, as animals and humans could share identical clones, this research highlights the importance of monitoring antibiotic resistance profiles in companion animals for a targeted intervention reducing the spread and transmission of MDR and XDR *Acinetobacter* species.

5. Conclusions

Acinetobacter isolates belonging to the ACB and non-ACB complex are of particular concern in companion animals since these species are also associated with the clinical setting. Particularly, *A. baumannii* has become an important pathogen in veterinary as well as in human medicine. To the best of our knowledge, an interesting finding is represented by the isolation, for the first time in Italy, of canine MDR *A. lactucae*, belonging to ACB-complex. Moreover, the detection of MDR, XDR and carbapenem-resistant strains observed both among ACB and not-ACB complex members reinforces the notion that these zoonotic pathogens could represent a worrying public health hazard.

In conclusion, the results obtained suggest that antimicrobial resistance control is required for clinical *Acinetobacter* species, belonging to ACB and non-ACB complex, in both in-visiting and hospitalized companion animals.

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CRediT authorship contribution statement

Anna-Rita Attili: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Luisa De Martino:** Writing – review & editing, Supervision, Conceptualization. **Vincenzo Cuteri:** Writing – review & editing, Supervision. **Eleonora Bonacucina:** Writing – review & editing, Investigation. **Marina C.T. Meligrana:** Writing – review & editing, Supervision, Investigation. **Claudia Cerracchio:** Writing – review & editing. **Filomena Fiorito:** Writing – review & editing. **Victor Ngu Ngwa:** Investigation. **Martina Linardi:** Investigation. **Martina Sisto:** Investigation. **Francesca Paola Nocera:** Writing – original draft, Formal analysis. **Francesca Gigli:** Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.cimid.2024.102185.

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