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Original Article
Small Animal Diseases

Acinetobacter calcoaceticus-Acinetobacter baumannii complex in animals: identification and antimicrobial resistance profile[1]

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ABSTRACT.- Holmström T.C.H., David L.A., Motta C.C., Rocha-de-Souza C.M., Maboni G., Coelho I.S., Melo D.A. & Souza M.M.S. 2022. ***Acinetobacter calcoaceticus-Acinetobacter baumannii* complex in animals: identification and antimicrobial resistance profile**. *Pesquisa Veterinária Brasileira 42:e07043, 2022*. Laboratório de Bacteriologia Veterinária, Departamento de Microbiologia e Imunologia Veterinária, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, BR-465 Km 7, Seropédica, RJ 23890-000, Brazil. E-mail: theresseholmstrom@yahoo.com.br

Acinetobacter spp. is emerging as an important human and veterinary pathogen, mostly due to intrinsic and acquired resistance to antimicrobials. Despite its public health relevance, little is known about the prevalence, role of different *Acinetobacter* species and antimicrobial resistance profile of animal-origin isolates. Traditional phenotypic tests may fail to discriminate *Acinetobacter* species, therefore molecular analyses are often required as a complementary approach. The objectives of this study were to evaluate the occurrence of strains of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* (*Acb*) complex isolated from animal infections including urinary tract infections, otitis, piodermitis and pododermatitis, and its resistance profile against different antimicrobial classes, including carbapenems. All Gram-negative coccobacilli isolates were characterized by MALDI-TOF and multiplex PCR, and the disk diffusion test was used to investigate multi-drug resistance (MDR) and carbapenem resistance genes by PCR as preconized by the standard guidelines. MALDI-TOF technique identified 21 strains belonging to the *Acb* complex (10 *A. pittii,* 8 *A. baumannii,* 3 *A. nosocomialis,* 1 *A. ursingii*, and 1 *A. venetianus*). Multiplex PCR confirmed the results of MALDI-TOF for 20 strains. Eight strains (34.78%) were classified as MDR, being 50% (4/8) *A. baumannii*, 37.5% (3/8) *A. pittii*, and 12.5% (1/8) *A. nosocomialis*. None of the isolates presented phenotypic carbapenemase production. Considering the carbapenem resistance genes, 26.09% (6/23) of the isolates presented one or more carbapenemase genes. From these, 50% (3/6) presented only bla_{VIM}, 33.33% (2/6) presented only bla_{IMP}, and 16.67% (1/6) presented bla_{IMP} e $bla_{VIM,}$ simultaneously. These genes were detected among *A. pittii* isolates mostly (66.67%, 4/6). This study provides further insights into the occurrence and resistance profile of *Acinetobacter* of animal origin.

INDEX TERMS: *Acinetobacter* complex, antimicrobial resistance, carbapenemase production.

RESUMO.- [Complexo *Acinetobacter calcoaceticus-Acinetobacter baumannii* em animais: identificação e perfil de resistência antimicrobiana.] *Acinetobacter* spp. está emergindo como um importante patógeno humano e veterinário, principalmente devido à resistência intrínseca e adquirida aos antimicrobianos. Apesar de sua relevância

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para a saúde pública, pouco se sabe sobre a prevalência, o papel das diferentes espécies de *Acinetobacter* e o perfil de resistência antimicrobiana de isolados de origem animal. Testes fenotípicos tradicionais podem falhar em discriminar espécies de *Acinetobacter*, portanto, análises moleculares são frequentemente necessárias como uma abordagem complementar. Os objetivos deste estudo foram avaliar a ocorrência de cepas do complexo *Acinetobacter calcoaceticus-Acinetobacter baumannii* (*Acb*) isolados de infecções de animais, incluindo infecções do trato urinário, otite, piodermite e pododermatite, e seu perfil de resistência a diferentes classes de antimicrobianos, incluindo os carbapenêmicos. Todas as cepas cocobacilos Gram-negativas foram caracterizados por MALDI-TOF e PCR multiplex, e o teste de difusão em disco foi usado para investigar genes de resistência a múltiplas drogas (MDR) e resistência a carbapenêmicos por PCR conforme preconizado pelas diretrizes padrão. A técnica MALDI-TOF identificou 21 cepas pertencentes ao complexo *Acb* (10 *A. pittii,* 8 *A. baumannii,* 3 *A. nosocomialis,* 1 *A. ursingii* e 1 *A. venetianus*). Multiplex PCR confirmou os resultados de MALDI-TOF para 20 cepas. Oito cepas (34.78%) foram classificadas como MDR, sendo 50% (4/8) *A. baumannii*, 37.5% (3/8) *A. pittii* e 12.5% (1/8) *A. nosocomialis*. Nenhum dos isolados apresentou produção fenotípica de carbapenemases. Considerando os genes de resistência a carbapenemas, 26.09% (6/23) dos isolados apresentaram um ou mais genes de carbapenemases. Destes, 50% (3/6) apresentaram apenas bla_{VIM}, 33.33% (2/6) apresentaram apenas bla_{IMP} e 16.67% (1/6) apresentaram bla_{IMP} e bla_{VIM}, simultaneamente. Esses genes foram detectados principalmente entre os isolados de *A. pittii* (66.67%, 4/6). Este estudo fornece mais informações sobre a ocorrência e perfil de resistência de *Acinetobacter* de origem animal.

TERMOS DE INDEXAÇÃO: Complexo *Acinetobacter,* resistência antimicrobiana, produção de carbapenemase.

INTRODUCTION

In the last decade, *Acinetobacter* species have emerged as one of the most clinically important pathogens. *Acinetobacter* spp. are associated with increased mortality, being considered a high priority nosocomial pathogen, mainly at intensive care units, featuring a challenge to infection management practices (Chen et al. 2018). The genus *Acinetobacter* comprises 74 species, being 23 effectively but not validly published named species[5]. The most relevant clinical species are from the *Acinetobacter calcoaceticus-Acinetobaceter baumannii* (*Acb*) complex: *A. baumannii, A. calcoaceticus, Acinetobacter pittii, Acinetobacter nosocomialis,* and the recently added *Acinetobacter seifertii* and *Acinetobacter dijkshoorniae. A. baumannii* is considered the most important species in human infections (van der Kolk et al. 2019). Due to the broad profile of intrinsic resistance, carbapenems are an excellent alternative for the treatment of infections by these pathogens, however, increasing resistance to carbapenems is one of the major challenges of the health system by the World Health Organization (Rodríguez et al. 2018). Multidrug-resistant *A. baumannii* is classified as an ESKAPE organism. ESKAPE is a group of pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumannii, Pseudomonas aeruginosa,* and *Enterobacter* spp.) with a high rate of antimicrobial resistance implicated in human nosocomial infections (Tuttobene et al. 2021). In veterinary medicine, knowledge about the occurrence of *Acinetobacter* spp., its clinical relevance, and potential zoonotic concerns is scarce. A recent study reported high prevalence of multidrug resistant-*A. baummannii a*ssociated with clinical disease in animals in North America (Maboni et al. 2020), however, to our knowledge, there is no current information for Brazilian isolates.

The phenotypic identification of these agents requires more than 36 different biochemical tests and does not guarantee the identification of *Acb* complex once these species are very closely related, showing similar phenotypic and biochemical properties, thus leading to an inappropriate identification. The accurate identification of the clinically important members of this group can be achieved by mass spectrometry matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) and PCR (Vijayakumar et al. 2019); however, these techniques are not always available in veterinary diagnostic laboratories, especially in low-middle income countries (Wisplinghoff et al. 2012). MALDI-TOF is a rapid and highly discriminatory method for the identification of bacteria (Welker & Moore 2011, Šedo et al. 2013, Wang et al. 2014), but it relies on frequent software and spectral databases updates (Martiny et al. 2012), so the reliability of the identification may vary due to the characteristics of the database used for interpretation. Molecular analysis by PCR is another alternative to characterize the *Acb* complex species. According to the protocol established by Chen et al. (2018), a multiplex PCR targeting species-specific genes is capable of differentiating three clinically relevant species of the *Acb* complex (*A. baumannii*, *A. pittii* and *A. nosocomialis)*.

In this context, the objectives of this study were to evaluate the occurrence of *Acb* complex associated with clinical disease in animals, and to characterize the antimicrobial resistance profile of these strains by phenotypic and molecular methods, including carbapenem resistance identification.

MATERIALS AND METHODS

Sampling. The isolates were obtained from companion and domestic animal clinical specimens (17 dogs, 5 cats and 1 horse) sent for routine diagnostic service at Veterinary Microbiological Diagnostic Laboratory from "Universidade Federal Rural do Rio de Janeiro", Brazil. Ninety-one non-fermenting and oxidase negative Gram-negative coccobacilli strains were isolated from urinary tract infections, otitis, piodermitis and pododermatitis. The present study was submitted to the Animal Experimentation and Use Committee (protocol number: CEUA 01076198732).

DNA Extraction. Isolates were cultured in 1.5mL of BHI broth at 35°C for 24 hours. Microtubes were centrifuged for 2 min at 8000g and the supernatant discarded, this procedure was repeat three times. Cells were resuspended in 200µL of ultrapure water and vortexed, being incubated at 100°C for 10 min. Microtubes were cooled at room temperature and centrifuged for 2 min at 8000g. Approximately 180µL of the supernatant was transferred to a new microtube (600uL) and stored at -20°C (Buyukcangaz et al. 2013).

Characterization of *Acb complex* species by genotypic analyses. A multiplex PCR was performed to identify the main species of *Acb* complex targeting the following genes: *rec*A *(Acinetobacter* spp.),

[5] Available at <www.bacterio.net/Acinetobacter.html> Accessed on Jan. 2022.

*gyr*B (*A. baumannii* and *A. nosocomialis*), *ITS* region (*A. baumannii*) and *ITS* region (*Acinetobacter pittii*) as recommended by Chen et al. (2014). Amplicons were detected by 1.5% agarose gel, stained with SYBR Green (Invitrogen) and examined under UV transilluminator (UvTrans).

Characterization of phenotypic resistance profile of *Acb* complex species. The disk diffusion test was performed for the following antimicrobials: ampicillin (AMP 30µg), aztreonam (ATM 30µg), ampicillin + sulbactam (ASB 20µg), amikacin (AMI), ceftazidime (CAZ), ciprofloxacin (CIP 5µg), cefepime (CPM), gentamicin (GEN 10µg), imipenem (IMP 10µg), levofloxacin (LEV), doxycycline (DOX 30µg), tetracycline (TET 30µg), azithromycin (AZI 15µg), cefoxitin (CFO 30µg), cefotaxime (CTX 30µg), aztreonam (ATM 30µg), cefepime (CPM 30µg) and amoxicillin + clavulanic acid (AMC 30µg), sulfamethoxazole + trimethoprim (SUT 25µg), tetracycline (TET 30µg) and meropenem (MPM 10µg). All antimicrobials were obtained from LaborClin® laboratory.

Isolates were considered multidrug resistant if they were resistant to three or more classes of antimicrobials, as previously described for *Acinetobacter* isolates (Maboni et al. 2020).

Phenotypic carbapenemase detection. The CarbaNP, mCIM and eCIM tests were carried out to identify and classify phenotypic carbapenem-resistance adapted from CLSI (2020-adapted).

CarbaNP. The bacteria sample were inoculated on a Mueller Hinton agar plate or blood agar and incubated for 18 to 24 hours at 37°C. Then, 100µL of the NaCl (5.0M) and one calibrated handle (10µL) of the bacterial inoculum were added to a 1.5mL microtube, vortexed for 1 min and incubated for 30 min at room temperature. After incubating, 100µL of Carba NP (zinc sulfate monohydrate 10mM, phenol red to 0.5%, ultra-pure water, NaOH 1N, pH 7.8) + imipenem solution (12mg/mL imipenem/cilastatin) was added in the same tube and incubated for 2 hours at 37°C. Readings were taken in 15 min, 1 and 2 hours of incubation (CLSI 2020-adapted).

mCIM. Each isolate was added to a disk impregnated with 10µg of meropenem and incubated at 35±2°C for about 4 hours. The bacterial suspension was prepared in sterile saline according to Mac Farland 0.5 scale. The *Escherichia coli* ATCC 25922 strain (sensitive to meropenem - MPM 10µg) was used to the diffusion disk procedure according to CLSI (2020).

eCIM. This analysis must be compared to mCIM tubes to detect a metalo-beta-lactamase carbapenemase. In a second tube of Trypticase Soy Broth, the same procedure described at mCIM was repeated adding 20µL of 0.5M EDTA solution. Results of both tests must be compared (CLSI 2020).

Detection of carbapenemase genes. Genotypic investigation of metallo-type carbapemases (*bla*) in strains of the *Acb* complex was performed by detecting the following carbapenemase genes in multiplex: bla_{IMP} and bla_{VIM} (Fallah *et al*. 2014).

RESULTS

Acb complex identification

All Gram-negative coccobacilli isolates, which tested negative for oxidase and motility were analyzed by MALDI-TOF as potential *Acinetobacter* spp. strains. The MALDI-TOF technique identified twenty-three strains as *Acinetobacter* spp.: 43.48% (10/23) *Acinetobacter pittii*, 34.78% (8/23) *Acinetobacter baumannii*, 17.39% (3/23) *Acinetobacter nosocomialis* and 4.35% (1/23) *Acinetobacter ursingii* and *Acinetobacter veneitianus*. Therefore, in total, 91.3% (21/23) belonged to *Acb* complex. After proteomic identification, strains were submitted to genotypic assays and the multiplex PCR technique identified twenty-three strains as *Acinetobacter* spp.: 43.47% (10/23) *A. pittii*; 34.78% (8/23), *A. baumannii*, 13.04% (3/23) *A. nosocomialis* and 8.69% (2/23) *Acinetobacter* level. From this identification 91.30% (21/23) belong to *Acb* complex. It was observed that 73.91% (17/23) of the strains were from dogs, 21.74% (5/23) from cats and 4.34% (1/23) of equine origin (Table 1).

Discordant results between MALDI-TOF and PCR were observed for three isolates (17, 20 and 23). The prevalence of *A. pittii* was observed, mostly from urine samples (14/23) of pet animals, specially from dogs. *A. baumannii* and *A. nosocomialis* were also frequently detected in infection sites from different animal species (Table 1).

Acb complex resistance profile

Eight strains (34.78%) were classified as multidrug-resistant (isolates 5, 6, 7, 8, 12, 18, 19 and 23), 50% (4/8) were *A. baumannii*, 37.5% (3/8) *A. pittii* and 12.5% (1/8) *A. nosocomialis*. Although 21.73% (5/23) of the isolates were resistant to meropenem, there was no detection of carbapenemases by the adapted phenotypic methods evaluated (CarbaNP, mCIM and eCIM).

Considering the carbapenem resistance genes search 26.09% (6/23) of the isolates presented one or more carbapenemase genes. From these, 50% (3/6) presented only bla_{VIM}, 33.33% (2/6) presented only bla_{IMP}, and 16.,67% (1/6) presented bla_{IMP} e bla_{VIM} simultaneously. The prevalence of these genes was detected in *A. pittii*, 66.67% (4/6). The phenotypic and

Table 1. Characteristics of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex isolates regarding animal species, specimen of origin and identification based on proteomic and genotypic analyses

Isolate	Origin	Specimen	Species identification	
			MALDI-TOF	Multiplex PCR
1	Dog	Urine	*A. pittii*	*A. pittii*
2	Cat	Urine	*A. pittii*	*A. pittii*
3	Dog	Urine	*A. pittii*	*A. pittii*
4	Dog	Urine	*A. pittii*	*A. pittii*
5	Cat	Tissue	*A. baumannii*	*A. baumannii*
6	Dog	Skin swab	*A. baumannii*	*A. baumannii*
7	Horse	Limb swab	*A. baumannii*	*A. baumannii*
8	Dog	Urine	*A. nosocomialis*	*A. nosocomialis*
9	Dog	Urine	*A. pittii*	*A. pittii*
10	Cat	Urine	*A. pittii*	*A. pittii*
11	Dog	Urine	*A. pittii*	*A. pittii*
12	Dog	Otologic swab	*A. baumannii*	*A. baumannii*
13	Dog	Urine	*A. pittii*	*A. pittii*
14	Dog	Urine	*A. baumannii*	*A. baumannii*
15	Dog	Urine	*A. pittii*	*A. pittii*
16	Dog	Urine	*A. baumannii*	*A. baumannii*
17	Cat	Urine	*A. ursingii*	*Acinetobacter* spp.
18	Dog	Oral swab	*A. baumannii*	*A. baumannii*
19	Dog	Nasal swab	*A. pittii*	*A. pittii*
20	Dog	TVT swab	*A. venetianus*	*A. nosocomialis*
21	Dog	Otologic swab	*A. nosocomialis*	*A. nosocomialis*
22	Dog	Otologic swab	*A. baumannii*	*A. baumannii*
23	Cat	Urine	*A. nosocomialis*	*Acinetobacter* spp.

genotypic correlation considering carbapenem resistance was observed in 33.33% (2/6), however, 66.67% (4/6) of the isolates presented genes that were not detected phenotypically.

The prevalence of resistance in *Acb* complex according to each antimicrobial evaluated was 95.65% (22/23) to ampicillin + sulbactam, 86.96% (20/23) to aztreonam, 73.91% (17/23) to cefotaxime, 39.13% (9/23) to sulfamethoxazole + trimethoprim and amoxicillin + sulbactam, 34.78% (8/23) to gentamicin, 30.44% (7/23) to levofloxacin and ciprofloxacin, 26.09% (6/23) to imipenem and ceftazidime, 21.74% (5/23) to meropenem, 17.39% (4/23) to cefepime, and 8.69% (2/23) to tetracycline and amikacin. The resistance profile is displayed in Table 2.

DISCUSSION

Acb complex identification

The identification of coccobacilli non-fermentative bacteria is challenging. The *Acb* complex species have slight differences between them which cannot be distinguished by conventional methods used in the clinical laboratory (Vijayakumar et al. 2019). Many studies report that the genus *Acinetobacter* is highly diverse, comprising oxidase-positive and negative, non-pigmented, Gram-negative coccobacilli in more than 50 similar species (Sousa et al. 2014, Wong et al. 2017). Phenotypic identification of *Acb* complex at species-level is not consistent and reliable, and this characterization is essential for the understanding of the role of these microorganisms, mainly in veterinary medicine. MALDI-TOF and multiplex PCR might be useful techniques to overcome some of these challenges.

The most prevalent species identified in this study were *Acinetobacter pittii, Acinetobacter baumannii and Acinetobacter nosocomialis,* respectively. Our findings corroborate with those from a previous study highlighting that species of the *Acb* complex are of great veterinary clinical relevance (Chen et al. 2018). *A. baumannii* is the most well-studied species in humans and it is associated with bacteremia, ventilator-associated pneumonia, urinary tract infections, skin and soft tissue infections (Vijayakumar et al. 2019, Weinberg et al. 2020). In animals, *A. baumannii* is associated with different infections, at hospitals or clinical settings, affecting dogs, cats, horses among other animal species (Maboni et al. 2020). In the same study, *A. baumannii* was found as the most prevalence species isolated from a wide range of animal infections.

The non-*baumannii Acinetobacter* species has emerged in recent decades as a clinically relevant pathogen causing a wide range of nosocomial and community-acquired infections in humans. The identification of non-*baumannii* species in animals is increasing thanks to technological advances such as the introduction of MALDI-TOF and molecular analysis in the clinical setting (Al Atrouni et al. 2016, Maboni et al. 2020). *A. pittii* was identified as a significant non-*baumannii* species in livestock, horses and small pets (Rafei et al. 2015). At the present study, the *A. pittii* was the most prevalent species identified, mostly in dogs presenting urinary tract infection. As *A. pittii*, another non-*baumannii* species, *A. nosocomialis,* has emerged causing infections in human and animals. In a retrospective study by Chusri et al. (2014), out of 25 infections caused by non-*baumannii Acinetobacter* species in humans, *A. nosocomialis* was identified in 17 cases followed by *A. pittii* in

Table 2. Characteristics of *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex isolates

Isolate/*Acinetobacter* spp.	Phenotypic resistance profile	Genotypic resistance profile
1 (*A. pittii*)	CTX	bla_{IMP} and bla_{VIM}
2 (*A. pittii*)	ATM, AMP, SUT, TET	-
3 (*A. pittii*)	ATM, AMP, SUT, CTX(I)	bla_{VIM}
4 (*A. pittii*)	ATM, AMP, CTX, MER, LEV	bla_{VIM}
5 (*A. baumannii*)	ATM, AMP, SUT, CTX, IMP, CPM, GEN, LEV, CIP, ASB	-
6 (*A. baumannii)*	ATM, AMP, SUT, CTX, LEV, CIP, CAZ, AMI	bla_{VIM}
7 (*A. baumannii)*	ATM, AMP, SUT, CTX, LEV, CIP, CAZ, GEN	-
8 (*A. nosocomialis*)	AMP, SUT, CTX, CPM, LEV, CIP, CAZ, ASB	-
9 (*A. pittii*)	AMP, CTX, CPM, GEN, ASB	-
10 (*A. pittii*)	ATM, AMP, CTX, IMP, ASB	bla_{IMP}
11 (*A. pittii*)	ATM, AMP, CTX, IMP, ASB	-
12 (*A. baumannii*)	ATM, AMP, CTX, GEN, IMP, ASB	-
13 (*A. pittii*)	ATM, AMP, SUT, TET, CTX, CIP, GEN	-
14 (*A. baumannii*)	ATM, AMP, MER	
15 (*A. pittii*)	ATM, AMP, CTX, MER, GEN, ASB	-
16 (*A. baumannii*)	ATM, AMP, CTX, MER, GEN, ASB	bla_{IMP}
17 (*Acinetobacter* spp.)	ATM, AMP	-
18 (*A. baumannii*)	ATM, AMP, CAZ, GEN	-
19 (*A. pittii*)	AMP, ATM, CAZ	-
20 (*A. nosocomialis*)	ATM, AMP, SUT, CTX, IPM, CPM, CIP, CAZ, ASB	-
21 (*A. nosocomialis*)	ATM, AMP, SUT, CTX, MER, IPM, CIP, LEV	-
22 (*A. baumannii*)	ATM, AMP, CTX, LEV	-
23 (*Acinetobacter* spp.)	ATM, AMP, AMI	-

CAZ = ceftazidime, CPM = cefepime, GEN = gentamicin, IPM = imipenem, SUT = sulfamethoxazole + trimethoprim, CIP = ciprofloxacin, LEV = levofloxacin, AMP = ampicillin + sulbactam, AMI = amikacin, ATM = aztreonam, ASB = amoxicillin + sulbactam, MER = meropenem, CTX = cefotaxime, TET = tetracycline.

7 cases. Considering the discordant results in the identification of the isolates 17 (*Acinetobacter ursingii*), 20 (*Acinetobacter venetianus*) and 23 (*A. nosocomialis*), it is likely that MALDI-TOF failed to identity these isolates at species level, therefore, we considered the results obtained from the multiplex PCR as the most precise identification.

MALDI-TOF or multiplex PCR: what is more useful?

All isolates (n=23) were identified at species level by MALDI-TOF and 21 isolates by multiplex PCR; these two strains were not identified by multiplex PCR because they do not belong to the *Acb* complex, and this assay only identifies *Acinetobacter* species belonging to this complex. MALDI-TOF and multiplex PCR have advantage and disadvantage, but both are simple, fast and reliable (Vijayakumar et al. 2019). MALDI-TOF is the fastest laboratory technique for identification of microorganisms at genus, species and in some cases subspecies level, and it has revolutionized the clinical microbiology diagnostic laboratory in the past decade (Álvarez-Buylla et al. 2012, Ge et al. 2017). The biggest advantage of MALDI-TOF is the possibility to identify uncommon *Acinetobacter* species in clinical isolates, which were previously unidentified. However, some limitations include the need for constant software update, mainly for veterinary isolates. The MALDI-TOF databases aimed primarily at identifying strains from human infectious and may potentially misidentify animal isolates, possibly showing different results for *Acb* complex members. Thus, it is important to highlight that the use of MALDI-TOF in a clinical setting depends on the standardization and regular updating of its database (Guardabassi et al. 2017), which leads to challenges for its adoption in middle-low-income countries.

The main limitation observed for the multiplex PCR assay used in this study was that it only characterizes species belonging to the *Acb* complex, but it is still a great tool for characterization at the genus level (Chen et al. 2018). Therefore, considering that MALDI-TOF is an expensive technique to be introduced and maintained at a routine laboratory, the PCR assay could be a more practical alternative to identify and discriminate the most prevalent species of the *Acb* complex, contributing with the knowledge of the role of these species in animal infections.

***Acb* complex resistance profile**

All strains identified as MDR (n=8) were resistant to three or more antimicrobial classes, according with CLSI (2020). Besides the MDR phenotype, the *Acb* complex isolates are intrinsically resistant to ampicillin, amoxicillin, amoxicillin + c*lavulanic* acid, aztreonam, ertapenem, trimethoprim, chloramphenicol and fosfomycin (CLSI 2020), which impose additional challenges to treat *Acinetobacter*-associated infections. In addition, there are only a few antimicrobials with specific cut-off points described for the disk diffusion testing in CLSI or EUCAST manuals. It is important to highlight that VET-CLSI (2020) considers intrinsic resistance to cefotaxime at *Acb* complex showing the real challenge of working with these species and generating an accurate diagnosis.

Our results along with those from recent studies raise concerns about the considerable high prevalence of MDR-*Acinetobacter* associated with animal infections, and that it may potentially be transmitted to the human environment or vice-versa (van der Kolk et al. 2019, Maboni et al. 2020). The MDR pattern is related to the expression of several resistance mechanisms, including β-lactamases, multidrug efflux pumps, aminoglycoside modifying enzymes, permeability defects and alteration of target sites (Gallagher & Baker 2020). In a recent student, high resistance rates of MDR to the major antimicrobials were found for *Acinetobacter* isolates from animals, except for imipenem to which all isolates tested were susceptible (Maboni et al. 2020). Our results differ from those found by Maboni et al. (2020), since they found MDR *A. baumannnii* as the most prevalent among north American animals, but we found *A. pittii*; geographical differences might be one of the factors associated with these results.

For clinic routine, carbapenemase are a good therapeutic choice to treat *Acb* complex infections (Zahra et al. 2021) raising concerns when the bacteria are resistant to this class. So, in addition to MDR, resistance to carbapenems was researched and the choice of carbapenem antimicrobial was meropenem, because they have more specificity (CLSI 2020). No carbapenemase-producing at phenotypic enzymatic test was detected in this study, CLSI (2020) do not preconize the methodology CarbaNP, mCIM and eCIM to analyze *Acb* complex because there are poor of specificity and reproducibility, being necessary the genotypic detection to characterize this kind of resistance.

The correlation between the resistance strains and the carbapenemase gene was low. In cases where there was resistance to meropenem, but no bla_{IMP} or bla_{VIM}, it may be that they have other carbapenem resistance genes that were not evaluated in the study. In the strains that presented the gene, but were not resistant to meropenem, some factor prevented their phenotypic expression, and this should be elucidated in future studies. Resistance to carbapenems is associated with increased mortality, it is difficult to determine the independent influence of MDR *A. baumannii* on morbidity and mortality (Weinberg et al. 2020).

Metallo-lactamases are frequently involved in carbapenem resistance (Zahra et al. 2021). A recent study from a hospital in Tanzania observed prevalence of bla_{IMP} 91,30% (21/23). Another study was detected bla_{IMP} e bla_{VIM} genes in animal, but sample was not *discriminative* reported (Waltenburg et al. 2022). Girija et al. (2018) found 34,34% (25/73) of bla_{VIM} in severe urinary tract infection in human. There is very difficult detected these genes in animal studies. It is worth noting that the transmission of antibiotic-resistant bacteria from animals to humans and vice versa is possible (Nocera et al. 2021). More studies should More studies are needed to better investigate the bottlenecks found.

CONCLUSIONS

Acinetobacter pittii followed by *Acinetobacter baumannii* was the most prevalent *Acinetobacter calcoaceticus-Acinetobaceter baumannii* (*Acb*) complex species identified from animal clinical specimens.

The MDR profile was detected mainly in *A. baumannii and A. pittii* isolates, and the presence of carbapenemase genes was observed for both species. MALDI-TOF or PCR assays are necessary to reliably identify *Acinetobacter* at species level, and in this study, the performance of both techniques was fairly similar. Practical implications such as costs and availability of these techniques should be considered as potential challenges, mostly in low-middle income settings.

This study alerts for a potential increase of resistant *Acb* complex isolates, reinforcing the need for complete and precise diagnostics, and continuous monitoring of *Acinetobacter* associated with animal infections.

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REFERENCES

Al Atrouni A., Joly-Guillou M.-L., Hamze M. & Kempf M. 2016. Reservoirs of non-*baumannii Acinetobacter* species. Frontiers Microbiol. 7:49. <https://dx.doi.org/10.3389/fmicb.2016.00049> <PMid:26870013>

Álvarez-Buylla A., Culebras E. & Picazo J.J. 2012. Identification of *Acinetobacter* species: is Bruker biotyper MALDI-TOF mass spectrometry a good alternative to molecular techniques? Infect. Genet. Evol. 12(2):345-349. <https://dx.doi.org/10.1016/j.meegid.2012.01.002> <PMid:22266021>

Buyukcangaz E., Velasco V., Sherwood J.S., Stepan R.M., Koslofsky R.J. & Logue C.M. 2013. Molecular typing of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) isolated from animals and retail meat in North Dakota, United States. Foodborne Pathog. Dis. 10(7):608-617. <https://dx.doi.org/10.1089/fpd.2012.1427> <PMid:23638848>

Chen L., Yuan J., Xu Y., Zhang F. & Chen Z. 2018. Comparison of clinical manifestations and antibiotic resistances among three genospecies of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex. PLoS One 13(2):e0191748. <https://dx.doi.org/10.1371/journal.pone.0191748> <PMid:29389980>

Chen T.-L., Lee Y.-T., Kuo S.-C., Yang S.-P., Fung C.-P. & Lee S.-D. 2014. Rapid identification of *Acinetobacter baumannii*, *Acinetobacter nosocomialis* and *Acinetobacter pittii* with a multiplex PCR assay. J. Med. Microbiol. 63(Pt 9):1154-1159. <https://dx.doi.org/10.1099/jmm.0.071712-0> <PMid:24965800>

Chusri S., Chongsuvivatwong V., Rivera J.I., Silpapojakul K., Singkhamanan K., McNeil E. & Doi Y. 2014. Clinical outcomes of hospital-acquired infection with *Acinetobacter nosocomialis and Acinetobacter pittii.* Antimicrob. Agents Chemother. 58(7):4172-4179. <https://dx.doi.org/10.1128/AAC.02992-14> <PMid:24820079>

CLSI 2020. Clinical Laboratory Standards Institute: CLSI Guideline, Performance Standards for Antimicrobial Susceptibility Testing. CLSI/ NCCLS M100, 2020 <ISSN 2162-2914>

Fallah F., Noori M., Hashemi A., Goudarzi H., Karimi A., Erfanimanesh S. & Alimehr S. 2014. Prevalence of blaNDM, blaPER, blaVEB, blaIMP, and blaVIM genes among *Acinetobacter baumannii* isolated from two hospitals of Tehran, Iran. Scientifica, Cairo, 2014:245162. <https://dx.doi.org/10.1155/2014/245162> <PMid:25133013>

Gallagher P. & Baker S. 2020. Developing new therapeutic approaches for treating infections caused by multi-drug resistant *Acinetobacter baumannii*: *Acinetobacter baumannii* therapeutics. J. Infect. 81(6):857-861. <https://dx.doi.org/10.1016/j.jinf.2020.10.016> <PMid:33115656>

Ge M.-C., Kuo A.-J., Liu K.-L., Wen Y.-H., Chia J.-H., Chang P.-Y., Lee M.-H., Wu T.-L., Chang S.-C. & Lu J.-J. 2017. Routine identification of microorganisms by matrix-assisted laser desorption ionization time-of-flight mass spectrometry: Success rate, economic analysis, and clinical outcome. J. Microbiol. Immunol. Infect. 50(5):662-668. <https://dx.doi.org/10.1016/j.jmii.2016.06.002> <PMid:27426930>

Girija S.A., Jayaseelan V.P. & Arumugam P. 2018. Prevalence of VIM-and GIM-producing *Acinetobacter baumannii* from patients with severe urinary tract infection. Acta Microbiol. Immunol. Hungarica 65(4):539-550. <https://dx.doi.org/10.1556/030.65.2018.038> <PMid:30111160>

Guardabassi L., Damborg P., Stamm I., Kopp P.A., Broens E.M., Toutain P.-L. & ESCMID Study Group for Veterinary Microbiology. 2017. Diagnostic microbiology in veterinary dermatology: present and future. Adv. Vet. Dermatol. 28(1):146-e30. <https://dx.doi.org/10.1111/vde.12414> <PMid:28133869>

Maboni G., Seguel M., Lorton A. & Sanchez S. 2020. Antimicrobial resistance patterns of *Acinetobacter* spp. of animal origin reveal high rate of multidrug resistance. Vet. Microbiol. 245:108702. <https://dx.doi.org/10.1016/j.vetmic.2020.108702> <PMid:32456823>

Martiny D., Busson L., Wybo I., El Haj R.A., Dediste A. & Vandenberg O. 2012. Comparison of the Microflex LT and Vitek MS systems for routine identification of bacteria by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. 50(4):1313-1325. <https://dx.doi.org/10.1128/JCM.05971-11> <PMid:22322345>

Nocera F.P., Attili A.-R. & De Martino L. 2021. *Acinetobacter baumannii*: Its clinical significance in human and veterinary medicine. Pathogens 10(2):127. <https://dx.doi.org/10.3390/pathogens10020127> <PMid:33513701>

Rafei R., Hamze M., Pailhoriès H., Eveillard M., Marsollier L., Joly-Guillou M.-L., Dabboussi F. & Kempf M. 2015. Extra-human epidemiology of *Acinetobacter baumannii* in Lebanon. Appl. Environ. Microbiol. 81(7):2359-2367. <https://dx.doi.org/10.1128/AEM.03824-14> <PMid:25616788>

Rodríguez C.H., Nastro M. & Famiglietti A. 2018. Carbapenemases in *Acinetobacter baumannii*. Review of their dissemination in Latin America. Revta Arg. Microbiol. 50(3):327-333. <https://dx.doi.org/10.1016/j.ram.2017.10.006> <PMid:29548732>

Šedo O., Nemec A., Křížová L., Kačalová M. & Zdráhal Z. 2013. Improvement of MALDI-TOF MS profiling for the differentiation of species within the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex. Syst. Appl. Microbiol. 36(8):572-578. <https://dx.doi.org/10.1016/j.syapm.2013.08.001> <PMid:24054697>

Sousa C., Botelho J., Silva L., Grosso F., Nemec A., Lopes J. & Peixe L. 2014. MALDI-TOF MS and chemometric based identification of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex species. Int. J. Med. Microbiol. 304(5/6):669-677. <https://dx.doi.org/10.1016/j.ijmm.2014.04.014> <PMid:24877727>

Tuttobene M.R., Pérez J.F., Pavesi E.S., Perez Mora B., Biancotti D., Cribb P., Altilio M., Müller G.L., Gramajo H., Tamagno G., Ramírez M.S., Diacovich L. & Mussi M.A. 2021. Light modulates important pathogenic determinants and virulence in ESKAPE pathogens *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. J. Bacteriol. 203(5):e00566-20. <https://dx.doi.org/10.1128/JB.00566-20> <PMid:33288627>

van der Kolk J.H., Endimiani A., Graubner C., Gerber V. & Perreten V. 2019. *Acinetobacter* in veterinary medicine, with an emphasis on *Acinetobacter baumannii*. J. Glob. Antimicrob. Resist. 16:59-71. <https://dx.doi.org/10.1016/j.jgar.2018.08.011> <PMid:30144636>

Vijayakumar S., Biswas I. & Veeraraghavan B. 2019. Accurate identification of clinically important *Acinetobacter* spp.: an update. Future Science OA 5(7):FSO395. <https://dx.doi.org/10.2144/fsoa-2018-0127> <PMid:31285840>

Waltenburg M.A., Shugart A., Loy J.D., Tewari D., Zhang S., Cole S.D., CRO Veterinary Diagnostic Laboratory Investigation Group, Walters M.S. & Nichols M. 2022. A survey of current activities and technologies used to detect carbapenem resistance in bacteria isolated from companion animals at veterinary diagnostic laboratories - United States, 2022. J. Clin. Microbiol. 60(3):e0215421. <https://dx.doi.org/10.1128/JCM.02154-21> <PMid:34985981>

Wang J., Ruan Z., Feng Y., Fu Y., Jiang Y., Wang H. & Yu Y. 2014. Species distribution of clinical *Acinetobacter* isolates revealed by different identification techniques. PloS One 9(8):e104882. <https://dx.doi.org/10.1371/journal.pone.0104882> <PMid:25120020>

Weinberg S.E., Villedieu A., Bagdasarian N., Karah N., Teare L. & Elamin W.F. 2020. Control and management of multidrug resistant *Acinetobacter baumannii*: A review of the evidence and proposal of novel approaches. Infect. Prev. Pract. 2(3):100077. <https://dx.doi.org/10.1016/j.infpip.2020.100077> <PMid:34368717>

Welker M. & Moore E.R.B. 2011. Applications of whole-cell matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry in systematic microbiology. System. Appl. Microbiol. 34(1):2-11. <https://dx.doi.org/10.1016/j.syapm.2010.11.013> <PMid:21288677>

Wisplinghoff H., Paulus T., Lugenheim M., Stefanik D., Higgins P.G., Edmond M.B., Wenzel R.P. & Seifert H. 2012. Nosocomial bloodstream infections due to *Acinetobacter baumannii*, *Acinetobacter pittii* and *Acinetobacter nosocomialis* in the United States. J. Infect. 64(3):282-290. <https://dx.doi.org/10.1016/j.jinf.2011.12.008> <PMid:22209744>

Wong D., Nielsen T.B., Bonomo R.A., Pantapalangkoor P., Luna B. & Spellberg B. 2017. Clinical and pathophysiological overview of *Acinetobacter* infections: a century of challenges. Clin Microbiol Rev. 30(1):409-447. <https://dx.doi.org/10.1128/CMR.00058-16> <PMid:27974412>

Zahra N., Zeshan B., Qadri M.M.A., Ishaq M., Afzal M. & Ahmed N. 2021. Phenotypic and genotypic evaluation of antibiotic resistance of *Acinetobacter baumannii* bacteria isolated from surgical intensive care unit patients in Pakistan. Jundishapur J. Microbiol. 14(4):e113008. <https://dx.doi.org/10.5812/jjm.113008>