



Dynamics of *bla*_{OXA-23} gene transmission in *Acinetobacter* spp. from contaminated veterinary environmental surfaces: an emerging One Health threat?

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SUMMARY

Background: Carbapenem-resistant *Acinetobacter baumannii* is a common pathogen associated with healthcare-acquired infections, and robust infection prevention and control protocols exist in human healthcare settings. In contrast, infection prevention and control (IPC) standards are limited in veterinary medicine, necessitating further investigation.

Aim: Examine the possible transmission of carbapenem-resistant *Acinetobacter* spp. in a veterinary practice where a cat was diagnosed with an OXA-23-producing *A. baumannii* ST2 strain.

Methods: Environmental samples together with nasal and hand swabs from the veterinary personnel were collected. All swabs were screened for the presence of extended-spectrum-β-lactamase- and carbapenemase-producing Enterobacterales, meticillin-resistant staphylococcus and multi-drug-resistant *Acinetobacter* spp. Whole-genome sequencing was performed for carbapenemase-producing strains.

Results: Of the veterinary staff, 60% carried meticillin-resistant *Staphylococcus epidermidis*. Environmental evaluation showed that 40% ($N=6/15$) of the surfaces analysed by contact plates and 40% ($N=8/20$) by swabs failed the hygiene criteria. Assessment of the surfaces revealed contamination with five OXA-23-producing *Acinetobacter* spp. strains: an OXA-23-producing *Acinetobacter schindleri* on the weight scale in the waiting room;

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and four OXA-23-producing *Acinetobacter lwoffii* strains, on different surfaces of the treatment room. The *bla*_{OXA-23} gene was located on the same plasmid-carrying Tn2008 across the different *Acinetobacter* spp. strains. These plasmids closely resemble a previously described OXA-23-encoding plasmid from a human Portuguese nosocomial *Acinetobacter pittii* isolate. Distinctly, the OXA-23-producing *A. baumannii* ST2 clinical strain had the resistant gene located on Tn2006, possibly inserted on the chromosome.

Conclusion: The detection of an OXA-23-producing *A. baumannii* ST2 veterinary clinical strain is of concern for companion animal health and infection, prevention and control. This study established the dynamic of transmission of the plasmid-mediated *bla*_{OXA-23} gene on critical surfaces of a small animal veterinary practice. The genetic resemblance to a plasmid found in human nosocomial settings suggests a potential One Health link.

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Introduction

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) infections are of great concern in healthcare human setting, with mortality surpassing 40% in critically ill patients [1]. According to the latest European Centre for Disease Prevention and Control report on healthcare-associated infections (HAIs) acquired in intensive care units [2], carbapenem resistance was observed on 82% of *A. baumannii* recovered. Additionally, *Acinetobacter schindleri* and *Acinetobacter lwoffii* have also been described as causative agents of nosocomial infections, regardless of their carbapenem resistance status [3,4]. The need for infection, prevention and control (IPC) policies in human medicine is clear and is mandatory in preventing outbreak situations and in controlling healthcare-associated infections caused by multi-drug-resistant (MDR) and biofilm-producing organisms, such as methicillin-resistant *Staphylococcus aureus* and *Acinetobacter* spp. [5–7].

In the past decade, the number of companion animals per family has risen, which consequently leads to the increasing number of veterinary hospitals and practices. This has led to an increased development around small-animal intensive care facilities [8]. Furthermore, the number of animal patients subjected to invasive procedures is expected to increase, and consequently a rise in the preventive use of antibiotics.

In veterinary medicine, carbapenemase-producing Enterobacterales and *Acinetobacter* spp. clinical strains from companion animals are emerging in Portugal and other European countries [9–11]. Subsequently, colonization of staff with carbapenemase-producing bacteria is occurring and spread of OXA-48-producing Enterobacterales inside a veterinary practice is, nowadays, a reality [12,13]. These recent studies draw a parallel between human and veterinary medicine, indicating that it is necessary to comprehend the level of contamination by MDR bacteria in veterinary practices to better guide antimicrobial stewardship practices and IPC programmes.

In the last week of April 2022, in a medium-size veterinary practice in the central region of Portugal, a hospitalized cat was diagnosed with a skin and soft tissue infection, secondary to a post-traumatic lesion in the posterior aspect of its body, caused by OXA-23-producing *A. baumannii*. To evaluate the possible environmental contamination by carbapenem-resistant *Acinetobacter* spp. and other bacteria, samples from different surfaces of the clinic were taken one week after

the cat's stay. Nasal and hand carriage of MDR bacteria were also evaluated in the veterinary personnel. Possible transmission of carbapenem-resistance was identified by whole-genome bacterial sequencing.

Methods

Clinical isolate

A clinical swab collected from a cat with skin and soft tissue infection was sent to a diagnostic laboratory for microbiological culture analysis and minimum inhibitory concentration (MIC) determination. The swab was plated on Columbia Blood agar (Thermofisher Scientific), MacConkey agar, Brilliance™ ESBL agar and Brilliance™ MRSA 2 agar (Thermofisher Scientific) for phenotypic identification of the causative agent of infection. MIC panel testing for 22 antibiotics (nalidixic acid, amikacin, ampicillin, amoxicillin in combination with clavulanic acid, aztreonam, cefoxitin, cephalothin, ceftazidime, ciprofloxacin, colistin, doripenem, ertapenem, gentamicin, imipenem, levofloxacin, meropenem, nitrofurantoin, norfloxacin, piperacillin, tetracycline, tobramycin, trimethoprim – sulfamethoxazole) using MicroSan NEG44 plates (Beckman Coulter, USA) was performed and interpreted according to EUCAST breakpoints [14]. Species identification was performed using 16S rRNA [15] and detection of *bla*_{OXA-23} gene was carried out by polymerase chain reaction (PCR) detection [16].

On-site collection

Contact plates (with 28.26 cm² of Plate Count Agar area) and surface swabs (TS/5–42 with 10 mL neutralizing buffer, TSC Ltd) were taken from different critical surfaces of the clinic. Samples using surface swabs were limited to an area of 100 cm² using a template square of 10 × 10 cm. Flat surfaces were sampled by both methods, while irregular ones were only sampled by surface swabs. Samples were taken from locations as they were, i.e., no cleaning procedure was specifically performed prior to sampling. Additionally, surfaces where an animal has just been in contact with or that was currently in use, were not considered for collection as this would lead to biased results.

The veterinary practice had no record of the locations where the infected cat had passed, apart from the treatment room table. As such, we decided to analyse all critical surfaces — areas which are critically important in a veterinary

practice such as high-touch and high-contact surfaces. Regarding detergents and disinfectants in use at the clinic, the detergents routinely used were purchased at the supermarket and the only disinfectant used was bleach diluted with water at an unknown concentration. [Supplementary Table S1](#) summarizes all locations sampled and by which method.

Two nasal swabs (one per nostril) and one swab sampling both hands were taken from each member of staff (five veterinary doctors, three nurses and two support staff), with a written consent form having been signed prior to collection. A questionnaire was used to assess demographic and general human health data, professional situation and previous antibiotic treatment.

To reduce potential bias, hand swabs were taken during the daily procedures, when the workload permitted. All samples were carefully coded and placed in a cooler until processing.

Sample analysis

Contact plates were placed directly at 37 °C and colony forming units (cfu) were counted at 24 and 48 h of incubation. Evaluation of efficacy of hygiene and disinfection protocols was interpreted in accordance with the criteria established by Mulvey *et al.* [17] – a growth >2.5 cfu/cm² fails the efficacy cleaning criteria for aerobic colony count (ACC).

To evaluate the efficacy of cleaning regimen by surface swabs, a criterion of >1 cfu/cm² was applied [18] for the growth observed on non-selective media Brain Heart Agar (BHA) (Biokar Diagnostics, France). Following an enrichment step on Brain Heart Infusion broth (Biokar Diagnostics, France) overnight at 37 °C, samples were plated on MacConkey agar supplement with 1.5 mg/mL of cefotaxime, MacConkey agar supplemented with 1.5 mg/mL of meropenem (ThermoFisher Scientific), CHROMagar™ *Acinetobacter* supplemented with CHROMagar™ MDR Selective (CR102, Chromagar) and Brilliance™ MRSA 2 agar (ThermoFisher Scientific).

One randomly selected nasal swab was placed overnight on buffered peptone water (Biokar Diagnostics, France) at 37 °C for the enrichment procedure, and then plated on MacConkey agar supplemented with 1.5 mg/mL of cefotaxime for the selective growth of extended-spectrum-β-lactamase-producing bacteria, on MacConkey agar supplemented with 1.5 mg/mL of meropenem for carbapenem-resistant bacteria and on CHROMagar™ *Acinetobacter* supplemented with CHROMagar™ MDR Selective (CR102, Chromagar). The other nasal swab was placed overnight on sodium chloride supplement with 13% tryptone soy broth and plated on Mannitol Salt Agar (Biokar Diagnostics, France) and Brilliance™ MRSA 2 agar for the selective growth of methicillin-resistant staphylococci.

Hand swabs followed an enrichment procedure with peptone water, followed by plating on the non-selective (Brain Heart Agar and Mannitol Salt Agar) and the selective media mentioned previously for nasal swab analysis.

In all cases, up to three isolates with similar phenotypical appearance were further selected for analysis. MIC testing was performed as described previously [13].

Resistance gene identification and sequencing

DNA was extracted from pure cultures using a boiling extraction method, and a series of multiplex PCRs was performed, as previously described for detection of β-lactamase

and carbapenemase genes [19]. Sanger sequencing was performed to identify the amplified β-lactamase and carbapenemase genes. For staphylococci strains, presence of the *mecA* gene was evaluated [20]. Confirmation of species identification was performed by sequencing 16S rRNA as previously described [15]. Multi-locus sequence typing (MLST) was performed for methicillin-resistant staphylococci isolates according to the scheme published by Thomas *et al.* [21,22].

Whole-genome sequencing analysis

One representative resistant strain from each surface harbouring carbapenemase genes was selected for whole-genome sequencing (WGS). Whole DNA was extracted from RNase-treated lysates via NZY Tissue gDNA Isolation kit (NZYTech, Lisbon, Portugal). All libraries for WGS were prepared using TruSeq DNA PCR-Free preparation kit (Illumina, San Diego, CA, USA). DNA sequencing was performed using Illumina NovaSeq platform with 2×150 bp paired-end reads. *De novo* assembled genomes were obtained using a previously described pipeline [19]. ResFinder 4.1 (available at the Centre of Genomic Epidemiology – <https://www.genomic epidemiology.org/>) and CARD database (available at <https://card.mcmaster.ca/home> [23]) were used for screening the novel generated assemblies for the identification of antimicrobial resistance genes. Single-nucleotide polymorphism (SNP) analysis was conducted using Parsnp v1.2 for multiple sequence alignment of generated assemblies plus reference genome for each *acinetobacter* species (reference *A. baumannii* – Genbank Acc. GCF_003264275.1; reference *A. lwoffii* – Genbank Acc. GCF_019787625.1). All *de novo* assemblies have been submitted to NCBI under the accession number PRJNA1000421.

ISAb1 amplification

A set of primers was designed using NCBI Primer Blast server (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>) to amplify the nucleotide sequence of ISABA1 (ISABA1_Forward – 5'-TCCTATCAGGTTCTGCCTTC-3'; ISABA1_Reverse – 5'-ACGGGTGAATGGCAACATGA-3'). The reaction mix contained 25 µL Supreme NZY Taq II 2× Green Master Mix (Nzytech, Portugal), 10 pmol/µL of each primer for a volume of 1 µL and 5 µL of DNA in a final volume of 50 µL. Initial denaturation (94 °C for 4 min) was followed by 35 cycles of amplification. Each cycle consisted of 90 °C at 30 s, 60 °C for 40 s and 72 °C for 1 min. A last extension step (72 °C for 5 min) completed the amplification. PCR product was amplified by Sanger sequencing to complete the missing gaps in WGS.

Plasmid comparison

Using BRIG analysis tool [24], obtained contigs which contained antimicrobial resistance genes were aligned to a reference plasmid on NCBI database (GenBank Acc. MF078634).

Ethics statement

Ethical approval for the study was obtained (CEBEA011/2021) at the Faculty of Veterinary Medicine, University of Lisbon.

Results

Surface hygiene evaluation by direct culture

A total of 20 surfaces were analysed throughout the clinic, 15 with contact plates and surface swabs, five with surface swabs alone (Supplementary Table S1). According to contact plate evaluation, 40% of the surfaces ($N=6/15$) failed the cleaning efficacy assessment when interpreted in accordance with the Mulvey *et al.* criterion of >2.5 cfu/cm² [17] (Table I). Yet, when evaluating the total surfaces by swabs, 40% ($N=8/20$) failed the criterion established by Dancer *et al.* of >1 cfu/cm² [18]. Incidentally, a susceptible *Acinetobacter radioresistens* strain was identified based on its peculiar phenotypic appearance on the BHA plate from the ultrasound's keyboard.

Environmental contamination with MDR bacteria after enrichment

Of the 20 surfaces analysed, five (25%) also showed environmental contamination with MDR organisms (Table 2), namely *Acinetobacter* spp. ($N=4$) and an ST554 meticillin-resistant *Staphylococcus epidermidis* (MRSE) ($N=1$), that were recovered from acinetobacter- and meticillin-resistant-staphylococcus-selective plates, respectively. Four of these surfaces had failed the cleaning efficacy criteria (Table I).

Further analysis of these *Acinetobacter* spp. MDR strains classified them as *A. schindleri* (B1E8A1, on the weight scale waiting room) and *A. lwoffii* on different surfaces of the treatment room (B4Z4A1, B4Z8A1, B11Z4A1 and B12Z8A1) (Table II). Moreover, MIC determination confirmed their resistance to fluoroquinolones and carbapenems (Table III). Molecular analysis confirmed that all *Acinetobacter* spp. strains harboured the *bla*_{OXA-23} carbapenemase gene.

Veterinary personnel carriage with MDR bacteria after enrichment

All team members ($N=10$) worked exclusively in the clinic, except for one veterinarian. Three members had taken antibiotics in the last six months prior to sampling, albeit only two classes were labelled – one took the combination of amoxicillin with clavulanic acid, while the other took a fluoroquinolone (not specified). The third member did not fill out which antibiotic had been taken.

Of the nasal samples collected, 20% were negative ($N=2/10$). Among the positive samples, no *Acinetobacter* spp. strains were recovered and 60% ($N=6/10$) of veterinary staff carried meticillin-resistant *S. epidermidis* (MRSE) (ST32, ST487, ST54 and ST59), recovered from MRS selective plates. The remaining employees only carried meticillin-susceptible *S. aureus* (MSSA), recovered from mannitol plates.

Table I

Results of surface evaluation based on contact plates and environmental swabs on non-selective media

Surfaces	Contact plates	Criterion <2.5 cfu/cm ^{2a}	Non-selective media	Criterion <1 cfu/cm ^{2b}
Surgery room				
Surgery table – window side	1.77	Passed	–	Passed
Wood stool	Uncountable	Failed	0.01	Passed
Stainless-steel supporting table	Uncountable	Failed	0.01	Passed
Thermal blanket	0.50	Passed	Uncountable	Failed
Surgery bed	0.88	Passed	Uncountable	Failed
Surgery table – door side	0.88	Passed	0.02	Passed
Anaesthetic device buttons	NA	NA	–	Passed
Oxygen balloon	NA	NA	–	Passed
Treatment room				
Stainless-steel tray	Uncountable	Failed	Uncountable	Failed
Black plastic tapete on treatment table	0.07	Passed	–	Passed
Treatment table grills	0.04	Passed	–	Passed
Weight scale treatment room	Uncountable	Failed	Uncountable	Failed
Keyboard computer	NA	NA	Uncountable	Failed
Isolation unit				
Cage 01	0.78	Passed	–	Passed
Cage 02	0.00	Passed	0.05	Passed
Ultrasound room				
Ultrasound screen	Uncountable	Failed	0.5	Passed
Ultrasound table	NA	NA	0.5	Passed
Ultrasound keyboard	NA	NA	Uncountable	Failed
Others				
Consultation room – table	0.28	Passed	Uncountable	Failed
Waiting room weight scale	Uncountable	Failed	Uncountable	Failed

cfu were considered uncountable if >300 . NA, not applicable.

^a Cut-off value defined by Mulvey *et al.*, 2011 [17].

^b Cut-off value defined by Dancer 2004 [18].

Table II
Multi-drug-resistant bacteria found at the surfaces analysed and number of cfu/cm2

Surfaces	Isolates	Selective growth media	Cfu/100 cm ²	Bacterial strains
Waiting room weight scale	B1E8A1	CHROMagar™ <i>Acinetobacter</i>	50	MDR <i>Acinetobacter schindleri</i>
Weight scale treatment room	B4Z4A1	MCK + 1.5 mg/mL of MEM	50	MDR <i>Acinetobacter lwoffii</i>
	B4Z8A1	CHROMagar™ <i>Acinetobacter</i>	8	MDR <i>A. lwoffii</i>
Stainless steel tray	B11Z4A1	MCK + 1.5 mg/mL of MEM	25	MDR <i>A. lwoffii</i>
Keyboard computer	B12Z8A1	CHROMagar™ <i>Acinetobacter</i>	>100	MDR <i>A. lwoffii</i>
Ultrasound table	B19Z9S1	Brilliance™ MRSA 2 agar	50	Meticillin-resistant <i>Staphylococcus epidermidis</i>

As for hand swab analysis, MRSE was also detected on two veterinarians, however the clones were different from those colonizing the nares (ST278 and ST510). No *Acinetobacter* spp. strains were found.

Acinetobacter spp. whole-genome analysis

WGS was performed to evaluate the core- and accessory genome of the MDR *Acinetobacter* strains, namely the genetic environment of the bla_{OXA-23} carbapenemase genes. Properties on the whole-genome-sequenced strains are listed in Supplementary Table S2.

All environmental strains (B1E8A1, B4Z4A1, B4Z8A1, B11Z4A1 and B12Z8A1) possessed the operon czc, which encodes proteins responsible for resistance to cobalt, zinc and cadmium. However, only *A. lwoffii* strains possessed the mer operon, that encodes for mercury resistance.

Intrinsically carbapenem-hydrolysing class D β-lactamase bla_{OXA-134}-like non-expressed genes were identified on *A. lwoffii* and *A. schindleri* species, as previously described [25,26]. Specifically, the bla_{OXA-276} gene was present on strain B1E8A1 (*A. schindleri*) and the bla_{OXA-285} gene was present on three *A. lwoffii* strains (B4Z4A1, B11Z4A1 and B12Z8A1). Strain B4Z8A1 harboured the bla_{OXA-362} gene. In addition, all strains

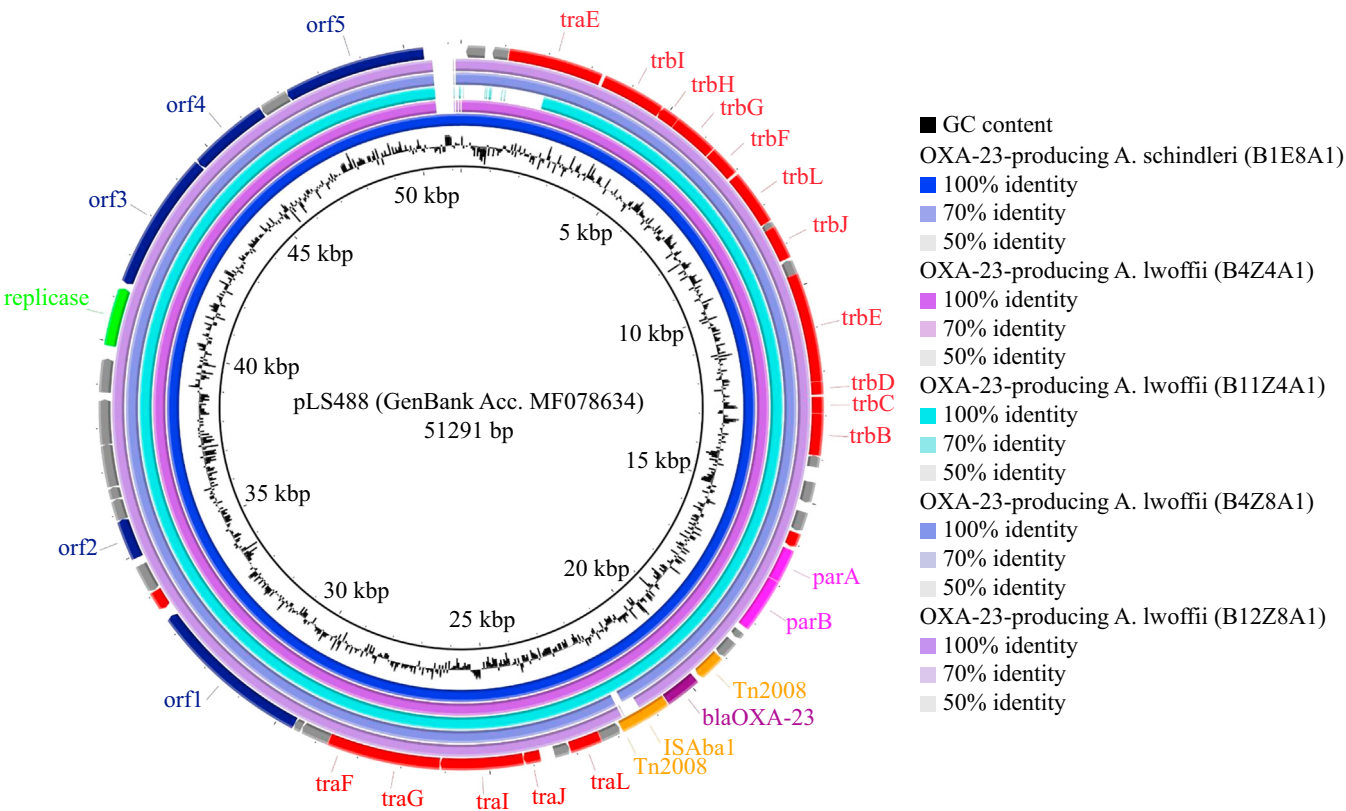


Figure 1. Plasmid alignment comparison between de-novo assembled plasmids and Portuguese nosocomial reference (GenBank Acc. MF078634). From inner ring to outer ring: p_B1E8A1 (dark blue, weight scale waiting room); p_B4Z4A1 (pink, weight scale treatment room); p_B4Z8A1 (purple, weight scale treatment room); p_B11Z4A1 (turquoise, stainless steel tray in the treatment room); p_B12Z8A1 (lilac, computer's keyboard treatment room). Genes are represented by coloured blocks: purple, resistance genes; blue and green, DNA replication, regulation, and restriction systems; red, conjugation-association genes; fuchsia, genes associated with partition and stability systems; orange, transposons, insertion sequences (ISs) and transposase genes; grey, hypothetical proteins. Image generated using BRIG 0.95, available at <http://brig.sourceforge.net/>.

Table III
OXA-23-producing *Acinetobacter* spp. molecular characteristics and susceptibility profile

Environmental surface isolation	Bacterial species	Representative strain	Resistance genes	Mutations	Antibiotic resistance profile ^a						
					Amikacin (R>8)	Gentamicin (R>4)	Ciprofloxacin (R<1)	Doripenem (R>2)	Imipenem (R>4)	Meropenem (R>8)	
Waiting room weight scale	<i>Acinetobacter schindleri</i>	B1E8A1	<i>bla</i> _{OXA-23} <i>sul2</i> <i>aph(3'')-Ib</i> <i>aph(6)-Id</i>		<8	≤2	>2	4	8	4	
Weight scale (treatment room)	<i>Acinetobacter lwoffii</i>	B4Z4A1	<i>bla</i> _{OXA-23} <i>tet39</i>		<8	≤2	2	4	8	4	
Stainless steel tray (treatment room)	A. <i>lwoffii</i>	B4Z8A1	<i>bla</i> _{OXA-23}		<8	≤2	>2	4	8	8	
	A. <i>lwoffii</i>	B11Z4A1	<i>bla</i> _{OXA-23}		<8	≤2	2	2	8	4	
Keyboard computer (treatment room)	A. <i>lwoffii</i>	B12Z8A1	<i>tet39</i>		<8	≤2	2	2	4	4	
Infection	<i>Acinetobacter baumannii</i>	10854	<i>bla</i> _{OXA-23} <i>tet39</i> <i>bla</i> _{TEM-1} ; <i>bla</i> _{OXA-23} ; <i>aph(3'')-Ib</i> <i>parC</i> (S84L, <i>aph(6)-Id</i> ; <i>tet(B)</i> V104I, D105E)	<i>gyrA</i> (S81L) <i>parC</i> (S84L, V104I, D105E)	<8	>2	>2	>2	>8	>8	

^a Breakpoints determined in accordance with EUCAST breakpoint guidelines 2023.

produced OXA-23 oxacillinases, which were responsible for the carbapenem resistance observed phenotype (Table III). As for additional antibiotic resistance genes, strains B4Z4A1, B11Z4A1 and B12Z8A1 possessed the *tet39* gene and B1E8A1 harboured the *sul2*, *aph(3'')-Ib* and *aph(6)-Id* genes (Table III).

Three of the four MDR A. *lwoffii* strains had a range from 12 to 22 different SNPs (Supplementary Table S3), while the A. *lwoffii* B4Z8A1 strain had a difference greater than >6000 SNPs. Thus, two different clones of MDR A. *lwoffii* strains were circulating in the veterinary practice environment.

For the OXA-23-producing A. *baumannii*, the intrinsically *bla*_{OXA-66} gene was identified, as well as *tet(B)*, *sul2* and *bla*_{TEM-1}. Mutations conferring resistance to fluoroquinolones were also identified on *gyrA* (S81L) and *parC* (S84L, V104I, D105E). In-depth analysis did not yield any environmental heavy metal resistance genes (Table III). This strain was classified as OXA-23-producing A. *baumannii* ST2, belonging to the Global Clone 2.

WGS analysis on A. *radioresistens* only confirmed the presence of intrinsically *bla*_{OXA-23} non-expressed gene and the operon *czc*.

Dynamics of plasmid-mediated transmission of the *bla*_{OXA-23} genes

On all studied environmental strains (B1E8A1, B4Z4A1, B4Z8A1, B11Z4A1 and B12Z8A), the *bla*_{OXA-23} gene was located on transposon Tn2008, which is characterized by the presence of a single copy of ISAbal and absence of seven base pairs between ISAbal and the beginning of the *bla*_{OXA-23} gene [27].

When using a formerly described plasmid containing the *bla*_{OXA-23} gene (GenBank Acc. MF078634) from an *Acinetobacter pittii* Portuguese human nosocomial strain [28], a high degree of homology was observed with p_B1E8A1, p_B4Z4A1, p_B4Z8A1, p_B11Z4A1 and p_B12Z8A from the present study. Figure 1 depicts the complete alignment of the plasmids found on the studied strains and the reference plasmid.

The cat's clinical strain A. *baumannii*, *bla*_{OXA-23} was present on transposon Tn2006. This transposon is characterized by the additional seven base pairs before the beginning of the *bla*_{OXA-23} gene and two copies of the ISAbal [27]. An in-depth analysis of the bacterial genome confirmed the absence of genes commonly associated with the plasmid conjugation system, which would suggest that the *bla*_{OXA-23} gene was located on the chromosome.

Data availability

All *de novo* assemblies have been deposited on Bioproject PRJNA1000421 in GenBank (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1000421/>).

Discussion

Following the stay of a cat infected with an OXA-23-producing A. *baumannii* human Global Clone 2, evaluation of critical surfaces from the veterinary practice revealed contamination with five OXA-23-producing *Acinetobacter* spp. strains. Furthermore, the evaluation of the genetic environment of the *bla*_{OXA-23} gene showed that it was located on the same transposon Tn2008 and on the same plasmid in the different *Acinetobacter* spp. found in the veterinary

environment. Additionally, these plasmids were homologous with a carbapenemase-encoding plasmid also harbouring the *bla*_{OXA-23} gene from a clinical human emergent *A. pittii* strain isolated in a Portuguese hospital [28].

Our study investigated the hygiene of clinical surfaces by direct culture and found that some failed the hygiene criteria established for contact plates, despite meeting the criteria established for surface swabs. This fact may be explained because contact plates are designed to permit the growth of any type of micro-organism (e.g., fungi and yeast), whereas surfaces swabs with a universal neutralizing liquid buffer are specific for bacterial growth. Surface swab evaluation revealed that eight surfaces were unclean, having failed the criteria established by Dancer [18]. Furthermore, 25% of the surfaces were contaminated with carbapenem-resistant *Acinetobacter* spp.: *A. schindleri* (on the weight scale waiting room) and *A. lwoffii* on different surfaces of the treatment room (weight scale of the treatment room, stainless-steel tray and keyboard computer). In contrast, a Swiss study [8] reported, for a veterinary practice of the same size as our work, that 33% of the analysed surfaces were contaminated with meticillin-resistant staphylococci. Yet, the same study described carbapenem-resistant bacteria only in large veterinary hospitals. As such, according to our research, the criteria to establish IPC guidelines cannot be based on case numbers because carbapenem-resistant bacteria may be contaminating the environment regardless of the veterinary practice dimension.

Regarding veterinary personnel carriage with MDR bacteria after enrichment, we found that MRSE was far more common (60%) than MSSA carriage. Despite the absence of meticillin-resistant *S. aureus*, susceptible *S. aureus* can acquire resistance to β -lactams by acquisition of *mecA* gene through horizontal gene transfer by MRSE [29]. Thus, the screening of veterinary personnel for MRSE carriage may be useful to in the prevention of *mecA* dissemination and in the resistance to spreading.

All five OXA-23-producing *Acinetobacter* spp. strains (one *A. schindleri* and four *A. lwoffii*) from the veterinary practice environment underwent WGS. *A. lwoffii* and *A. schindleri* are commonly found in the environment [30,31], but they are also opportunistic pathogens, with cases of New Delhi metallo-beta-lactamase-1 (NDM-1)-producing *A. lwoffii* and *A. schindleri* infections in immunocompromised patients being reported [3,32]. To the best of our knowledge, only one report of OXA-23-producing *A. lwoffii* has been made in an inpatient [32]. Conversely, in veterinary medicine, infections caused by *A. lwoffii* are rarely reported [33] and none caused by *A. schindleri* have been described.

The incidental finding of a susceptible *A. radioresistens* strain on the ultrasound keyboard is relevant, as this species is considered the source of *bla*_{OXA-23} gene [16,27]. However, the gene is not expressed in this species as it lacks the promoter region of ISAbA1. Reports of infection in humans and animals have occurred [33,34]. Thus far, no human *A. radioresistens* nosocomial strains harbouring carbapenemase genes have been reported. However, in veterinary medicine, a hospitalized dog was colonized with NDM-1-producing *A. radioresistens* in Italy [10]. Although the authors of this study did not evaluate the environmental contamination, this result demonstrates the importance of applying IPC guidelines to veterinary healthcare facilities and of performing active surveillance screening for this *Acinetobacter* species.

Both in *A. lwoffii* strains and *A. schindleri*, the *bla*_{OXA-23} gene was located on Tn2008. An in-depth analysis of the plasmid revealed that the same plasmid was present on all strains found on the contaminated surfaces. The plasmid used as reference was previously described in *A. pittii* Portuguese human nosocomial strain [28]. Also, the plasmids here described are conjugative which contributes to easy horizontal gene transfer across different species [35]. These results are worrisome as they show that a probable dissemination of the *bla*_{OXA-23} gene across the different surfaces of the veterinary practice was occurring through plasmid dissemination. Moreover, they also suggest the transmission of this carbapenemase-encoding gene in the transposon Tn2008 through a homologous plasmid from the human hospital healthcare setting to the veterinary healthcare setting in Portugal. Further studies are needed to establish this important One Health link. Hospital–human environmental and human clinical strains from *A. baumannii* which carried *bla*_{OXA-23} gene on the Tn2008 have also been reported, showing the ability of bacteria carrying this transposon to colonize different settings [36].

An OXA-23-producing *A. baumannii* ST2 strain was identified as the causative agent of a skin and soft tissue infection in a cat. ST2 lineage (which is part of Global Clone 2) has been described worldwide and is associated to *bla*_{OXA-23} carbapenemase gene [37]. A previous 2009 report in Portugal also characterized an OXA-23-producing *A. baumannii* ST2 clinical strain with the *bla*_{OXA-23} of feline origin in the veterinary healthcare setting [38]. While this clone was prevalent in Portugal in 2010 [39], lack of updated information of circulating MLST lineages of clinical *A. baumannii* strains and their resistance genes make it difficult to ascertain whether this clone is still in circulation in the human healthcare setting in Portugal, as it has been shown that the dominant clone tends to evolve with time [40]. The *bla*_{OXA-23} gene is commonly associated to Tn2006 in the *A. baumannii* ST2 lineage, whether it is chromosome or plasmid inserted [41]. In our strain, Tn2006 is possibly located on the chromosome as no conjugative elements from plasmids were identified. This is in agreement with the previously described Portuguese veterinarian clinical strain [38], in which Tn2006 is located on the chromosome.

It would have been interesting to evaluate clinical samples from the animals hospitalized at the time of the environmental sampling, but owner consent would have had to be obtained. Thus, this study has the limitation of being unable to establish whether any of the strains described here were linked to a possible outbreak.

The detection of an infection in an admitted cat to a veterinary practice with an OXA-23-producing *A. baumannii* ST2 strain is of concern for companion animal health and IPC programmes. The whole-genome sequencing performed on the environmental carbapenem-resistant *Acinetobacter* spp. strains allowed us to observe the occurrence of equal mobile genetic elements – transposon- and plasmid-carrying *bla*_{OXA-23} gene on different *Acinetobacter* spp. strains found on distinguished surfaces of the small-animal veterinary practice. Additionally, the homology with a plasmid of nosocomial human origin in Portugal is worrisome as it might suggest the transmission between the human hospital healthcare and the veterinary healthcare settings. Additional studies are required to comprehend this important One Health link. The need for implementation of IPC guidelines directed at antimicrobial-resistance in veterinary medicine is urgent. Regular

surveillance, IPC protocols and antimicrobial stewardship are key to preventing the dissemination of these MDR bacteria on to humans and pets.

Author contributions

J.M.S. and C.P. designed the study. J.M.S., J.M. and L.F. collected and analysed the data. J.M.S. and A.A. analysed W.G.S. data. J.M.S., C.M., D.T., S.C.C. and C.P. wrote, revised and approved the manuscript.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jhin.2024.02.001>.

References

- [1] Mediolini F, Bacca E, Faltoni M, Burastero GJ, Volpi S, Menozzi M, et al. Is it possible to eradicate carbapenem-resistant *Acinetobacter baumannii* (CRAB) from endemic hospitals? *Antibiotics* 2022;11:1015.
- [2] ECDC. Healthcare-associated infections acquired in intensive care units Annual Epidemiological Report 2019. 2019.
- [3] Montaña S, Palombarani S, Carulla M, Kunst A, Rodriguez CH, Nastro M, et al. First case of bacteraemia due to *Acinetobacter schindleri* harbouring bla_{NDM-1} in an immunocompromised patient. *New Microbes New Infect* 2018;21:28–30.
- [4] Tega L, Raieta K, Ottaviani D, Russo GL, Blanco G, Carraturo A. Catheter-related bacteremia and multidrug-resistant *Acinetobacter lwoffii*. *Emerg Infect Dis* 2007;13:355–6.
- [5] WHO. Improving infection prevention and control at the health facility: Interim practical manual supporting implementation of the WHO Guidelines on Core Components of Infection Prevention and Control Programmes. Geneva: World Health Organization; 2018. Available at: <https://iris.who.int/handle/10665/279788> [last accessed June 2023].
- [6] WHO Regional Office for Europe/European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2020–2022. 2022. Available at: <https://doi.org/10.2900/112339> [last accessed September 2023].
- [7] Samia NI, Robicsek A, Heesterbeek H, Peterson LR. Methicillin-resistant *Staphylococcus aureus* nosocomial infection has a distinct epidemiological position and acts as a marker for overall hospital-acquired infection trends. *Sci Rep* 2022;12:17007.
- [8] Schmidt JS, Kuster SP, Nigg A, Dazio V, Brilhante M, Rohrbach H, et al. Poor infection prevention and control standards are associated with environmental contamination with carbapenemase-producing Enterobacterales and other multidrug-resistant bacteria in Swiss companion animal clinics. *Antimicrob Resist Infect Control* 2020;9:1–13.
- [9] Moreira da Silva J, Menezes J, Marques C, Pomba CF. Companion animals—an overlooked and misdiagnosed reservoir of carbapenem resistance. *Antibiotics* 2022;11:533.
- [10] Gentilini F, Turba ME, Pasquali F, Mion D, Romagnoli N, Zambon E, et al. Hospitalized pets as a source of carbapenem resistance. *Front Microbiol* 2018;9:1–9.
- [11] van der Kolk JH, Endimiani A, Graubner C, Gerber V, Perreten V. *Acinetobacter* in veterinary medicine, with an emphasis on *Acinetobacter baumannii*. *J Glob Antimicrob Resist* 2019;16:59–71.
- [12] Schmitt K, Biggel M, Stephan R, Willi B. Massive spread of OXA-48 carbapenemase-producing Enterobacteriaceae in the environment of a Swiss companion animal clinic. *Antibiotics* 2022;11:213.
- [13] Endimiani A, Brilhante M, Bernasconi OJ, Perreten V, Schmidt JS, Dazio V, et al. Employees of Swiss veterinary clinics colonized with epidemic clones of carbapenemase-producing *Escherichia coli*. *J Antimicrob Chemother* 2020;75:766–8.
- [14] Eucast. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. 2023., Version 13.0. Available at: <http://www.eucast.org>. n.d. [last accessed June 2023].
- [15] Srinivasan R, Karaoz U, Volegova M, MacKichan J, Kato-Maeda M, Miller S, et al. Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. *PLoS One* 2015;10:1–22.
- [16] Poirel L, Figueiredo S, Cattoir V, Carattoli A, Nordmann P. *Acinetobacter* radioresistens as a silent source of carbapenem resistance for *Acinetobacter* spp. *Antimicrob Agents Chemother* 2008;52:1252–6.
- [17] Mulvey D, Redding P, Robertson C, Woodall C, Kingsmore P, Bedwell D, et al. Finding a benchmark for monitoring hospital cleanliness. *J Hosp Infect* 2011;77:25–30.
- [18] Dancer SJ. How do we assess hospital cleaning? A proposal for microbiological standards for surface hygiene in hospitals. *J Hosp Infect* 2004;56:10–5.
- [19] Menezes J, Moreira da Silva J, Frosini S-M, Loeffler A, Weese S, Perreten V, et al. mcr-1 colistin resistance gene sharing between *Escherichia coli* from cohabiting dogs and humans, Lisbon, Portugal, 2018 to 2020. *Eurosurveillance* 2022;27.
- [20] Rodrigues AC, Belas A, Marques C, Cruz L, Gama LT, Pomba C. Risk factors for nasal colonization by methicillin-resistant *Staphylococci* in healthy humans in professional daily contact with companion animals in Portugal. *Microb Drug Resist* 2018;24:434–46.
- [21] Thomas JC, Vargas MR, Miragaia M, Peacock SJ, Archer GL, Enright MC. Improved multilocus sequence typing scheme for *Staphylococcus epidermidis*. *J Clin Microbiol* 2007;45:616–9.
- [22] Jolley KA, Bray JE, Maiden MCJ. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications [version 1; referees: 2 approved]. *Wellcome Open Res* 2018;3:124.
- [23] Alcock BP, Huynh W, Chalil R, Smith KW, Raphenya AR, Wlodarski MA, et al. CARD 2023: expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database. *Nucleic Acids Res* 2023;51:D690–9.
- [24] Alikhan NF, Petty NK, Ben Zakour NL, Beatson SA. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. *BMC Genomics* 2011;12:402.
- [25] Périchon B, Goussard S, Walewski V, Krizova L, Cerqueira G, Murphy C, et al. Identification of 50 class D β -lactamases and 65 *Acinetobacter*-derived cephalosporinases in *Acinetobacter* spp. *Antimicrob Agents Chemother* 2014;58:936–49.
- [26] Kamolvit W, Higgins PG, Paterson DL, Seifert H. Multiplex PCR to detect the genes encoding naturally occurring oxacillinases in *Acinetobacter* spp. *J Antimicrob Chemother* 2014;69:959–63.
- [27] Nigro SJ, Hall RM. Structure and Context of *acinetobacter* transposons carrying the oxa23 carbapenemase gene. *J Antimicrob Chemother* 2016;71:1135–47.

- [28] Silva L, Mourão J, Grosso F, Peixe L. Uncommon carbapenemase-encoding plasmids in the clinically emergent *Acinetobacter pittii*. *J Antimicrob Chemother* 2018;73:52–6.
- [29] Wielders C, Vriens M, Brisse S, de Graaf-Miltenburg L, Troelstra A, Fleer A, et al. Evidence for in-vivo transfer of *mecA* DNA between strains of *Staphylococcus aureus*. *Lancet* 2001;357:1674–5.
- [30] Kee C, Junqueira ACM, Uchida A, Purbojati RW, Houghton JN, Chénard C, et al. Complete genome sequence of *Acinetobacter schindleri* SGAir0122 isolated from Singapore Air. *Genome Announc* 2018;6.
- [31] Rakitin AL, Ermakova AY, Beletsky AV, Petrova M, Mardanov AV, Ravin NV. Genome analysis of *Acinetobacter lwoffii* strains isolated from permafrost soils aged from 15 thousand to 1.8 million years revealed their close relationships with present-day environmental and clinical isolates. *Biology (Basel)* 2021;10:871.
- [32] Chatterjee S, Datta S, Roy S, Ramanan L, Saha A, Viswanathan R, et al. Carbapenem resistance in *Acinetobacter baumannii* and other *acinetobacter* spp. causing neonatal sepsis: focus on NDM-1 and its linkage to ISAb125. *Front Microbiol* 2016;7:1126.
- [33] Maboni G, Seguel M, Lorton A, Sanchez S. Antimicrobial resistance patterns of *Acinetobacter* spp. of animal origin reveal high rate of multidrug resistance. *Vet Microbiol* 2020;245:108702.
- [34] Wang T, Costa V, Jenkins SG, Hartman BJ, Westblade LF. *Acinetobacter radioresistens* infection with bacteremia and pneumonia. *IDCases* 2019;15:e00495.
- [35] Che Y, Yang Y, Xu X, Brinda K, Polz MF, Hanage WP, et al. Conjugative plasmids interact with insertion sequences to shape the horizontal transfer of antimicrobial resistance genes. *Proc Natl Acad Sci U S A* 2021;118:e2008731118.
- [36] Royer S, Amaral de Campos P, Araújo BF, Ferreira ML, Gonçalves IR, William da Fonseca Batistão D, et al. Molecular characterization and clonal dynamics of nosocomial blaOXA-23 producing XDR *Acinetobacter baumannii*. *PLoS One* 2018;13:e0198643.
- [37] Hamidian M, Nigro SJ. Emergence, molecular mechanisms and global spread of carbapenem-resistant *acinetobacter baumannii*. *Microb Genom* 2019;5:e000306.
- [38] Pomba C, Endimiani A, Rossano A, Saial D, Couto N, Perreten V. First report of OXA-23-mediated carbapenem resistance in sequence type 2 multidrug-resistant *Acinetobacter baumannii* associated with urinary tract infection in a cat. *Antimicrob Agents Chemother* 2014;58:1267–8.
- [39] Grosso F, Quinteira S, Peixe L. Understanding the dynamics of imipenem-resistant *Acinetobacter baumannii* lineages within Portugal. *Clin Microbiol Infect* 2011;17:1275–9.
- [40] Silva L, Grosso F, Rodrigues C, Ksiezarek M, Ramos H, Peixe L. The success of particular *Acinetobacter baumannii* clones: accumulating resistance and virulence inside a sugary shield. *J Antimicrob Chemother* 2021;76:305–11.
- [41] Findlay J, Nordmann P, Bouvier M, Kerbol A, Poirel L. Dissemination of ArmA- and OXA-23-co-producing *Acinetobacter baumannii* Global Clone 2 in Switzerland, 2020–2021. *Eur J Clin Microbiol Infect Dis* 2023 July 20. <https://doi.org/10.1007/s10096-023-04643-4> [Epub ahead of print].