Comparative Immunology, Microbiology and Infectious Diseases 70 (2020) 101471

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

Detection of a novel clone of *Acinetobacter baumannii* isolated from a dog with otitis externa

Francesca Paola Nocera[a], Luciana Addante[b], Loredana Capozzi[b], Angelica Bianco[b], Filomena Fiorito[a], Luisa De Martino[a,*], Antonio Parisi[b]

[a] Department of Veterinary Medicine and Animal Production, University of Naples "Federico II", Via F. Delpino 1, 80137 Naples, Italy
[b] Istituto Zooprofilattico Sperimentale di Puglia e Basilicata, Sezione di Putignano, Contrada San Pietro Piturno, 70017, Putignano, BA, Italy

ARTICLE INFO

Keywords:
Acinetobacter baumannii
Antibiotic resistance patterns
Canine otitis externa
Transmission and prevention
Whole genome sequencing

ABSTRACT

In this study, the isolation of *Acinetobacter baumannii* in a dog with clinical bilateral otitis externa is described. Moreover, to investigate the zoonotic potential of the isolate, microbiological examinations on the family members were performed. An *A. baumanniis*train was isolated from nasal swab in one of the dog owners. The identity of bacterial strains, either from dog and owner, was confirmed by phenotypic and molecular typing (wgMLST). Furthermore, to assess the pathogenic potential of the isolates a deep characterization of virulence and antibiotic resistance genes was done by Whole Genome Sequencing (WGS). Finally, the susceptibility towards a wide panel of antimicrobials was investigated. In our knowledge, this is the first recorded case of *A. baumanniii*solation from canine auricular swabs in Italy. And interestingly, this study underlines the possible spread of this microorganism from human to animal.

1. Introduction

Otitis externa is an inflammation of the external auditory canal from the pinna to the tympanic membrane commonly observed in canine patients. It is one of the main reasons of dog presentation to veterinary examination. Otitis externa has a complex etiology that includes primary or secondary causes and predisposing factors. Primary causes of otitis include allergies/hypersensitivities, autoimmune diseases, keratinization disorders, foreign bodies and parasites in the ear canal. Allergy, especially atopic dermatitis, is the most common primary trigger for otitis externa [1–3]. However, these cases frequently evolve into chronic or recurrent otitis, that require specific treatments.

Otitis can be perpetuated by yeasts and bacterial infections. Both commensal (i.e. *Staphylococcus* spp. and *Malassezia pachydermatis*) or environmental (i.e. *Pseudomonas* spp.) microorganisms can be involved. Recently, we reported an update on microbiological causes of canine otitis externa in Campania Region (Italy), demonstrating a higher prevalence of bacterial species (74.6 %), (predominately *Staphylococcus pseudintermedius*) compared to yeast species (25.4 %), (predominately *Malassezia pachydermatis*) [4]. To the best of our knowledge, *A. baumannii* associated with microbiological causes of canine otitis externa has never been reported in literature.

A. baumannii is a gram negative coccobacillus, strictly aerobic, nonfermenting, non fastidious, nonmotile, catalase-positive, oxidase-negative bacterium [5]. Multidrug-resistant (MDR) *A. baumannii* causing nosocomial infections with high mortality have been raising serious concerns in humans [6,7]. Thanks to its virulence properties, MDR *A. baumannii* has emerged as one of the most troublesome pathogens for health care institutions globally. *A. baumannii,* together with *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Enterobacter* spp., belongs to ESKAPE group, pathogens exhibiting several virulence factors. These bacteria are common causes of life-threatening nosocomial infections amongst critically ill and immunocompromised individuals and are characterized by increased antibiotic resistance [8].

In veterinary medicine, data regarding *A. baumannii* from animals are still scarce, even if some cases have been recently reported [9,10]. In particular, this bacterial strain has been isolated from pets from various sites of infection, e.g. urinary tract infections, otitis, abscess, pneumonia and sepsis [10–13]. Moreover, *Acinetobacter* spp can survive on canine healthy skin, where they may be potential reservoirs for infection. The natural reservoir of *A. baumannii* is actually unknown, but recent studies detected the presence of acquired carbapenemases in clinical isolates from pets, suggesting that they may be linked to human isolates [5,13].

This study describes the isolation and characterization of *A.*

* Corresponding author at: Department of Veterinary Medicine and Animal Production, University of Naples "Federico II", Naples, Italy.
E-mail address: luisa.demartino@unina.it (L. De Martino).

https://doi.org/10.1016/j.cimid.2020.101471
Received 15 January 2020; Received in revised form 28 February 2020; Accepted 13 March 2020

baumannii from dog and its owner, by using phenotypical, molecular and antibiotic resistance typing.

2. Materials and methods

2.1. Dog case presentation

In September 2018, a 10-years-old Cavalier King Charles Spaniel male, with a history of chronic dermatitis for at least four years, was taken to visit to a private Veterinary Clinic of Naples, where a bilateral severe, chronic and purulent otitis externa was diagnosed. The dog did not receive any antibiotic therapy, because initially a food-allergic otitis was suspected. Canine auricular swabs were sent from the private Veterinary Clinic to the Veterinary Microbiology Laboratory of the Department of Veterinary Medicine and Animal Production (University of Naples Federico II, Italy).

2.2. Microbiological investigation

For bacteriological examination, each swab was streaked on blood agar base supplemented with 5% sheep blood, Columbia naladixic acid agar (CNA), mannitol salt agar (MSA), MacConkey agar (MCA), and incubated aerobically at 37 °C for 24–48 h. Sabouraud dextrose agar (SDA) was also used for fungal flora evaluation and was kept at 30 °C for 7 days. Plates were all purchased from Oxoid Ltd, Uk.

Bacteria were identified by API 20E System (Biomerieux, Italy). Species identification was also confirmed by matrix-assisted laser desorption ionization- time of flight/mass spectrometry (MALDI-TOF/MS) analysis (Bruker Daltonics, Germany) performed at the Unit of Microbiology and Virology of the University of Study of Campania "Luigi Vanvitelli", Naples, Italy.

The isolates were tested for susceptibility to 22 antimicrobial agents by the Kirby–Bauer disc diffusion method on Mueller–Hinton agar incubated at 37 °C, in accordance with the principles described in The European Committee on Antimicrobial Susceptibility Testing [14]. Antibiotic resistance profile of the human *A. baumannii* isolate was defined according to human CLSI [15] and EUCAST [16] guidelines. Discs of amoxicillin and clavulanic acid (30 μg), ampicillin (10 μg), cefotaxime (30 μg), ceftriaxone (30 μg), cephalexin (30 μg), clindamycin (2 μg), ciprofloxacin (5 μg), colistin sulfate (10 μg), doxycycline (30 μg), gentamicin (10 μg), imipenem (10 μg), lincomycin (15 μg), meropenem (10 μg), neomycin (10 μg), pradofloxacin (5 μg), tetracycline (30 μg), penicillin G (10 IU), cefadroxil (30 μg), fusidic acid (10 μg), enrofloxacin (5 μg), marbofloxacin (5 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg) were tested.

After measuring the inhibition diameters, the strains were categorized as susceptible, intermediate or resistant to the drugs. All antibiotic resistance determinations were performed twice.

2.3. Specimen collection from pet owners

As the microbiological examination from the canine samples allowed to isolate an *A. baumanni*, frequently involved in human disease, a survey investigating the colonization of the pet owners living in the family was performed. Nasal swabs were collected from two owners, sharing the same house. Strict instruction was given for self-collection of nasal swabs. In particular, a sterile swab soaked with normal saline was inserted into the nares and then gently rotated to guarantee an adequate contact with the nasal septum. All the swabs were placed in transport media and stored at 4 °C until culture was done within 8 h of collection. The dog's owners provided written, informed consent at the time of enrolment into the study. Microbiological examination was performed as described above.

2.4. Whole-genome sequencing

Genomic DNA from human and animal *A. baumannii* isolates was purified using Dneasy Blood and Tissue Kit (Qiagen, Germany), according to manufacturer's protocol. An indexed genomic library for each isolate was prepared using the Nextera XT library preparation workflow (Illumina, CA), following the manufacturer's instructions. Whole genome sequencing was performed on an Illumina MiSeq platform generating tagged paired-end reads (2 × 250 bp). The paired-end raw reads (fastq) were filtered and trimmed using Trimmomatic [17], a flexible trimmer for Illumina sequence data (https://github.com/timflutre/trimmomatic). De novo genome assembly was performed with SPAdesv. 3.12 [18]. The species identification was confirmed for both the isolates using Average Nucleotide Identity based on Blast (ANIb) by online available service JSpacesWS (http://jspecies.ribohost.com/jspeciesws/) as previously described [19].

Isolates were typed by Multi-Locus Sequence Typing (MLST) submitting whole genome sequences to the MLST database (https://pubmlst.org/abaumannii/), according to the scheme described by Diancourt et al. [20], commonly referred as the Pasteur scheme and the other described by Bartual et al. [21], commonly referred as Oxford scheme. The new alleles and Sequence Types (ST) were assigned by the curators of the MLST databases.

Whole genome MLST (wgMLST) analysis were performed on the BioNumerics 7.6 (Applied Maths, Belgium) calculation engine using default settings. The main virulence-associated genes were predicted by submitting assembled genomes to BacWGSTdb [22] platform (http://bacdb.org/BacWGSTdb/Tools.php). The acquired antibiotic-resistance genes were identified by using ABRicate pipeline (https://github.com/tseemann/abricate), ARG-ANNOT [23], NCBI, CARD [24] and ResFinder [25] as reference databases.

3. Results

The Diff-Quik® staining, performed usually for the routine ear swab cytology in the private clinic, allowed the observation of numerous rods, flooring of neutrophilic granulocytes and pus, some erythrocytes, few *Malassezia*. Bacterial cultures were set up and a pure and conspicuous growth of gram negative bacteria was observed. Based on the morphological characterization and biochemical behavior, as non-lactose fermenter on MacConkey agar and oxidase negative coccobacilli, the canine isolates, from both the ears, were identified by using API 20E system as *A. baumannii* with an excellent identification level(98.7 %). The species identification was also confirmed by MALDI-TOF MS with a log(score) of ≥2.2.

Only one owner, the one having the closest contact with the dog and with a documented prolonged stay in hospital, resulted colonized by *A. baumannii,* which showed an excellent identification level of 92.8 % by API 20E. Also this isolate was further identified as *A. baumannii* by MALDI-TOF MS with a log(score) of ≥2.2.

The draft genome sequences of two *A. baumannii* strains, respectively ACB004 isolated from dog and ACB003 from its owner, consisted both of 167 contigs comprising an average of 3.677.006 bp. The average coverage was estimated at ~ 100 × . The overall G + C content of two isolates was 39.15 %. Based on average nucleotide identity (ANIb) values, obtained by online available service JSpacesWS, the isolates ACB003 and ACB004 showed a nucleotide identity > 99.94 % and were closely related to *A. baumannii* reference strain ATCC 17978 with an ANI value > 97 %.

Analysis of MLST genes, based either on the Pasteur and the Oxford schemes, detected the same allelic profiles for both strains, although they represented new Sequence Types, ST1386 and ST2042 respectively. The allelic numbers for each of the seven loci observed in the two isolates are shown in Table 1.

The *de novo* assembled genomes, submitted to calculation engine by BioNumerics 7.6 produced a wgMLST profile including 3.092 alleles.

Table 1
Multi-Locus Sequence Typing (MLST) for both strains (ACB003-ACB004) obtained submitting whole genome sequences to the MLST database, according with the Pasteur scheme and the other referred as Oxford scheme.

	Strain	ST	MLST						
			cpn60	*fusA*	*gltA*	*pyrG*	*recA*	*rplB*	*rpoB*
Pasteur	ACB003	1386	3	3	2	4	5	4	63
	ACB004	1386	3	3	2	4	5	4	63
			cpn60	*gdhB*	*gltA*	*gpi*	*gyrB*	*recA*	*rpoD*
Oxford	ACB003	2042	1	213	1	346	63	11	6
	ACB004	2042	1	213	1	346	63	11	6

The comparison of characters showed a similarity value of 99.8 % for the two isolates.

The presence of virulence factor genes was assessed by BacWGSTdb, which identified a total of 74 genes for ACB003 and 72 genes for ACB004 with a high average percentage of identity > 95 % (Table 2).

Among these virulence factors, many of them were identified. Precisely, the following genes were detected: five genes that encoding for pilus assembly proteins (*csuA/B, csuB, csuC, csuD*and*csuE*); sixteen genes involved in *A. baumannii* high-affinity iron acquisition systems, necessary to survive under iron-limited host environments (*basA, basB, basC, basD, basF, basG, basH, basI*and*basJ* encoding for acinetobactin biosynthesis proteins; *bauA, bauB, bauC, bauD, bauE, bauF* encoding for the proteins of ferric-acinetobactin complexes receptor; *entE* involved in the bacterial iron homeostasis control) and five genes encoding for efflux pumps and other multi-drug resistance systems (*adeF, adeG, adeH, barA*and *barB*).

Using ABRicate pipeline, 1 aminoglycoside resistance gene, 10 penicillin resistance genes and 13 genes involved in multidrug resistance mechanisms were predicted. These genes and the relative percentages of identity are shown in Table 3. With slight exceptions, the same antibiotic resistance genes were predicted for both *A. baumannii* isolates, although several differences were identified among the reference databases (Table 3).

The strains ACB003 and ACB004 displayed the same broad antimicrobial resistance pattern as shown in Table 4. The isolates were resistant to penicillins (amoxicillin/clavulanic acid, ampicillin, penicillin), cephalosporins (cefadroxil, cefotaxime,cefalexin), carbapenems (imipenem, meropenem) and lincosamides (clindamycin, lincomycin), whereas they were susceptible to several tested antibiotics such as fluoroquinolones (nalidixic acid, ciprofloxacin, enrofloxacin and marbofloxacin), aminoglycosides (gentamicin, neomycin and streptomycin), sulphonamides and tetracycline.

Regarding the bacterial susceptibility, marbofloxacin and gentamicin were prescribed, topically for 14 days. Follow-up was performed with weekly checks for two months. The treatment allowed the resolution of the chronic otitis externa and the dog remained free of clinical signs already after two weeks from the start of the therapy.

4. Discussion

Among *Acinetobacter* species, *A. baumannii* is the most important member associated with human hospital-acquired infections worldwide. It is a potential pathogen responsible for opportunistic infections of lungs, skin, bloodstream, urinary tract, and other soft tissues mostly in immunocompromised patients [5,26]. Little data about this emerging pathogen are reported in Veterinary medicine [10], and connection between human and veterinary strains has not been described yet. The treatment of these infections in critically ill patients hospitalized in the intensive care unit (ICU) is a worldwide challenge. Noteworthy, MDR *A. baumannii* strains are considered as major threat among microbial pathogens by the World Health Organization (WHO) and besides methicillin-resistant *S. aureus* (MRSA), MDR *A. baumannii* is one of the most frequently isolated bacteria during outbreaks in burn units, where they are also funded from staff and environmental samples [13].

In this study the same *A. baumannii* strain has been isolated from chronic otitis externa in a dog and from a nasal swab of one of its pet owners. It is worth noting that this person with an asymptomatic nasal colonization of *A. baumannii* was hospitalized for a long period before the onset of the symptoms in the dog. It is possible to speculate that, during the hospital stay, the owner has become an *A. baumannii* reservoir, and consequently able to transmit the strain to the dog having a very close contact.

Here, we investigated the clonal relatedness of the two *A. baumannii* isolates by wgMLST, comprising a set of 3.092 genes, showing a similarity value of 99.8 %, which confirmed their genetic identity. Using both the standardized MLST schemes, they shared the same allelic profiles identified as new STs. In Italy, the most frequent isolated human strains is the “Italian strain” ST78. Besides the ST78, the ST2 strain, belonging to International Clonal lineage II, has gradually outbreak in hospitals as cause of epidemics and it is even replacing the ST78 strains [11]. However, the new ST1386 was very different from both the described ST78 and ST2.

Several virulence factors have been identified by genomic analyses, including pilus, outer membrane porins, phospholipases, proteases, lipopolysaccharides (LPS), capsular polysaccharides, protein secretion systems, and iron-chelating systems. Both strains shared genes related to great ability to adhere to cells, to invade and survive as well as to form biofilm on abiotic surface [11,27]. OmpA (an outer membrane porin), a well characterized virulence factor, was present in both the strains analyzed. A random mutagenesis screen showed that the *A. baumannii* OmpA mutant is defective in inducing apoptosis in human epithelial cells. OmpA also plays a major role in adherence and invasion of epithelial cells by interacting with fibronectin. Moreover, OmpA is also involved in antimicrobial resistance and biofilm formation in *A. baumannii* [26,27].

Biofilm and motility are important and interconnected capacities that enable bacteria to persist in the environment and colonize the host. In some bacteria, motility has been associated to increased biofilm production and virulence (i.e. *Pseudomonas aeruginosa*). Our strains showed this ability thanks to OmpA, pili assembly virulence genes (*csu*/A, *csu*/B, *csu*/C, *csu*/D, *csu*/E) and *eps*A/ptK genes, involved in the production of lipopolysaccharides (LPS) [26]. Furthermore, biofilm producers *A. baumannii* strains are able to survive on inanimate surfaces, such as nitrile gloves or equipment, making possible an indirect transmission [28].

Iron is one of the essential nutrients for bacteria, that need the metal as an essential cofactor for growth. *A. baumannii* requires iron to successfully cause infections and persist in the host [29]. In aerobic environments at neutral pH, iron occurs in an insoluble, oxidized, ferric form, which limits the availability of free iron significantly below the concentration required for optimal growth [26]. To overcome iron limitations in both the environment and the host, *A. baumannii* can express acinetobactin, a high-affinity siderophore-mediated iron acquisition system. A change in acinetobactin biosynthesis and transport functions results in a significantly reduction in the ability of *A. baumannii* ATCC 19606 to persist within epithelial cells and cause cell damage [26,29,30]. We identified sixteen genes involved in *A. baumannii* high-affinity iron acquisition systems (Table 2) [30].

Both non-enzymatic and enzymatic mechanisms of antimicrobial resistance have been described in *A. baumannii*. The non-enzymatic mechanism relies mostly on the upregulation of the efflux systems, but also through alterations of target penicillin-binding proteins, and changes in the outer membrane protein (OMP) composition [5,26,27]. Different AMR genes, involving all the AMR mechanisms have been identified in our isolates. Moreover, *A. baumannii* strains are naturally resistant to extended spectrum cephalosporins, as they code the AmpC cephalosporinase [26].

Efflux pumps are associated with resistance against many different

Table 2
Virulence factor genes profiles for ACB003 and ACB004 predicted by submitting assembled genomes to BacWGSTdb. The data reported refers to percentage of identity (%Id) of strains, with a high average > 95 %.

Class	Virulence gene	%ID		Functional annotation
		ACB003	ACB004	
Enzyme	A1S_0050	94.6	94.6	protein tyrosine phosphatase
	A1S_0059	–	–	glycosyltransferase
	A1S_0063	99.4	99.4	UDP-glucose 6-dehydrogenase
	abaI	99.4	99.4	N-acyl-L-homoserine lactone synthetase
	ABK1_0099	94.9	94.9	WeeH
	ABK1_0100	98.2	98.2	galU
	ABK1_0102	98.5	98.5	glucose-6-phosphate isomerase
	ABSDF0074	90.3	90.3	polysaccharide biosynthesis protein
	ABTW07_0082	96.9	96.9	UDP-N-acetyl-D-mannosaminuronate dehydrogenase
	ACICU_00071	95.8	95.8	ATPase
	ACICU_00075	97.7	97.7	nucleoside-diphosphate sugar epimerase
	ACICU_00076	97.1	97.1	pyridoxal phosphate-dependent enzyme
	ACICU_00077	97.1	97.1	CMP-N-acetylneuraminic acid synthetase
	ACICU_00080	89.9	89.9	sialic acid synthase
	BJAB07104_00106	98.6	98.6	Phosphomannomutase
	bap	98.3	98.3	hemolysin-type calcium-binding domain-containing protein
	entE	97.8	97.8	Peptide arylation enzyme
	BJAB0868_00090	98.73	98.7	Periplasmic protein involved in polysaccharide export
	galE	98.4	98.4	UDP-glucose 4-epimerase
	hemO	99.8	99.8	heme oxygenase
	lpsB	97.5	97.5	glycosyltransferase
	lpxA	99.0	98.0	UDP-N-acetylglucosamine acyltransferase
	lpxB	99.2	99.2	lipid-A-disaccharide synthase
	lpxC	100	100	UDP-3-O-3-hydroxymyristoyl N-acetylglucosamine deacetylase
	lpxD	99.2	99.2	UDP-3-O-3-hydroxymyristoyl glucosamine N-acyltransferase
	lpxL	99.1	99.1	lipid A biosynthesis lauroyl acyltransferase
	lpxM	99.9	99.9	Lauroyl/myristoyl acyltransferase
	ompA	99.3	99.3	Outer membrane protein-related peptidoglycan-associated (lipo)protein
	pbpG	99.1	99.1	D-alanyl-D-alanine endopeptidase
	pgaB	98.6	98.58	xylanase/chitin deacetylase
	pgaC	98.9	98.9	Glycosyltransferase, probably involved in cell wall biogenesis
	plcD	99.2	99.2	Phosphatidylserine/phosphatidylglycerophosphate/cardiolipin synthase-related enzyme
	adeF	98.5	98.5	RND family efflux transporter
	adeG	97.5	97.5	Cation/multidrug efflux pump
	adeH	99.1	99.1	multidrug efflux system outer membrane protein
	barA	96.9	96.9	ABC transporter family protein
	barB	99.0	99.0	ABC-type multidrug transport system, ATPase and permease component
Iron acquisition	basB	96.9	96.9	non-ribosomal peptide synthetase module
	basC	98.4	98.4	lysine/ornithine N-monooxygenase
	basD	97.5	97.5	High-molecular-weight protein 2 (HMWP2)
	basF	97.1	97.1	acinetobactin biosynthesis protein
	basG	97.8	97.8	basG
	basH	97.5	97.5	acinetobactin biosynthesis protein
	basI	99.8	99.8	hypothetical protein
	basJ	98.4	98.4	isochorismate synthetase
	bauA	98.8	98.8	Outer membrane receptor protein, mostly Fe transport
	bauB	98.0	98.0	bauB
	bauC	96.8	96.8	bauC
	bauD	97.6	97.6	ferric acinetobactin transport system permease
	bauE	99.0	99.0	ferric acinetobactin transport system ATP-binding protein
	bauF	97.8	97.8	Vulnibactin utilization protein viuB
	BJAB0715_01026	93.5	93.5	Fe2 + -dicitrate sensor, membrane component
Pili assembly	csuA/B	98.9	–	protein CsuA/B; secreted protein related to type I pili
	csuB	98.8	98.8	protein CsuB
	csuC	98.5	98.5	P pilus assembly protein, chaperone PapD
	csuD	98.5	98.5	CsuD
	csuE	99.2	99.2	CsuE
Lipase	plc	98.3	98.3	phospholipase C
	plc	98.4	98.4	phospholipase C
Hypothetical protein	A1S_0060	97.4	97.4	hypothetical protein
	AB57_0987	96.9	96.9	hypothetical protein
	AB57_0992	100	100	hypothetical protein
	ABZJ_01015	99.7	99.7	hypothetical protein
	ACICU_00881	97.1	97.1	hypothetical protein
	BJAB0715_01029	99.7	99.7	hypothetical protein
	BJAB0715_01030	99.6	99.6	hypothetical protein
	pgaA	98.9	98.9	hypothetical protein
	pgaD	99.3	99.3	hypothetical protein

(*continued on next page*)

Table 2 (*continued*)

Class	Virulence gene	%ID		Functional annotation
		ACB003	ACB004	
Regulation	A1S_0917	96.0	96.0	transcriptional regulator
	abaR	99.6	99.6	eR transcriptional regulator
	AB57_0985	97.6	97.6	RNA polymerase sigma factor FecI
	bfmR	99.7	99.7	OmpR family response regulator
	bfmS	98.5	98.5	Signal transduction histidine kinase

classes of antibiotics. There are different classes of efflux pumps *(i.e.the resistance-nodulation-division superfamily, the multidrug and toxic compound extrusion family, the major facilitator superfamily, and the small multidrug resistance family transporters)* [5,26]. One of the most important efflux pumps is the AdeFGH, which belongs to the resistance-nodulation-division superfamily. AdeFGH expression is regulated by the LysR-type transcriptional regulator AdeL and the TetR-type transcriptional regulator AdeN [31]. A recent result describes that low-dose antimicrobial therapy increases the expression of AdeFGH and biofilm production [26,32]. The genomic analyses revealed the presence of both *ade*FGH encoding genes and their regulators *ade*L and *ade*R, unfolding the wide antimicrobial resistance.

Inactivation of β-lactams by β-lactamases is the major antibiotic resistance mechanism in *A. baumannii* [5,26]. β-lactamases are grouped into molecular classes, A, B, C, and D [33]. All four classes of β-lactamases were identified in *A. baumannii*, nevertheless, class D β-lactamases, also known as oxacillinases (OXA), are the most widely β-lactamases found in *A. baumannii* clinical isolates [27]. β-OXA-type lactamases are a family of extended spectrum β lactamases (ESBLs) that are so named thanks to their ability to hydrolyze oxacillin. They confer resistance to ampicillin and cephalotin, have high hydrolytic activity against oxacillin and cloxacillin and are weakly inhibited by clavulanic acid [33].

All strains of *A. baumannii* have an intrinsic chromosomal blaOXA-51-like alleles encoding more than 95OXA-51-like variants [5,27,34]. We found blaOXA-93 and blaOXA385 genes, that belong to OXA-51-like group.

The worldwide interest about *A. baumannii* is increasing dramatically because of its rising clinical importance. Recent interest about *A. baumannii* is mostly due to its seemingly endless capacity to acquire antibiotic resistance. Moreover, the rapid spread of MDR *A. baumannii* as pathogen in critical care unit poses a serious hazard in the clinical challenge [13].

Not only in human medicine, but also in veterinary health care, outbreaks by multidrug resistant *A. baumannii* in dogs and cats are potentially highly fatal and difficult to eradicate [35]. It has been stated that animals can be a potential reservoir of *A. baumannii* and contribute to the dissemination of new emerging strains, including those resistant to carbapenems [11,13,36]. The situation may be different between food-producing animals and companion animals, which are in more direct contact with humans [13]. This emphasizes the requirement for molecular typing to trace potential sources of the isolates and to implement infection control interventions. In this study a comparison of human and animal isolates was done using a wgMLST approach that allowed to confirm the identity of the strains even if it remains hard to define exactly the source of *A. baumannii* transmission, if owner - dog or *vice-versa*.

On the other hand, two different approaches are essential to limit the spread of antimicrobial-resistant *A. baumannii*, that are infection control and antimicrobial control programs. The first requires strict environmental cleaning, usefulness sterilization of medical equipment and attention on proper hand hygiene practices. The second is also

Table 3
Percentages of identity (%Id) of the acquired antibiotic-resistance genes involved in the aminoglycoside, penicillin and multidrug resistance for both *A. baumannii* isolates (ACB003-ACB004) using ResFinder (RESF.), ARG-ANNOT (ARGA.), NCBI and CARD as reference databases.

		ACB003				ACB004			
		%Id				%Id			
Antimicrobial class	Genes	RESF.	ARGA.	NCBI	CARD	RESF.	ARGA.	NCBI	CARD
Aminoglycoside	ant(3")-Iia	–	–	99.8	99.8	–	–	99.8	99.8
Penicillin	bla A1	–	90.2	–	–	–	90.2	–	–
	blaA2	–	97.3	–	–	–	–	–	–
	Zn dependent hydrolase	–	98.2	–	–	–	98.8	–	–
	ADC-25-1	96.8	–	–	–	96.8	–	–	–
	ACD-58	–	98.3	–	–	–	98.3	–	–
	ADC-163	–	–	99.1	–	–	–	99.1	–
	OXA-93	–	98.9	–	–	–	98.9	–	–
	Mbl	–	97.3	–	–	–	97.3	–	–
	Oxa-385	98.9	–	98.9	98.9	98.9	–	98.9	98.9
	AmpC beta-lactamase	–	–	–	98.0	–	–	–	98.0
Multidrug	adeH	–	–	–	99.2	–	–	–	99.2
	adeF	–	–	–	97.1	–	–	–	97.1
	adeG	–	–	–	98.5	–	–	–	98.5
	adeL	–	–	–	98.3	–	–	–	98.3
	abeS	–	–	–	98.8	–	–	–	98.8
	abeM	–	–	–	99.3	–	–	–	99.3
	adeN	–	–	–	98.8	–	–	–	98.8
	adeK	–	–	–	99.3	–	–	–	99.3
	adeJ	–	–	–	99.4	–	–	–	99.4
	adeB	–	–	–	79.7	–	–	–	79.7
	adeA	–	–	–	79.8	–	–	–	79.8
	adeR	–	–	–	78.6	–	–	–	78.6

Table 4
antibiotic susceptibility to 22 antimicrobial agents by the Kirby–Bauer disc diffusion method for ACB003 and ACB004 strains.

Antibiotics classes	Antibiotics	S/I/R	
		ACB003	ACB004
Penicillin	Amoxicillin + clavulanic acid (30 μg)	R	R
	Ampicillin (10 μg)	R	R
	Penicillin (10 UI)	R	R
Cephalosporins	Cefadroxil (30 μg)	R	R
	Cefotaxime (30 μg)	R	R
	Ceftriaxone (30 μg)	I	I
	Cefalexin (30 μg)	R	R
Carbapenems	Imipenem (10 μg)	R	R
	Meropenem (10 μg)	R	R
Fluorochinoloni	Ciprofloxacin (5 μg)	S	S
	Pradofloxacin (5 μg)	I	I
	Enrofloxacin (5 μg)	S	S
	Marbofloxacin (5 μg)	S	S
Aminoglycoside	Gentamicin (10 μg)	S	S
	Neomicin (30 μg)	S	S
Tetracycline	Doxycycline (30 μg)	S	S
	Tetracycline (30 μg)	S	S
Lincosamides	Clindamycin (2 μg)	R	R
	Lincomycin (2 μg)	R	R
Miscellaneous agents	Colistine sulfate (10 μg)	R	R
	Fusidic acid (10 μg)	R	R
	Trimethoprim/sulfamethoxazole (25 μg)	S	S

S = Susceptible; I = intermediate; R = resistant.

important and requires observing the rules concerning the prudent use of antibiotics in the clinical practices to hinder the growth of new MDR strains [11].

5. Conclusion

A. baumannii, belonging to ESKAPE pathogens group (including *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), is of growing interest worldwide. In this study *A. baumannii* was isolated from a dog with otitis externa. As in human medicine, *A. baumannii* can be considered an emerging opportunistic pathogen also in veterinary medicine. Furthermore, it can be considered a zoonotic pathogen and this clinical case demonstrates a possible transmission between human and dog. As the person with an asymptomatic nasal colonization of *A. baumannii* was hospitalized for a long period before the onset of the symptoms in the dog, we hypothesize that the pet owner could have been the potential reservoir for the canine infection. Therefore, further experimental studies are needed in order to understand if this was an isolated case or if *A. baumannii* can successfully infect both pets and owners.

Ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received.

Funding

The authors received no financial support for the research, authorship, and publication of this article.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

The authors wish to thank Dr. R. Lucà Veterinary Clinic Cilea, Vico Acitillo 164/168, 80127 Naples for providing canine auricular samples and human nasal swabs collection for this study.

References

[1] M.N. Saridomichelakis, R. Farmaki, L.S. Leontides, A.F. Koutinas, Aetiology of canine otitis externa: a retrospective study of 100 cases, Vet. Dermatol. 18 (2007) 341–347, https://doi.org/10.1111/j.1365-3164.2007.00619.x.
[2] G. Zur, B. Lifshitz, T. Bdolah-Abram, The association between the signalment, common causes of canine otitis externa and pathogens, J. Small Anim. Pract. 52 (2011) 254–258, https://doi.org/10.1111/j.1748-5827.2011.01058.x.
[3] S. Paterson, Discovering the causes of otitis externa, In Pract. 38 (2016) 7–11, https://doi.org/10.1136/inp.i470.
[4] L. De Martino, F.P. Nocera, K. Mallardo, S. Nizza, E. Masturzo, F. Fiorito, G. Iovane, P. Catalanotti, An update on microbiological causes of canine otitis externa in Campania Region, Italy, Asian Pac. J. Trop. Biomed. 6 (2016) 384–389, https://doi.org/10.1016/j.apjtb.2015.11.012.
[5] A.Y. Peleg, H. Seifert, D.L. Paterson, *Acinetobacter baumannii*: emergence of a successful pathogen, Clin. Microbiol. Rev. 21 (2008) 538–582, https://doi.org/10.1128/CMR.00058-07.
[6] P.G. Higgins, C. Dammhayn, M. Hackel, H. Seifert, Global spread of carbapenem-resistant Acinetobacter baumannii, J. Antimicrob. Chemother. 65 (2010) 233–238, https://doi.org/10.1093/jac/dkp428.
[7] F. Perez, A. Endimiani, A.J. Ray, B.K. Decker, C.J. Wallace, K.M. Hujer, D.J. Ecker, M.D. Adams, P. Toltzis, M.J. Dul, A. Windau, S. Bajaksouzian, M.R. Jacobs, R.A. Salata, R.A. Bonomo, Carbapenem-resistant Acinetobacter baumannii and Klebsiella pneumoniae across a hospital system: impact of post-acute care facilities on dissemination, J. Antimicrob. Chemother. 65 (2010) 1807–1818, https://doi.org/10.1093/jac/dkq191.
[8] S. Santajit, N. Indrawattana, Mechanisms of antimicrobial resistance in ESKAPE pathogens, Biomed Res. Int. 2016 (2016) 1–8, https://doi.org/10.1155/2016/2475067.
[9] S. Müller, T. Janssen, L.H. Wieler, Multidrug resistant Acinetobacter baumannii in veterinary medicine–emergence of an underestimated pathogen? Berl. Munch. Tierarztl. Wochenschr. 127 (2014) 435–446.
[10] Y. Kimura, K. Harada, T. Shimizu, T. Sato, A. Kajino, M. Usui, Y. Tamura, Y. Tsuyuki, T. Miyamoto, A. Ohki, M. Watarai, Species distribution, virulence factors and antimicrobial resistance of *Acinetobacter* spp. isolates from dogs and cats: a preliminary study: *Acinetobacter* spp. from dogs and cats, Microbiol. Immunol. 62 (2018) 462–466, https://doi.org/10.1111/1348-0421.12601.
[11] F. Gentilini, M.E. Turba, F. Pasquali, D. Mion, N. Romagnoli, E. Zambon, D. Terni, G. Peirano, J.D.D. Pitout, A. Parisi, V. Sambri, R.G. Zanoni, Hospitalized pets as a source of carbapenem-resistance, Front. Microbiol. 9 (2018) 2872, https://doi.org/10.3389/fmicb.2018.02872.
[12] K.E. Mitchell, J.F. Turton, D.H. Lloyd, Isolation and identification of *Acinetobacter* spp. from healthy canine skin, Vet. Dermatol. 29 (2018), https://doi.org/10.1111/vde.12528 240-e87.
[13] J.H. van der Kolk, A. Endimiani, C. Graubner, V. Gerber, V. Perreten, Acinetobacter in veterinary medicine, with an emphasis on Acinetobacter baumannii, J. Glob. Antimicrob. Resist. 16 (2019) 59–71, https://doi.org/10.1016/j.jgar.2018.08.011.
[14] EUCAST, The European Committee on Antimicrobial Susceptibility Testing Breakpoint Tables for Interpretation of MICs and Zone Diameters 7.1 (2017).
[15] CLSI, M100-S25, Performance Standards for Antimicrobial Susceptibility Testing: Twenty-fifth Informational Supplement, (2015).
[16] EUCAST, The European Committee on Antimicrobial Susceptibility Testing Breakpoint Tables for Interpretation of MICs and Zone Diameters 6 (2016).
[17] A.M. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data, Bioinformatics 30 (2014) 2114–2120, https://doi.org/10.1093/bioinformatics/btu170.
[18] A. Bankevich, S. Nurk, D. Antipov, A.A. Gurevich, M. Dvorkin, A.S. Kulikov, V.M. Lesin, S.I. Nikolenko, S. Pham, A.D. Prjibelski, A.V. Pyshkin, A.V. Sirotkin, N. Vyahhi, G. Tesler, M.A. Alekseyev, P.A. Pevzner, SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing, J. Comput. Biol. 19 (2012) 455–477, https://doi.org/10.1089/cmb.2012.0021.
[19] M. Richter, R. Rosselló-Móra, Shifting the genomic gold standard for the prokaryotic species definition, PNAS 106 (2009) 19126–19131, https://doi.org/10.1073/pnas.0906412106.
[20] L. Diancourt, V. Passet, A. Nemec, L. Dijkshoorn, S. Brisse, The population structure of Acinetobacter baumannii: expanding multiresistant clones from an ancestral susceptible genetic pool, PLoS One 5 (2010) e10034, , https://doi.org/10.1371/journal.pone.0010034.
[21] S.G. Bartual, H. Seifert, C. Hippler, M.A.D. Luzon, H. Wisplinghoff, F. Rodriguez-Valera, Development of a multilocus sequence typing scheme for characterization of clinical isolates of Acinetobacter baumannii, J. Clin. Microbiol. 43 (2005) 4382–4390, https://doi.org/10.1128/JCM.43.9.4382-4390.2005.
[22] Z. Ruan, Y. Feng, BacWGSTdb, a database for genotyping and source tracking bacterial pathogens, Nucleic Acids Res. 44 (2016) D682–D687, https://doi.org/10.1093/nar/gkv1004.
[23] S.K. Gupta, B.R. Padmanabhan, S.M. Diene, R. Lopez-Rojas, M. Kempf, L. Landraud, J.-M. Rolain, ARG-ANNOT, a new bioinformatic tool to discover antibiotic

resistance genes in bacterial genomes, Antimicrob. Agents Chemother. 58 (2014) 212–220, https://doi.org/10.1128/AAC.01310-13.
[24] A.G. McArthur, N. Waglechner, F. Nizam, A. Yan, M.A. Azad, A.J. Baylay, K. Bhullar, M.J. Canova, G. De Pascale, L. Ejim, L. Kalan, A.M. King, K. Koteva, M. Morar, M.R. Mulvey, J.S. O'Brien, A.C. Pawlowski, L.J.V. Piddock, P. Spanogiannopoulos, A.D. Sutherland, I. Tang, P.L. Taylor, M. Thaker, W. Wang, M. Yan, T. Yu, G.D. Wright, The comprehensive antibiotic resistance database, Antimicrob. Agents Chemother. 57 (2013) 3348–3357, https://doi.org/10.1128/AAC.00419-13.
[25] K.A. Kleinheinz, K.G. Joensen, M.V. Larsen, Applying the ResFinder and VirulenceFinder web-services for easy identification of acquired antibiotic resistance and *E. coli* virulence genes in bacteriophage and prophage nucleotide sequences, Bacteriophage 4 (2014), https://doi.org/10.4161/bact.27943 e27943.
[26] C.-R. Lee, J.H. Lee, M. Park, K.S. Park, I.K. Bae, Y.B. Kim, C.-J. Cha, B.C. Jeong, S.H. Lee, Biology of *Acinetobacter baumannii*: pathogenesis, antibiotic resistance mechanisms, and prospective treatment options, Front. Cell. Infect. Microbiol. 7 (2017), https://doi.org/10.3389/fcimb.2017.00055.
[27] C. Ambrosi, D. Scribano, M. Aleandri, C. Zagaglia, L. Di Francesco, L. Putignani, A.T. Palamara, Acinetobacter baumannii virulence traits: a comparative study of a novel sequence type with other italian endemic international clones, Front. Microbiol. 8 (2017) 1977, https://doi.org/10.3389/fmicb.2017.01977.
[28] H. Takoi, K. Fujita, H. Hyodo, M. Matsumoto, S. Otani, M. Gorai, Y. Mano, Y. Saito, M. Seike, N. Furuya, A. Gemma, Acinetobacter baumannii can be transferred from contaminated nitrile examination gloves to polypropylene plastic surfaces, Am. J. Infect. Control (2019), https://doi.org/10.1016/j.ajic.2019.04.009 S0196655319302378.
[29] W.F. Penwell, L.A. Actis, Isolation and characterization of the acinetobactin and baumannoferrin siderophores produced by Acinetobacter baumannii, in: I. Biswas, P.N. Rather (Eds.), Acinetobacter Baumannii, Springer, New York, New York, NY, 2019, pp. 259–270, , https://doi.org/10.1007/978-1-4939-9118-1_24.
[30] T. Hasan, C.H. Choi, M.H. Oh, Genes involved in the biosynthesis and transport of acinetobactin in *Acinetobacter baumannii*, Genomics Inform. 13 (2015) 2, https://doi.org/10.5808/GI.2015.13.1.2.
[31] C. Xu, S.R. Bilya, W. Xu, adeABC efflux gene in Acinetobacter baumannii, New Microbes New Infect. 30 (2019) 100549, , https://doi.org/10.1016/j.nmni.2019.100549.
[32] X. He, F. Lu, F. Yuan, D. Jiang, P. Zhao, J. Zhu, H. Cheng, J. Cao, G. Lu, Biofilm formation caused by clinical acinetobacter baumannii isolates is associated with overexpression of the AdeFGH Efflux pump, Antimicrob. Agents Chemother. 59 (2015) 4817–4825, https://doi.org/10.1128/AAC.00877-15.
[33] C. Al-Bayssari, F. Dabboussi, M. Hamze, J.-M. Rolain, Detection of expanded-spectrum β-lactamases in gram-negative bacteria in the 21st century, Expert Rev. Anti. Ther. 13 (2015) 1139–1158, https://doi.org/10.1586/14787210.2015.1066247.
[34] B.A. Evans, S.G.B. Amyes, O.X.A. -lactamases, Clin. Microbiol. Rev. 27 (2014) 241–263, https://doi.org/10.1128/CMR.00117-13.
[35] S. Kuzi, S.E. Blum, N. Kahane, A. Adler, O. Hussein, G. Segev, I. Aroch, Multi-drug-resistant *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex infection outbreak in dogs and cats in a veterinary hospital: nosocomial Acinetobacter infection outbreak, J. Small Anim. Pract. 57 (2016) 617–625, https://doi.org/10.1111/jsap.12555.
[36] R. Rafei, M. Hamze, H. Pailhoriès, M. Eveillard, L. Marsollier, M.-L. Joly-Guillou, F. Dabboussi, M. Kempf, Extrahuman epidemiology of Acinetobacter baumannii in Lebanon, Appl. Environ. Microbiol. 81 (2015) 2359–2367, https://doi.org/10.1128/AEM.03824-14.