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Acinetobacter pittii from Companion Animals Coharboring bla_{OXA-58}, the *tet*(39) Region, and Other Resistance Genes on a Single Plasmid

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Besides *Acinetobacter baumannii*, *Acinetobacter pittii* is an important nosocomial pathogen (1, 2). Carbapenems are the antimicrobial agents of choice for the treatment of infections with multidrug-resistant *Acinetobacter* spp. However, the incidence of carbapenem-resistant strains has risen over the last decade, mainly because of the acquisition of oxacillinases (OXA-23, -40, -58, -143, and -235) and less frequently of metallo-beta-lactamases (e.g., NDM, GIM, IMP) and the overexpression of the intrinsic OXA-51-like oxacillinase (2, 3). Also for human *A. pittii* isolates, different carbapenemase genes have been determined, including bla_{OXA-58} and bla_{NDM-1} (4–12). However, in animals, there is only one report of a carbapenem-resistant *A. pittii* isolate, namely, an OXA-40-positive strain from a rabbit in Lebanon (13).

In the present study, a PCR-based screening of 200 clinical *A. pittii* isolates from dogs ($n = 110$), cats ($n = 48$), rabbits ($n = 13$), horses ($n = 10$), and other animals ($n = 19$) collected between 2008 and 2017 for β-lactamase genes $bla_{OXA-23-like}$, $bla_{OXA-40-like}$, $bla_{OXA-58-like}$, $bla_{VIM-like}$, $bla_{NDM-like}$, and $bla_{IMP-like}$ (14, 15) revealed five (2.5%) bla_{OXA-58}-positive strains (Table 1). All of the carriers had different owners and had been admitted to two epidemiologically unlinked veterinary clinics in Germany between February 2014 and September 2016. Pulsed-field gel electrophoresis (15) divided the strains into pulsotypes A and B according to their origin from veterinary clinics A and B, respectively. This suggests the maintenance of pulsotype A strains in clinic A for >2 years and pulsotype B strains in clinic B for about 1 month.

On the basis of whole-genome sequencing data and the use of MLST (multilocus sequence type) Finder 1.8 (https://cge.cbs.dtu.dk/services/), all isolates were assigned to sequence type 93 (ST93) according to the Pasteur scheme (http://pubmlst.org/abaumannii). A maximum common genome (MCG) analysis based on 2,770 orthologous genes of 17 publicly available genomes of ST93 and OXA-58-positive *A. pittii* isolates from humans and our isolates was performed (16, 17). Pulsotype A isolates clustered separately from pulsotype B isolates but together with strains isolated from human patients in Germany and the United States (Fig. 1). The high relatedness (88 single nucleotide polymorphisms in the MCG) of feline OXA-58 strain IHIT29469 to GIM-1-positive clinical strain UKK-0555, which was isolated in 2011 in Germany, indicates that humans and companion animals may be infected by similar *A. pittii* genotypes, which has already been reported for other bacteria, including methicillin-resistant *Staphylococcus aureus*, extended-spectrum beta-lactamase producers, and multidrug-resistant *A. baumannii* (15, 18, 19).

We detected *aacC2*, *aph(3′)-Ic*, *strA*, *strB*, *sul2*, and *tet*(39) as additional resistance genes in our *A. pittii* isolates (https://cge.cbs.dtu.dk/services/ResFinder/). They all

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TABLE 1 Characteristics of OXA-58-producing *A. pittii* isolates from companion animals in Germany

Strain	Date of isolation	Host	Sample (disease)	Other bacteria isolated	Veterinary clinic	Pulsotype	ST	Antimicrobial resistance[a]	Resistance genes
IHIT24944	2/2014	Dog 1	Nose swab (rhinitis, sinusitis)	*Acinetobacter baumannii*, *Bacillus* sp. *Micrococcus* sp.	A	A	93	FEP, CTZ, FEP, CFP, CTU, GEN, CIP, MOX, ENR, MAR, TET, TOB, NIT, SXT	*aacC2*, *aph(3′)-Ic*, *strA*, *strB*, *sul2*, $bla_{OXA-500}$, $bla_{ADC-18-like}$, bla_{OXA-58}, *tet*(39)
IHIT32473	7/2016	Dog 2	Bronchial fluid (respiratory tract infection)	*Acinetobacter johnsonii*, *Pseudomonas* sp., *Candida* sp.	A	A	93	FEP, CTZ, FEP, CFP, CTU, CPD, GEN, CIP, MOX, ENR, MAR, TET, TOB, NIT, SXT	*aacC2*, *aph(3′)-Ic*, *strA*, *strB*, *sul2*, $bla_{OXA-500}$, $bla_{ADC-18-like}$, bla_{OXA-58}, *tet*(39)
IHIT32685	9/2016	Rabbit	Nose (none, screening)	*Bacillus* sp., *Candida* sp., *Streptococcus* sp.	A	A	93	FEP, CTZ, FEP, CFP, CTU, CPD, GEN, CIP, MOX, ENR, MAR, TET, TOB, NIT, SXT	*aacC2*, *aph(3′)-Ic*, *strA*, *strB*, *sul2*, $bla_{OXA-500}$, $bla_{ADC-18-like}$, bla_{OXA-58}, *tet*(39)
IHIT29469	5/2015	Cat 1	Nose swab (suppurating rhinitis)	*Bordetella bronchiseptica*, *Staphylococcus simulans*, *Micrococcus* sp.	B	B	93	CPD, CTU, GEN, CIP, MOX, ENR, TET, NIT, SXT	*aacC2*, *aph(3′)-Ic*, *strA*, *strB*, *sul2*, $bla_{OXA-500}$, $bla_{ADC-18-like}$, bla_{OXA-58}, *tet*(39)
IHIT29592	6/2015	Cat 2	Skin swab (purulent dermatitis)	*Acinetobacter* sp., *Bacillus* sp., *Pasteurella multocida*	B	B	93	CPD, CTU, CFP, GEN, CIP, MOX, ENR, TET, NIT, SXT	*aacC2*, *aph(3′)-Ic*, *strA*, *strB*, *sul2*, $bla_{OXA-500}$, $bla_{ADC-18-like}$, bla_{OXA-58}, *tet*(39)

[a]All isolates were resistant to ampicillin (AMP), amoxicillin-clavulanate (AMC), chloramphenicol (CHL), fosfomycin (FOS), cephalexin (LEX), and piperacillin (PIP) in accordance with the definitions of intrinsic resistance for members of the *Acinetobacter baumannii* complex set by EUCAST and CLSI. All isolates were susceptible to amikacin (AMK), imipenem (IPM), meropenem (MER), polymyxin B (PMB), tigecycline (TGC), tobramycin (TOB), and piperacillin/tazobactam (TZP). CPD, cefpodoxime; CTZ, ceftazidime; FEP, cefepime; CFP, cefpirome; CTU, ceftiofur; ENR, enrofloxacin; GEN, gentamicin; MAR, marbofloxacin; TET, tetracycline; SXT, sulfamethoxazole-trimethoprim; CIP, ciprofloxacin; MOX, moxifloxacin; FOS, fosfomycin; NIT, nitrofurantoin; CHL, chloramphenicol; TZP, piperacillin-tazobactam.

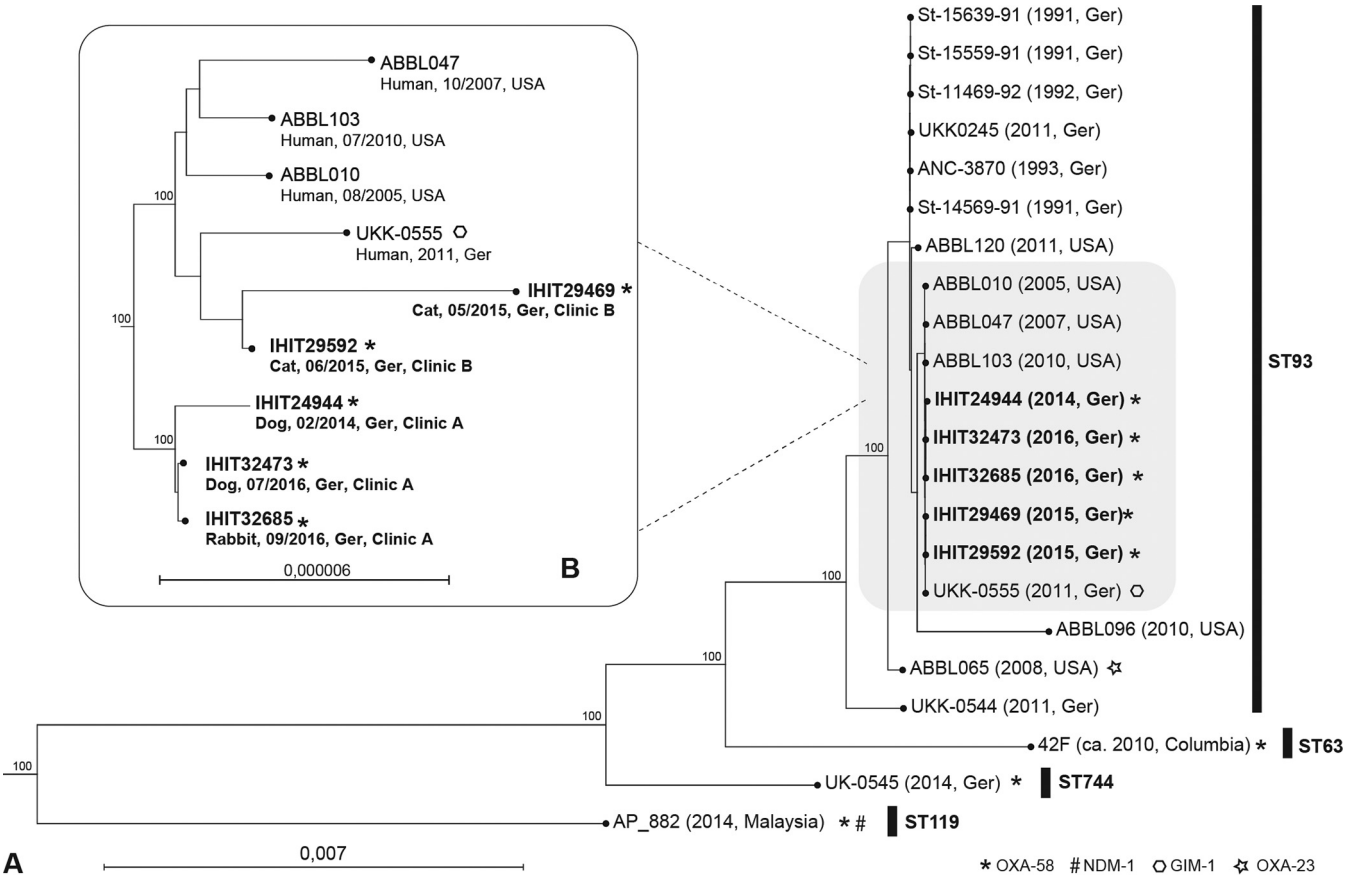

FIG 1 (A) Maximum-likelihood tree (based on 2,770 orthologous genes) of 17 publicly available genomes of ST93 and/or OXA-58-positive *A. pittii* isolates from humans (lightface) and five animal isolates (bold) from this study. (B) Excerpt of the tree in panel A providing higher resolution of closely related animal and human strains. The host, isolation date, and country are shown with the strain number. Scales indicate the numbers of nucleotide substitutions per site. Bootstrap values are based on 1,000 iterations. Ger, Germany.

showed resistance to ampicillin, amoxicillin-clavulanate, piperacillin, cephalexin, ceftiofur, gentamicin, enrofloxacin, marbofloxacin, tetracycline, nitrofurantoin, and sulfamethoxazole-trimethoprim but were susceptible to imipenem (VITEK2, bioMérieux, AST-card GN38), as interpreted according to breakpoints defined for human *Acinetobacter* spp. by either EUCAST or CLSI (20, 21). The hydrolytic activity of oxacillinases is normally low, and OXA-58 only confers a resistance phenotype when its expression is enhanced via insertion elements (22). Nevertheless, in *A. pittii*, OXA-58 does not always confer resistance, even when associated with insertion elements (9). We reassessed the MIC of imipenem after serial passages in broth containing increasing concentrations of meropenem (Sigma-Aldrich, Munich, Germany). The imipenem MICs for the OXA-58 isolates increased >8-fold (from ≥1 to 8 mg/liter), while this was not the case for an *A. pittii* isolate without an acquired oxacillinase gene, which served as a control.

Only one kind of genetic context of bla_{OXA-58}, containing IS*Aba3*, *araC*, and *lysE*, was present among the *A. pittii* isolates (Fig. 2). The direct surroundings of bla_{OXA-58} in strain IHIT24944 resemble those of carbapenem-susceptible *A. pittii* AG304 (23) but differ from those of carbapenem-resistant *A. baumannii* strain WH8144 (Fig. 2). In strain IHIT24944, bla_{OXA-58} was localized on a 53-kb contig that could be circularized by PCR (see Table S1 in the supplemental material) and sequencing. Plasmid pAP24944 (GenBank accession no. KY888886.1) was 53,802 bp in size, was non-self-transmissible, and additionally harbored genes for streptomycin (*strA* and *strB*), aminoglycoside (*aacC2*), sulfonamide (*sul2*), and tetracycline [*tetA*(39)/*tetR*(39)] resistance. We could determine OXA-58 plasmids similar in size and structure in all of the strains, and they

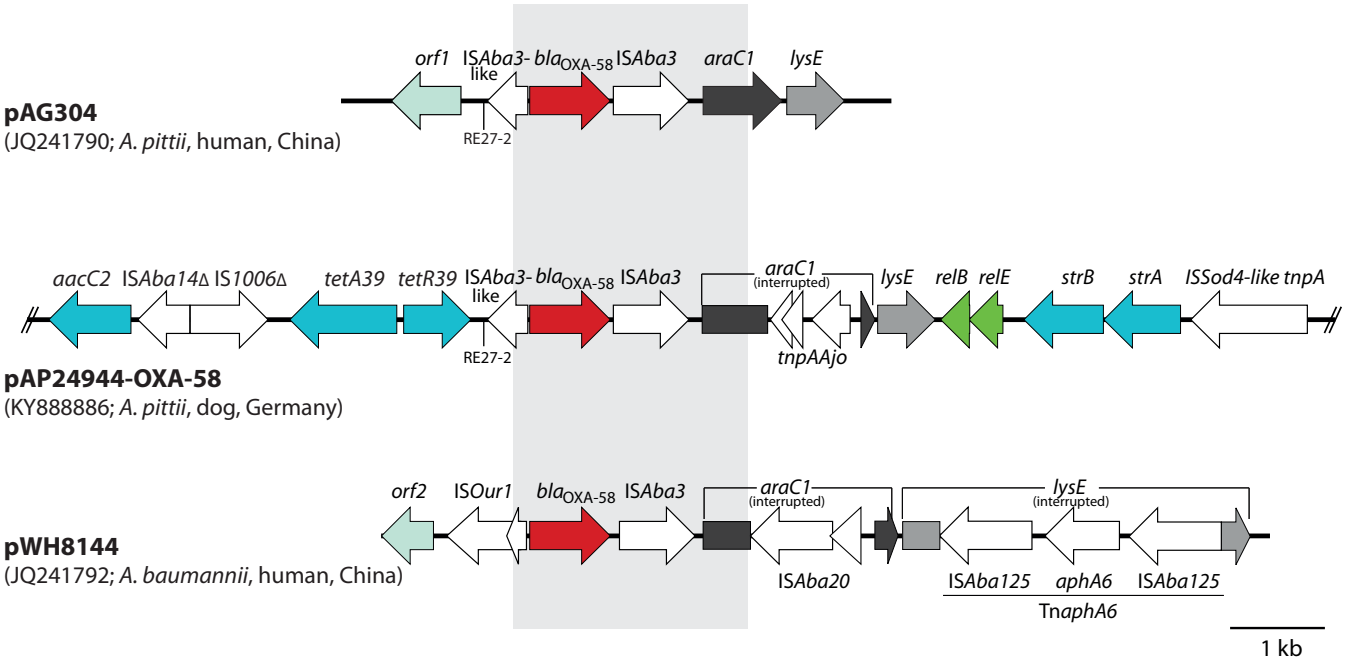

FIG 2 Comparison of the genetic region surrounding bla_{OXA-58} in pAP24944-OXA-58 (GenBank accession no. KY888886.1; only a partial sequence is shown) with the corresponding regions of pAG304 (JQ241790) of *A. pittii* strain AG304 and pWH8144 (JQ241792) of *A. baumannii* strain WH8144. Arrows indicate the extent and direction of genes and open reading frames. The genes (and encoded proteins) shown are *araC1* (transcription-regulating protein), *lysE* (threonine excretion pump protein), *aacC2* (aminoglycoside-3-*N*-acetyltransferase), *tetA*(39)/*tetR*(39) (tetracycline efflux pump), *relB*/*relE* (putative toxin-antitoxin system), and *strA*/*strB* (aminoglycoside 3′-phosphotransferases). *orf1*, DNA-binding response regulator gene; *orf2*, putative exodeoxyribonuclease VII subunit. The region with significant DNA sequence identity is shown as a gray box. Re27-2 regions (ATTTAACATAATGGCTGTTATACGAAA) are indicated by vertical lines. The images are drawn to scale from the GenBank entries indicated in parentheses.

only partially overlapped published *Acinetobacter* sp. plasmid sequences, as identified by a BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Fig. S1).

This study provides the first evidence of the presence of OXA-58 plasmids in carbapenem-susceptible *A. pittii* isolates from pets. Colocalization of various resistance genes on these plasmids might enable their spread despite the rare use of carbapenems in these animals.

Accession number(s). The whole genome sequences of *A. pittii* strains have been assigned accession numbers NWWC01000000 (IHIT24944), NWWB01000000 (IHIT29469), NWWA01000000 (IHIT29592), NWVZ01000000 (IHIT32473), and NWVY01000000 (IHIT32685). The complete sequence of pAP24944-OXA-58 is available from the GenBank database under accession number KY888886.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01993-17.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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