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Dogs Are a Reservoir of Ampicillin-Resistant *Enterococcus faecium* Lineages Associated with Human Infections▽

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Ampicillin resistance is a marker for hospital-associated *Enterococcus faecium*. Feces from 208 dogs were selectively screened for the occurrence of ampicillin-resistant *E. faecium* (AREF). AREF was detected in 42 (23%) of 183 dogs screened in a cross-sectional study in the United Kingdom and in 19 (76%) of 25 dogs studied longitudinally in Denmark. AREF carriage was intermittent in all dogs studied longitudinally. Multilocus sequence typing of 63 canine AREF isolates revealed the presence of 13 distinct sequence types. Approximately 76% of the isolates belonged to hospital-adapted clonal complex 17 (CC17), including those of sequence types ST-78 and ST-192, which are widespread in European and Asian hospitals. Longitudinal screening of 18 healthy humans living in contact with 13 of the dogs under study resulted in the identification of a single, intermittent CC17 carrier. This person carried one of the sequence types (ST-78) recovered from his dog. Based on PCR and Southern hybridization analyses, the putative virulence gene cluster from *orf903* to *orf907* was widespread in canine AREF isolates (present in 97%), whereas *orf2351* (present in 26% of isolates) and *orf2430* (present in 31%) were strongly associated with CC17-related sequence types ($P < 0.05$). Surprisingly, *esp* and *hyl* were not detected in any of the isolates. The antimicrobial resistance profiles of canine AREF isolates generally differed from those previously described for clinical human isolates. The results indicate that dogs are frequent carriers of CC17-related lineages and may play a role in the spread of this nosocomial pathogen. The distinctive virulence and antimicrobial resistance profiles observed among canine AREF isolates raise interesting questions about the origin and evolution of the strains causing human infections.

Enterococci are opportunistic pathogens and form part of the normal gastrointestinal flora in humans and animals. Over the last two decades, nosocomial infections caused by enterococci have emerged and their incidence has increased rapidly, first in the United States and recently in Europe (25, 26, 29). Although *Enterococcus faecalis* is the causative agent in most enterococcal infections, a shift toward infections caused by multidrug-resistant *E. faecium* has been noted in the last years, and presently, up to one-third of enterococcal infections in some countries are attributed to this species (17). This shift may be explained by changes in the patterns of antimicrobial usage, which may have resulted in the emergence of a distinct genogroup of hospital-associated ampicillin-resistant *E. faecium* (AREF) strains, currently labeled clonal complex 17 (CC17) (33). CC17 isolates are characterized by resistance to ampicillin and fluoroquinolones, as well as by the presence in most isolates of putative virulence genes encoding the enterococcal surface protein (*esp*) and hyaluronidase (*hyl*) and five recently described open reading frames (ORFs; *orf903*, *orf904.5*, *orf906.7*, *orf2351*, and *orf2430*) encoding LPXTG surface proteins, which are found less frequently among other *E. faecium* lineages (15, 20, 27).

Based on the results of multilocus sequence typing (MLST) (28) and amplified fragment length polymorphism analysis (34), *E. faecium* isolates of animal origin seem to be host specific and generally unrelated to human lineages of clinical importance. Prior to this study, AREF CC17 strains have been isolated only sporadically from animals, including pigs (2, 10) and more recently dogs (8). Following these unexpected findings, the present study was designed to investigate the prevalence and shedding patterns of AREF in dogs. A cross-sectional study and two longitudinal studies involving a total of 208 dogs and 479 canine fecal samples were conducted in the United Kingdom and in Denmark, respectively. Canine isolates were characterized by MLST, antimicrobial susceptibility testing, and putative virulence gene profiling to assess the genetic relationship between human and canine AREF strains.

MATERIALS AND METHODS

Sampling. The occurrence of AREF in 183 dogs screened as part of a cross-sectional study in Cheshire, United Kingdom, in 2006 (32) and in 25 dogs studied longitudinally in the region of Zealand, Denmark, in 2007 was investigated. Fecal samples or swabs from freshly voided feces were collected in sterile containers and submitted to the laboratory by the dog owners. Samples were kept frozen at −80°C whenever bacteriological analysis could not be performed within 72 h after collection. Samples from Danish dogs were collected as part of two separate longitudinal studies investigating intrafamily bacterial transmission and antimicrobial effects on fecal microflora, respectively. In the first longitudinal study (study A) (Table 1), samples from 18 human and 13 canine members of eight family households were obtained on 12 occasions over a 6-month period. In the second longitudinal study (study B) (Table 2), samples from 12 dogs with pyoderma that were undergoing treatment with different β-lactam compounds (i.e., amoxicillin-clavulanic acid, cephalexin, and cefovecin) were collected at 14 time points over the course of 1 month. Human samples consisted of rectal swabs. The

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TABLE 1. Longitudinal carriage of putative AREF strains in healthy dogs in longitudinal study A

Dog identification no.	ST of AREF isolate or result[a] for sample from day:											
	1	2	3	4	11	18	25	48	90	120	150	180
A1	−	−	−	−	78	−	19	−	396	+	+	+
A2	−	−	−	−	−	−	−	−	−	−	−	−
A3	−	−	−	−	−	−	−	−	−	−	−	−
A4	NR	NR	NR	NR	NR	NR	NR	−	−	121	−	+
A5	−	−	−	−	−	−	−	−	−	−	−	78
A6	−	−	−	−	−	−	−	−	−	78	−	+
A7	+	+	+	+	−	−	+	78	−	−	−	+
A8	−	−	78	−	+	+	−	+	−	−	−	−
A9	+	−	−	−	−	−	−	−	266	−	−	−
A10	−	−	−	−	−	−	−	−	−	−	−	397
A11	−	−	−	−	−	−	−	−	−	−	−	−
A12	−	−	−	−	−	−	−	192	−	−	+	+
A13	−	−	−	−	−	−	−	78	−	+	+	−

[a] Samples from 13 healthy dogs (A1 to A13) from a family study were analyzed. Numbers refer to STs of isolates verified as AREF. +, putative AREF isolate, not characterized; −, no growth on Slanetz-Bartley agar with ampicillin; NR, sample not received.

protocol for obtaining samples from human participants was approved by the Danish National Committee on Biomedical Research Ethics (license number H-KF-2007-0007).

Bacterial isolation and identification. All fecal samples were streaked onto plates of Slanetz-Bartley agar (Oxoid, Basingstoke, United Kingdom) supplemented with 32 μg/ml of ampicillin, and the plates were incubated for 48 h at 44°C. One putative AREF isolate from each culture-positive dog and human was randomly selected and identified by a species-specific PCR method (12). Isolates confirmed to be AREF were subjected to further analyses. As part of longitudinal study B, total and relative numbers of putative AREF bacteria in all culture-positive samples were determined in duplicate. One gram of feces was mixed in a stomacher with 9 ml of sterile Milli-Q water. Plate counts were then performed by streaking 10-fold dilutions of samples onto Slanetz-Bartley agar with and without ampicillin. Following 48 h of incubation at 44°C, putative AREF bacteria and total enterococci on selective and nonselective plates, respectively, were counted, and bacterial concentrations (in CFU per gram) were calculated based on the plate counts.

Antimicrobial susceptibility. Antimicrobial susceptibility testing was performed by the disk diffusion method according to the CLSI breakpoints for isolates from humans (5). Disks with the following antimicrobials were used: ampicillin (10 μg), ciprofloxacin (5 μg), erythromycin (15 μg), gentamicin (120 μg), linezolid (30 μg), quinupristin-dalfopristin (15 μg), rifampin (5 μg), tetracycline (30 μg), and vancomycin (30 μg).

MLST. One AREF isolate from each dog was chosen randomly and subjected to MLST according to the protocol described by Homan et al. (16). Three isolates collected from a single dog (A1) at different sampling times were selected to get information on AREF diversity over time. Alleles were analyzed and sequence types (STs) were assigned using the database available at http://www.mlst.net and the software CLC Combined Workbench 3 (CLC bio A/S, Aarhus, Denmark). STs obtained for AREF isolates were analyzed and compared to the entries in the existing *E. faecium* MLST database by using the eBURST algorithm. New STs were classified as belonging to CC17 if they were single-locus variants of STs within this complex.

Detection of putative virulence genes. The presence of the genes *esp*, *hyl*, *orf903*, *orf905*, *orf907*, *orf2351*, and *orf2430* was investigated by PCR using the primers and conditions described in previous studies (15, 20, 27) with the exception that template DNA was extracted using the DNeasy kit (Qiagen Inc., Venlo, The Netherlands). *E. faecium* E1162 (14) and *E. faecium* C68 (27) were included as positive controls. The PCR results were confirmed by Southern blot analysis. In brief, chromosomal DNA was digested with EcoRI for 3 h at 37°C and DNA fragments were separated overnight on a 1% agarose gel. Upon exposure to UV light for 5 min, the gel was washed for 15 min in 0.25 M HCl and then subjected to two separate 15-min washes in 0.4 M NaOH. DNA fragments were transferred onto a Hybond N^+ nylon membrane by vacuum blotting, and the membrane was fixated in 0.4 M NaOH for 2 min and neutralized in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). Membranes were hybridized overnight at 42°C with a 100-ng probe. Probes specific for each gene were amplified from the chromosomal DNA of *E. faecium* strain DO (accession no. AAAK00000000), except those for *esp* and *hyl*, which were amplified from *E. faecium* E1162 DNA and *E. faecium* C68 DNA, respectively. Probes were purified using the QIAquick

TABLE 2. Longitudinal carriage of putative AREF strains in dogs with pyoderma in longitudinal study B

Dog identification no.	ST of AREF isolate or result[a] for sample from day:													
	1	2	3	4	6	8	10	12	14	16	18	20	22	29
B1[b]	−	192	+	+	+	+	+	+	+	+	+	+	−	+
B2[b]	−	−	−	−	−	−	−	−	−	−	−	−	−	−
B3[b]	−	−	−	−	−	+	+	−	78	+	−	−	+	NR
B4[c]	+	78	+	+	+	−	+	−	−	+	NR	+	−	+
B5[c]	192	−	−	−	−	−	−	−	+	+	NR	−	−	+
B6[c]	−	NR	-	19	+	+	+	+	+	+	+	+	+	+
B7[c]	−	−	−	−	−	−	−	−	−	−	−	−	NR	−
B8[c]	−	−	NR	−	−	−	−	NR	NR	−	−	−	NR	NR
B9[d]	−	19	NR	+	+	+	+	+	−	+	−	−	−	+
B10[d]	−	19	+	NR	+	−	+	−	−	−	−	NR	−	−
B11[d]	NR	78	+	−	−	−	−	−	−	NR	−	NR	−	NR
B12[d]	−	−	NR	−	−	−	78	−	−	NR	NR	NR	−	−

[a] Samples from 12 dogs (B1 to B12) with pyoderma being treated with β-lactams were analyzed. Numbers refer to STs of isolates verified as AREF. +, putative AREF isolate, not characterized; −, no growth on Slanetz-Bartley agar with ampicillin; NR, sample not received.
[b] Dog was treated daily with amoxicillin-clavulanic acid tablets from day 1 until day 14.
[c] Dog was treated daily with cephalexin tablets from day 1 until day 14.
[d] Dog was treated by cefovecin injection on day 1.

TABLE 3. Antimicrobial susceptibilities of 61 canine AREF isolates

Antimicrobial	No. (%) of isolates		
	Susceptible	Intermediately resistant	Resistant
Ciprofloxacin	5 (8)	41 (67)	15 (25)
Erythromycin	2 (3)	48 (79)	11 (18)
Gentamicin	58 (95)	0	3 (5)
Linezolid	53 (87)	6 (10)	2 (3)
Quinupristin-dalfopristin	52 (85)	8 (13)	1 (2)
Rifampin	28 (46)	1 (2)	32 (52)
Tetracycline	7 (11)	3 (5)	51 (84)
Vancomycin	61 (100)	0	0

PCR purification kit (Qiagen Inc.). Labeling with horseradish peroxidase, hybridization, and detection were done according to the instructions of the manufacturer of the enhanced chemiluminescence nucleic acid labeling kit (GE Healthcare, Diegem, Belgium).

The ORFs *orf905* and *orf907* are not putative virulence genes since their products lack the typical N-terminal signal peptide sequence of cell wall-anchored proteins (CWAPs). Isolates positive for these genes were therefore subjected to PCR amplification and sequencing of the regions at the *orf904-orf905* and *orf906-orf907* junctions to identify the possible existence of the merged *orf904.5* and *orf906.7* genes, which both encode potentially functional CWAPs (15).

Statistical analyses. Geographical clustering of STs and statistically significant differences in the prevalence of virulence genes among CC17 and non-CC17 isolates were determined by comparing proportions in EpiCalc 2000 (version 1.02 [http://www.brixtonhealth.com]).

Nucleotide sequence accession number. The novel DNA sequence identified in the junction region of *orf906.7* has been deposited in the GenBank nucleotide sequence database under accession number FJ481924.

RESULTS

AREF prevalence in and patterns of shedding from dogs. A total of 479 fecal samples and swabs from dogs were received and analyzed. Presumptive AREF was isolated from 42 (23%) of the 183 dogs screened as part of the cross-sectional study in the United Kingdom. Among the 25 dogs evaluated longitudinally in Denmark, 19 dogs (76%) had at least one sample positive for a presumptive AREF strain during the study period. The patterns of shedding from most of the dogs appeared to be extremely variable, as indicated by the intermittent detection of AREF in their feces (Tables 1 and 2). AREF concentrations in feces varied substantially among individuals and among samples collected from the same individual at different time points as part of longitudinal study B. Total and relative numbers of putative AREF bacteria varied from 10 CFU/g and less than 1% of the total enterococci up to 10^6 CFU/g and 89% of enterococci (data not shown). All presumptive canine AREF isolates selected for further pheno- and genotypic characterization were confirmed to be *E. faecium* by PCR. Overall, AREF was detected at least once in 61 (29%) of the 208 dogs studied. A total of 203 fecal samples were received from the 18 healthy family household members who were screened concurrently with their dogs in longitudinal study A. Only one 10-year-old boy, living with dog A1 (Table 1), was positive for AREF on a single occasion.

Antimicrobial susceptibility. Ampicillin resistance was confirmed for all isolates. More than half of the 61 AREF isolates tested displayed intermediate or full resistance to ciprofloxacin (92% of isolates), erythromycin (97%), tetracycline (89%), and rifampin (54%). Lower prevalences of resistance toward certain first- or second-line agents currently used for the treatment of enterococcal infections, such as gentamicin (5%), linezolid (3%), and streptogramins (2%), were observed. Resistance to vancomycin was not detected. Notably, only a small percentage of isolates were fully resistant to erythromycin (Table 3).

TABLE 4. Multilocus STs of 63 canine AREF isolates

Genogroup	ST	No. with indicated ST among:	
		United Kingdom isolates (n = 42)	Danish isolates (n = 21[c])
CC17 isolates (n = 48)	ST-19	2	4
	ST-78	6	10[b]
	ST-121	0	1
	ST-192	5	3
	ST-397[a]	0	1
	ST-398[a]	14[b]	0
	ST-399[a]	1	0
	ST-400[a]	1	0
Non-CC17 isolates (n = 15)	ST-157	1	0
	ST-266	9	1
	ST-396[a]	0	1
	ST-401[a]	2	0
	ST-402[a]	1	0

[a] New ST.
[b] This result shows a significant association between the ST and the country of origin ($P < 0.05$).
[c] Three Danish isolates belonging to ST-78, ST-192, and ST-396 originated from the same dog, whereas other isolates were from distinct dogs.

MLST. MLST analysis of 63 canine AREF isolates (including 3 from the same dog) revealed the occurrence of 13 STs, including seven novel STs (ST-396 to ST-402) (Table 4). The most frequent clone was ST-78, which was found in 16 isolates (25%) and was significantly associated with Danish origin (P = 0.011). Another common clone (ST-398) was found solely in isolates from the United Kingdom (P = 0.007). Four STs (ST-19, ST-78, ST-121, and ST-192) had been associated previously with CC17, and four of the novel STs (ST-397 to ST-400) were single-locus variants of ST-78 and ST-192 and therefore also considered to belong to CC17. Overall, 48 (76%) of the 63 canine AREF isolates belonged to CC17 (Table 4). The AREF strain isolated from a 10-year-old boy belonged to the same ST (ST-78) previously detected in the boy's dog. MLST analysis of two other isolates from the same dog (A1) revealed the presence of AREF ST-19 and ST-396 on days 25 and 90, respectively (Table 1). The genetic relatedness of STs to those listed in the central database (http://www.mlst.net) is depicted in Fig. 1.

Occurrence of putative virulence genes. The results obtained by PCR and Southern hybridization matched for all isolates. Table 5 shows the distributions of putative virulence genes in isolates classified as CC17 and non-CC17 strains. None of the 62 analyzed canine isolates carried *esp* or *hyl*. The ORFs *orf903*, *orf905*, and *orf907* occurred simultaneously in 60 isolates (97%) and were not statistically associated with CC17. No variation in the sequence of the *orf904-orf905* junction region relative to the publicly available *E. faecium* DO sequence was observed, indicating that *orf904* and *orf905* were not merged

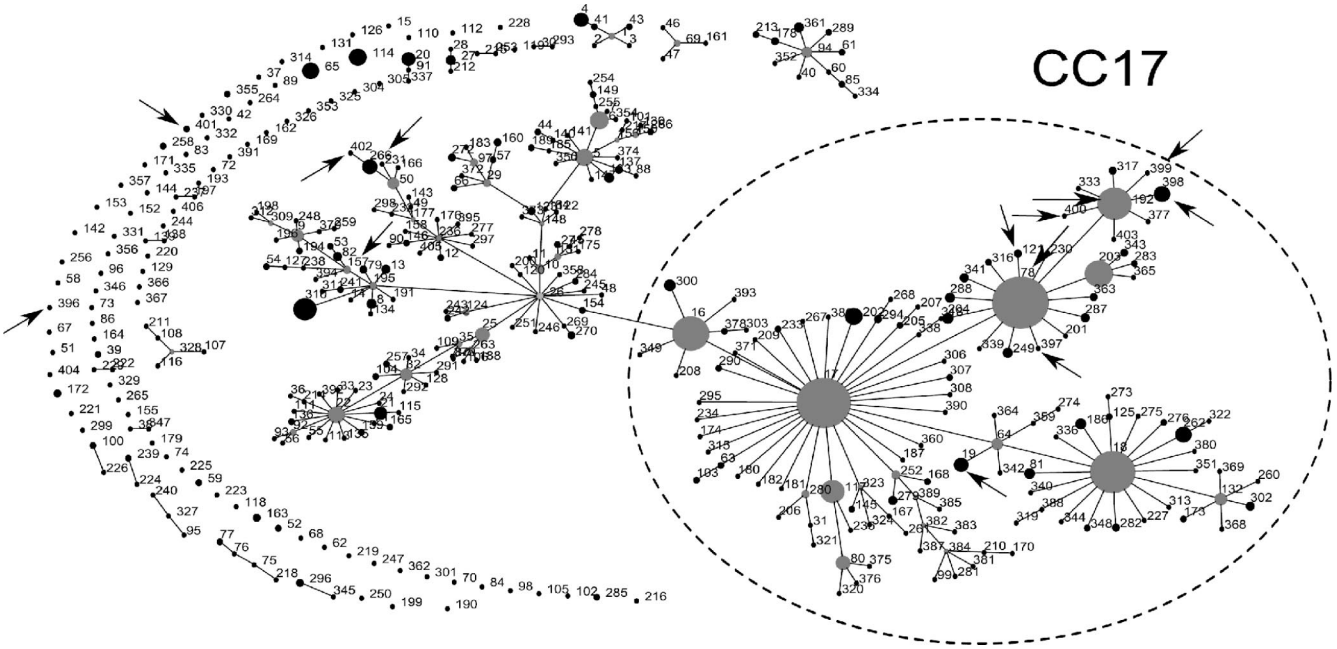

FIG. 1. Clustering of the MLST profiles as depicted by the eBURST algorithm. The 13 MLST profiles identified for canine isolates are indicated by arrows in the figure, which is based on MLST profiles of 1,449 *E. faecium* isolates from the central database (http://www.mlst.net). Each ST is represented as a node; the size of each node indicates the relative frequency of a particular ST. Each line indicates a single-locus difference between STs.

into *orf904.5* in any of the canine isolates. A more diverse picture was evident from the sequencing of the *orf906-orf907* junction region; in 20 isolates, the sequence was identical to that in *E. faecium* DO; 3 isolates had a 101-bp oligonucleotide deletion before the stop codon of *orf906*, resulting in various premature stop codons; and 36 isolates had a 19-bp oligonucleotide insertion (TTTATAACCCGAATTCATC) just before the *orf906* stop codon, which resulted in a frameshift and the merging of *orf906* and *orf907* into *orf906.7*, encoding an intact CWAP. This 19-bp insertion was identical to one reported previously (15) and occurred more commonly in non-CC17 isolates (86%) than in CC17-isolates (51%) (Table 5). In contrast to the almost ubiquitously present *orf903*-to-*orf907* cluster, *orf2351* and *orf2430* were present in only 15 isolates (25%) and 18 isolates (30%), respectively, and both genes were significantly (*P*, 0.037 and 0.015) associated with CC17. Furthermore, *orf2430* was specific to ST-78 and its two single-locus variants, ST-121 and ST-397.

TABLE 5. Distribution of putative virulence genes among 61 canine AREF isolates

Gene	Occurrence (no. [%] of isolates with gene) in:	
	CC17 group (n = 47)	Non-CC17 group (n = 14)
esp	0	0
hyl	0	0
orf903[b]	47 (100)	12 (86)
orf904.5	0	0
orf906.7	24 (51)	12 (86)
orf2351	15 (32)[a]	0
orf2430	18 (38)[a]	0

[a] This result shows that the gene is significantly associated with CC17 isolates ($P < 0.05$).
[b] All isolates positive for *orf903* also contained *orf905* and *orf907*.

DISCUSSION

We describe for the first time the widespread occurrence of hospital-associated AREF lineages in dogs. Remarkably, two of the STs most frequently isolated from dogs (ST-78 and ST-192) are among the most common AREF lineages causing infections in European and Asian hospitals (3, 18, 29, 31).

This finding was surprising considering the general perception that *E. faecium* strains are host specific and cluster according to the species of origin (19, 34). Approximately one in every four dogs harbored AREF CC17 bacteria; hence, dogs seem to be an important reservoir for these bacteria of medical interest. On the contrary, only 1 of the 18 healthy humans tested was found to be positive for *E. faecium*. One previous study failed to detect AREF in healthy humans despite the use of selective isolation media (9). However, the human carriage rate observed needs to be confirmed on a larger scale, since the low community prevalences of AREF among healthy people (0 to 6%) reported in other studies (1, 2, 11, 23) might be influenced by the use of nonselective isolation methods.

AREF CC17 has spread rapidly in hospitals across the world (25, 29, 33). The widespread occurrence of ST-78, ST-192, and other CC17-related clones in dogs is worrisome since these animals may provide a vehicle for the spread of AREF among humans. In line with this hypothesis, AREF isolates displaying the same ST (ST-78), virulence level, and resistance pattern were obtained from a dog and a boy living within the same household. We were informed that the boy had close contact with the dog and that this contact included frequent kissing

and petting. Such a close relationship may have enhanced the opportunity for the transmission of the strain between the dog and the boy. Possible links between canine and human pathogenic enterococci have been addressed previously by other authors; genetic similarities between vancomycin-resistant *E. faecium* isolates of canine and human origins were observed by Willems et al. (34) using amplified fragment length polymorphism; a vancomycin-resistant *E. faecalis* isolate in a case of canine mastitis in New Zealand, described by Manson et al. (22), displayed a pulsed-field gel electrophoresis profile indistinguishable from that of a common human pathogenic clone in New Zealand.

The putative virulence gene content of canine AREF isolates differed considerably from that usually observed in CC17 isolates from human infections. In particular, two putative virulence genes associated with CC17, *esp* and *hyl* (3, 6, 30), were completely absent among canine AREF isolates. Similarly, *orf2351* and *orf2430* occurred less frequently (in <30% of isolates) than previously reported for human vancomycin-resistant and -sensitive CC17 isolates (>70% of which carried the genes) (15). The difference between canine and human clinical isolates may reflect an evolutionary multistep process during which *E. faecium* CC17 sequentially acquired a number of virulence and antibiotic resistance properties before becoming the most successful hospital-adapted lineage. As previously suggested by Leavis et al. (19), ampicillin resistance is likely one of the first properties that was acquired by a diverse group of clones and lineages currently included within CC17. Canine AREF isolates may therefore represent an early evolutionary ancestor of human clinical CC17 strains, which may have evolved and adapted to hospital environments by acquiring virulence genes such as *esp* and *hyl*. Alternatively, human AREF strains may be ancestors of canine strains, which may have evolved by the loss of these putative virulence factors outside hospital settings. The observation that the *orf903*-to-*orf907* gene cluster was present in almost all canine AREF isolates, irrespective of their genetic backgrounds, suggests that the acquisition of this gene cluster may have been an early event in the evolutionary development of CC17. It is noteworthy that the *orf906.7* fusion, resulting in a potentially functional CWAP with similarities to the biofilm enhancer in enterococcus 3 protein of *E. faecalis*, was predominant in non-CC17 isolates and generally occurred more frequently in canine AREF isolates (60%) than previously reported for human AREF isolates (24%) (15). This finding suggests that the gene cluster became partly obsolete upon the transmission of *E. faecium* to humans and may have a minor role in the pathogenesis of human *E. faecium* infections. Despite the atypical putative virulence gene profile of canine isolates, the virulence and thereby the potential of canine isolates to cause human infections should not be underestimated, since *esp* and *hyl* occur only in a proportion of human AREF CC17 strains (7, 27, 35) and may therefore not be necessary for establishing infection in the human host. In addition, *orf2351* and *orf2430* occurred more or less specifically in ST-78, suggesting that the most common ST in dogs may also be one of the most pathogenic.

Data on shedding patterns were obtained by the longitudinal studies conducted on 25 dogs in Denmark. Although 76% of the Danish dogs carried AREF at least once during the study period of 1 to 6 months, none of the dogs were found to be positive at all sampling times (Tables 1 and 2). This result suggests that the enterococcal flora in dogs is subject to frequent shifts and that most dogs are only transiently colonized with AREF or that AREF colonization dropped below the detection level. Plate counts performed as part of longitudinal study B indicated a high degree of variation in the concentrations of AREF bacteria among and within dogs screened at different time points. This variability suggests that the inclusion of a preenrichment step to enhance the detection of AREF in dogs may be advisable. Interestingly, dogs appear to shed distinct AREF strains over time, as indicated by dog A1's being colonized by three distinct STs (ST-19, ST-78, and ST 396) over a period of 3 months (Table 1).

The levels of resistance of canine AREF strains to some of the most clinically relevant antimicrobials were relatively low (Table 3). Most importantly, vancomycin resistance was not detected and high-level gentamicin resistance was rare (occurring in 5% of isolates). Both vancomycin and gentamicin are first-line drugs for the treatment of enterococcal diseases, either separately or in combination with β-lactams (4). Two of the most frequently used second-line drugs, linezolid and streptogramins, were also included in our panel, and the majority of isolates (>85%) were susceptible to both agents. Canine isolates displayed some atypical resistance patterns in comparison with data previously reported for CC17 isolates from human infections, including vancomycin-resistant and -susceptible variants (6, 13, 24). In particular, the prevalences of macrolide and streptogramin resistance were low (18 and 2%, respectively) whereas the frequency of tetracycline resistance was unexpectedly high (82%). High-level resistance to fluoroquinolones was not as common as that reported previously for human CC17 isolates (21). Altogether, these differences support the notion that human and canine AREF strains may represent two distinct bacterial populations, despite the genetic similarities observed by MLST.

In conclusion, healthy dogs are frequent carriers of human hospital-associated AREF CC17. Dogs may therefore play a role in the spread of this nosocomial pathogen in the community, and a risk of zoonotic transfer exists, as indicated by the possible case of transmission between a boy and his dog. Although the distinct putative virulence gene profiles suggest that canine isolates represent early evolutionary ancestors of human pathogenic strains, further research is needed to assess the virulence of canine strains in comparison with that of human strains and, more generally, to quantify the magnitude of this possible emerging zoonotic problem. The Centers for Disease Control and Prevention have stated immunocompromised groups, for example, people with human immunodeficiency virus infection, organ transplant patients, and young children, to be at risk for infection with canine zoonotic agents (http://www.cdc.gov/healthypets/animals/dogs.htm). The professional use of pets to promote the recovery of patients (pet therapy) may pose a risk to such patients if the dogs are not previously screened for the presence of AREF and other zoonotic pathogens. The occurrence of AREF in dogs and other domestic animals could be addressed by national programs for the surveillance of antimicrobial resistance in animals in order to explore the importance of the animal reservoir in the evolution of human hospital-associated enterococci.

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