

## Dispersion of Multidrug-Resistant *Enterococcus faecium* Isolates Belonging to Major Clonal Complexes in Different Portuguese Settings<sup>▽</sup>

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**The population structure of 56 *Enterococcus faecium* isolates selected from a collection of enterococci from humans, animals, and the environment in Portugal (1997 to 2007) was analyzed by multilocus sequence typing. We identified 41 sequence types clustering into CC17, CC5, CC9, CC22 and CC94, all clonal lineages comprising isolates from different hosts. Our findings highlight the role of community-associated hosts as reservoirs of enterococci able to cause human infections.**

Enterococci are organisms widely distributed in nature that are recognized as one of the leading causes of nosocomial infections (1, 20). Despite their ubiquity, the population structure of *Enterococcus faecium* comprises a diversity of sequence types, with overrepresentation of particular clonal complexes (CCs) associated with swine (CC5), poultry (CC9), veal calf (CC1), or humans (CC17, CC22, and CC94) (1, 5, 15, 17, 30, 37). Particular host-specific human clonal lineages are considered high-risk CCs (HiRCCs) since they are recovered mostly from hospitalized patients (17). CC17 is the only recognized *E. faecium* HiRCC, nowadays globally disseminated, which has been sporadically isolated from nonhospitalized humans (3, 8–10). Different studies have analyzed the population structure of local enterococci, but they are focused mainly on clinical strains with a specific phenotype, such as vancomycin resistance, generally isolated in a short temporal frame (2, 7, 16, 18, 27, 29, 36).

We analyzed 56 representative *E. faecium* isolates from a Portuguese collection comprising 1,700 enterococci from different geographical locations (1997 to 2007), some included in previous studies (22–26). They were recovered from hospitalized patients in five hospitals of different regions ( $n = 20$ ), swine excrement ( $n = 6$ ) and environmental piggery samples ( $n = 16$ ), retail poultry ( $n = 6$ ) of four national commercial brands, feces from healthy humans ( $n = 5$ ), hospital wastewater ( $n = 2$ ), and the estuary of the river Douro ( $n = 1$ ). Susceptibility testing with 15 antibiotics was performed following CLSI guidelines (6). Species identification and detection of genes encoding vancomycin resistance were performed by us-

ing multiplex PCR (11). Clonal relatedness was established by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) as described previously (15, 22, 28). Sequence types (STs) differing in one or two of the seven housekeeping genes were considered single-locus variants (SLVs) and double-locus variants, respectively. Clusters of related STs differing in  $\leq 2$  loci that were thought to be descendants from a common ancestor were grouped into CCs by using the eBURST software program (12, 13) (<http://www.mlst.net>). Genes coding for virulence factors such as enterococcal surface protein (encoded by *esp*), hyaluronidase (encoded by *hyl*), cytotoxin (encoded by *cyl*), gelatinase (encoded by *gel*), and aggregation substance (encoded by *agg*) and the backbone structure of Tn1546 harbored by vancomycin-resistant *E. faecium* (VRE) were investigated by using PCR (21, 34, 38).

The isolates studied (35 VRE isolates and 21 vancomycin-susceptible *E. faecium* [VSE] isolates) corresponded to 49 PFGE types and 41 different STs, including 24 newly identified STs, which clustered into CC5, CC9, CC17, CC22, and CC94 (Fig. 1 and Table 1).

CC17 was identified in 24 isolates (18 VRE isolates and 6 VSE isolates) from hospitals, healthy volunteers, swine, piggeries, and the environment in different regions from 1997 to 2007. In agreement with other studies, the CC17 meroclone consisted of a high diversity of STs (ST16, ST18, ST78, ST80, ST125, ST132, ST280, ST368, ST369, ST390, ST393, ST430, and ST431), particularly enriched by ST18 (7, 18, 29, 36, 37) (Table 1). All CC17 isolates were resistant to ampicillin and erythromycin, and most of them were associated with resistance to glycopeptides (75%) and ciprofloxacin (71%) and a high level of resistance to kanamycin (58%) and streptomycin (50%). *esp* was detected among isolates from most sources (38%), while *hyl* (17%) was mostly associated with the clinical setting. The detection of CC17 among nonclinical sources might indicate a hospital input of community strains with different genetic contents besides contamination from the hospi-

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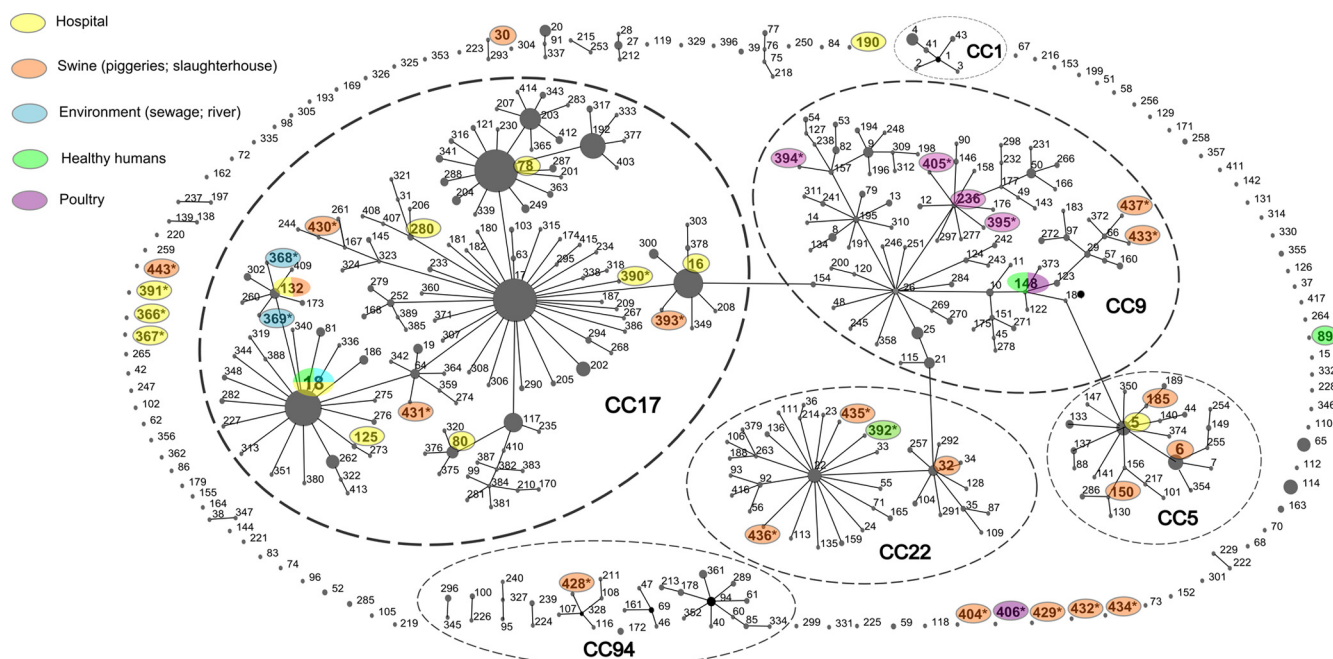


FIG. 1. Clustering of 41 *E. faecium* STs by use of eBURST. STs are indicated by colored circles, representing 56 isolates from the present study, with 439 MLST profiles representing 1,358 *E. faecium* isolates from the database ([www.mlst.net](http://www.mlst.net)). Each ST is represented as a node; the relative size of each node is indicative of its prevalence among the isolates, and lines connect SLVs. Colors indicate isolation sources, and CCs are represented by dashed circles (CC1, CC5, CC9, CC17, CC22, and CC94).

tal setting and could explain its high prevalence and global spread (3, 8–10).

Clonal dissemination and maintenance of particular resistant *E. faecium* strains among food animals have previously been documented, but MLST data are absent in almost all of these works (14, 19, 26, 32, 33). Although swine and poultry are considered potential reservoirs of pathogenic *E. faecium*, transmission to humans has been demonstrated in a few cases and is always linked to persons in close contact with farms (4, 19, 32, 33). CC5 comprised isolates of ST5 ( $n = 1$ ), ST6 ( $n = 1$ ), ST150 ( $n = 1$ ), and ST185 ( $n = 2$ ). Isolates classified as ST5 and ST185, which is an SLV of ST5, were recovered from two hospitals over 2 years (22) and from soil of an extensive piggyery, respectively. They were clonally related to the ST6 isolate which represents the VRE strain “A” widespread among swine of four European countries since 1997 (26). CC5 isolates did not contain the tested putative virulence factors and exhibited various resistance phenotypes (Table 1).

CC9 included eight clonally unrelated isolates from community (ST148), swine (ST433 and ST437), and poultry (ST236, ST394, ST395, and ST405), generally resistant to ciprofloxacin (88%) and vancomycin (62%). Most of the CC9 poultry strains were persistently recovered from commercial brands of retail poultry products over the years and corresponded to different ampicillin-resistant VRE or VSE strains (23). All isolates lacked the virulence genes tested. CC9 strains associated with *purK6* (ST236 and their SLVs, ST395 and ST405) or *purK3* (ST148) have also been detected in chickens from Korea and Spain, respectively, which might mirror particular globally disseminated poultry lineages (<http://efaecium.mlst.net/>).

CC22 (ST32, ST392, ST435, and ST436) comprised isolates

mostly resistant to tetracycline and ciprofloxacin and highly resistant to kanamycin from different piggyeries and from human feces. This genogroup was recently associated with isolates from human origin (5, 17), with this study representing the first description of isolates among swine.

The remaining STs were recovered from humans and animals in nine different locations and corresponded to ST30, ST89, ST190, ST366, ST367, ST391, ST404, ST406, ST428 (CC94), ST429, ST432, ST434, and ST443, mostly identified as singletons. It is of note that ST366/ST367 and ST391 from hospitals are double-locus variants of STs belonging to CC17 and were resistant to ampicillin and contained *esp*. Also, ST443 from swine harbored *purK1*, which is associated with CC17, and contained the *esp* and *hyl* genes.

Tn1546 among CC17 isolates was highly diverse, with a number of variants containing *ISEf1*. Tn1546 types A, D, and S, lacking insertion sequences and associated with animals, were detected in the non-CC17 VRE isolates CC5 and CC9 (21, 38; this study).

In summary, this is the first study describing the population structure of *E. faecium* from different origins and locations in Portugal, one of the European nations with the highest VRE rates in the nosocomial setting (see the EARSS Annual Report 2006 [<http://www.rivm.nl/earss>]) (35). Our results pose new insights into *E. faecium* host specificity, since all the identified clonal complexes comprised isolates from different host origins. The frequent recovery from the community of HiRCC17 and the emergence of CC5 in the hospital setting highlight the role of nonhospital hosts as possible reservoirs of pathogenic enterococci. This community reservoir of multidrug-resistant strains able to cause human infections might contribute to their

TABLE 1. Epidemiological features of *Enterococcus faecium* isolates from human, swine, poultry, and environmental samples in Portugal

CC <sup>a</sup>	ST	PFGE type <sup>b</sup>	Epidemiology/origin	Source (product) <sup>c</sup>	Region/yr of isolation	Antibiotic resistance profile <sup>de</sup>	Virulence trait(s) <sup>e</sup>	Tn/546 type <sup>f</sup>	Reference
CC17	16	74	Clinical isolate	HP <sub>A</sub> (catheter)	Center/2001	VAN, TEC, AMP, TET, ERY, STR	esp	PP-9	22
CC17	18	108	Clinical isolate	HP <sub>A</sub> (blood)	Center/2000	VAN, TEC, AMP, TET, ERY, CIP, STR, KAN	esp	PP-4	22
CC17	18	E	Hospital sewage (different places)	HW <sub>F</sub> (wastewater)	North/2001	VAN, TEC, AMP, ERY, CIP, GEN, STR, KAN, OD	hyl	PP-16	24
CC17	18	59	Community surveillance	HV (fecal swab)	North/2001	VAN, TEC, AMP, TET, ERY, CIP, GEN, KAN, CHL, OD	None	PP-5	25
CC17	18	78	Outbreak strain ( <i>n</i> = 17 isolates) disseminated in 2 hospitals (2001–2003)	HP <sub>A</sub> (liver fluid)	Center/2002	VAN, TEC, AMP, ERY, CIP, KAN, NIT	None	PP-4	22
CC17	18	70	Outbreak strain ( <i>n</i> = 14 isolates) disseminated in 3 hospitals (2001–2003)	HP <sub>B</sub> (unknown)	Center Eastern/2002	VAN, TEC, AMP, TET, ERY, CIP, OD	None	PP-2b	22
CC17	18	97	Clinical isolate	HP <sub>A</sub> (unknown)	Center	VAN, TEC, AMP, ERY, CIP	None	PP-4	22
CC17	18	69	Community surveillance	HV (fecal swab)	North/2004	VAN, TEC, AMP, TET, ERY, STR, KAN	None	D	25
CC17	18	125	Clinical isolate	HP <sub>D</sub> (catheter)	North/2007	VAN, TEC, AMP, ERY, CIP	None	PP-5	This study
CC17	18	128	Clinical isolate	HP <sub>D</sub> (pus)	North/2007	VAN, TEC, AMP, ERY, CIP, NIT	None	PP-5	This study
CC17	78	127	Clinical isolate	HP <sub>D</sub> (urine)	North/2007	AMP, ERY, CIP, GEN, KAN, NIT	None	-	This study
CC17	80	80	Clinical isolate	HP <sub>A</sub> (urine)	Center/1997	VAN, TEC, AMP, ERY, CIP	esp	PP-4	22
CC17	125	126	Clinical isolate	HP <sub>A</sub> (urine)	North/2007	VAN, TEC, AMP, ERY, CIP, NIT	esp, hyl	PP-5	This study
CC17	132	88	Outbreak strain disseminated in two hospitals (1999–2001)	HP <sub>D</sub> (blood)	Center/1999	VAN, TEC, AMP, TET, ERY, CIP, GEN, STR, KAN, OD	None	PP-4	22
CC17	132	119	Clinical isolate	HP <sub>C</sub> (urine)	North/2002	VAN, TEC, AMP, ERY, CIP, GEN, KAN, OD	esp	PP-13	22
CC17	132	119.5	Environmental isolate from an intensive piggery	PE <sub>in</sub> (liquid manure)	South/2007	VAN, TEC, AMP, ERY, GEN, KAN	esp	PP-31	This study
CC17	280	100 (2)	Outbreak strain disseminated in two hospitals (2002–2003)	HP <sub>B</sub> (urine, <i>n</i> = 2)	Center Eastern/2002–2003	(VAN), (TEC), AMP, (TET), ERY, CIP, GEN, (STR), KAN	(esp), hyl	PP-5	This study
CC17	368	H	Hospital sewage (different places)	HW <sub>C</sub> (wastewater)	North/2001	VAN, TEC, AMP, ERY, CIP, GEN, STR, KAN	esp	PP-20	24
CC17	369	RP5	River Douro (different places)	R (water sample)	North/2003	VAN, TEC, AMP, ERY, CIP, STR, KAN, OD	esp	X	24
CC17	390	122	Clinical isolate	HP <sub>B</sub> (unknown)	Center Eastern/2002	AMP, TET, ERY, STR, KAN, OD	None	-	This study
CC17	393/431	SN208 (2)	From one intensive piggery	SW <sub>in</sub> (solid manure)	South/2006–2007	AMP, TET, ERY, STR, NIT	None	-	This study
CC17	430	SN211	From an intensive piggery	PE <sub>in</sub> (dust)	South/2007	AMP, TET, ERY, STR, NIT	None	-	This study
CC5	6	A	Strain from a pig slaughterhouse spread in 4 European countries (1997–2000)	SW (feces)	Center/1997	VAN, TEC, TET, ERY, KAN	None	D	26
CC5	5	A <sub>3</sub>	Clinical strain disseminated in 2 hospitals during 2001–2002	HP <sub>C</sub> (pus)	North/2002	VAN, TEC, ERY	None	D	22
CC5	185	A <sub>5</sub> (2)	From the soil of an extensive piggery	PE <sub>v1</sub> (soil)	South/2007	VAN, TEC, AMP, TET	None	D	This study
CC5	150	SN216	From an intensive piggery	SW <sub>v</sub> (feces)	North/2007	AMP, TET, ERY, CIP, STR, KAN	None	-	This study
CC9	148	36	HLR-Gm strain from HV and poultry	HV (fecal swab)	2001	AMP, TET, ERY, CIP, GEN, STR, KAN	None	-	25
CC9	236	1	Strain ( <i>n</i> = 18 isolates) identified in 5 different national brands during 3 yr	RP (carcass)	North/1999	VAN, TEC, TET, ERY, CIP, NIT, OD	None	A	23
CC9	236	4	Strain ( <i>n</i> = 18 isolates) identified in 3 different national brands during 3 yr	RP (carcass)	North/1999	VAN, TEC, TET, ERY, CIP, KAN, NIT, OD	None	A	23
CC9	394	8	Retail poultry isolate	RP (carcass)	North/1999	VAN, TEC, TET, ERY, CIP, NIT	None	A	23
CC9	395	2	Strain ( <i>n</i> = 11 isolates) identified in 4 different national brands during 3 yr	RP (carcass)	North/2001	VAN, TEC, TET, ERY, CIP, NIT, OD	None	A	23
CC9	405	12	Retail poultry isolate	RP (carcass)	North/1999	VAN, TEC, ERY, CIP, STR, NIT, OD	None	S	23
CC9	433	SN214	From one facility of an intensive piggery	PE <sub>v</sub> (air)	North/2007	AMP, TET	None	-	This study
CC9	437	SN219	From an intensive piggery	PE <sub>iv</sub> (food)	North/2007	AMP, TET, ERY, CIP, STR, KAN	None	-	This study
CC22	32	SN210	From antibiotic preparation given in an intensive piggery	PE <sub>in</sub> (antiseptic)	South/2006	TET, KAN	None	-	This study

CC22	32	SN213	From an extensive piggyery	PE <sub>V1</sub> (water for consumption)	South/2007	TET, CIP, NIT	None	-	This study
CC22	392	41	Community surveillance	HV (fecal swab)	Center/2001	TET, ERY, CIP, GEN, STR, KAN, OD	None	-	25
CC22	435	SN217	From swine excrements of intensive piggyery	SW <sub>V</sub> (feces)	North/2007	TET, ERY, CIP, GEN, STR, KAN	None	-	This study
CC22	436	SN220	Animal feed isolate	PE <sub>V</sub> (stock food)	North/2007	TET, CIP, KAN	agg, cyl, esp	-	This study
CC94	428	SN207	From the surroundings of intensive piggyery	PE <sub>1</sub> (waste lagoon)	South/2006	TET, ERY, GEN, KAN	None	-	This study
ST	30	SN221 (2)	Swine and animal feed isolates from an intensive piggyery	SW <sub>in</sub> (feces)/ PE <sub>in</sub> (food)	South/2006	TET, ERY, CIP, GEN, KAN	None	-	This study
CS	89	58	Community surveillance	HV (fecal swab)	North/2001	VAN, TEC, ERY, STR, OD	None	D	25
CS	190	98	Clinical isolate	HP <sup>A</sup> (urine)	Center/1998	VAN, TEC, AMP, TET, ERY, CIP, STR, KAN	None	A	22
CS	366	99	Clinical strain recovered during 1999–2000 in hospital A	HP <sup>A</sup> (urine)	Center/1999	VAN, TEC, AMP, ERY, CIP, GEN, STR, KAN	esp	PP-5	22
CS	367	84	Clinical isolate	HP <sup>A</sup> (catheter)	Center/2000	VAN, TEC, AMP, ERY, CIP, GEN, STR, KAN	esp	X	22
CS	391	124	Clinical isolate	HP <sup>E</sup> (pus)	Center Eastern/2007	VAN, TEC, AMP, ERY, OD	esp	PP-5	This study
CS	404	D	From a pig slaughterhouse	SW (feces)	Center/1997	VAN, TEC, TET, ERY, CIP	None	A	This study
CS	406	3	Strain identified in 3 different national brands during 1999–2001	RP (carcass)	North/2001	VAN, TEC, TET, ERY, CIP, GEN, KAN, STR	None	A	23
CS	429	SN209	From swine excrements of intensive piggyery	PE <sub>in</sub> (solid manure)	South/2006	TET, ERY, STR, KAN	gel	-	This study
CS	432	SN212	From swine excrements of intensive piggyery	PE <sub>V1</sub> (liquid manure)	South/2007	TET, ERY, OD	None	-	This study
CS	434	SN215	From one facility of an intensive piggyery	PE <sub>IV</sub> (air)	North/2007	TET, ERY, STR	None	-	This study
CS	443	SN221	From a swine in the final facility before the abattoir	PE <sub>V</sub> (rectal swab)	North/2007	VAN, TEC, TET, ERY, NIT	esp, hyl	A	This study

<sup>a</sup> CCs are shown according to eBURST clustering. CS, singletons.

<sup>b</sup> Persistent and/or disseminated PFGE types are in bold. Strains identified with different PFGE subtypes were included in some cases, and their number is designated in parentheses.

<sup>c</sup> HP, hospitalized patients; HV, healthy volunteers; RP, retail poultry; SW, swine; PE, piggyery environment; HW, hospital sewage; R, river. The different hospitals are designated by capital letters (A to E) and piggyeries by roman numerals (I to VI). Capital letters represent the cities where hospitals are located: A, Coimbra; B, Viseu; C and F, Porto; D, Matosinhos; E, Covilhã.

<sup>d</sup> VAN, vancomycin; TEC, teicoplanin; AMP, ampicillin; TET, tetracycline; ERY, erythromycin; CIP, ciprofloxacin; HLR, high level of resistance; GEN, gentamicin; STR, streptomycin; KAN, kanamycin; NIT, nitrofurantoin; CHL, chloramphenicol; OD, quinupristin-dalfopristin.

<sup>e</sup> Variable presence of a given antibiotic and virulence trait among isolates belonging to the same PFGE type appear in parentheses.

<sup>f</sup> Tn1546 designation is based on the results obtained by a PCR overlapping assay as described previously (21, 38); PP-31 appears in bold because this is a new Tn1546 type accordingly to the scheme described by Woodford et al. (38).



spread in hospitals and counteract any containment measure at the hospital level.

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AUTHOR’S CORRECTION

Dispersion of Multidrug-Resistant *Enterococcus faecium* Isolates Belonging to Major Clonal Complexes in Different Portuguese Settings

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Volume 75, no. 14, p. 4904–4908. Page 4904, Abstract, line 3: “clustering into CC17, CC5, CC9, CC22, and CC94” should read “clustering into CC17, CC5, CC9, and CC22.”  
Page 4904, column 2, paragraph 2, line 4: “which clustered into CC5, CC9, CC17, CC22, and CC94” should read “which clustered into CC5, CC9, CC17, and CC22.”  
Page 4905: Figure 1 should appear as shown below. (ST428 should not be considered as belonging to CC94.)  
Page 4905, column 2, paragraph 2, lines 3 and 4: “ST428 (CC94)” should read “ST428.”  
Page 4907, Table 1, column 1, line 8: “CC94” should read “ST.”

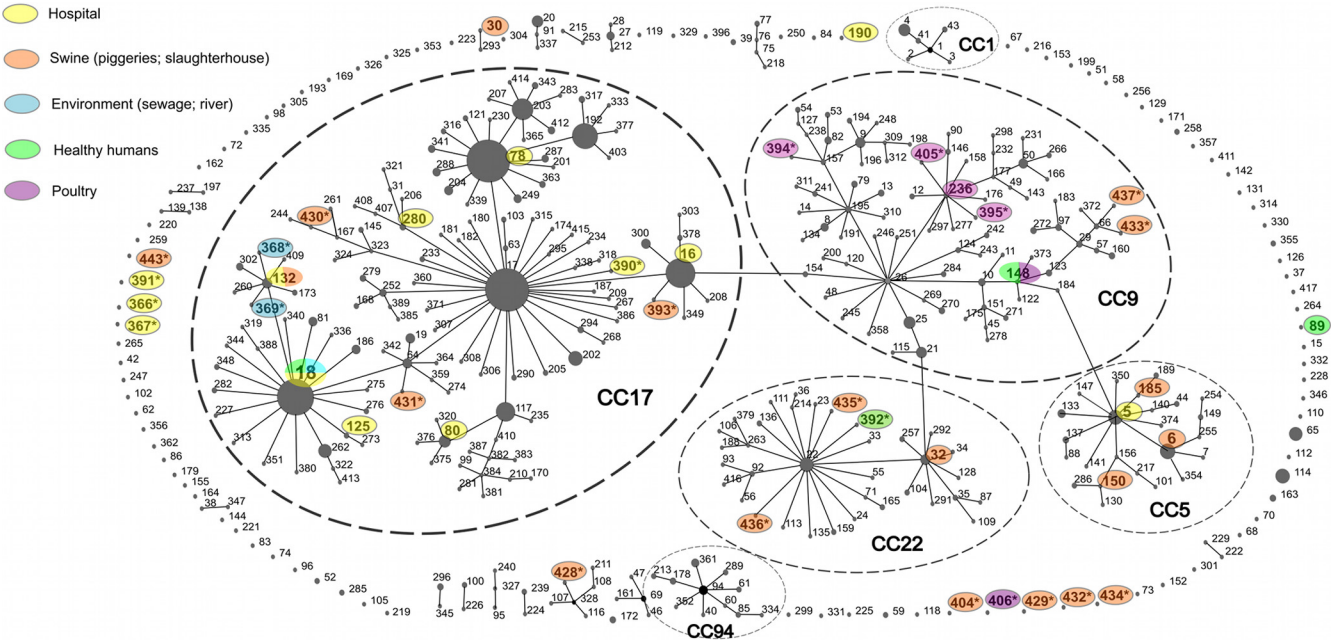


FIG. 1.