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Insight into antimicrobial susceptibility and population structure of contemporary human *Enterococcus faecalis* isolates from Europe

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Objectives: To investigate antimicrobial susceptibility and clonal relatedness of *Enterococcus faecalis* human isolates recovered recently (2006–09) in six European countries.

Methods: Antimicrobial susceptibility of 386 isolates from Denmark, Germany, Norway, Poland, Spain and The Netherlands, from hospital infections (223 isolates), carriage (82 isolates) and from colonization in the community (81 isolates) was determined by the broth microdilution method. Clonal relatedness of isolates was assessed by multilocus sequence typing.

Results: All isolates were susceptible to benzylpenicillin, ampicillin, linezolid, tigecycline and daptomycin. Non-susceptibility to tetracycline (77.6%), rifampicin (57.3%), ciprofloxacin (51.2%), aminoglycosides (43.3% high-level gentamicin resistance, 40.0% high-level streptomycin resistance) was frequent among hospital isolates, while non-susceptibility to glycopeptides was rare and associated mostly with *vanA*. Multidrug resistance was found in 59.7% of hospital isolates and 16.1% of community isolates. Isolates were classified into 105 sequence types (STs), of which 21 STs, representing more than half of the collected isolates (53.9%), grouped with 6 large *E. faecalis* clonal complexes (CCs; CC2, CC16, CC21, CC30, CC40 and CC87). Two of these, CC2 (frequently recovered in Spain and The Netherlands) and CC87 (prevalent in Poland), were found almost exclusively in hospitals and included the highest proportion of multiresistant isolates.

Conclusions: While hospital-acquired *E. faecalis* in Europe remains susceptible to ampicillin and glycopeptides, the high prevalence of strains that are highly resistant to aminoglycosides excludes these antibiotics from combination therapies. Genotyping revealed that nosocomial infections by multiresistant *E. faecalis* are largely caused by only a few hospital-associated clones.

Keywords: *E. faecalis*, hospital, community, antibiotic, resistance, typing, high-risk clonal complex

Introduction

Enterococci have for a long time been recognized as common gastrointestinal commensals in a wide variety of hosts, and are found in diverse environments, such as sewage, soil, water and food. Today, enterococci represent increasingly important nosocomial pathogens, causing endocarditis, bacteraemia and (rarely) meningitis, as well as post-operative wound and urinary tract infections (UTIs).[1] Their ability to survive under a wide range of physicochemical conditions, such as drying, extreme temperatures, high osmolarity and the presence of disinfectants,[2] allows them to persist on various surfaces in hospitals and on the hands of healthcare workers.[3] Recently, enterococci were ranked as the third most common nosocomial pathogen (12%) after coagulase-negative staphylococci and *Staphylococcus aureus*, and the second most common responsible for intensive care

Table 1. Geographical origin and source of isolation of *E. faecalis* isolates

Source of isolation	Number of isolates per country						Total number of isolates
	Denmark	Germany	Norway	Poland	Spain	The Netherlands	
Hospital							
invasive	20	20	15	25	18	20	118
non-invasive	20	20	15	23	7	20	105
carriage	0	20	0	27	16	19	82
Community	28	0	12	23	18	0	81
Total number of isolates	68	60	42	98	59	59	386

unit-acquired bacteraemia.[4,5] The majority (up to 90%) of enterococcal infections was and still is due to *Enterococcus faecalis*;[1] however, the numbers of infections of *Enterococcus faecium* have increased over the last decade in the USA,[4] Europe[6,7] and China,[8] leading to a growing burden of enterococcal infections in general, and to a decreased ratio of *E. faecalis* to *E. faecium* infections (down to 60:40). Although *E. faecalis* remains generally more susceptible to antibiotics than *E. faecium*, antimicrobial resistance in the former poses an increasing challenge to therapy.[9,10]

Sequence-based typing methods, in particular multilocus sequence typing (MLST), have gained an important role in the study of the long-term epidemiology of several bacterial species and their population structure.[11] For *E. faecalis*, the proposed approaches were based on sequencing of virulence-associated loci[12,13] and house-keeping genes.[14] Studies using the latter scheme have revealed the epidemic population structure of the pathogen, which is composed of several clonal complexes (CCs) and singletons with relatively high rates of recombination.[14–16] So far, the *E. faecalis* MLST scheme has proved its value in studying the hospital epidemiology of *E. faecalis* clones colonizing and causing infections in patients.[14–17] However, the European-wide perspective on the epidemiology and clonal composition of *E. faecalis* infecting humans is limited. To fill this gap, we collected recent *E. faecalis* isolates from nosocomial invasive and non-invasive infections, and from carriage in hospitals and in the community, from six European countries (Denmark, Germany, Norway, Poland, Spain and The Netherlands), and analysed their antimicrobial susceptibility and clonal relatedness by MLST. This provided important up-to-date information on the population structure of antibiotic-resistant and susceptible *E. faecalis* from humans in Europe.

Materials and methods

Isolate collection

Enterococcal isolates were collected from six countries (Denmark, Germany, Norway, Poland, Spain and The Netherlands), from community-dwelling individuals and from hospitalized patients. Enterococci were isolated by collaborating healthcentres according to standard microbiological procedures and shipped to a central laboratory in each of six countries; the isolates were re-identified as *E. faecalis* and stored at −70°C until further analysis. All isolates were from 2006 to 2009 except the 40 German hospital isolates from non-invasive infections and carriage, which were from 1998 to 2008. Altogether, 386 isolates were included in this study, of which 68 originated from Denmark, 60 from Germany, 42 from Norway, 98 from Poland, 59 from Spain and 59 from The Netherlands (Table 1); these isolates were derived from colonization and invasive and non-invasive infections in hospitals, as well as from human non-hospitalized carriers (details below).

Antimicrobial susceptibility testing

Antimicrobial susceptibility was tested for the following antibiotics: penicillin, ampicillin, gentamicin, streptomycin, vancomycin, teicoplanin, tetracycline (minocycline in the case of isolates from Spain), tigecycline (Denmark, Germany, Norway, Poland and The Netherlands), ciprofloxacin (levofloxacin for the Spanish isolates), linezolid, rifampicin, and chloramphenicol (Denmark, Germany, Norway, Poland and The Netherlands), using the broth microdilution method as recommended by the CLSI. Interpretations were made according to CLSI- and EUCAST-approved clinical breakpoints and epidemiological cut-off values (ECOFFs).[18,19] *E. faecalis* strain ATCC 29212 was used as a control during testing. Susceptibility to daptomycin was evaluated by the Etest (bioMérieux SA, Marcy l'Étoile, France), following the manufacturer's recommendations. Multidrug-resistant (MDR) isolates were defined as those with acquired non-susceptibility (i.e. intermediately susceptible and resistant) to any compound belonging to three or more classes of the antimicrobial agents tested. High-level streptomycin resistance (HLSR) and high-level gentamicin resistance (HLGR) were considered as resistance to two classes.[20] Vancomycin-resistance determinants *vanA* and *vanB* were detected by PCR as described previously.[21]

MLST and data analysis

MLST was performed as described previously;[14] allele numbers and sequence types (STs) were assigned with the use of the *E. faecalis* MLST database (http://efaecalis.mlst.net/; 20 October 2011, date last accessed). New alleles and allelic profiles were submitted to the database. MLST data were analysed with eBURST software[22] (http://eburst.mlst.net/; 20 October 2011, date last accessed), with each CC defined as a group of STs that shared 6 out of 7 MLST loci. Additionally, the MLST data obtained in this study were analysed by comparative eBURST against the whole dataset for *E. faecalis* (316 STs, 773 isolates; 27 June 2011). The diversity index (DI) with 95% CI was calculated as described previously.[23] Differences in distributions were evaluated using the χ^2 test, with P values ≤ 0.05 considered to be significant.

Results

Susceptibility to antimicrobial agents

The sources and susceptibility profiles of the 386 isolates studied are summarized in Table 1 and Table 2, respectively. A total of

Table 2. Susceptibility to antimicrobial agents of 386 *E. faecalis* isolates from six European countries; the CLSI breakpoints were used for interpretation unless indicated otherwise

Source of isolation and antibiotic	Number of non-susceptible/HLGR/HLSR/MDR isolates (%) per country						*P* value	Total number (%) of non-susceptible/ HLGR/HLSR/MDR isolates (CLSI breakpoints)	Total number (%) of non-susceptible/HLGR/ HLSR isolates (EUCAST clinical breakpoints/ ECOFF values)
	Denmark	Germany	Norway	Poland	Spain	The Netherlands			
Hospital									
tetracycline	29 (72.5)	47 (79.7)	22 (73.3)	65 (86.7)	25 (61.0)[a]	42 (71.2)	0.2[b]	205 (77.9)[b]	NA/205 (77.9)[b]
rifampicin	23 (57.5)	22 (36.7)	15 (50.0)	54 (72.0)	10 (24.4)	51 (86.4)	<0.0001	175 (57.3)	NA/77 (25.2)
ciprofloxacin	13 (32.5)	29 (49.2)	9 (30.0)	40 (53.3)	21 (51.2)[c]	44 (74.6)	0.0001[b]	135 (51.3)[b]	NA/97 (36.9)[b]
HLGR	9 (22.5)	17 (28.3)	11 (36.7)	40 (53.3)	23 (56.1)	32 (54.4)	0.0005	132 (43.3)	132 (43.3)
HLSR	15 (37.5)	16 (26.7)	10 (33.3)	35 (46.7)	24 (58.5)	22 (37.3)	0.03	122 (40.0)	147 (48.2)
chloramphenicol	13 (32.5)	10 (16.7)	16 (53.3)	41 (54.7)	NA	15 (25.4)	0.0001[b]	95 (36.0)[b]	NA/59 (22.3)[b]
vancomycin	0	13 (21.7)	0	1 (1.3)	0	0	NC	14 (4.6)	14 (5.3)
teicoplanin	0	5 (8.3)	0	1 (1.3)	0	0	NC	6 (2.0)	11 (4.2)
MDR	18 (45.0)	30 (50.0)	14 (46.7)	55 (73.3)	24 (58.5)	41 (69.5)	0.006	182 (59.7)	NC
Total number of hospital isolates	**40**	**60**[d]	**30**	**75**	**41**	**59**		**305**	
Community									
tetracycline	12 (42.9)	NA	7 (58.3)	16 (69.6)	4 (22.2)[a]	NA	NC	35 (55.6)[e]	NA / 35 (55.6)[e]
rifampicin	12 (42.9)	NA	7 (58.3)	6 (26.1)	5 (27.8)	NA	NC	30 (37.0)	NA/18 (22.2)
ciprofloxacin	3 (10.7)	NA	1 (8.3)	0	2 (11.1)[c]	NA	NC	4 (6.3)[e]	NA/1 (1.6)[e]
HLGR	0	NA	0	4 (17.4)	3 (16.7)	NA	NC	7 (8.6)	7 (8.6)
HLSR	3 (10.7)	NA	1 (8.3)	6 (26.1)	5 (27.8)	NA	NC	15 (18.5)	15 (18.5)
chloramphenicol	0	NA	1 (8.3)	4 (17.4)	NA	NA	NC	5 (7.9)[e]	NA/4 (6.4)[e]
MDR	4 (14.3)	NA	1 (8.3)	5 (21.7)	3 (16.7)	NA	0.8	13 (15.9)	NC
Total number of community isolates	**28**	**0**	**12**	**23**	**18**	**0**		**81**	
Total number of isolates	**68**	**60**	**42**	**98**	**59**	**59**		**386**	

NA, data/breakpoint not available; NC, not calculated.
[a]Minocycline.
[b]For Denmark, Germany, Norway, Poland and The Netherlands.
[c]Levofloxacin.
[d]A single isolate was not available for determination of the MIC of tetracycline and ciprofloxacin.
[e]For Denmark, Norway and Poland.

118 isolates (30.6%) were from invasive infections (116 isolates from blood, 1 from CSF and 1 from a biopsy specimen); 105 isolates (27.2%) were from non-invasive infections (90 from UTIs, 11 from wounds and the remaining 4 from catheters, an ear swab and a bronchoalveolar lavage), and 82 were faecal-carrier isolates (21.2%) were from hospital patients. The 81 isolates (21.0%) from the community represented 72 faecal-carrier isolates, 6 isolates from UTIs and 3 wound isolates. All tested isolates were susceptible to benzylpenicillin, ampicillin, linezolid, tigecycline and daptomycin. For other agents, differences were typically observed between hospital and community isolates (Table 2). Among hospital isolates, the highest rates of non-susceptibility (CLSI breakpoints) were observed for tetracycline, followed by rifampicin, ciprofloxacin, HLGR and HLSR. Although isolates from invasive infections showed higher resistance to both gentamicin and streptomycin (48.3% and 44.9%, respectively) than isolates from UTIs (36.7% and 36.8%, respectively), these differences were not statistically significant. Rates of non-susceptibility to the majority of antibiotics varied significantly between countries. For example, non-susceptibility to ciprofloxacin was significantly lower in Norway (30.0%) than in The Netherlands (74.6%; $P=0.0001$), while HLGR was significantly lower in Denmark (22.5%) than in Spain (56.1%; $P=0.004$), and HLSR was significantly lower in Germany (26.7%) than in Spain (58.5%; $P=0.001$). Interpretation of susceptibility data using the EUCAST criteria (Table 2) increased the number of HLSR isolates and decreased the ratio of rifampicin, ciprofloxacin and chloramphenicol non-susceptible isolates. For these three antimicrobials, ECOFF values were applied due to the lack of clinical breakpoints.

Non-susceptibility to vancomycin was found in 14 hospital isolates (4.6%; 13 resistant isolates and one with intermediate susceptibility); of these, 6 were also resistant to teicoplanin (VanA phenotype) according to the CLSI criteria. Applying EUCAST breakpoints, however, increased the total number of teicoplanin-resistant isolates to 11, thus yielding 11 VanA phenotype isolates and 3 VanB phenotype isolates. PCR confirmed the presence of the *vanA* gene in the 11 isolates with the VanA phenotype (defined according to the EUCAST criteria) and the presence of the *vanB* gene in the 3 strains with the VanB phenotype. It should be noted that 10 of the vancomycin non-susceptible isolates belonged to the group of earlier German isolates, while the remaining 4 were obtained during the main collection period (2006–09): 3 UTI isolates from Germany and a single carriage isolate from Poland.

Compared with hospital isolates, community isolates showed higher susceptibility to all tested antibiotics, with the highest level of non-susceptibility observed for tetracycline and rifampicin, followed by HLSR, and HLGR, with non-susceptibility for chloramphenicol and ciprofloxacin. In this case, the use of the ECOFF values lowered the ratio of isolates non-susceptible to ciprofloxacin and rifampicin. All community isolates retained full susceptibility to glycopeptides, benzylpenicillin, ampicillin, linezolid, tigecycline and daptomycin.

Among 195 MDR isolates (50.5% of all isolates, based on CLSI breakpoints), significantly more isolates were from hospital patients [182 (59.7%)] than from the community [13 (16.1%); $P<0.001$]. The level of MDR hospital isolates differed significantly among countries (45.0% in Denmark to 73.3% in Poland). A similar trend, although non-significant, was also evident among the four countries that collected community isolates (8.3% in Norway to 21.7% in Poland).

Population structure of E. faecalis isolates from six European countries

Among the studied isolates, 105 different STs were found (3.8 isolates per ST, on average). Thirty-six new allelic profiles, representing 50 isolates, were found, with 59 STs (56.2%) represented by single isolates. The eBURST analysis revealed 20 groups for 301 isolates (78.0% of the studied collection) and 50 singletons (85 isolates; 22.0%). Clustering of STs in this study with the entire MLST database revealed that most of the analysed isolates (208; 53.9%) grouped into six CCs: CC2 (with the major ST6 as a double-locus variant of ST2), CC16, CC21, CC30, CC40 and CC87 (Table 3 and Figure 1). Some of these CCs showed country-specific distribution. Representatives of CC2 were more prevalent in Spain and in The Netherlands compared with the four remaining countries, while CC87 was significantly associated with Poland and was not found at all among the German and Spanish isolates (Table 3). CC2 and CC87 were significantly highly represented among hospital isolates; both showed a higher frequency of HLGR, HLSR and quinolone and tetracycline resistance; moreover, CC2 was significantly associated with invasive infections (Table 4). Significantly, in contrast, CC30 and CC40 were not associated with invasive infections and were significantly more susceptible to gentamicin. Significantly more isolates belonging to CC16 were susceptible to ciprofloxacin, but resistant to tetracycline. Isolates within particular groups displayed different combinations of resistance phenotypes to antimicrobial agents and several different non-susceptibility profiles were found for each group. Three CCs (CC2, CC16 and CC87) were significantly associated with the MDR phenotype. The genotypically divergent subset of strains not associated with the six CCs (CC2, CC16, CC21, CC30, CC40 and CC87) represented significantly fewer MDR isolates (31.5% versus 66.8% in the six CCs; $P<0.0001$). In agreement with this finding, the prevalence rates for HLGR (18.0% versus 51.4%), HLSR (20.2% versus 51.4%) and tetracycline non-susceptibility (53.4% versus 71.6%) were also lower among these isolates.

Vancomycin non-susceptibility was associated with 11 different STs (6, 16, 25, 27, 40, 47, 55, 82, 88, 116 and 207), indicating an independent acquisition of *van* determinants by genotypically unrelated strains. In the community isolates set, HLGR was almost exclusively associated with ST16 (6 out of 7 isolates with this phenotype), while the HLSR isolates were more diverse and included isolates of ST16 ($n=5$) as well as STs 6, 21, 23, 41, 55 and 211 ($n=1$). Resistance to rifampicin and tetracycline in the community isolates was due to strains with several STs, although this was most prevalent in strains of ST16. Altogether, 6 out of 10 isolates of ST16 showed the MDR phenotype, constituting 46.2% of the MDR community isolates.

The DI for the entire strain set was 95.2% (95% CI, 94.0–96.3) and did not differ significantly between the participating countries, with the exception of isolates from Spain, which exhibited a significantly lower diversity than isolates from Norway (Table 3). This was due to a high proportion of isolates from CC2 among isolates from Spain. Community isolates tended to be more diverse (DI=96.8%; 95% CI, 95.1–98.4) than hospital isolates (DI=93.9%; 95% CI, 92.3–95.6), although the difference

Table 3. Genogroup and genetic diversity of *E. faecalis* isolates from six European countries: distribution among MLST CCs, number of STs and DI with 95% CI

Genogroups and genetic diversity	Denmark	Germany	Norway	Poland	Spain	The Netherlands	Total
CC2[a]	5	7	7	9	21 (31.3)[b]	18 (26.9)[c]	67
CC16	6	2	4	17	9	8	46
CC21	8	1	3	7	2	3	24
CC30	8	5	4	7	4	2	30
CC40	5	7	5	3	1	1	22
CC87[a]	1	0	1	13 (68.4)[d]	0	4	19
Other[a]	35 (51.5)	38 (63.3)	18 (42.9)	42 (42.9)	22 (37.3)	23 (39.0)	178 (46.1)
Number of STs	35	34	24	31	26	28	105
DI (%)	96.5	96.3	96.6	93.9	86.3	91.0	95.2
95% CI	94.0–96.3	94.2–98.3	94.9–98.4	91.7–96.2	78.3–94.3	85.5–96.5	94.0–96.3

[a]The percentage of isolates in a country is given in brackets.
[b]$P<0.0001$.
[c]$P=0.0001$.
[d]$P<0.0001$.

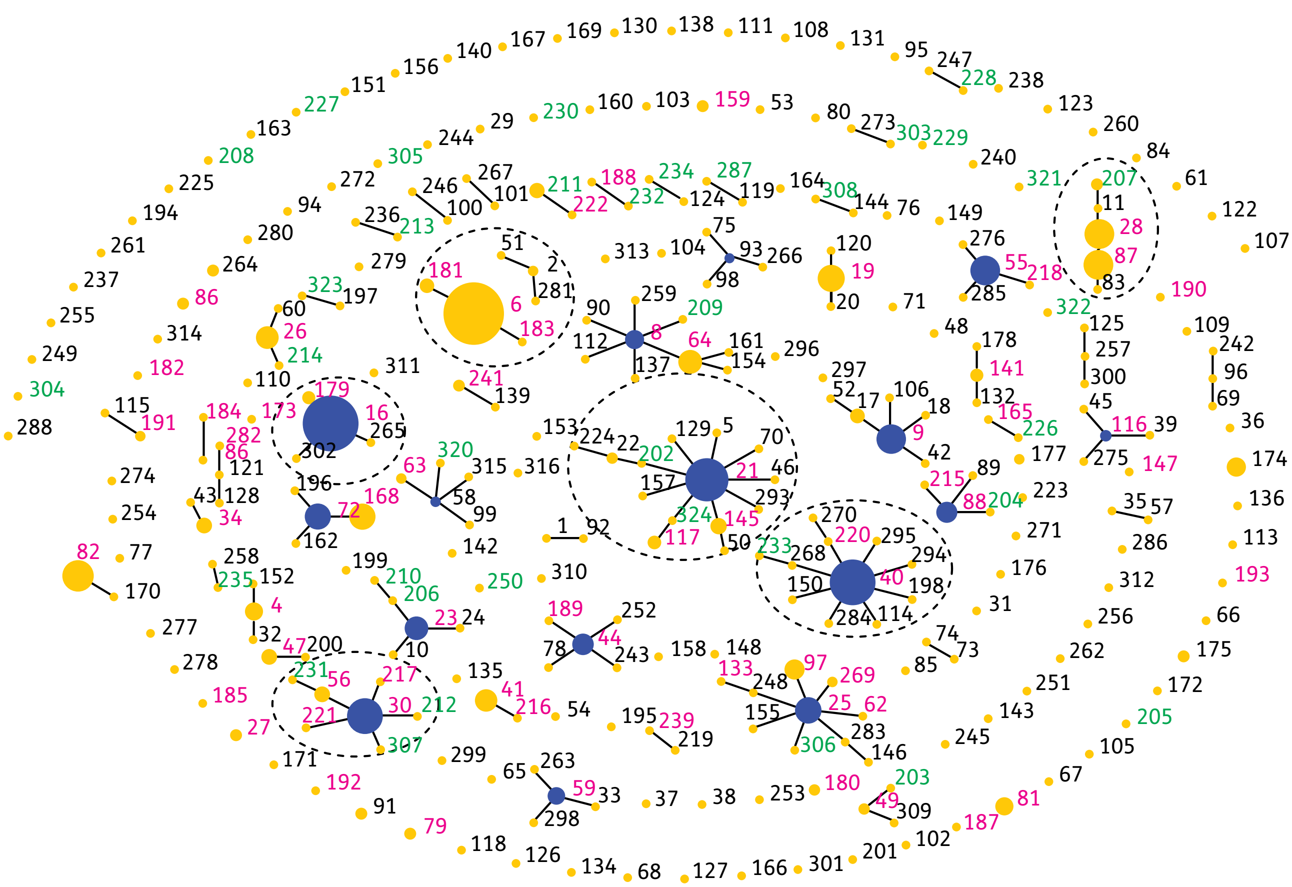

Figure 1. Comparative electronic Based Upon Related Sequence Types (eBURST) analysis of the studied group of *E. faecalis* isolates and the MLST database reference dataset. Blue circles, presumed ancestors of CCs; yellow circles, other STs (circle size is proportional to the number of isolates); lines, SLV links; numbers in black, STs from the database; pink numbers, STs common to both sets; green numbers, new STs found in this study. The six main clonal groups identified in the study are indicated by broken circles. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Table 4. Properties of the major *E. faecalis* CCs

CC	No. of isolates	No. of hospital isolates	No. of invasive isolates	No. of HLGR isolates	No. of HLSR isolates	No. of ciprofloxacin-non-susceptible isolates[a]	No. of tetracycline-non-susceptible isolates[b]	No. of MDR isolates
CC2	67	66 ($P<0.0001$)	34 ($P<0.0001$)	64 ($P<0.0001$)	59 ($P<0.0001$)	66 ($P<0.0001$)	61 ($P<0.0001$)	66 ($P<0.0001$)
CC16	46	34	15	23	15	9 ($P=0.02$)[c]	42 ($P<0.0001$)	36 ($P=0.0001$)
CC21	24	17	7	1	5	6	14	7
CC30	30	18	3 ($P=0.02$)[d]	2 ($P=0.001$)[e]	5 ($P=0.04$)[f]	4	20	6
CC40	22	19	2 ($P=0.04$)[d]	1 ($P=0.003$)[e]	5	3	19	6
CC87	19	19 ($P=0.04$)	7	16 ($P<0.0001$)	12 ($P=0.02$)	19 ($p<0.0001$)	18 ($P=0.007$)	18 ($P=0.0001$)
Other	178	132	50	32 ($P<0.0001$)[e]	36 ($P<0.0001$)[f]	55	95 ($P=0.003$)[g]	56 ($P<0.0001$)[h]
Total	386	305	118	139	137	162	269	195

Isolates of groups significantly associated with hospitals, invasive infections and antimicrobial resistance are underlined.
[a]Levofloxacin for Spain.
[b]Minocycline for Spain.
[c]Significantly susceptible to ciprofloxacin.
[d]Significantly under-represented in invasive disease.
[e]Significantly susceptible to gentamicin.
[f]Significantly susceptible to streptomycin.
[g]Significantly susceptible to tetracycline.
[h]Significantly less MDR isolates.

was not statistically significant. Among hospital isolates there were no significant differences in genetic diversity between isolates from bloodstream infections, UTIs or carriage isolates.

Discussion

The aim of this study was to obtain insight into antimicrobial resistance levels and the population structure of contemporary European *E. faecalis* isolates from various human sources. Differences in observed susceptibility depended on the clinical origin of isolates, with hospital isolates being typically much more resistant than community isolates. Also, susceptibility percentages differed substantially among the six participating countries for some agents, as did the prevalence rates of MDR hospital isolates, with the lowest rates detected in the two participating Scandinavian countries. Molecular typing showed that STs belonging to six CCs (CC2, CC16, CC21, CC30, CC40 and CC87) played a predominant role in the spread of antimicrobial resistance in hospitals and contributed to higher resistance rates in some countries (Poland, Spain and The Netherlands) than in the others (Denmark, Germany and Norway). Antimicrobial use is undoubtedly driving the spread of such clones, although the six predominant CCs showed remarkable heterogeneity with respect to their resistance phenotypes. Likely, the epidemic properties of particular clones represent an additional factor for the dissemination of resistance.[24,25]

Enrichment of STs belonging to CC2 among hospital-associated isolates was recognized in earlier studies in a global *E. faecalis* collection,[14] among Polish isolates from 1996–2005,[15] and among recent isolates from Spain[26] and Portugal.[27] A single representative of CC2 was reported also from a farm animal (pig).[28] Isolates belonging to CC21 and ST16 have been frequently observed in the global *E. faecalis* collection among isolates of diverse origin, including hospitalized patients, non-hospitalized individuals, meat and farm animals,[14,29] while CC87 has been frequently encountered in Europe since the 1980s (Patricia Ruiz-Garbajosa, unpublished data; http://efaecalis.mlst.net/, last accessed on the 20 October 2011) and among Polish clinical isolates from 1996–2005.[15] Isolates belonging to CC40 have been commonly found in Europe and the USA among isolates from humans, animals and food since the early 1960s.[14–16,30] Also, *E. faecalis* ST30 isolates have been reported previously in global *E. faecalis* collections,[14,16,30] among Polish clinical isolates,[15] and in single isolates in Spain[26] and Portugal.[27] In all these studies, ST30 was associated with humans and dates back to at least the 1950s.[16] In contrast to these ubiquitous *E. faecalis* clones and CCs, which were well represented in our collections, some CCs previously described in European countries, including CC10 and the hospital-associated CC9,[14,15,26] were poorly represented (two isolates with ST9) or even absent (ST10) in the current study. This suggests a dynamic circulation of *E. faecalis* clones and reflects further differences in the local epidemiology of *E. faecalis*.

Some of the STs found in this study are associated with CCs (CC2, CC9 and CC87) that bear features typical for the so-called high-risk enterococcal CCs (HiRECCs, i.e. CCs enriched with MDR colonizing or invasive isolates from hospitalized patients).[31] CC2 probably emerged in the early 1980s[16] and is known for its increased pathogenic potential, being involved in serious outbreaks and cases of invasive infections like septicemias and endocarditis.[32] A representative strain of this CC, V583 (ST6), is the first enterococcus whose genome was fully sequenced[33] and which, compared with the unrelated strain OG1RF (ST1),[34] contained several mobile elements, a pathogenicity island and an operon for capsular polysaccharide biosynthesis. The latter feature has been shown to play a role in resistance to phagocytic

killing.[35] Another factor that differed between the two sequenced enterococcal genomes was the presence of two clustered regularly interspaced short palindromic repeat (CRISPR) loci in OG1RF.[34] CRISPR loci are involved in the defence of bacterial cells against incoming foreign DNA, such as prophages, transposons and plasmids. Lack of CRISPR loci may facilitate acquisition of new genes, including determinants of antimicrobial resistance.[36] Comparative genomic hybridization and comparisons with published *E. faecalis* genomes identified a number of genes enriched among the CC2 isolates, typically encoding surface-located structures [microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), WxL domain proteins and proteins of the internalin family].[30]

Another CC associated with the hospital environment and antimicrobial resistance is CC87. This CC has spread to different hospitals in Poland since 1996.[15] Isolates belonging to CC87 typically produce cytolysin and are *asa*- and *esp*-positive,[15] but uniformly show a gelatinase-negative phenotype due to a 23.9 kb deletion of most of the *fsr* regulatory locus.[37] It was shown recently that a lack of gelatinase results in increased amounts of the collagen-binding MSCRAMM Ace protein on the surface of bacteria, thus improving their adherence to host tissues.[38]

In conclusion, the current high antibiotic-resistance prevalence rates of *E. faecalis* recovered from humans in Europe limit the options for successful treatment of infections. Especially, the relatively high prevalence rates of HLSR and HLGR undermine the use of a combination of a β-lactam with an aminoglycoside antibiotic for empirical therapy. For infections with these multiresistant *E. faecalis* strains only ampicillin, vancomycin, linezolid, daptomycin and tigecycline remain currently available, although resistance to these antimicrobial agents has already been reported in this species.[39–44] Increasing resistance to antimicrobial agents in *E. faecalis* is significantly linked to the selection and expansion of certain CCs that are either found enriched among hospitalized patients (CC2 and CC87) or well-represented among both hospital and community isolates (CC16). Persistent use of antimicrobial agents in hospitals, in the community and in animal husbandry will certainly further promote the selection and spread of MDR clones. Therefore, ongoing surveillance and improved infection control procedures at hospitals are indispensable to curb further spread of multiresistant *E. faecalis*.

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Transparency declarations

None to declare.

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