The multifaceted lifestyle of enterococci: genetic diversity, ecology and risks for public health

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Running title: The multifaceted lifestyle of enterococci

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Abstract

Enterococci are long-standing members of the gastrointestinal tract of humans and many animals and they are also ubiquitously distributed in natural environments. Classically as harmless bacteria, two main species (namely *Enterococcus faecalis* and *Enterococcus faecium*) have become a leading cause of human infections, especially in hospital settings, with the worldwide spread of multidrug-resistant isolates, especially vancomycin-resistant enterococci. In this review, it will be summarized what is known about genetic diversity and ecology of enterococci with a focus on *E. faecalis* and *E. faecium* from human and non-human habitats and related risks for public health.

Introduction

Enterococci are low-GC Gram-positive cocci belonging to the phylum *Firmicutes* [1], and the genus currently comprises up to 60 different species until now (www.bacterio.net). These microorganisms are remarkably resistant to numerous environmental stresses (e.g., temperature, pH, 6.5% NaCl, 40% bile salts, desiccation) allowing them to survive and grow in harsh environments [2•]. Consequently, they are ubiquitously distributed in nature with a large variety of habitats such as the gastrointestinal tract (GIT) of humans and nearly all terrestrial animals (mammals, reptiles, birds and insects) as well as plants, soil and sediments, fresh and marine waters and different types of foods (including dairy products, fermented vegetables, meat, fish and sea foods) (Figure 1) [3-5].

While many *Enterococcus* spp. are generally commensal bacteria with coevolution with their hosts for hundreds of millions of years [5], several species have been described as human opportunistic pathogens [6]. Of them, *Enterococcus faecalis* and *Enterococcus faecium* are by far the most frequent species responsible for human infections, especially in hospital settings (Figure 1) [7]. Of the greatest concern, is the worldwide dissemination of multidrug-resistant (MDR) enterococci, especially vancomycin-resistant enterococci (VRE), for which limited therapeutic options remain [8]. The human GIT can harbor additional enterococcal species (notably *E. avium*, *E. casseliflavus*, *E. durans*, *E. gallinarum*, *E. hirae*, *E. mundtii* and *E. raffinosus*) that are rarely isolated from human infections (Figure 1) [9•]. Whereas enterococci can also cause infections in animals, they are not considered as food- or waterborne pathogens. However, their ingestion can lead to the asymptomatic colonization of the human GIT that is usually the first step in the development of invasive infections [10]. Outside of the hospital, they can be transmitted to humans by various ways,

including contaminated food and water, underlying that animal and environmental enterococci may potentially serve as a reservoir of MDR strains and resistance genes.

This review will describe the up-to-date knowledge on genetic diversity and ecology of enterococci from human and non-human habitats with a focus on *E. faecalis* and *E. faecium* animal/environmental isolates and the related risks for public health.

Genome plasticity of enterococci

Both *E. faecalis* and *E. faecium* have reduced genomes but an important accessory genome (up to 38% in *E. faecium*) underlying their remarkable genome plasticity (Table 1) [11-13]. Besides recombination that plays a predominant role over mutation for their evolution, enterococci are also particularly adept at acquiring new genes through horizontal gene transfer (HGT) of mobile genetic elements (MGEs) including plasmids and transposons [14-19]. This is notably true for *E. faecium* for which antimicrobial resistance (AMR) genes are major drivers for selection and spread in the hospital environment [6,20]. Importantly, the high genome plasticity in the majority of clinical isolates is sustained by the frequent lack of genome defence mechanisms limiting HGT such as CRISPR-Cas and restriction-modification systems while the most frequently reported mechanism for foreign DNA acquisition is conjugation (Table 1) [9•,21,22].

Genetic diversity of enterococci

From an evolution point of view, *E. faecalis* and *E. faecium* are at the opposite ends of the phylogenetic tree of enterococci (Table 1). The former occurs in one of the oldest branches of the genus whereas the latter arose more recently [5]. However, their shared occurrence

78 in the human GIT and in hospital settings means that they possess common features that
79 allow them to inhabit similar ecological niches.
80 Genetic evolution of *E. faecium* populations has been deeply studied since the emergence
81 of VRE clinical isolates in the early 1990s. A subpopulation associated with hospital
82 outbreaks and human infections, named 'clonal complex 17' (CC17), was early identified in
83 2000s [15]. Since then, many other studies confirmed the worldwide dissemination of
84 hospital-adapted *E. faecium* clones [19,23]. More recent WGS-based studies have split the
85 *E. faecium* population into two well-differentiated lineages occupying mostly non-
86 overlapping ecological niches, including the hospital-associated (HA) lineage (clade A) and
87 the community-associated (CA) lineage (clade B) [11,24]. Clade A was then subdivided into
88 clade A1 (mostly associated to human outbreaks and infections, and comprising 'CC17'
89 isolates) and clade A2 (mostly animal-related) [16]. Whereas more recent studies using
90 larger collections of animal isolates also supported the phylogenetic distinction between
91 clades A and B, they do not longer support the split of clade A [18,25,26••]. Animal isolates
92 represent multiple lineages that diverged prior to the emergence of hospital-associated *E.*
93 *faecium* isolates, which could have been emerged from an ancestor lineage associated with
94 animals, with hospital-associated populations evolving faster than animal ones [18,25]. A
95 recent study also showed that plasmid contents (referred as plasmidomes) were more
96 informative than chromosomes for source specificity of *E. faecium*, suggesting that the
97 distribution of plasmid-mediated genes significantly contributes to niche adaptation
98 [26••]. Interestingly, HA *E. faecium* isolates possess a very diverse accessory genome and
99 larger chromosomes and plasmidomes than non-clinical isolates [16,26••]. These strains
100 are especially enriched in a variety of determinants enabling them to adapt to the hospital
101 environment and to better succeed during host colonization and infection [25,26••].

As opposed to *E. faecium*, phylogenetic diversity is limited in *E. faecalis* with a dispersion of single genotypes from different origins and countries revealing the absence of clade structure and a generalist lifestyle of this species [11,27]. Note that a few lineages show adaptation to the hospital environment with some CCs (e.g., CC2, CC16, CC87) more associated with MDR strains and enriched for MGEs while CC2 and CC87 are almost exclusively identified from nosocomial infections [27-29]. The generalist nature of *E. faecalis* is also clearly supported by pangenomic clustering showing no prominent host specialization with stable genome sizes across isolation years and sources and no correlation between strain isolation habitat and phylogeny [30,31••]. In addition, AMR genes are highly prevalent in *E. faecalis* isolates from both non-hospital settings and non-human origin while the oldest hospital-associated clusters (mid-19th century) predate the introduction of antibiotics, altogether suggesting survival and selection across multiple niches [31••].

Enterococci in humans

Enterococcus spp. are minority members of the normal flora of the human GIT, representing less than 1% of the intestinal microbiota of an adult [32]. Primarily regarded as harmless commensal bacteria, they have become major opportunistic pathogens [7]. Indeed, *E. faecalis* and *E. faecium* are currently a leading cause of hospital-acquired infections (e.g., urinary tract and intra-abdominal infections, bacteremia, endocarditis), especially in critically-ill and immunocompromised patients with long-course antimicrobial treatments and/or prolonged hospital stays [1,6]. Historically, *E. faecalis* caused the majority of enterococcal infections (80-90%) but *E. faecium* is of increasing importance as it is usually much more resistant to antimicrobials (Table 1) [9•]. Importantly, HA *E. faecium*

is known to efficiently outcompete other bacteria (including clade B CA strains) and dominate the gut microbiota in long-stay hospitalized patients with antimicrobial-exposed GIT where MDR strains (especially VRE) quickly replace susceptible populations before bloodstream invasion [33,34]. It has been proposed that differential carbohydrate utilization and biofilm production could be the main drivers for the divergent evolution of HA *E. faecium* [35••]. Also, *E. faecium* remains viable for extended periods of time on inanimate surfaces (from several days to several months), which is in relation with their persistence in the hospital environment and their implication in hospital outbreaks even if no clear transmission chains of direct acquisition is demonstrated [17,36,37].

Enterococci in animals and food

Apart from humans, GIT of animals likely represent the greatest reservoir for enterococci, in which they also can cause infections while large amounts of antimicrobial agents are used in animal production [38]. The most commonly encountered enterococcal species in the gut of mammals are *E. faecalis*, *E. faecium*, *E. hirae* and *E. durans* while *E. cecorum* is an important poultry pathogen [39]. The major concern with animal enterococci is the potential transmission of MDR enterococci to humans since they can be exposed by direct contact with animals and animal-contaminated environments or indirectly, through consumption of contaminated food of animal origin and vegetables from crops treated with animal manure (Table 1) [4]. Once acquired, strains of animal origin may transiently colonize the human GIT and potentially transfer AMR-carrying MGEs to the indigenous microbiota, including bacteria other than enterococci [38].

E. faecium and *E. faecalis* are generally the most frequently encountered species in food products. Contamination of raw meat occurs during the evisceration process at

slaughterhouses, with fecal enterococci contaminating over 90% of food products of animal origin [39]. Nevertheless, there is limited evidence of the direct role of food-producing animals in the dissemination of VRE among humans suggesting that animal MDR strains have a limited zoonotic potential (Table 1) [38,39]. Only some sporadic cases have been described showing overlapping between animal and human isolates such as the existence of clonal relationships between HA and swine-associated VRE [40], the recovery of human-adapted CCs from farm and companion animals (e.g., *E. faecium* CC17, *E. faecalis* ST6) or the isolation of animal-associated CCs in humans (e.g., *E. faecium* CC5, *E. faecalis* ST16) [38,39,41]. Interestingly, a recent (2014-2015) cross-sectional survey in the UK including farm animals (but not pets) found limited sharing of strains and resistance genes between livestock and humans except for some pig isolates that were genetically related to HA strains [40,42]. This suggests that livestock is unlikely to play a major role in the persistence of vancomycin resistance in human invasive isolates. By contrast, dogs may be a reservoir of HA *E. faecium* clones and may form a higher risk for zoonotic transfer to humans [23].

Very interestingly, a recent one-health investigational study conducted in Southern Alberta (Canada) showed that throughout a human-agriculture-environment continuum a clear delineation of species present in different environments with *E. faecium* and *E. faecalis* being the predominant species associated with humans (hospital and urban wastewaters) while *E. hirae* was the predominant species isolated from cattle feces and associated feedlot catch-basins [43••]. This confirms a minimal transmission of *Enterococcus* spp. from animals and animal-associated environments to humans with a negligible role in enterococcal human infections [43••,44]. In the same way, significant differences were

observed between isolates from dairy products and humans, suggesting that dairy isolates exist as independent lineages rather than a product of fecal contamination [45].

Enterococci in the environment and water

In most extra-enteric environments, both growth and persistence of enterococci are generally limited due to many abiotic and biotic stressors, such as sunlight, salinity, competition for nutrients, or predation by indigenous microorganisms (i.e., protozoa, bacteriophages) [3]. However, they are able to survive for extended periods of time in the environment since they develop numerous mechanisms allowing them to cope with these adverse conditions [2•].

Environmental and water samples, especially those contaminated by sewage or fecal wastes, often contain enterococci. They have been widely used as bacteriological indicators of fecal contamination (fecal indicator bacteria; FIB), especially to monitor the quality of recreational waters [46]. Indeed, there is a strong positive correlation with enterococcal concentrations in marine and fresh waters determined by culturable or qPCR methods and the risk of gastroenteritis associated with swimming [46]. Nonetheless, 'fecal' species have also been detected in various environmental samples with no obvious sources of contamination while environmental enterococci have been isolated from both human and animal feces [47].

Even though their actual prevalence in many non-clinical contexts is likely underestimated since most studies focus only on clinical isolates, wastewater is often reported as a reservoir for HA *E. faecium* (Table 1). Recently conducted in the East of England, a systematic genomic survey on *E. faecium* isolates collected from wastewater confirmed that HA lineages of VRE were widespread in wastewater and showed that wastewater treatment did not prevent downstream environmental contamination, with the majority of

plants releasing MDR *E. faecium* into the environment [48••]. The detection of VRE at all treatment plants is consistent with the widespread dissemination of MDR lineages in the community, with potential sources including the environment and the food chain [48••]. Interestingly, recent data from the same group also showed that VRE in the food chain differed genetically from human and wastewater VRE, suggesting that environmental isolates come from anthropogenic pollution and human isolates are not originated from the food chain [42]. The release of VRE into the environment should be controlled by improving wastewater decontamination both at the hospital and municipal waste level [48••].

Risk for public health

Even though they are one of the traditional bacterial markers of fecal contamination of food and water for human consumption, enterococci are generally not considered as food- or waterborne pathogens causing diarrhea [6]. By contrast, enterococci are potential vectors of AMR genes from the environment to humans, risk aggravated by the capacity of disseminate those genes to the host microbiome by HGT [49]. In the community, MDR enterococci may reach humans by several ways, including direct contact with farm personnel, via wastewater and surface water, or by contact with or consumption of food animals and food of animal origin (Figure 2) [50].

The most typical example is that of VRE, which have been identified mid-1980s in Europe and then have rapidly spread worldwide especially in the United States [51]. Epidemiological differences between the US and Europe presumably resulted from the massive antibiotic use in US hospitals (most notably of vancomycin and cephalosporins) while initial reports of VRE in Europe reported strains frequently isolated from healthy

people, farm animals, pets, and retail food products [52]. It was demonstrated that this large community reservoir in Europe was related to the extensive and widespread use of avoparcin (a vancomycin-like glycopeptide never used in the US) as growth promoter in animal husbandry, which subsequently led to the colonization of healthy humans by VRE via the food chain [38]. Due to the VRE emergence, use of avoparcin was banned in Europe in 1997, leading to a rapid decrease of the prevalence of VRE fecal carriage in food-producing animals and healthy humans. Nonetheless, VRE colonization never completely disappeared from livestock after the avoparcin withdrawal, likely related to the co-selection of *vanA*-harboring plasmids carrying other resistance genes by other compounds (e.g., tylosin, copper) or the presence of toxin-antitoxin systems located on these plasmids [38,39]. Note that this community reservoir seemed absent in the US where the detection of VRE from food-producing animals has been exceptionally reported.

Another major concern is the emergence and diffusion of transferable linezolid resistance genes since oxazolidinones are pivotal last-line drugs for the treatment of infections caused by VRE and methicillin-resistant staphylococci [53]. To date, five mobile oxazolidinone resistance genes (namely *cfr*, *cfr*(B), *cfr*(D), *optrA* and *poxtA*) have been identified among human and animal enterococci on various conjugative and non-conjugative plasmids [54,55]. The emergence of linezolid-resistant enterococci (LRE) of animal origin carrying *optrA*-positive plasmids underlines the role in the co-selection of MDR enterococci by antibiotics commonly used in animals (e.g., phenicols, tetracyclines, lincosamides, aminoglycosides) and the risk of transmission from food-producing animals to humans via the food chain [39].

Conclusions

Enterococci have an extraordinary genome plasticity and metabolic versatility that enable them to thrive in many diverse environments. Therefore, clinically-relevant species (i.e., *E. faecalis* and *E. faecium*) are found ubiquitously in non-human habitats such animals and the environment, which constitute important secondary reservoirs. There is then a potential risk of transfer of MDR enterococci and AMR genes into the food chain and the environment that could potentially pose a threat to public health. Whereas contamination through the food chain seems to be negligible, the environment (especially that related to human activities) may play a critical role in the acquisition and the dissemination of AMR in humans. Indeed, the highest concentrations HA lineages of MDR *E. faecalis* and *E. faecium* are found in hospital and municipal wastewaters, for which the decontamination process in wastewater treatment plants should be improved.

Conflict of interest statement

Nothing declared.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

** of outstanding interest

1. Murray BE: **The life and times of the *Enterococcus***. *Clin Microbiol Rev* 1990, **3**:46-65.
2. Gaca AO, Lemos JA: **Adaptation to adversity: the intermingling of stress tolerance and pathogenesis in enterococci**. *Microbiol Mol Biol Rev* 2019, **83**.

• Excellent review summarizing mechanisms by which enterococci respond to and survive diverse environmental stresses with a focus on the role of stress tolerance in biology and pathogenesis.

3. Byappanahalli MN, Nevers MB, Korajkic A, Staley ZR, Harwood VJ: **Enterococci in the environment**. *Microbiol Mol Biol Rev* 2012, **76**:685-706.
4. Staley C, Dunny GM, Sadowsky MJ: **Environmental and animal-associated enterococci**. *Adv Appl Microbiol* 2014, **87**:147-186.
5. Lebreton F, Manson AL, Saavedra JT, Straub TJ, Earl AM, Gilmore MS: **Tracing the enterococci from paleozoic origins to the hospital**. *Cell* 2017, **169**:849-861 e813.
6. Arias CA, Murray BE: **The rise of the *Enterococcus*: beyond vancomycin resistance**. *Nat Rev Microbiol* 2012, **10**:266-278.
7. Van Tyne D, Gilmore MS: **Friend turned foe: evolution of enterococcal virulence and antibiotic resistance**. *Annu Rev Microbiol* 2014, **68**:337-356.
8. Bender JK, Cattoir V, Hegstad K, Sadowy E, Coque TM, Westh H, Hammerum AM, Schaffer K, Burns K, Murchan S, et al.: **Update on prevalence and mechanisms of resistance to linezolid, tigecycline and daptomycin in enterococci in Europe: towards a common nomenclature**. *Drug Resist Updat* 2018, **40**:25-39.
9. Garcia-Solache M, Rice LB: **The *Enterococcus*: a model of adaptability to its environment**. *Clin Microbiol Rev* 2019, **32**.

• Comprehensive review on enterococci presenting a general overview of the genus: basic biology, clinical relevance, genetics, virulence, antimicrobial resistance and therapeutic options.

10. Sorensen TL, Blom M, Monnet DL, Frimodt-Moller N, Poulsen RL, Espersen F: **Transient intestinal carriage after ingestion of antibiotic-resistant *Enterococcus faecium* from chicken and pork**. *N Engl J Med* 2001, **345**:1161-1166.
11. Palmer KL, Godfrey P, Griggs A, Kos VN, Zucker J, Desjardins C, Cerqueira G, Gevers D, Walker S, Wortman J, et al.: **Comparative genomics of enterococci: variation in *Enterococcus faecalis*, clade structure in *E. faecium*, and defining characteristics of *E. gallinarum* and *E. casseliflavus***. *mBio* 2012, **3**:e00318-00311.
12. Lam MM, Seemann T, Bulach DM, Gladman SL, Chen H, Haring V, Moore RJ, Ballard S, Grayson ML, Johnson PD, et al.: **Comparative analysis of the first complete *Enterococcus faecium* genome**. *J Bacteriol* 2012, **194**:2334-2341.
13. Kim EB, Marco ML: **Nonclinical and clinical *Enterococcus faecium* strains, but not *Enterococcus faecalis* strains, have distinct structural and functional genomic features**. *Appl Environ Microbiol* 2014, **80**:154-165.
14. Paulsen IT, Banerjei L, Myers GS, Nelson KE, Seshadri R, Read TD, Fouts DE, Eisen JA, Gill SR, Heidelberg JF, et al.: **Role of mobile DNA in the evolution of vancomycin-resistant *Enterococcus faecalis***. *Science* 2003, **299**:2071-2074.
15. Willems RJ, Top J, van Santen M, Robinson DA, Coque TM, Baquero F, Grundmann H, Bonten MJ: **Global spread of vancomycin-resistant *Enterococcus faecium* from distinct nosocomial genetic complex**. *Emerg Infect Dis* 2005, **11**:821-828.
16. Lebreton F, van Schaik W, McGuire AM, Godfrey P, Griggs A, Mazumdar V, Corander J, Cheng L, Saif S, Young S, et al.: **Emergence of epidemic multidrug-resistant *Enterococcus faecium* from animal and commensal strains**. *mBio* 2013, **4**.
17. van Hal SJ, Ip CLC, Ansari MA, Wilson DJ, Espedido BA, Jensen SO, Bowden R: **Evolutionary dynamics of *Enterococcus faecium* reveals complex genomic relationships between isolates with independent emergence of vancomycin resistance**. *Microb Genom* 2016, **2**.

18. Raven KE, Reuter S, Reynolds R, Brodrick HJ, Russell JE, Torok ME, Parkhill J, Peacock SJ: **A decade of genomic history for healthcare-associated *Enterococcus faecium* in the United Kingdom and Ireland**. *Genome Res* 2016, **26**:1388-1396.

19. van Hal SJ, Willems RJL, Gouliouris T, Ballard SA, Coque TM, Hammerum AM, Hegstad K, Westh HT, Howden BP, Malhotra-Kumar S, et al.: **The global dissemination of hospital clones of *Enterococcus faecium***. *Genome Med* 2021, **13**:52.

20. Guzman Prieto AM, van Schaik W, Rogers MR, Coque TM, Baquero F, Corander J, Willems RJ: **Global Emergence and dissemination of enterococci as nosocomial pathogens: attack of the clones?** *Front Microbiol* 2016, **7**:788.

21. Palmer KL, Gilmore MS: **Multidrug-resistant enterococci lack CRISPR-cas**. *mBio* 2010, **1**.

22. van Schaik W, Top J, Riley DR, Boekhorst J, Vrijenhoek JE, Schapendonk CM, Hendrickx AP, Nijman IJ, Bonten MJ, Tettelin H, et al.: **Pyrosequencing-based comparative genome analysis of the nosocomial pathogen *Enterococcus faecium* and identification of a large transferable pathogenicity island**. *BMC Genomics* 2010, **11**:239.

23. Willems RJ, Hanage WP, Bessen DE, Feil EJ: **Population biology of Gram-positive pathogens: high-risk clones for dissemination of antibiotic resistance**. *FEMS Microbiol Rev* 2011, **35**:872-900.

24. Galloway-Pena J, Roh JH, Latorre M, Qin X, Murray BE: **Genomic and SNP analyses demonstrate a distant separation of the hospital and community-associated clades of *Enterococcus faecium***. *PLoS One* 2012, **7**:e30187.

25. Rios R, Reyes J, Carvajal LP, Rincon S, Panesso D, Echeverri AM, Dinh A, Kolokotronis SO, Narechania A, Tran TT, et al.: **Genomic epidemiology of vancomycin-resistant *Enterococcus faecium* (VREfm) in Latin America: revisiting the global VRE population structure**. *Sci Rep* 2020, **10**:5636.

26. Arredondo-Alonso S, Top J, McNally A, Puranen S, Pesonen M, Pensar J, Marttinen P, Braat JC, Rogers MRC, van Schaik W, et al.: **Plasmids shaped the recent emergence of the major nosocomial pathogen *Enterococcus faecium***. *mBio* 2020, **11**.

•• This is the first comprehensive population-wide analysis of the pan-plasmidome of *Enterococcus faecium* (1,644 isolates from hospital, commensal, and animal sources) that demonstrates that plasmidomes rather than chromosomes are most informative for source specificity.

27. Neumann B, Prior K, Bender JK, Harmsen D, Klare I, Fuchs S, Bethe A, Zuhlke D, Gohler A, Schwarz S, et al.: **A core genome multilocus sequence typing scheme for *Enterococcus faecalis***. *J Clin Microbiol* 2019, **57**.

28. Kuch A, Willems RJ, Werner G, Coque TM, Hammerum AM, Sundsfjord A, Klare I, Ruiz-Garbajosa P, Simonsen GS, van Luit-Asbroek M, et al.: **Insight into antimicrobial susceptibility and population structure of contemporary human *Enterococcus faecalis* isolates from Europe**. *J Antimicrob Chemother* 2012, **67**:551-558.

29. Raven KE, Reuter S, Gouliouris T, Reynolds R, Russell JE, Brown NM, Torok ME, Parkhill J, Peacock SJ: **Genome-based characterization of hospital-adapted *Enterococcus faecalis* lineages**. *Nat Microbiol* 2016, **1**.

30. He Q, Hou Q, Wang Y, Li J, Li W, Kwok LY, Sun Z, Zhang H, Zhong Z: **Comparative genomic analysis of *Enterococcus faecalis*: insights into their environmental adaptations**. *BMC Genomics* 2018, **19**:527.

31. Pontinen AK, Top J, Arredondo-Alonso S, Tonkin-Hill G, Freitas AR, Novais C, Gladstone RA, Pesonen M, Meneses R, Pesonen H, et al.: **Apparent nosocomial adaptation of *Enterococcus faecalis* predates the modern hospital era**. *Nat Commun* 2021, **12**:1523.

•• This is a large population genomic analysis investigating a collection of 2,207 *Enterococcus faecalis* isolates recovered from widely different sources that demonstrates the generalist nature of this species with no prominent host specialization. Findings also suggest that oldest hospital-associated lineages predate the introduction of antibiotics.

32. Banla LI, Salzman NH, Kristich CJ: **Colonization of the mammalian intestinal tract by enterococci**. *Curr Opin Microbiol* 2019, **47**:26-31.

33. Montealegre MC, Singh KV, Murray BE: **Gastrointestinal tract colonization dynamics by different *Enterococcus faecium* clades**. *J Infect Dis* 2016, **213**:1914-1922.

34. Ravi A, Halstead FD, Bamford A, Casey A, Thomson NM, van Schaik W, Snelson C, Goulden R, Foster-Nyarko E, Savva GM, et al.: **Loss of microbial diversity and pathogen domination of the gut microbiota in critically ill patients**. *Microb Genom* 2019, **5**.

35. Chilambi GS, Nordstrom HR, Evans DR, Ferrolino JA, Hayden RT, Maron GM, Vo AN, Gilmore MS, Wolf J, Rosch JW, et al.: **Evolution of vancomycin-resistant *Enterococcus faecium* during colonization and infection in immunocompromised pediatric patients**. *Proc Natl Acad Sci U S A* 2020, **117**:11703-11714.

•• This study demonstrates how vancomycin-resistant *Enterococcus faecium* evolve during colonization of the antibiotic-perturbed intestinal microbiota and invasion in the bloodstream in immunocompromised pediatric patients. By genomic and phenotypic analysis, they notably showed changes in carbohydrate utilization and biofilm production.

36. Kramer A, Schwebke I, Kampf G: **How long do nosocomial pathogens persist on inanimate surfaces? A systematic review**. *BMC Infect Dis* 2006, **6**:130.

37. Gouliouris T, Coll F, Ludden C, Blane B, Raven KE, Naydenova P, Crawley C, Torok ME, Enoch DA, Brown NM, et al.: **Quantifying acquisition and transmission of *Enterococcus faecium* using genomic surveillance**. *Nat Microbiol* 2021, **6**:103-111.

38. Hammerum AM, Lester CH, Heuer OE: **Antimicrobial-resistant enterococci in animals and meat: a human health hazard?** *Foodborne Pathog Dis* 2010, **7**:1137-1146.

39. Torres C, Alonso CA, Ruiz-Ripa L, Leon-Sampedro R, Del Campo R, Coque TM: **Antimicrobial resistance in *Enterococcus* spp. of animal origin**. *Microbiol Spectr* 2018, **6**.

40. Freitas AR, Coque TM, Novais C, Hammerum AM, Lester CH, Zervos MJ, Donabedian S, Jensen LB, Francia MV, Baquero F, et al.: **Human and swine hosts share vancomycin-resistant *Enterococcus faecium* CC17 and CC5 and *Enterococcus faecalis* CC2 clonal clusters harboring Tn*1546* on indistinguishable plasmids**. *J Clin Microbiol* 2011, **49**:925-931.

41. Freitas AR, Pereira AP, Novais C, Peixe L: **Multidrug-resistant high-risk *Enterococcus faecium* clones: can we really define them?** *Int J Antimicrob Agents* 2021, **57**:106227.

42. Gouliouris T, Raven KE, Ludden C, Blane B, Corander J, Horner CS, Hernandez-Garcia J, Wood P, Hadjirin NF, Radakovic M, et al.: **Genomic surveillance of *Enterococcus faecium* reveals limited sharing of strains and resistance genes between livestock and humans in the United Kingdom**. *mBio* 2018, **9**.

43. Zaheer R, Cook SR, Barbieri R, Goji N, Cameron A, Petkau A, Polo RO, Tymensen L, Stamm C, Song J, et al.: **Surveillance of *Enterococcus* spp. reveals distinct species and antimicrobial resistance diversity across a One-Health continuum**. *Sci Rep* 2020, **10**:3937.

•• Pioneering one-health investigational study conducted in Southern Alberta, Canada, showing that specific *Enterococcus* spp. are tightly associated with specific host or environmental niches, and that enterococci from beef cattle may not play a significant role in human infections.

44. Iversen A, Kuhn I, Rahman M, Franklin A, Burman LG, Olsson-Liljequist B, Torell E, Mollby R: **Evidence for transmission between humans and the environment of a nosocomial strain of *Enterococcus faecium***. *Environ Microbiol* 2004, **6**:55-59.

45. Zhong Z, Kwok LY, Hou Q, Sun Y, Li W, Zhang H, Sun Z: **Comparative genomic analysis revealed great plasticity and environmental adaptation of the genomes of *Enterococcus faecium***. *BMC Genomics* 2019, **20**:602.

46. Harwood VJ, Staley C, Badgley BD, Borges K, Korajkic A: **Microbial source tracking markers for detection of fecal contamination in environmental waters: relationships between pathogens and human health outcomes**. *FEMS Microbiol Rev* 2014, **38**:1-40.

47. Lee T, Pang S, Abraham S, Coombs GW: **Antimicrobial-resistant CC17 *Enterococcus faecium*: the past, the present and the future**. *J Glob Antimicrob Resist* 2019, **16**:36-47.

48. Gouliouris T, Raven KE, Moradigaravand D, Ludden C, Coll F, Blane B, Naydenova P, Horner C, Brown NM, Corander J, et al.: **Detection of vancomycin-resistant *Enterococcus faecium* hospital-adapted lineages in municipal wastewater treatment plants indicates widespread distribution and release into the environment**. *Genome Res* 2019, **29**:626-634.

•• Cross-sectional survey conducted in the East of England (2014-2015) showing the widespread distribution of hospital-adapted vancomycin-resistant *Enterococcus faecium* lineages beyond acute healthcare settings with extensive release into the environment and limited efficacy of the decontamination process in wastewater treatment plants in both urban and rural settings.

49. Manaia CM: **Assessing the risk of antibiotic resistance transmission from the environment to humans: non-direct proportionality between abundance and risk**. *Trends Microbiol* 2017, **25**:173-181.

50. Daniel DS, Lee SM, Dykes GA, Rahman S: **Public health risks of multiple-drug-resistant *Enterococcus* spp. in Southeast Asia**. *Appl Environ Microbiol* 2015, **81**:6090-6097.

51. Bonten MJ, Willems R, Weinstein RA: **Vancomycin-resistant enterococci: why are they here, and where do they come from?** *Lancet Infect Dis* 2001, **1**:314-325.

52. Cattoir V, Leclercq R: **Twenty-five years of shared life with vancomycin-resistant enterococci: is it time to divorce?** *J Antimicrob Chemother* 2013, **68**:731-742.

53. Zahedi Bialvaei A, Rahbar M, Yousefi M, Asgharzadeh M, Samadi Kafil H: **Linezolid: a promising option in the treatment of Gram-positives**. *J Antimicrob Chemother* 2017, **72**:354-364.

54. Sadowy E: **Linezolid resistance genes and genetic elements enhancing their dissemination in enterococci and streptococci**. *Plasmid* 2018, **99**:89-98.

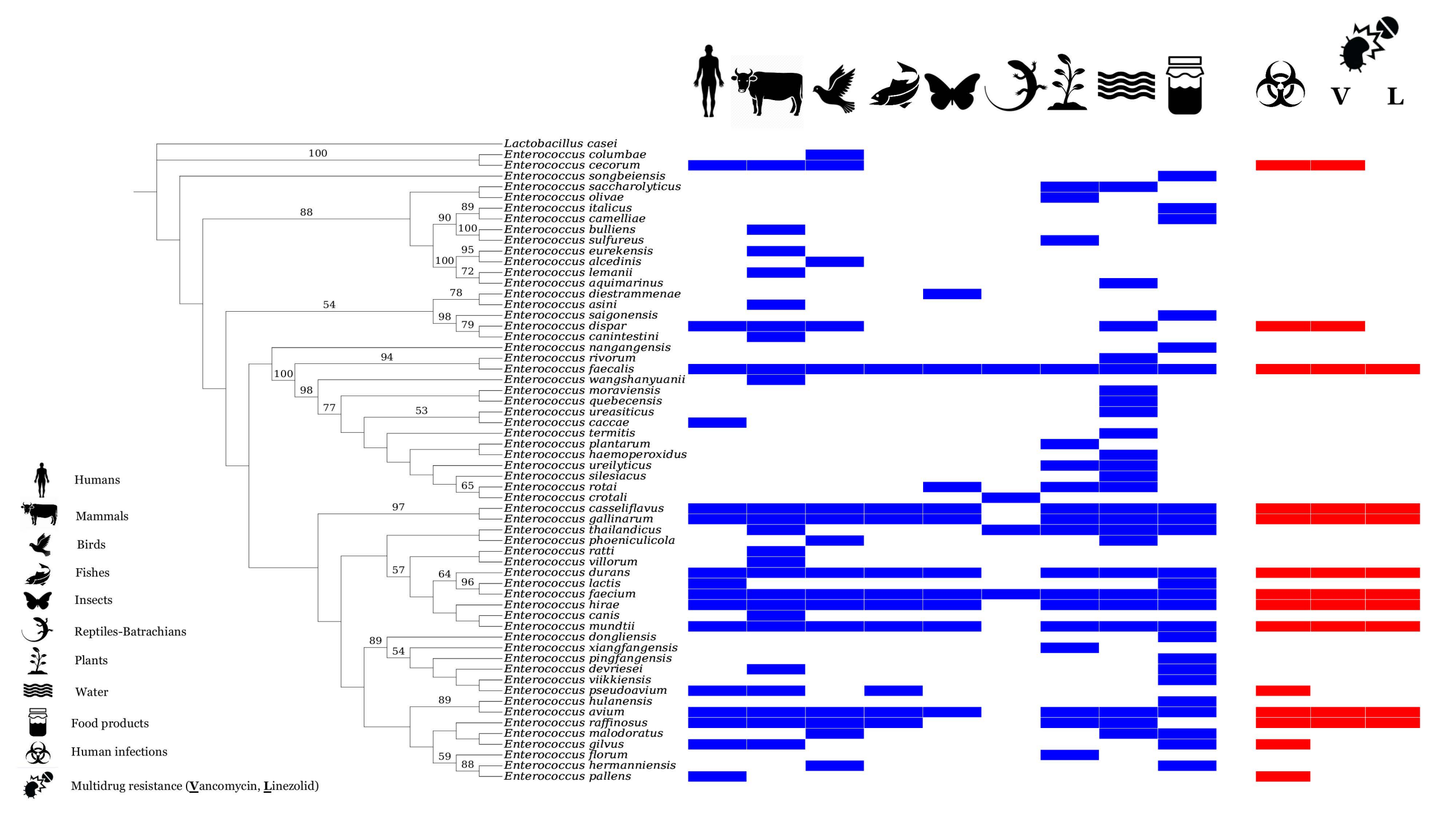
55. Bi R, Qin T, Fan W, Ma P, Gu B: **The emerging problem of linezolid-resistant enterococci**. *J Glob Antimicrob Resist* 2018, **13**:11-19.

Legend of the figures

Figure 1. Phylogenetic tree of *Enterococcus* species and their corresponding habitats. The 16S rRNA sequences of the 59 enterococcal type strains were used to construct the maximum-likelihood tree with *Lactobacillus casei* as an outgroup. The tree was constructed using Mega X with 1,000 bootstrap iterations and only bootstraps >50 are showed. The heatmap with corresponding habitats was combined to the phylogenetic tree by using the iTOL online program (https://itol.embl.de/). Species involved in human infections and reported as multidrug resistant (vancomycin, linezolid) are also indicated in the heatmap.

Figure 2. Possible routes for transfer of enterococci or AMR genes (especially VRE) among different reservoirs.

Strains can move between ecological niches in the environment, animal and/or human animal hosts, carrying with them plasmid-mediated AMR genes. Strains can be transferred from the environment to humans/animals via contact or consumption of contaminated water sources or vegetables; between humans and animals via contact or food consumption; and from hosts back to the environment via effluents or wastewaters. The risk level of transfer is represented by the width of arrows. The figure was obtained by using the Biorender online program (https://biorender.com/).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Enterococcus olivae
Enterococcus italicus
Enterococcus camelliae
Enterococcus bulliens
Enterococcus sulfureus
Enterococcus eurekensis
Enterococcus alcedinis
Enterococcus lemanii
Enterococcus aquimarinus
Enterococcus diestrammenae
Enterococcus asini
Enterococcus saigonensis
Enterococcus dispar
Enterococcus canintestini
Enterococcus nangangensis
Enterococcus rivorum
Enterococcus faecalis
Enterococcus wangshanyuanii
Enterococcus moraviensis
Enterococcus quebecensis
Enterococcus ureasiticus
Enterococcus caccae
Enterococcus termitis
Enterococcus plantarum
Enterococcus haemoperoxidus
Enterococcus ureilyticus
Enterococcus silesiacus
Enterococcus rotai
Enterococcus crotali
Enterococcus casseliflavus
Enterococcus gallinarum
Enterococcus thailandicus
Enterococcus phoeniculicola
Enterococcus ratti
Enterococcus villorum
Enterococcus durans
Enterococcus lactis
Enterococcus faecium
Enterococcus hirae
Enterococcus canis
Enterococcus mundtii
Enterococcus dongliensis
Enterococcus xiangfangensis
Enterococcus pingfangensis
Enterococcus devriesei
Enterococcus viikkiensis
Enterococcus pseudoavium
Enterococcus hulanensis
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Humans
Mammals
Birds
Fishes
Insects
Reptiles-Batrachians
Plants
Water
Food products
Human infections
Multidrug resistance (Vancomycin, Linezolid)

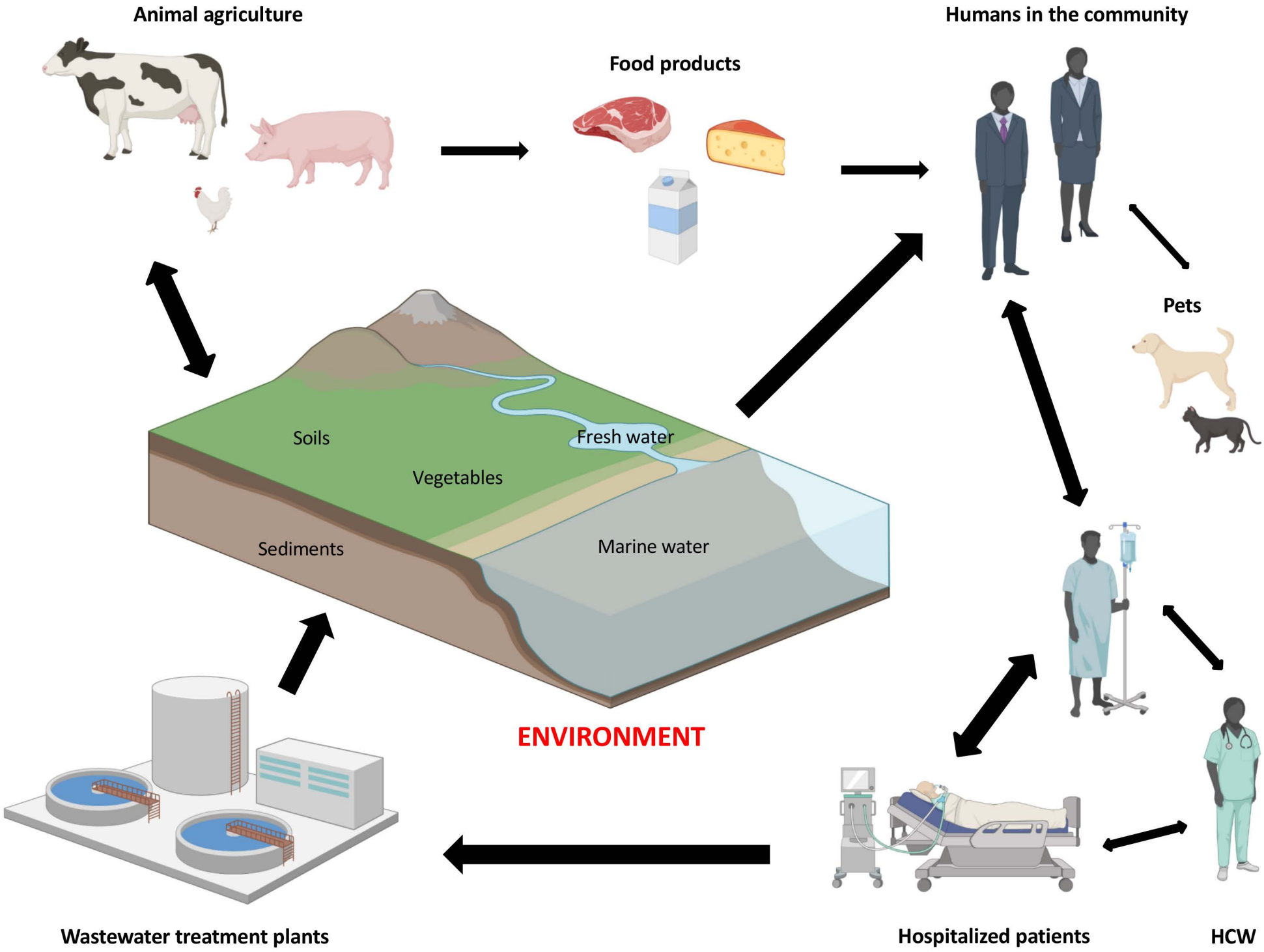
Animal agriculture
Food products
Humans in the community
Pets
Soils
Vegetables
Fresh water
Sediments
Marine water
ENVIRONMENT
Wastewater treatment plants
Hospitalized patients
HCW

Table 1. Genetic and ecological similarities and differences between *E. faecalis* and *E. faecium* species (4,5,9•,13,26••,31••,39,41,42,47).

	E. faecalis	***E. faecium***
Reservoirs	Human GIT (healthy individuals)	Human GIT (hospitalized patients)
	Animal GIT (dog, cat, chicken, pig, calf, cow, wild birds, invertebrates) Food (meat, vegetables, milk, cheese) Soil and sediments, fresh and marine water, vegetation	
Genome plasticity	Small genome size (3.00 Mb on average) Large accessory genome (up to 25 %)	Small genome size (2.85 Mb on average) Large accessory genome (up to 38 %)
	Homologous recombination, HGT (conjugation) Usually lack of CRISPR-Cas and restriction-modification systems	
Genetic evolution	No clear clade structure with limited phylogenetic diversity Generalist lifestyle No host specialization	Clade structure with separation between clinical and commensal isolates Distinct hospital-associated lineage Niche ecology driven by plasmidome
Human pathogens	Yes (ca. 75 %) (both CAI and HAI)	Yes (ca. 25 %) (quasi-exclusively HAI)
MDR phenotype and hospital outbreaks	Occasionally	Frequent (VRE+++)
Animal pathogens and food contaminants	Yes (poultry, pigs, cattle)	
Risk of zoonotic transfer of AMR to humans	Low (*optrA*-mediated linezolid resistance?)	Low (except from pig isolates?)
Contamination of the environment by clinical isolates	Possible	Frequent (VRE in hospital and municipal wastewaters)
Role in environmental dissemination of AMR	Possible	Important (VRE, *van* genes)

AMR, antimicrobial resistance; CAI, community-associated infections; GIT, gastro-intestinal tract; HAI, hospital-acquired infections; HGT, horizontal gene transfer; VRE, vancomycin-resistant enterococci.