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REVIEW ARTICLE

Commensal gut bacteria: distribution of *Enterococcus* species and prevalence of *Escherichia coli* phylogenetic groups in animals and humans in Portugal

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Abstract The gastrointestinal tract is continuously in contact with commensal bacteria that are composed of more than 500 different species, and has an important role in human nutrition and health, by promoting nutrient supply, preventing pathogen colonization and shaping and maintaining normal mucosal immunity. The present review demonstrates the distribution of the intestinal commensal bacteria *Enterococcus* spp. and the prevalence of *Escherichia coli* phylogenetic groups in animals and humans in Portugal. The enterococcal population described in this review includes 1,909 enterococcal isolates recovered from a series of fecal samples of different animals (horses, swine, ostriches, partridges, mullet fish, garden dormice, seagulls, pets, poultry, wild boars, birds of prey, and wild rabbits) and healthy and clinical humans. We also compared the phylogenetic groups of *Escherichia coli* isolates (n=203) recovered from healthy humans and animals (poultry, ostriches, seagulls, wild boars, birds of prey, and pigs). Phenotypic and molecular analysis allowed the identifying of *Enterococcus faecium* as the predominant species followed by *Enterococcus faecalis*. In addition, the *Escherichia coli* data from different studies showed that isolates of the A and B1 phylogenetic groups are predominant in the gut flora of animal origin and the phylogenetic group B2 isolates were the most common in healthy human samples.

Keywords Gastrointestinal tract · *Enterococcus* spp. · *Escherichia coli* · Portugal

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Introduction

A large number of commensal bacteria colonize the gastrointestinal tract of mammals. When the bacteria invade the host, the intestine immune system recognizes commensal bacteria from pathogenic ones, discriminates between safe and dangerous, and attacks only those that are hazardous to the host. Although the commensal bacteria are identified as non-host antigens, these organisms are able to reside in the gut without being eliminated, playing an important role in human nutrition and health, by promoting nutrient supply, preventing pathogen colonization and shaping, and maintaining the homeostasis of the intestinal immune system. Thus, the immune system and the commensal bacteria form the symbiotic system in the intestine (Takahashi 2010; Xu et al. 2003).

Enterococci are commonly found in the gastrointestinal tract of healthy humans and animals (Vankerckhoven et al. 2004). They are Gram-positive facultative anaerobic bacteria, spherical, which occur singly, in pairs or short chains and fit within the general definition of lactic acid bacteria (Ciftci et al. 2009). These bacteria can be brought into the

environment by human and animal fecal material (Kühn et al. 2000). Most enterococci are not virulent and are considered relatively harmless, with little potential for human infection. However, they have also been identified as nosocomial opportunistic pathogens with increased resistance to antimicrobial approved agents (Chenoweth and Schaberg 1990). *Enterococcus faecalis* represent 80–90% of human clinical enterococcal infections, while 5–15% are caused by *E. faecium*. However, other species including *E. hirae*, *E. durans*, *E. gallinarum*, or *E. casseliflavus* are occasionally identified in clinical isolates (Patterson et al. 1995; Ruoff et al. 1990). Common enterococcal infections include those of the urinary tract, bloodstream, endocardium and wounds (Shepard and Gilmore 2002).

Enterococci species show significant differences in the incidence of virulence factors. Generally, *E. faecalis* appears to harbor more virulence traits while *E. faecium* strains were generally free of virulence factors (Eaton and Gasson 2001). In addition, considering the distribution of the antibiotic resistance according to the species, the *E. faecium* possessed a higher level of resistance than *E. faecalis* (Franz et al. 2001; Gin and Zhanel 1996).

Accurate species identification of enterococci has become important, in particular because some species have been recognized as human pathogens following the wide prevalence of acquired antibiotic resistance (Tyrrell et al. 1997).

Escherichia coli is the head of the large bacterial family, Enterobacteriaceae, the enteric bacteria, which are facultatively anaerobic Gram-negative, and is commonly found in the intestinal tract of a wide variety of animals and humans (Sorum and Sunde 2001). This intestinal bacterium can be easily disseminated in different ecosystems. For this reason, fecal *Escherichia coli* is considered to be an important indicator for the selective pressure exerted by the use of antimicrobials on intestinal populations of bacteria (van den Bogaard and Stobberingh 2000). The emergence of multiresistant *Escherichia coli* has been previously reported in humans and in different animal species, increasing the public health concern (Saenz et al. 2004). On the other hand, the production of extended-spectrum beta-lactamases (ESBLs) by Enterobacteriaceae, specifically by *Escherichia coli*, has caused a major concern in several countries, being frequently implicated in human infections. Previous reports have described ESBL-containing *Escherichia coli* strains in healthy animals (Pinto et al. 2010; Poeta et al. 2009).

Escherichia coli can be classified into four main phylogenetic groups (A, B1, B2 and D) that were initially identified by the allelic variation of strains associated with enzymes that could be detected by multilocus enzyme electrophoresis (Herzer et al. 1990). Usually, the commensal strains are placed into the phylogenetic groups A and B1. On the other hand, the *Escherichia coli* strains causing extraintestinal infections are known to mainly belong to group B2 and, to a lesser extent, group D (Clermont et al. 2000). The intestinal pathogenic strains are usually assigned to groups A, B1 and D (Pupo et al. 1997). More recently, a rapid and simple method for the determination of *Escherichia coli* phylogenetic groups, based on a triplex PCR strategy, has been reported (Clermont et al. 2000).

The aim of the present review is to illustrate the distribution of the gut enterococci species as well as the prevalence of *Escherichia coli* phylogenetic groups in animals and humans in Portugal, in order to evaluate our knowledge about the diversity of enterococcal species and *Escherichia coli* phylogenetic groups from different sources, and therefore compare this diversity in Portugal with other countries.

Enterococci species distribution

In the last decade, several studies involving enterococci, recovered from different animal and human origins, were performed in Portugal. Using phenotypic and molecular methods, a total of 1,909 enterococcal isolates were identified to species level. The distribution of enterococcal species in human and animal fecal samples is shown in Fig. 1 and Table 1.

For the majority of the isolates, one fecal sample per individual (human or animal) was collected, plated onto Slanetz-Bartley agar and incubated at 37°C for 48 h. Colonies with typical enterococcal morphology were identified to the genus level by cultural characteristics, Gram-staining, catalase test and the bile-aesculin reaction. Species identification was performed using the BBL Crystal Gram-Positive ID System® (Hamilton-Miller and Shah 1999) and was confirmed by polymerase chain reaction (PCR) using primers and conditions for the different enterococcal species with appropriate controls (Dutka-Malen et al. 1995; Miele et al. 1995; Robredo et al. 1999).

Molecular and biochemical approaches allowed us to identify the predominance of *E. faecium* (965 isolates), followed by *E. faecalis* (679 isolates), *E. hirae* (187 isolates), *E. avium* (43 isolates) *E. durans* (18 isolates), *E. gallinarum* (14 isolates), and *E. casseliflavus* (3 isolates). Similar species distribution was identified among enterococcal population in different geographical regions from different ecological habitats (Kühn et al. 2003). Although *E. faecium* was detected in all samples, the same situation does not occur with *E. faecalis* and *E. hirae* isolates. *E. gallinarum* was present in four different origins, *E. casseliflavus* was detected in two different sample origins and *E. avium* was detected only in healthy pets. *E. faecium*

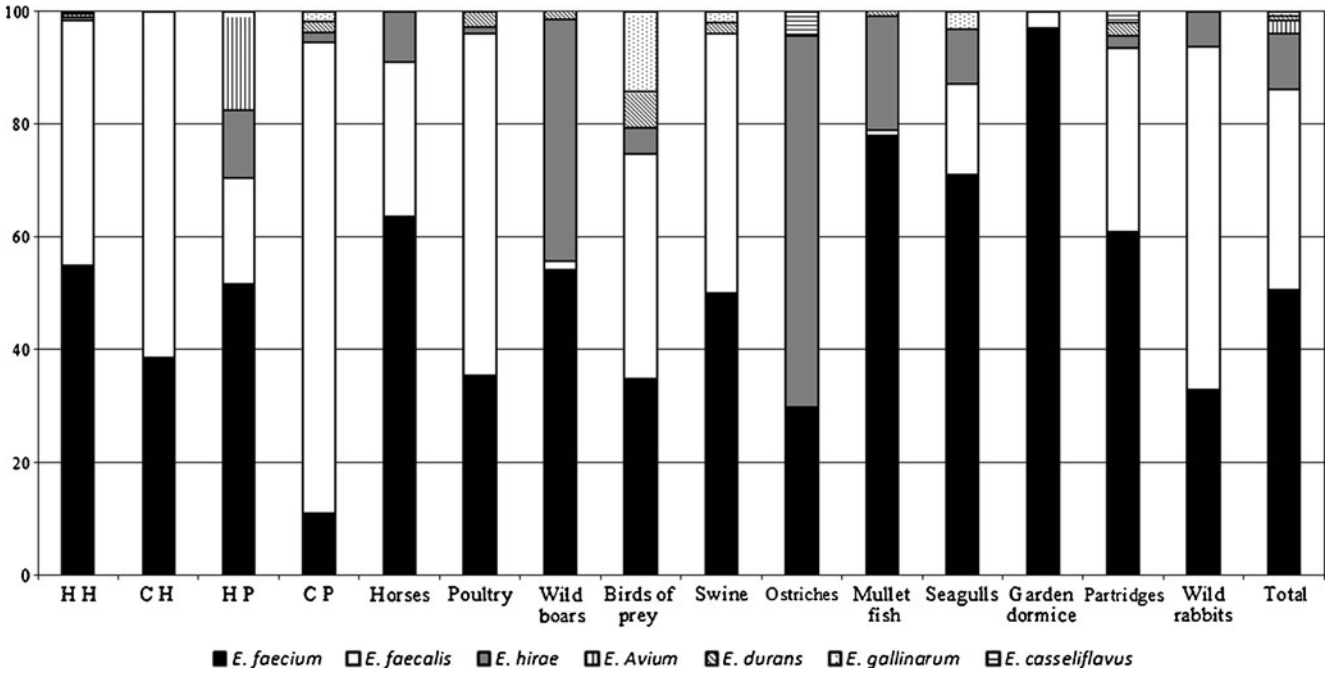

Fig. 1 Distribution of enterococcal species in human and animal recovery from fecal samples. Enterococci were isolated from the following samples: *HH* healthy humans (n=574) (Barreto et al. 2009; Guimaraes et al. 2009; Novais et al. 2003, 2006; Poeta et al. 2005a, 2006a), *CH* clinical humans (n=208) (Novais et al. 2003, 2008), *HP* healthy pets (n=246) (Poeta et al. 2005a, 2006a; Rodrigues et al. 2002), *CP* clinical pets (n=55) (Delgado et al. 2007), horses (n=110) (Moura et al. 2010), poultry (n=152) (Poeta et al. 2005a; 2006a, b), wild boars (n=126) (Poeta et al. 2007a, b), birds of prey (n=63) (Poeta et al. 2005b, 2007b), swine (n=50) (Novais et al. 2003, 2008), ostriches (n=47) (Gonçalves et al. 2010a), mullet fish (n=104) (Araújo et al. 2011), seagulls (n=31) (Radhouani et al. 2010b), garden dormice (n=33), partridges (n=46) (Silva et al. 2011), wild rabbits (n=64) (Silva et al. 2010)

and *E. faecalis* are predominant in healthy human samples in different studies with an average of 54.9% of *E. faecium* and 43.4% of *E. faecalis* (Barreto et al. 2009; Guimaraes et al. 2009; Novais et al. 2003, 2006; Poeta et al. 2005a, 2006a). A different distribution of the enterococcal species was observed in clinical human samples, where *E. faecalis* was the predominant species (Novais et al. 2003, 2008). This difference may be due to the fact that human patients may have been subjected to treatment with antibiotics, and also due to a much higher sampling in the cause of human health compared with the clinical samples. The detection of these enterococcal species in human clinical specimens is common. Although *E. faecalis* is the most frequent enterococcal species detected in human infections (Desai et al. 2001; Murray 1990; Schouten et al. 1999), the results found in Portugal isolates showed a relatively low occurrence of *E. faecalis* compared with *E. faecium* in human fecal isolates. Both species have long been known to be significantly important as human pathogens that are especially responsible for nosocomial infections (Murray 1990; Schaberg et al. 1991). *E. hirae* (0.9%) and *E. durans* and *E. gallinarum* (0.6%) were also detected in human fecal samples. These three enterococcal species were not detected in clinical samples.

Isolates from fecal samples of healthy pets, in different studies in Portugal (Poeta et al. 2005a, 2006a; Rodrigues et al. 2002) presented the following species: *E. faecium* (51.6%), *E. faecalis* (18.7%), *E. avium* (17.5%) and *E. hirae* (12.2%). *E. faecalis* and *E. hirae* were predominant in pet's anal swabs in another study performed in Europe (Devriese et al. 1992a). Enterococci isolates recovered from clinical pets in Portugal showed a different species distribution when compared with healthy pets, where most of the enterococcal isolates belong to *E. faecalis* with 83.6% of the total of isolates (Delgado et al. 2007).

In the horse fecal samples, the species identified were *E. faecium* (63.6%), *E. faecalis* (27.3%) and *E. hirae* (9.1%) (Moura et al. 2010). Similar to the results found in Portugal, *E. faecium* was identified as the predominant enterococcal species in horse fecal samples in Slovakia farms (Laukova et al. 2008). However, a study performed in Idaho (US) found that *E. casseliflavus* was most predominant in fresh and dry manure horse samples, and only 8–9% of the isolates were identified as *E. faecium* (Graves et al. 2009). No enterococcal isolates recovered from horse fecal samples in Portugal were identified as *E. casseliflavus*.

Although *E. faecium* was the most prevalent species found in the total fecal samples recovered from different sources in Portugal, *E. faecalis* was dominant in poultry (Poeta et al. 2005a, 2006a, b), in birds of prey (Poeta et al. 2005a, 2007b), and in wild rabbits (Silva et al. 2010), while *E. hirae* was dominant in ostrich fecal samples (Gonçalves

Table 1 Distribution of *Enterococcus* species in human and animal recovery from fecal samples

Source	Number (%) of isolates of the different enterococcal species							References
	E. faecium	*E. faecalis*	*E. hirae*	*E. avium*	*E. durans*	*E. gallinarum*	*E. casseliflavus*	
Healthy humans (*n*=574)	315 (54.9)	249 (43.4)	4 (0.7)	-	3 (0.5)	3 (0.5)	-	(Barreto et al. 2009; Guimaraes et al. 2009; Novais et al. 2003, 2006; Poeta et al. 2005a, 2006a)
Clinical humans (*n*=208)	80 (38.5)	128 (61.5)	-	-	-	-	-	(Novais et al. 2003, 2008)
Healthy pets (*n*=246)	127 (51.6)	46 (18.7)	30 (12.2)	43 (17.5)	-	-	-	(Poeta et al. 2005a, 2006a; Rodrigues et al. 2002)
Clinical pets (*n*=55)	6 (10.9)	46 (83.6)	1 (1.8)	-	1 (1.8)	1 (1.8)	-	(Delgado et al. 2007)
Horses (*n*=110)	70 (63.6)	30 (27.3)	10 (9.1)	-			-	(Moura et al. 2010)
Poultry (*n*=152)	54 (35.5)	92 (60.5)	2 (1.3)	-	4 (2.6)	-	-	(Poeta et al. 2005a, 2006a, b)
Wild boars (*n*=126)	68 (54.0)	2 (1.6)	54 (42.9)	-	2 (1.6)	-	-	(Poeta et al. 2007a, 2007b)
Birds of prey (*n*=63)	22 (34.9)	25 (39.7)	3 (4.8)	-	4 (6.3)	9 (14.3)	-	(Poeta et al. 2005b, 2007b)
Swine (*n*=50)	25 (50.0)	-	23 (46.0)	-	1 (2.0)	1 (2.0)	-	(Novais et al. 2003, 2008)
Ostriches (*n*=47)	14 (29.8)	-	31 (65.9)	-	-	-	2 (4.3)	(Gonçalves et al. 2010a)
Mullet fish (*n*=104)	81 (77.9)	1 (1.0)	21 (20.2)	-	1 (1.0)	-	-	(Araújo et al. 2011)
Seagulls (*n*=31)	22 (71.0)	5 (16.1)	3 (9.7)	-	1 (3.2)	-	-	(Radhouani et al. 2010b)
Garden dormice (*n*=33)	32 (97.0)	1 (3.0)	-	-	-	-	-	[a]
Partridges (*n*=46)	28 (60.9)	15 (32.6)	1 (2.2)	-	1 (2.2)	-	1 (2.2)	(Silva et al. 2011)
Wild rabbits (*n*=64)	21 (32.8)	39 (60.9)	4 (6.3)	-	-	-	-	(Silva et al. 2010)
Total (*n*=1,909)	965 (50.6)	679 (35.6)	187 (9.8)	43 (2.3)	18 (0.9)	14 (0.7)	3 (0.2)	

[a] The enterococcal species distribution from garden dormice has not yet been submitted for publication

et al. 2010a). Isolates of poultry samples included: *E. faecalis* (60.5%), *E. faecium* (35.5%), *E. durans* (2.6%), and *E. hirae* (1.3%). *E. faecalis* was also found to be predominant in fecal poultry samples in studies performed in Spain and Denmark (Kühn et al. 2003). Another study carried out by Klein, in food and in the gastro-intestinal tract, showed the prevalence of *E. faecalis* and *E. faecium* while *E. durans* and *E. hirae* were found with low frequency (Klein 2003). It is interesting to note that the gastro-intestinal tract of the birds of prey presented the highest enterococcal species diversity detected: *E. faecalis* (39.7%), *E. faecium* (34.9%), *E. hirae* (4.8%), *E. durans* (6.3 %), and *E. gallinarum* (14.3%).

In swine fecal samples, the species identified were as follows: *E. faecium* (50.0%), *E. hirae* (46.0%), *E. durans* (2.0%), and *E. gallinarum* (2.0%) (Novais et al. 2003, 2008). This was similar to other studies carried out in Sweden, UK and Spain, where *E. faecium* and *E. hirae* were identified as the predominant enterococcal species in fecal swine samples (Kühn et al. 2003). Enterococci from ostriches included: *E. hirae* (65.9%), *E. faecium* (29.8%), and *E. casseliflavus* (4.3%) (Gonçalves et al. 2010a). In wild boars, the species detected were: *E. faecium* (54.0%), *E. hirae* (42.9%), *E. faecalis* (1.6%) and *E. durans* (1.6%) (Poeta et al. 2007a, b). A similar enterococcal species distribution was detected in the swine fecal samples. Mitochondrial DNA studies showed that the wild boar is the ancestor of the domestic pig (*Sus scrofa domesticus*) (Giuffra et al. 2000), and this relationship could explain the similarities observed in the distribution of enterococcal species in their intestinal microbial flora. Along with the birds of prey, the partridges group showed the highest diversity of enterococcal isolates. A total of six species were identified: *E. faecium* (60.9%), *E. faecalis* (32.6%), and *E. hirae*, *E. durans,* and *E. casseliflavus,* all with 2.2% (Silva et al. 2011). Mullet fish and seagulls isolates presented the same enterococcal species: *E. faecium* (77.9 and 71.0%, respectively), *E. faecalis* (1.0 and 16.1%, respectively), *E. hirae* (20.2 and 9.7%, respectively), and *E. durans* (1.0 and 3.2%, respectively) (Araújo et al. 2011; Radhouani et al. 2010b).

Only two enterococcal species were identified in the garden dormice fecal samples: *E. faecium* (97.0%) and *E. faecalis* (3.0%). *E. faecalis* was the most prevalent detected species (60.9%), in wild rabbits in Portugal, followed by *E. faecium* (32.8%) and *E. hirae* (6.3%) (Silva et al. 2010). The detection of *E. faecalis* and *E. faecium* as the predominant enterococcal species in the fecal samples of wild rabbits shows strong similarities with data previously reported for fecal enterococci of farmed rabbits (Linaje et al. 2004). Furthermore, the enterococcal isolates from partridges, mullet fish, garden dormice, seagulls and wild boars showed a higher prevalence of *E. faecium* species. It is important to underline that these results diverge slightly with the species distribution demonstrated in the enterococcal isolates from another study performed in wild animals, where the frequency of *E. faecium* and *E. faecalis* was more homogeneous (32.1 and 52.1%, respectively) (Poeta et al. 2005b).

Escherichia coli phylogenetic groups

Different studies in commensal *Escherichia coli* from animal and human origin were performed in Portugal (Table 2).

Fecal samples from human and animals were plated onto Levine agar and MacConkey agar and incubated at 37°C for 24 h. One colony per sample with typical *Escherichia coli* morphology was selected and identified by standard bacteriological tests (gram, catalase, oxidase, indol, methyl red/Voges-Proskauer, citrate and urease), by the API 20E system (BioMérieux, La Balme Les Grottes, France) (Radhouani et al. 2010a), or by API ID 32GN galleries (BioMérieux) and by the automated WIDER system (Fco. Soria Melguizo, Madrid, Spain) (Machado et al. 2008). *Escherichia coli* isolates were classified into one of the four main phylogenetic groups, A, B1, B2 and D, by PCR as described previously based on the presence or absence of *chu*A, *yja*A or *tsp*E4.C2 genes (Clermont et al. 2000).

The molecular approach allowed the identification of phylogenetic groups in 203 *Escherichia coli* isolates, 119 of them ESBL-containing *Escherichia coli*. The non-ESBL-containing *Escherichia coli* identified are isolates resistant to at least one of the tested antibiotics. In general, most of the isolates belonged to the phylogenetic group B1 (69 isolates) following by groups A (58) and B2 (57). Nineteen of the isolates were identified in the phylogenetic group D. It is interesting to note that most of the healthy human *Escherichia coli* isolates belong to the phylogenetic groups B2 and D (Barreto et al. 2009; Guimaraes et al. 2009), and they are the main cause for the large percentage of strains belonging to these two phylogenetic groups in the total number of isolates from different origins. The phylogenetic groups B2 and D have been reported to be associated with virulent isolates (Clermont et al. 2000). The high ratio of B2 isolates obtained from humans (65.5%) reveals great concern as a public health problem. While commensal isolates of phylogenetic groups A, B1 and D present a smaller number of virulence determinants than in the corresponding pathogenic strains, the strains of phylogenetic group B2 in the commensal flora appear to be potentially virulent (Duriez et al. 2001). The results found in healthy humans in Portugal diverges greatly from previous studies in human commensal *Escherichia coli*

Table 2 Phylogenetic group distribution among *Escherichia coli* isolates

Source	Number (%) of isolates of the different *Escherichia coli* phylogenetic groups				References
	A	B1	B2	D	
Healthy human (n=58)	5 (8.6)	6 (10.4)	38 (65.5)	9 (15.5)	(Barreto et al. 2009; Guimaraes et al. 2009)
Poultry (n=36)	12 (33.3)	17 (47.2)	-	7 (19.5)	(Costa et al. 2009; Machado et al. 2008)
Ostriches (n=3)	-	3	-	-	(Carneiro et al. 2010)
Pigs (n=16)	14 (87.5)	2 (12.5)	-	-	(Gonçalves et al. 2010b)
Seagulls (n = 40)	15 (37.5)	19 (47.5)	3 (7.5)	3 (7.5)	(Poeta et al. 2008; Radhouani et al. 2010a)
Wild boars (n=8)	2 (25.0)	3 (37.5)	3 (37.5)	-	(Poeta et al. 2009)
Birds of prey (n=42)	10 (23.8)	19 (45.2)	13(31.0)	-	(Pinto et al. 2010; Radhouani et al. 2010a)
Total (n=203)	58 (27.9)	69 (34.3)	57 (28.4)	19 (9.5)	

isolated from stools, where isolates belonging to the phylogenetic group B2 were only between 11 and 30% in studies performed in different geographic regions (Duriez et al. 2001; Lee et al. 2010). In fact, isolates of phylogenetic group B2 are more frequent among extra-intestinal pathogenic strains (40–72%) than among commensal strains (9–30%) (Bingen et al. 1998; Duriez et al. 2001; Gonçalves et al. 2010b; Hilali et al. 2000; Johnson et al. 1991; Picard et al. 1999). These differences in the distribution of the phylogenetic groups from human origin may be due to geographical and climatic conditions, by dietary factors and/or the use of antibiotics. About 80% of the ESBL-containing *Escherichia coli* isolates recovered from poultry in Portugal belonged to the B1 (47.2%) and A (33.3%) phylogenetic groups and none of them were included in the B2 group. The remaining isolates belonged to the phylogenetic group D (19.5%) (Costa et al. 2009; Machado et al. 2008). The prevalence of *Escherichia coli* of groups A and B1 was also observed in pigs from a Portuguese intensive swine farm with 87.5 and 12.5%, respectively (Gonçalves et al. 2010b), while in captive ostrich, all the three isolates belong to the phylogenetic group B1 (Carneiro et al. 2010). These results observed in Portugal are in agreement with a study performed in poultry and pig farms in Spain, where the phylogroup B1 (38.6%) was predominant among isolates from poultry farms and the phylogroup A (55.2%) was most frequently detected among isolates from pig farms (Cortes et al. 2010). The ESBL-containing *Escherichia coli* recovered from wild animals, wild boars (Poeta et al. 2009), and birds of prey (Pinto et al. 2010; Radhouani et al. 2010a), showed a similar distribution between the phylo-groups A, B1 and B2. Samples from seagulls showed that the isolates from the phylogenetic A (37.5%) and B1 (47.5%) groups are predominant (Poeta et al. 2008; Radhouani et al. 2009). Furthermore, in contrast to the other wild animals in which isolates from phylo-group D were not found, 7.5% of the *Escherichia coli* recovered from seagulls belonged to the phylogenetic group D.

Role of *Enterococcus* and *Escherichia coli* as commensal bacteria

Although enterococci and *Escherichia coli* are the most well-characterized bacteria and the most important indicator organisms of fecal contamination of food and water, relatively little is yet known about the structure of these populations in their different hosts.

Concerning the enterococcal population, the predominant species found in fecal samples of human and animal origin were *E. faecium*, followed by *E. faecalis*, and *E. hirae*. Other species, as *E. durans*, *E. gallinarum*, and *E. casseliflavus* were found with lower frequencies; however, the enterococcal species distribution among isolates from fecal samples varied between the different sources. Moreover, the origins of the differences in the enterococcal species distribution, when compared with other studies. are not clear, but may be a result of resistance and flexibility of *Enterococcus* spp., to differences related to the geographical regions, or to the diet (which may alter the composition of the intestinal microbial flora), or by incorrect species identification.

Enterococci may play a beneficial or a detrimental role in foods. They may cause spoilage or they may contribute to ripening and flavoring processes of certain foods. A special application concerns their use as indicator strains to detect fecal contamination of water. *Enterococcus* spp. also produce a large number of bacteriocins, the so-called enterocins (Franz et al. 2007), which are small peptides with antimicrobial activity towards closely related Gram-positive bacteria including spoilage or pathogenic bacteria, such as *Listeria* (Foulquie Moreno et al. 2006). Some enterococcal bacteriocins are also active against viruses. Bacteriocin production helps enterococci to colonize the gastrointestinal tract and this also contributes to the inhibition of pathogenic organisms in the intestine (Todorov et al. 2010; Wachsman et al. 1999).

Moreover, enterococci are nowadays used as probiotics (Franz et al. 1999, 2003). Probiotic consumption is reported

to have beneficial effects, including enhanced immune response, improving the intestinal microbial balance, reduction of fecal enzymes implicated in cancer initiation, vaccine adjuvant effects, treatment of diarrhea associated with travel and antibiotic therapy, control of rotavirus and *Clostridium difficile*-induced colitis, and prevention of ulcers related to *Helicobacter pylori*. Probiotics are also implicated in the reduction of serum cholesterol, the antagonism against food-borne pathogens and tooth decay organisms, as well as candidiasis and urinary tract infections (Saavedra 2001). However, at the same time, enterococci have been associated with a number of human infections. Several virulence factors have been described, and the number of vancomycin-resistant enterococci is increasing. The controversial nature of enterococci has prompted an enormous increase in scientific papers and reviews in recent years, in which researchers have been divided into two groups, namely pro and contra enterococci. For this reason, further studies are essential to determine the enterococci population not only in humans but also from animals and even from other origins.

The *Escherichia coli* data suggest that isolates of the A and B1 phylogenetic groups are predominant in the gut flora of animal origin and that these isolates must probably acquire virulence factors to become pathogenic. In contrast, the phylogenetic group B2 isolates were the most common in human samples and, because these are highly pathogenic and frequently responsible for extraintestinal infections in humans, may represent a major public health problem. The structure of *Escherichia coli* populations influences several aspects of public health. Pathogenic subtypes of *Escherichia coli* are known to cause illness around the world (Leclerc et al. 2001), and an increased understanding of the genetic variability of populations in animal reservoirs can inform epidemiological studies. Although the population structure of several pathogenic isolates has been extensively studied, little is known about the structure of commensal strain populations. It is therefore essential that there are further studies to determine the phylogenetic relationships of *Escherichia coli* isolates from the normal gut flora of healthy humans and animals, to establish a relationship between the commensal and pathogenic *Escherichia coli* strains.

Despite the vast information about this bacterium, the ecology of *Escherichia coli* in the intestine of humans is poorly understood. *Escherichia coli* is the major facultative anaerobic inhabitant of the human gut, and commensal *Escherichia coli* strains can outcompete against gut pathogens and seem to have a beneficial effect on several types of intestinal disorders. For example, the *Escherichia coli* strain Nissle 1917 is a commensal strain that has been used as a probiotic agent to treat gastrointestinal infections in humans since the early 1920s (Sartor 2005). This strain has the ability to compete with pathogenic strains during biofilm formation, a complex and heterogeneous matrix associated with bacterial infections (Hancock et al. 2010). Furthermore, *Escherichia coli* strain Nissle 1917 has been used as a probiotic in human inflammatory bowel disease (Kamada et al. 2005) to maintaining remission of ulcerative colitis (Kruis et al. 1997; Rembacken et al. 1999) and Crohn's disease (Malchow 1997). Moreover, the development of *Escherichia coli* probiotic strains may serve as the first line of defence in protecting humans against colonization by *Escherichia coli* intestinal pathogens (Leatham et al. 2009).

Analysis, perspectives and conclusions

Although the analysis described in this review is a comparison of *Enterococcus* spp. and *Escherichia coli* populations of different studies performed over a 10-year sampling period in Portugal, an identical methodological approach was used in these investigations, for the isolation and identification of *Enterococcus* species and also for *Escherichia coli* isolation and phylotyping.

Direct comparisons of *Enterococcus* and *Escherichia coli* population structure in different host animals are rare in the literature, and most of the studies have focused on only a few host species (Gordon 1997; Jenkins et al. 2003; Kühn et al. 2003; Layton et al. 2010). The aim of the present review was to generate knowledge about these bacterial population structures from different human and animal sources in Portugal, and to show the importance of them as normal inhabitants of the intestinal tract. Moreover, this study also presents a global overview of a possible link between the bacteria found in healthy human and certain animal species, and also if the enterococci and *Escherichia coli* from these sources can be found in human and animal infections.

Although is not possible to determine precisely the causes of the origin of the differences in the *Enterococcus* and *Escherichia coli* population distribution, there are several factors that may be related to these bacterial distribution rates.

Host specificity Certain host-specific variations in the occurrence of different species in different animal hosts are known to exist. In humans as well as in many other animal species, *E. faecalis* and *E. faecium* are found most frequently. The first is more common and usually occurs in larger numbers than the second (Murray 1990). *E. columbae* is specific for pigeons (Devriese et al. 1990), and *E. asini* for donkeys (de Vaux et al. 1998), whereas *E. cecorum* is a prominent member of the enterococcal flora of poultry and pigs (Devriese et al. 1991, 1994). *E. hirae* is a frequent inhabitant of the swine gut and may occur in poultry, cattle dogs and cats (Devriese et al. 1987).

Age variation In certain hosts, variations in the enterococcal flora according to age have been reported. Enterococci are among the dominant flora of the intestine in the very first days of life in many animals, but they decline to markedly lower levels after a few weeks of life. Age-dependent variations in species distribution have been observed in the enterococcal flora of chickens. *E. faecalis* and *E. faecium* have been found to constitute the dominant bacterial flora of the intestinal tract in 1-day-old chicks. However, with age, the dominant species is *E. faecium* following by *E. hirae* and *E. durans*. The latter species are gradually replaced by *E. cecorum* at the age of 12 weeks (Devriese et al. 2006). In cattle, age-dependent colonization has also been described (Devriese et al. 1992b). Also, in humans, *E. faecalis* largely outnumbers the other species in infants less than 1 week of age (Noble 1978).

Diet and the effects of environmental stress The composition of the diet or the exposure of animals to stressful environments can result in marked changes in the intestinal microflora (Tannock 1997). A well-known example of the influence food ingestion may have is the low enterococcal content of fecal samples from breastfed infants compared with formula-fed infants (Stark and Lee 1982). Moreover, food products may contain *Enterococcus* and *Escherichia coli* bacteria and influence the intestinal microbiota composition. However, the consumption of some dairy products such as the Camembert cheese, that does not contain either enterococci or enterobacteria, leads to a significant increase of the *E. faecalis* population in healthy humans guts, while the *Escherichia coli* population remains unaffected (Firmesse et al. 2007).

Variation in different compartments The enterococcal flora may differ in different compartments of the intestine, as has been documented in chickens. *E. durans* and *E. hirae* were part of the small intestinal flora of 3- to 4-week-old chicks but were not detected in the crop and the ceca of the same animals (Devriese et al. 1991).

Number of isolates recovered in fecal samples Normally, and particularly in epidemiological studies, a large number of samples are collected and cultivated, and only one or two isolates per sample are picked for further analysis. It is expected that the diversity values would be higher when only one isolate per sample is picked from many samples than when the same number of isolates is picked from fewer samples. However, a large number of isolates per individual may be required to adequately sample the bacteria populations (Anderson et al. 2006). Moreover, a study comparing the enterococcal populations in animals, humans, and the environment showed that using fewer samples but analyzing several isolates per sample may yield results that describe the total diversity of the bacterial population to be studied, as well as using more samples and only one isolate per sample (Kühn et al. 2003).

Temporal variability The diversity of bacterial populations may differ according to the collection period of the samples. Sampling of stream water for over a year at two separate farms where cattle have direct access to the streams, showed high proportions of *E. casseliflavus* and *E. faecalis* dominated the enterococcal community during spring and fall, respectively (Molina et al. 2007). On the other hand, *E. faecium* seemed to increase during winter. Moreover, a study of diversity suggest that the *Escherichia coli* populations in feces of individual humans, horses, and cows are not temporally stable and experience significant turnover on a monthly time scale but also that these population characteristics can differ among host individuals of the same species (Anderson et al. 2006).

On the other hand, there is also evidence that some enterococcal species are mainly associated with several virulence determinants and *Escherichia coli* isolates responsible for extraintestinal diseases belong mainly to the B2 group and, to a lesser extent, to the D group.

These factors highlight the potential importance not only on the differences between host species, but also differences in the animals age, diet, environment, etc., in the colonization with these bacteria in humans and animals.

Although there were a large number of strains isolated from different human and animals over a 10-year study period that strongly support the enterococcal and *Escherichia coli* population distribution, further studies should be conducted to corroborate with the findings of this review, taking into consideration a more robust sampling from a larger number of animals, the analysis of samples from environment sources such as from hospital, urban and farm sewage, and the analysis of several isolates per sample since fecal samples can show the presence of more than one enterococcal species or *Escherichia coli* phylogenetic group strains. Since this review only demonstrates the distribution of enterococcal and *Escherichia coli* populations from samples recovered in Portugal, a collaboration with other international groups, covering other European countries and the USA, should be taken in consideration to allow an overview of the distribution of these bacteria population at a global level. In addition, genotyping analysis through the use of multi-locus sequence typing (MLST) and/or pulsed-field gel electrophoresis (PFGE) should be performed to verify the clonal diversity among strains.

In conclusion, there is an urgent need to know more about enterococcal and *Escherichia coli* populations in humans, animals and in nature. Knowledge of the entero-

coccal species diversity and *Escherichia coli* phylogenetic groups in the gastro-intestinal tract of human and animals is substantially important since it has been suggested that *Enterococcus* spp. and *Escherichia coli* could act as a source of antimicrobial resistance and virulence determinants. The data presented in this review, collected over recent years from human and animal origins, can perform a basis for further research on an epidemiological approach.

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