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ORIGINAL ARTICLE

Isolation of vancomycin-resistant *Enterococcus* from apparently healthy human animal attendants, cattle and cattle wastes in Tanzania

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Abstract

Aim: The study aimed to isolate and characterize *Enterococcus* species from apparently healthy waste attendants, cattle and cattle waste in Tanzania. Emphasis was given to antimicrobial resistance and in particular occurrence of vancomycin (VA)-resistant enterococci.

Methods and Results: Faecal samples were collected from healthy cattle, cattle waste attendants and cattle house wastes, and isolation of *Enterococcus* species was performed using Slanetz Bartley agar. Isolates were characterized with regard to species, antimicrobial susceptibility and presence of VA resistance genes. *Enterococcus faecalis* was the most prevalent species from all sources of isolation (43·5%), followed by *Enterococcus faecium* (38·4%). Isolates of *E. faecium* showed a higher number of phenotypic antimicrobial resistance than isolates of *E. faecalis*. Fifty-eight isolates, which showed resistance or intermediate resistance to VA by disc diffusion test, were analysed for VA-resistant *Enterococcus* (VRE) by PCR. The *vanA* gene was detected in 14 isolates of *E. faecium* and 12 isolates of *E. faecalis*, while *vanB* was detected in three isolates. No isolates were found to carry *vanC1*-gene.

Conclusion: VRE was detected in both human and cattle samples, despite no known use of antimicrobial agents that can select for VRE in livestock in Tanzania. *Enterococcus faecalis* was the most commonly isolated species from cattle and humans.

Significance and Impact of the Study: The study provides information on the prevalence of VRE in human and nonhuman samples in Tanzania calling for further studies on the origin of VRE in such isolates, since no selection mechanism in Tanzania are known.

Introduction

Members of the genus *Enterococcus* are abundant in the normal gastro-intestinal microbiota of human, other mammals and avians (Manero and Blanch 1999; Harwood *et al.* 2005; Zhong *et al.* 2017). They are equipped with adaptive features to survive in various environment such as soil, plants and water (Layton *et al.* 2010), and their existence outside the intestine is considered a good indicator of faecal contamination (Devriese *et al.* 2006). While being considered a benign part of the normal flora, the members of the this genus has become a regular cause of nosocomial infection in immune-compromised individuals and are believed to play a potential role in spreading antimicrobial resistance in the community (Akova 2016). Disease complexes, where they have been implicated, include endocarditis, urinary tract infection, secondary infection in burn wounds, intra-abdominal and pelvic infections (Lawrence 2005; Murray 2010). *Enterococcus faecalis* and *Enterococcus faecium* are the

most important species of human health importance (Harwood *et al.* 2004; Ishihama *et al.* 2014).

The members of *Enterococcus* species are intrinsically resistant to many of the curative antimicrobials (Hayakawa *et al.* 2013), making the treatment of enterococcal infections difficult (Aamodt *et al.* 2015). A special concern is vancomycin (VA)-resistant *Enterococcus* (VRE), which may transfer their resistance genes to pathogenic bacteria, such as *Staphylococcus aureus* (Arias and Murray 2012; Novais *et al.* 2013). Vancomycin resistance is encoded by the *vanA* gene cluster, which is mostly observed in *E. faecium*, the *vanB*-cluster, which can be found in both *E. faecium* and *E. faecalis*, and which causes modest resistance, and the *vanC-1* type causing constitutive resistance in *E. gallinarum* (Jackson *et al.* 2004). VRE has been reported globally with *E. faecium* clonal complex ST17, which is commonly the cause of nosocomial infection in hospital settings (Gastmeier *et al.* 2014). In some parts of the world, selection has been related to present or past use of the glycopeptide antibiotic growth-promoter avoparcin *(Furtula et al.* 2013), which is an analogy to VA. This drug has never been licensed for use in Tanzania for growth promotion in livestock. VRE has been described in Tanzania in hospital isolates (Moyo *et al.* 2010; Aamodt *et al.* 2015), but little is known on the presence of community acquired VRE in humans and livestock. This study intended to isolate and determine prevalence of community acquired VRE among *Enterococcus* isolated from humans, cattle, and cattle waste in Tanzania.

Materials and methods

Study area

The study was conducted in Morogoro Urban and Peri-urban districts in Tanzania. These areas were chosen to represent a situation where a dense urban population is living in close contact with a rapidly expanding livestock sector.

Sample sources and sampling

The samples were collected from 110 randomly appointed cattle farms located in 13 different wards (local administrative units). The samples were obtained from apparently healthy cattle, one per farm ($n = 110$), using a gloved hand per rectum. Animal wastes ($n = 38$) were collected where waste heaps were available in the plot and the farmer gave permission. These samples were collected in sterile sample collection tubes around the animal house and dung heaps on the same location. Animal attendants from the farms were asked for their consent to provide stool. These samples ($n = 50$) were collected in sterile containers early in the morning. The collected samples were packed in a cool box and laboratory processing was carried within 6 h.

One gram of faecal sample was diluted serially in physiological saline and homogenized. Thereafter, 100 μl of the serially diluted sample aliquot (10^{-1} to 10^{-10}) was inoculated on sterile Petri dishes and molten Slatnetz Bartley (SB) Agar (Oxoid, Basingstoke, UK) at 45°C was poured into the plates, dried and incubated for 48 h at 44°C. The colonies were counted manually using illuminated colonial counter and colony forming unit was calculated basing on the highest dilution growth as described elsewhere (ISO 2004). Three to five presumptive *Enterococcus* colonies, which were red to mahogany of >0·5 cm diameter, were picked from each sample and stored at −80°C in 20% glycerol until further analysis could be performed.

Preserved isolates were subcultured on SB agar and incubated for 24 h at 37°C. A typical colony was picked using sterile inoculating loop, streaked onto brain heart infusion agar (BHIA; Oxoid) and incubated for 18 h at 37°C. Presumptive genus level identification was done by Gram stain, catalase test, bile aesculin hydrolysis test and growth in 6·5% NaCl tryptic soy broth (TSB). The isolates considered as enterococci were Gram-positive cocci, catalase negative, hydrolysed bile and grew in TSB with 6·5% NaCl.

Enterococcus speciation by multiplex PCR

A single colony from isolates on BHIA incubated overnight was diluted in 100 μl distilled water in Eppendorf tube, boiled on thermal block for 10 min at 95°C and centrifuged at 13 ***g*** for 5 min. The supernatant was pipetted and used on the same day as a template DNA in a PCR reaction, using published primers (Jackson *et al.* 2004). The primers have been extensively validated for species characterization in previous studies (Layton *et al.* 2010; Novais *et al.* 2013). In all the reactions, primers directed against 16s rRNA was used as an internal control. The multiplex PCR reactions were performed by predenaturation step of 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 50°C for 90 s, 72°C for 60 s and a final extension step of 72°C for 10 min. Ten microlitres of the PCR products were electrophoresed in 2% agarose Tris-borate-EDTA containing 0·5 μg ml^{-1} ethidium bromide for 60 min at 110V. A 1-kb DNA marker (ThermoFisher Scientific, Leicestershire, England) was used for band size determination.

Antimicrobial sensitivity testing and PCR detection of *van* genes

The isolates were subjected to antimicrobial susceptibility testing using the disc diffusion method (Bauer *et al.*

1966) and guided by EUCAST (2015). The antimicrobial discs included: ampicillin (30 μg), chloramphenicol (C 30 μg), enrofloxacin (ENR 5 μg), erythromycin (5 μg), gentamicin (CN 10 μg), rifampicin (RD 5 μg), sulphamethoxazole–trimethoprim (SXT 19 : 1), tetracycline (30 μg) and VA (30 μg). The reference strains *E. faecium* (35667) and *E. faecalis* (ATCC 19433) were used as internal controls. The results were classified basing on interpretive breakpoints suggested by EUCAST (2015). The occurrence of nonsusceptibility to at least one agent in three or more antimicrobial classes was counted as multidrug resistance (MDR) as suggested previously (Magiorakos *et al.* 2011).

Following the antimicrobial susceptibility testing, a total of 68 presumptive VRE isolates, 59 from humans, 6 from cattle and 3 from cattle waste (resistant or intermediate resistant according to EUCAST (2015) criteria) were characterized by PCR-analysis for the presence of resistance genes *vanA, vanB* and *vanC-1.* Primers and the protocol were as described (Dutka-Malen *et al.* 1995). In this analysis, *E. faecium* (35667) and *E. faecalis* (ATCC 19433) were used as positive controls, whereas RNAse-free water was used a negative control in all reactions.

Statistical analysis

Initially the data were entered into the MS-Excel for descriptive statistical analysis to determine the means, frequency, confidence interval and variances. Cross tabulation was carried out using statistical package for social sciences (SPSS version 2.0, Armonk, NY, USA) where chi-square and *t*-test were used to determine the statistical distribution of *Enterococcus* sp. with respect to source of isolation, antimicrobial resistance patterns and the correlation of VRE genes in relation to the source of isolation. A *P*-value of <0·05 was inferred as statistically significant.

Results

Colonies of enterococci were obtained from all samples. Average $\log_{10}$ CFU per gram of enterococci was determined and found to be significantly higher in samples from humans than in samples from cattle and cattle waste ($P < 0{\cdot}001$) (Table 1).

Typical colonies of *Enterococcus* on Slanetz Bartley agar were picked for further characterization. These putative *Enterococcus* isolates ($n = 227$ isolated from humans, $n = 165$ isolated from cattle, and $n = 38$ isolated from cattle waste) were subjected to multiplex PCR for speciation. *E. faecalis* (43·5%) was found to be the most common species, followed by *E. faecium* (38·4%), *E. gallinarum* (3·3%) and *E. avium* (2·6%), while 12·3% of the isolates did not produce an amplicon with any of the primer pairs used.

Table 1 Mean $\log_{10}$ colony-forming units (CFU) of *Enterococcus* in faecal samples from humans, cattle and cattle waste

Source	$\log_{10}$ CFU (mean)*	Standard deviations
Cattle	5·84[a]	0·43
Cattle waste	5·81[a]	0·37
Humans	6·19[b]	0·51

*Mean values marked with different letters are significantly different.

Statistically, the distribution of species differed significantly according to the source of isolation for *E. faecalis* and *E. faecium,* while the distribution was not significantly different for *E. gallinarum* and *E. avium* (Table 2).

Antimicrobial susceptibility testing was carried out using nine different antibiotics discs. The results showed that resistance differed statistically between sources of isolation except for C and ENR. Resistance to RD and gentamycin were higher than resistance to other drugs, irrespective of the source of isolation as shown in Table 3. Table 4 shows the resistance level according to the *Enterococcus* species. Generally, isolates of human origin had higher occurrence of antimicrobial resistance than isolates from the other groups, and the proportion of isolates showing MDR in this group (130 of 227 isolates) was significantly higher than among isolates from cattle (60 of 165 isolates ($P < 0{\cdot}0001$)). The number of isolates showing MDR in the different species was: *E. gallinarum* (11 of 14), *E. faecium* (82 of 165), *E. faecalis* (91 of 187), other *Enterococcus* (19 of 53) and *E. avium* (3 of 11). Supplementary table S1 lists the resistance per isolate according to source of isolation and species. Full details on resistance patterns per species and antimicrobial are depicted in the supplementary material (Table S2).

Sixty-eight (cattle = 6, human = 59, waste = 3) isolates with phenotypic resistance or intermediate resistance to vancomycin were detected. The isolates were analysed by PCR for presence of vancomycin resistance genes (*vanA*, *vanB* and *vanC1*). The *vanA* gene was detected in *E. faecium* of human (11 of 59), cattle (one of six) and cattle waste origin (two of three), while *vanB* was detected in human (8 of 59) and cattle (three of six) isolates of *E. faecalis* and *E. faecium*. *vanC1* was not detected.

Discussion

Prevalence of *Enterococcus* species according to source of isolation

This study describes isolation of *Enterococcus* species from cattle, human and cattle waste in Tanzania. *E. faecalis* was

Table 2 Multiplex PCR assignment of *Enterococcus* into species

Source	*Enterococcus* species according to source of isolation (%)				
	E. faecium	*E. faecalis*	*E. gallinarum*	*E. avium*	Other sp.
Cattle	65 (39·4)	77 (46·7)	4 (2·4)	6 (3·6)	13 (7·9)
Waste	12 (31·6)	18 (47·4)	2 (5·3)	0 (0·0)	6 (15·8)
Human	88 (38·8)	92 (40·5)	8 (3·5)	5 (2·2)	34 (15·0)
Total	165 (38·4)†	187 (43·5)†	14 (3·3)	11 (2·6)	53 (12·3)
P-value*	$P < 0{\cdot}0001$	$P < 0{\cdot}0001$	$P > 0{\cdot}98$	$P > 0{\cdot}99$	$P < 0{\cdot}1$

**P*-value for the null hypothesis that the frequency of a species was not statistically different between sample sources.
†The proportions of *E. faecalis* relative to the proportions of *E. faecium* were not significantly different from any of the three sources.

Table 3 Antimicrobial susceptibility of *Enterococcus* isolates according to source of isolation

Antimicrobial	Source of isolation			
	Cattle ($n = 165$)	Waste ($n = 38$)	Humans ($n = 227$)	*P*-value*
Amp				
R	1 (0·6)†	3 (7·9)	12 (5·3)	<0·01
I/S	164 (99·4)	35 (92·1)	215 (94·7)	
C				
R	6 (3·6)	3 (7·9)	22 (9·7)	0·10
I/S	159 (96·4)	35 (92·1)	205 (90·3)	
CN				
R	97 (58·8)	20 (52·6)	120 (52·9)	0·01
I/S	68 (41·2)	18 (47·4)	107 (47·1)	
E				
R	40 (24·2)	8 (21·1)	112 (49·3)	<0·01
I/S	125 (75·8)	30 (78·9)	115 50·7)	
ENR				
R	60 (36·4)	10 (26·3)	102 (44·9)	0·08
I/S	105 (63·6)	28 (73·7)	125 (55·1)	
RD				
R	99 (60·0)	30 (78·9)	179 (78·9)	<0·01
I/S	66 (40·0)	8 (21·1)	48 (21·1)	
SXT				
R	38 (23·0)	5 (13·2)	24 (10·6)	0·02
I/S	127 (77·0)	33 (86·8)	203 (89·4)	
TE				
R	66 (40·0)	8 (21·1)	59 (26·0)	0·01
I/S	99 (60·0)	30 (78·9)	168 (74·0)	
VA				
R	6 (3·6)	3 (7·9)	59 (26·0)	0·01
I/S	159 (60·0)†	35 (92·1)†	168 (74·0)†	

AMP, ampicillin; C, chloramphenicol; CN, gentamycin; E, erythromycin; ENR, enrofloxacin; RD, rifampicin; SXT, sulphamethoxazole; TE, tetracycline; VA, vancomycin; I/S, intermediate or susceptible; R, resistance.
**P*-value for the null hypothesis that the frequency of resistance is the same in all sources.
†Figures in brackets represent the percentage of isolates with this trait.

more prevalent than *E. faecium* in samples from all sources, however, statistically the differences were not significant. The results agree with other authors, who have reported *E. faecium* and *E. faecalis* to be the most common from mammals (Sreeja *et al.* 2012; Castillo-Rojas *et al.* 2013). Fisher and Phillips (2009) reported CFUs of *E. faecalis* and *E. faecium* in humans ranging between 10^5 and 10^7 CFU per gram and 10^4 to 10^5 CFU per gram, respectively, which are in good agreement with the results of the current study. However, the finding that *E. faecalis* was the most prevalent in the samples contrasts other published works from Tanzania. Moyo *et al.* (2010) and Aamodt *et al.* (2015) reported higher prevalence of *E. faecium* than *E. faecalis* in clinical isolates from humans, and Katakweba *et al.* (2015) showed a higher prevalence of *E. faecium* than *E. faecalis* in cattle and wildlife faecal samples. The findings are likely all reliable, since the technique used to characterize *Enterococcus* to species level has been shown to be accurate (Dutka-Malen *et al.* 1995; Layton *et al.* 2010). Hence, the differences may be related to the specific samples, and it is indicated to investigate how the distribution of *Enterococcus* species depends upon external and demographic factors, such as food/feeding, age and gender.

Antimicrobial resistance patterns in *Enterococcus* sp

Multiple drug resistance (MDR) to curative antimicrobial agents used in human medicine and food animals was higher in human isolates than in cattle isolates. The MDR finding is common in *Enterococcus* species and has been documented by other workers in various samples; Harwood *et al.* (2000) described MDR in *Enterococcus* sp. in samples collected from wild birds, cattle, chickens, dogs, pigs and raccoons. Diarra *et al.* (2010) quantified *Enterococcus* sp. and the MDR patterns in poultry faecal samples, whereas Novais *et al.* (2013) established MDR patterns in faeces sampled from pigs which were treated with antibiotics. Similarly, Sreeja *et al.* (2012) characterized the antibiogram in *Enterococcus* isolates from clinical samples from hospitals, and Katakweba *et al.* (2015) demonstrated MDR in faecal samples collected from wildlife ruminants. In the current study, most of the

Table 4 Antimicrobial susceptibility of *Enterococcus* isolates per species

	Species					
Antimicrobial	*E. faecalis*	*E. faecium*	*E. gallinarum*	*E. avium*	Other sp.	*P*-value*
Amp						
R	6 (3)†	7 (4)	1 (7)	1 (9)	1 (2)	0·76
I/S	181 (97)	158 (96)	13 (93)	10 (91)	52 (98)	
C						
R	10 (5)	15 (6)	3 (21)	1 (9)	2 (4)	0·07
I/S	177 (95)	150 (94)	11 (79)	10 (91)	51 (96)	
CN						
R	99 (53)	97 (59)	11 (79)	6 (55)	24 (45)	0·16
I/S	88 (47)	68 (41)	3 (21)	5 (45)	29 (55)	
E						
R	64 (34)	62 (38)	11 (79)	4 (36)	19 (36)	0·13
I/S	123 (66)	103 (62)	3 (21)	7 (64)	34 (64)	
ENR						
R	76 (41)	71 (43)	6 (43)	3 (27)	16 (30)	0·45
I/S	11 (59)	94 (57)	8 (57)	8 (73)	37 (70)	
RD						
R	135 (72)	118 (72)	12 (86)	5 (45)	38 (72)	0·08
I/S	52 (28)	47 (28)	2 (14)	6 (55)	15 (28)	
SXTSXT						
R	30 (16)	23 (14)	2 (14)	1 (9)	11 (21)	0·48
I/S	157 (84)	142 (86)	12 (86)	10 (91)	42 (79)	
TE						
R	56 (30)	59 (36)	05 (36)	2 (18)	11 (21)	0·26
I/S	131 (70)	106 (64)	09 (64)	9 (82)	42 (79)	
VA						
R	29 (16)	39 (24)	02 (14)	0 (0)	2 (4)	0·12
I/S	158 (84)	126 (76)	12 (86)	11 (100)	51 (96)	

AST, antimicrobial sensitivity test; St, status; AMP, ampicillin; C, chloramphenicol; CN, gentamycin; E, erythromycin; ENR, enrofloxacin, RD, rifampicin; SXT, sulphamethoxazole; TE, tetracycline; VA, vancomycin; I/S, intermediate or susceptible; R, resistance.
**P*-value for the null hypothesis that the frequency of this species was not statistically different between sample sources.
†The numbers in brackets are in percentages.

MDR isolates belonged to *E. faecalis* and *E. faecium*. Strains of these two species seem to maintain relative high levels of resistance and to be able to spread these traits to members of the same bacterial species or to other bacterial species through mobile genetic elements (Byappanahalli *et al.* 2012).

The susceptibility analysis demonstrated resistance to low concentrations of CN. With the concentration used in this study, more than 50% of the isolates were resistant, a finding which has also been reported by others (Sreeja *et al.* 2012). The *Enterococcus* species are intrinsically resistance to low concentration of aminoglycosides, leading to lower concentration of the drug in the intracellular space. This mechanism has been described to be chromosomally mediated due to genes encoding modification to the binding site of the antibiotic (Chow 2000; Miller *et al.* 2014).

It was further shown that the isolates, irrespective of source, were commonly resistant to RD. This observation is surprising, since the drug is strictly prescribed in humans against *Mycobacterium* infections (Enne *et al.* 2004). However, resistance to this drug in *Enterococcus* isolates has been reported to be widespread. Deshpande *et al.* (2007) reported RD resistance to be 65·9 and 67·5% of *E. faecium* isolates in United States and Europe respectively. The mechanism of RD resistance in *Enterococcus* is poorly described, but it has been suggested to be caused by mutation of the *rpoB* gene. This gene encodes the β-subunit of the RNA polymerase. Although RD is strictly prescribed in humans, the results showed that cattle isolates were commonly resistant to the drug; these findings could be explained by either transfer of human isolates to cattle, irregular use of drug RD for treatment of infections in cattle or a coselection pressure on the bacteria for other reasons.

Prevalence of VRE genes in the isolates

Enterococcus faecium is increasingly feared due to its ability to harbour and express VRE genes (Zhang *et al.*

2016). In the current study, *vanA* and *vanB* genes were detected more frequently in human isolates than in cattle isolates and isolates from cattle waste. The initial screening test for VRE in this study was the Kirby disc diffusion, which has been described to be less sensitive to quantify and qualify VRE strains (Sreeja *et al.* 2012; Vidyasagar *et al.* 2012). This might have caused false positives leading to high number of resistant and intermediate resistant isolates according to the phenotypic test, compared to the much lower prevalence detected by PCR. Nevertheless, the detection of VRE genes in nonhuman samples, as in the current study, should alert risk managers in Tanzania.

The VRE resistance has been described as being controlled either at chromosomal level (intrinsic), which is a finding that has been mostly described in *E. gallinarum,* or acquired through mutation and exogenous gene transfer as it occurs in *E. faecium* and *E. faecalis* (Willems *et al.* 2005; Aamodt *et al.* 2015; Akova, 2016). However, this work has demonstrated VRE genes in *E. faecium* and *E. faecalis* and not in *E. gallinarum.* The inducible *vanA* gene is commonly detected in *E. faecium.* It has crossed species boundaries, and it is now detected even in methicillin-resistant *Staphylococcus aureus*. This is a main public health concern, since vancomycin is currently a last resort drug in this species (Willems *et al.* 2005; Hayakawa *et al.* 2013). The *vanA*-type has been reported to be spread universally in a natural environment causing difficulty in determining its distribution and targeted source of isolation in the field (Gastmeier *et al.* 2014). The *vanB* gene is reported to induce relatively moderate resistance in all species of *Enterococcus*, whereas *vanC-1* is associated with an intrinsic resistance mechanism in *E. gallinarum* and *E. casseliflavus*, but is less common in clinical isolates (Courvalin 2004). The presence of VRE strains in the nonhuman samples in this study suggests a complex epidemiology. Further studies to understand the spread of such strains in Tanzania is warranted. This is because the vancomycin analogy, avoparcin, has not been licensed for use as growth promoter in animal feeds in Tanzania (Katakweba *et al.* 2012).

Summing up, the results of the present study have demonstrated that resistance levels are moderate in *Enterococcus* species from cattle and cattle waste in Tanzania compared to isolates from humans, and that VRE can be detected in nonhuman sources, despite avoparcin being not licensed for growth promotion in Tanzania. This finding together with a high number of strains isolated from cattle with RD resistance suggest that humans may actually transfer resistant strains to livestock, a conclusion that was also reached by Katakweba *et al.* (2015), when studying *Enterococc*us sp. from wildlife living in contact with livestock.

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Conflict of Interest

The authors declare that there is no conflict of interest.

References

Aamodt, H., Mohn, S.C., Maselle, S., Manji, K.P., Willems, R., Jureen, R., Langeland, N. and Blomberg, B. (2015) Genetic relatedness and risk factor analysis of ampicillin-resistant and high-level gentamicin-resistant *Enterococci* causing bloodstream infections in Tanzanian children. *BMC Infect Dis* **15**, 107.

Akova, M. (2016) Epidemiology of antimicrobial resistance in bloodstream infections. *Virulence* **7**, 252–266. Available at: http://dx.doi.org/10.1080/21505594.2016.1159366.

Arias, C.A. and Murray, B.E. (2012) The rise of the *Enterococcus*: beyond vancomycin resistance. *Nat Rev Microbiol* **10**, 266–278.

Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turck, M. (1966) Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* **45**, 493–496.

Byappanahalli, M.N., Nevers, M.B., Korajkic, A., Staley, Z.R. and Harwood, V.J. (2012) *Enterococcus* in the environment. *Microbiol Mol Biol Rev* **76**, 685–706.

Castillo-Rojas, G., Mazari-Hiríart, M., Ponce de León, S., Amieva-Fernández, R.I., Agis-Juárez, R.A., Huebner, J. and López-Vidal, Y. (2013) Comparison of *Enterococcus faecium* and *Enterococcus faecalis* strains isolated from water and clinical samples: antimicrobial susceptibility and genetic relationships. *PLoS ONE* **8**, 59491.

Chow, J.W. (2000) Special Section: Antimicrobial Resistance Aminoglycoside Resistance in Enterococci. *Clin. Infect. Dis* **31**, 586–589.

Courvalin, P. (2004) Vancomycin resistance in gram-positive cocci. *Clin Infect Dis* **24**, S25–S34.

Deshpande, L.M., Fritsche, T.R., Moet, G.J., Biedenbach, D.J. and Jones, R.N. (2007) Antimicrobial resistance and molecular epidemiology of vancomycin- resistant

enterococci from North America and Europe: a report from the SENTRY antimicrobial surveillance program. *Diagn Microbiol Infect Dis* **58**, 163–170.

Devriese, L.U.C., Baele, M. and Butaye, P. (2006) The genus Enterococcus: taxonomy. *Prokaryotes* **4**, 163–174.

Diarra, M.S., Rempel, H., Champagne, J., Masson, L., Pritchard, J. and Topp, E. (2010) Distribution of antimicrobial resistance and virulence genes in *Enterococcus* spp. and characterization of isolates from broiler chickens. *Appl Environ Microbiol* **76**, 8033–8043.

Dutka-Malen, S., Evers, S. and Courvalin, P. (1995) Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant *Enterococci* by PCR. *J Clin Microbiol* **33**, 24–27.

Enne, V.I., Delsol., A.A., Roe, J.M. and Bennett, P.M. (2004) Rifampicin resistance and its fitness cost in *Enterococcus faecium*. *J Antimicrob Chemother* **5353**, 203–207.

EUCAST. (2015) European committee on antimicrobial susceptibility testing. Available at http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST. 0–77 pp.

Fisher, K. and Phillips, C. (2009) The ecology, epidemiology and virulence of *Enterococcus*. *Microbiology* **155**, 1749–1757.

Furtula, V., Jackson, C.R., Farrell, E.G., Barrett, J.B., Hiott, L.M. and Chambers, P.A. (2013) Antimicrobial resistance in *Enterococcus* spp. isolated from environmental samples in an area of intensive poultry production. *Int J Environ Res Public Health* **10**, 1020–1036.

Gastmeier, P., Schroder, C., Behnke, M., Meyer, E. and Geffers, C. (2014) Dramatic increase in vancomycin-resistant *Enterococcus* in Germany. *J Antimicrob Chemother* **69**, 1660–1664.

Harwood, V.J., Whitlock, J. and Withington, V. (2000) Classification of Antibiotic Resistance Patterns of Indicator Bacteria by Discriminant Analysis : Use in Predicting the Source of Fecal Contamination in Subtropical Waters Classification of Antibiotic Resistance Patterns of Indicator Bacteria by Discrimin. *Appl. Environ. Microbiol* **66**, 3698–3704.

Harwood, V.J., Delahoya, N.C., Ulrich, R.M., Kramer, M.F., Whitlock, J.E., Garey, J.R. and Lim, D.V. (2004) Molecular confirmation of Enterococcus faecalis and E. faecium from clinical, faecal and environmental sources. *Lett Appl Microbiol* **38**, 476–482.

Harwood, V.J., Levine, A.D., Scott, T.M., Chivukula, V., Lukasik, J., Farrah, S.R. and Rose, J.B. (2005) Validity of the indicator organism paradigm for pathogen reduction in reclaimed water and public health protection. *AEM* **71**, 3163–3170.

Hayakawa, K., Marchaim, D., Palla, M., Gudur, U.M., Pulluru, H., Bathina, P., Alshabani, K., Govindavarjhulla, A. *et al.* (2013) Epidemiology of vancomycin-resistant *Enterococcus faecalis*: a case-case-control study. *Antimicrob Agents Chemother* **57**, 49–55.

Ishihama, A., Kori, A., Koshio, E., Yamada, K., Maeda, H., Shimada, T., Makinoshima, H., Iwata, A. *et al.* (2014) Intracellular concentrations of 65 species of transcription factors with known regulatory functions in Escherichia coli. *J. Bacteriol* **196**, 2718–2727.

ISO (2004) Microbiology of food and animal feeding stuffs. *Nat Lang Eng* **10**, 211–225.

Jackson, C.R., Fedorka-Cray, P.J. and Barrett, J.B. (2004) Use of a genus- and species-specific multiplex PCR for identification of *Enterococcus*. *J Clin Microbiol* **42**, 3558–3565.

Katakweba, A.A.S., Mtambo, M.M.A., Olsen, J.E. and Muhairwa, A.P. (2012) Awareness of human health risks associated with the use of antibiotics among livestock keepers and factors that contribute to selection of antibiotic resistance bacteria within livestock in Tanzania. *LRRD* **24**, 170.

Katakweba, A.A., Møller, K.S., Muumba, J., Muhairwa, A.P., Damborg, P., Rosenkrantz, J.T., Minga, U.M., Mtambo, M.M. *et al.* (2015) Antimicrobial resistance in faecal samples from buffalo, wildebeest and zebra grazing together with and without cattle in Tanzania. *J Appl Microbiol* **118**, 966–975.

Lawrence, J.G. (2005) Horizontal and vertical gene transfer: the life history of pathogens. *Contrib Microbiol* **12**, 255–271.

Layton, B.A., Walters, S.P., Lam, L.H. and Boehm, A.B. (2010) *Enterococcus* species distribution among human and animal hosts using multiplex PCR. *J Appl Microbiol* **109**, 539–547.

Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J.F. *et al.* (2011) Multidrug-resistance, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* **18**, 268–281.

Manero, A. and Blanch, A.R. (1999) Identification of *Enterococcus* spp. with a biochemical key. *AEM* **65**, 4425–4430.

Miller, W.R., Munita, J.M. and Arias, C.A. (2014) Mechanisms of antibiotic resistance in enterococci. *Expert Rev. Anti-infect. Ther* **12**, 1221–1236.

Moyo, S.J., Aboud, S., Kasubi, M. and Maselle, S.Y. (2010) Bacterial isolates and drug susceptibility patterns of urinary tract infection among pregnant women at Muhimbili National Hospital in Tanzania. *Tanzan J Health Res* **12**, 236–240.

Murray, P.R. (2010) Matrix-assisted laser desorption ionization time-of-flight mass spectrometry: usefulness for taxonomy and epidemiology. *Clin Microbiol Infect* **16**, 1626–1630.

Novais, C., Freitas, A.R., Silveira, E., Antunes, P., Silva, R., Coque, T.M. and Peixe, L. (2013) Spread of multidrug-resistant *Enterococcus* to animals and humans: an underestimated role for the pig farm environment. *J Antimicrob Chemother* **68**, 2746–2754.

Sreeja, S., Sreenivasa, B.P.R. and Prathab, A.G. (2012) The prevalence and the characterization of the *Enterococcus* species from various clinical samples in a tertiary care hospital. *JCDR* **6**, 1486–1488.

Vidyasagar, K., Nandan, T. M. and Ravikumar, R. (2012) Detection of vancomycin resistant enterococci (VRE) in hospitalized patients and comparision of Kirby-Bauer disc diffusion and vancomycin screen agar method. *JEMDS* **1**, 882–887.

Willems, R.J., Top, J., van Santen, M., Robinson, D.A., Coque, T.M., Baquero, F., Grundmann, H. and Bonten, M.J. (2005) Global spread of vancomycin-resistant *Enterococcus faecium* from distinct nosocomial genetic complex. *Emerg Infect Dis* **11**, 821–828.

Zhang, J., Wang, Y.Q., Li, J., Ma, Y.F., Lin, J., Xiao, Z.-R. and Yu, D.-J. (2016) Phenotypic and genotypic analysis of vancomycin-resistant enterococci strains isolated from different water sources. *AJMR* **8**, 8–13.

Zhong, Z., Zhang, W., Song, Y., Liu, W., Xu, H., Xi, X., Menghe, B., Zhang, H. *et al.* (2017) Comparative genomic analysis of the genus *Enterococcus*. *Microbiol Res* **196**, 95–105.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Number of resistance in *Enterococcus* sp. as per source of isolation and *Enterococcus* species.

Table S2 Antimicrobial resistance patterns of *E. faecalis*, *E. faecium*, *E. avium*, *E. gallinarum* and other *Enterococcus* species from cattle, cattle waste and animal waste attendants.