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Antibiotic Resistance Genes and Antibiotic Susceptibility of Oral *Enterococcus faecalis* Isolates Compared to Isolates from Hospitalized Patients and Food

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Abstract

Enterococcus faecalis, a commensal of the intestinal tract of humans and animals is of great significance as leading opportunistic pathogen, and also prevalent in oral diseases, such as endodontic infections, as well as the healthy oral cavity. To investigate the potential of oral E. faecalis to constitute a reservoir of antibiotic resistance, isolates from supragingival plaque/saliva and from endodontic infections were screened regarding their resistance to selected antibiotics in comparison to nosocomial and food isolates.

70 E. faecalis isolates were analyzed with PCR regarding their equipment with the resistance genes tetM, tetO, ermB, ermC, vanA, vanB and blaTEM. Additionally, they were tested for their phenotypic resistance to doxycycline, azithromycin, rifampicin, amoxicillin and streptomycin using the Etest.

High percentages of the plaque/saliva, nosocomial and food isolates were resistant to doxycycline and azithromycin, particularly plaque/saliva isolates (81%) and nosocomial isolates (73.3%) showed resistance to doxycycline, significantly more than among the food and endodontic isolates. Rifampicin resistance was widespread among isolates from plaque/saliva (52.4%), endodontic infections (50%) and nosocomial infections (40%); all isolates were susceptible to amoxicillin and all oral isolates to high-level streptomycin.

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TetM genes were detected in the majority of all isolates and ermB genes were present in many nosocomial and plaque/saliva isolates. Thirty percent of the endodontic isolates and 53% of the nosocomial isolates were equipped with blaTEM genes.

The results suggest that the oral cavity can harbor E. faecalis strains with multiple resistances against different antibiotics and thus be regarded as a potential source of resistance traits.

Keywords

Antibiotic resistance · Endodontic infections · Enterococci · Food · Nosocomial infections · Oral cavity · Plaque

1 Introduction

Enterococcus faecalis is of great importance as a leading opportunistic pathogen causing nosocomial infections (Arias and Murray 2012). Frequent infections include endocarditis, meningitis, urinary tract infections, wound infections and neonatal infections (Murray 1990). Furthermore, this bacterial species is of significance for the field of oral diseases. Even though *E. faecalis* is normally only found in low numbers in oral sites of healthy individuals (Portenier et al. 2003), its prevalence in the oral cavity substantially increases in many oral diseases, e.g. gingivitis, periodontitis, caries, endodontic infections and especially post-treatment apical periodontitis, where it is considered a main pathogen associated with endodontic failure (Dahlen et al. 2000; C. M. Sedgley et al. 2005b; C. Sedgley et al. 2006; Souto and Colombo 2008; Sun et al. 2009; Kouidhi et al. 2011; Anderson et al. 2013).

What contributes to its relevance as an opportunistic pathogen is the fact that *E. faecalis* is intrinsically resistant to several antibiotics and can harbor different acquired resistance traits (Van Tyne and Gilmore 2014). Despite its pathogenic potential, *E. faecalis* is typically found as a commensal in the gastrointestinal tract of humans and many animals (Arias and Murray 2012). Specific strains have been used as probiotics and some strains are found in food where they are responsible e.g. for the ripening of certain cheeses (Fisher and Phillips 2009; Franz et al. 2011; Hammerum 2012).

Its proficiency in efficiently acquiring and spreading genetic elements via horizontal gene transfer as well as its common ability to form biofilms have been well characterized for *E. faecalis* (Paulsen et al. 2003; Duggan and Sedgley 2007; Manson et al. 2010; Palmer et al. 2010; Paganelli et al. 2012) . Our group was able to demonstrate that *E. faecalis* originating from cheese is able to integrate into the oral biofilm *in vivo* (Al-Ahmad et al. 2010) and recently authors confirmed that *E. faecalis* can colonize a multi-species biofilm in a supragingival biofilm model (Thurnheer and Belibasakis 2015). These findings highlight the possibility of the oral cavity to constitute a reservoir for the antibiotic resistance genes of *E. faecalis* as well as other traits of clinical concern that could be spread within the oral biofilm. The information on antibiotic susceptibility characteristics of oral *E. faecalis* isolates is scarce apart from studies of endodontic isolates. In a previous study we investigated the virulence factors as well as the capacity for biofilm formation and susceptibility to some antibiotics of the *E. faecalis* isolates from different sources with a focus on the biofilm formation in association with virulence factors (Anderson et al. 2015). The aim of the present study was to take this analysis further focusing on selected antibiotic resistance genes as well as additional relevant phenotypic resistance to assess whether these strains can represent a reservoir for antibiotic resistance traits. For the determination of the antibiotic susceptibility, widely used phenotypic tests as well as PCR

were applied (Amsler et al. 2010; Jorgensen and Ferraro 2009; Tenover et al. 1996), which give an insight into the bacterial strains' equipment with resistance traits. This is advantageous in the attempt to determine if strains possessed genes that could be spread even without expressing the genes themselves.

2 Materials and Methods

2.1 Bacterial Isolates

A total of 70 *E. faecalis* strains, 20 isolates from endodontic retreatment, 21 oral isolates (saliva and supragingival plaque), 14 food isolates (raw milk) and 15 isolates from nosocomial infections (9 urinary tract infections, 1 wound infection, 1 intraoperative swab, 1 drainage secretion, 1 intraabdominal aspirate, 1 blood culture and 1 central venous catheter) were analyzed for their antibiotic susceptibility. The oral and endodontic isolates were gathered from 2011–2014 in the Department of Operative Dentistry and Periodontology (Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Germany). The plaque and saliva samples were taken from healthy individuals with the following exclusion criteria: no serious illness, no use of antibiotics until 6 months prior to the study, no pregnancy or lactation, healthy oral status and absence of carious lesions. The food isolates from raw milk samples from different cows were received in 2014 from the Bavarian Health and Food Safety Authority (Oberschleißheim, Germany) and the isolates from nosocomial infections were obtained from patients in 2013 from the Department of Medical Microbiology and Hygiene (Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Germany). All endodontic and clinical isolates were obtained after approval by the Ethics Committee (no. 140/09, University of Freiburg) and the collection of the endodontic samples followed the protocol from Schirrmeister et al. (2007). Prior to the antibiotic susceptibility testing, the isolates were confirmed to be *E. faecalis* by amplification of a species-specific 16S rDNA fragment (Table 1). The following strains were used as reference strains: *Klebsiella pneumoniae* 1230 and *Enterobacter cloacae* 472 (containing the bla_{TEM} gene), *Enterococcus faecium* 401, *E. faecium* 403 and *E. faecium* 643 (containing the *ermB*-gene), *Staphylococcus aureus* 2250, *S. aureus* 2223, *S. aureus* 4331 (containing the *ermC*-gene), *E. faecium* 633 and *E. faecium* 643 (containing the *vanA*-gene), *E. faecalis* V583, *E. faecium* BM4524, *E. faecium* 401 (containing the *vanB*-gene), *E. faecium* 633 (containing the *tetM*-gene) and a bacterial isolate from sewage MG (containing the *tetO*-gene). All reference strains were kindly provided by Prof. Daniel Jonas (Institute for Environmental Health Sciences and Hospital Infection Control, Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Germany).

2.2 Antibiotic Susceptibility Testing with the Etest

The Etest (Liofilchem, Roseto degli Abruzzi, Italy) was used to test the susceptibility of all the *E. faecalis* isolates to the following antibiotics: Doxycycline, azithromycin, amoxicillin, rifampicin and high-level streptomycin. The method was conducted according to the manufacturer's protocol as described earlier (Al-Ahmad et al. 2014). Specifically, material from pure colonies was taken from an overnight culture, suspended in sterile NaCl-solution (0.9%) to reach an inoculum turbidity of McFarland 0.5. Each sample was streaked on Mueller-Hinton agar plates and one Etest strip was placed on each agar plate using sterile tweezers. The results were interpreted as indicating

Table 1 Primers used for the detection of different antibiotic resistance genes of *E. faecalis* by PCR

Target	Primer	Primer sequence (5′–3′)	Amplicon size [bp]	References
E. faecalis	Efaec-F	GTTTATGCCGCATGGCATAAGAG	310	Siqueira and Rocas (2004)
	Efaec-R	CCGTACGGGGACGTTCAG		
blaTEM	blaTEM f	CCAATGCTTAATCAGTGAGG	858	Call et al. (2003)
	blaTEM r	ATGAGTATTCAACATTTCCG		
tetM	tetMf	AGTTTTAGCTCATGTTGATG	1862	Perez-Trallero et al. (2007)
	tetMr	TCCGACTATTTGGACGACGG		
tetO	tetO-f	GCGGAACATTGCATTTGAGGG	538	Perez-Trallero et al. (2007)
	tetO-r	CTCTATGGACAACCCGACAGAAG		
ermB	ermB-f	GAAAAGGTACTCAACCAAATA	639	Reinert et al. (2008)
	ermB-r	AGTAACGGTACTTAAATTGTTTAC		
ermC	ermC-f	AATCGGCTCAGGAAAAGG	562	Perreten et al. (2005)
	ermC-r	ATCGTCAATTCCTGCATG		
vanA	$vanA_1$	GGGAAAACGACAATTGC	732	Dutka-Malen et al. (1995)
	$vanA_2$	GTACAATGCGGCCGTTA		
vanB	vanB-B3-f	ACGGAATGGGAAGCCGA	647	Depardieu et al. (2004)
	vanB-B4-r	TGCACCCGATTTCGTTC		

Table 2 MIC reference values for *E. faecalis* strains for antimicrobial agents tested

Antimicrobial agent	MIC[a] (μg/mL)			References
	S[b]	I[b]	R[b]	
Amoxicillin	≤4		≥8	EUCAST (2016)[c]
Doxycycline	≤4	8	≥16	CLSI (2013)[c]
Rifampicin	≤1	2	≥4	CLSI (2013)[c]
Azithromycin	≤2		≥8	Fass (1993)
Streptomycin HL-R[d]			>512	EUCAST (2016)[c]

[a]MIC = Minimum inhibitory concentration
[b]S = Susceptible, I = Intermediate, R = Resistant
[c]CLSI Clinical and Laboratory Standard Institute, EUCAST European Committee on Antimicrobial Susceptibility Testing
[d]High-level Resistance

susceptible, intermediate or resistant categories according to the EUCAST (The European Committee on Antimicrobial Susceptibility Testing) breakpoints and, where EUCAST values were not available, to the CSLI (Clinical and Laboratory Standards Institute), both listed in Table 2 (EUCAST 2016; CLSI 2013). If these standards were not available, minimal inhibitory concentration (MIC) values were compared with values for similar strains in literature.

3 DNA Isolation

Material from pure cultures was used to extract total bacterial DNA with the DNeasy Blood and

Tissue Kit (Qiagen, Hilden, Germany). The DNA extraction was performed according to the manufacturer's protocol for Gram-positive bacteria. The DNA was eluted with 200 µl AE buffer (Qiagen) and stored at −20 °C.

3.1 PCR for the Detection of *E. faecalis* Antibiotic Resistance Genes

The isolated DNA was used as a template for the amplification of nine different antibiotic resistance genes from *E. faecalis*. The primers, annealing temperatures and corresponding references are listed in Table 1. To amplify the different fragments, initial denaturation was performed at 94 °C for 5 min, followed by 35 cycles with denaturation at 94 °C for 60 s, varying annealing times (Table S1), extension at 72 °C for 60s and a final extension at 72 °C for 10 min. The primer concentration, template amount and annealing temperature varied for the different PCR systems, therefore all respective information is listed in the supplementary material (Table S1). The amplification was performed in a total volume of 25 µl and all reaction mixtures contained 1x PCR buffer (Qiagen), 0.2 mM each of the four deoxyribonucleoside triphosphates (dNTPs; PEQLAB, Erlangen, Germany) and 2.5 U Taq-Polymerase (Qiagen) and the specific amount of forward and reverse primers as well as template DNA. A no-template control and a positive control were included in each set of PCR reactions. The amplified products were visualized by gel electrophoresis using a 1% agarose gel.

4 Statistical Analysis

The correlation of the antibiotic resistance genes and the phenotypic resistance characteristics with the respective origin of the *E. faecalis* isolates was analyzed using the Fisher's exact test and pairwise comparisons were performed with the chi-square test with Bonferroni correction. The level of significance was 0.05.

5 Results

A total of 70 *E. faecalis* isolates from four different origins (endodontic, plaque/saliva, food and nosocomial isolates) were analyzed for the presence of nine antibiotic resistance genes and for their antibiotic susceptibility to five different antibiotics.

5.1 Phenotypic Antimicrobial Susceptibility of *E. faecalis* Isolates

Table S2 (Supplementary material) shows the MIC values for the tested antibiotic agents for all isolates in detail and additionally includes the resistance phenotype analyzed by VITEK in a prior study (Anderson et al. 2015). All tested isolates were susceptible to amoxicillin. As shown in Fig. 1, a high percentage of the plaque/saliva isolates from healthy individuals (81.0%), of the food (78.6%) and the nosocomial isolates (73.33%) were resistant against doxycycline, as well as against azithromycin (81.0%; 85.6% and 86.7% resp.). Rifampicin resistance was detected in about half the plaque/saliva and endodontic isolates (52.4% and 50%), in 40% of the nosocomial isolates and in a lower percentage of the food isolates (14.3%). While none of the oral isolates showed resistance against high-level streptomycin, 46.7% of the nosocomial and 35.7% of the food isolates showed resistance.

5.2 Multidrug-Resistance among *E. faecalis* Isolates from Different Origins

According to the classification recommendations suggested by the European Center for Disease Prevention and Control (ECDC) only Enterococci resistant to ≥1 agent in ≥3 antimicrobial categories relevant for these species (Magiorakos et al. 2012) can be determined as multidrug-resistant (MDR). In our study this would correspond to a combined resistance

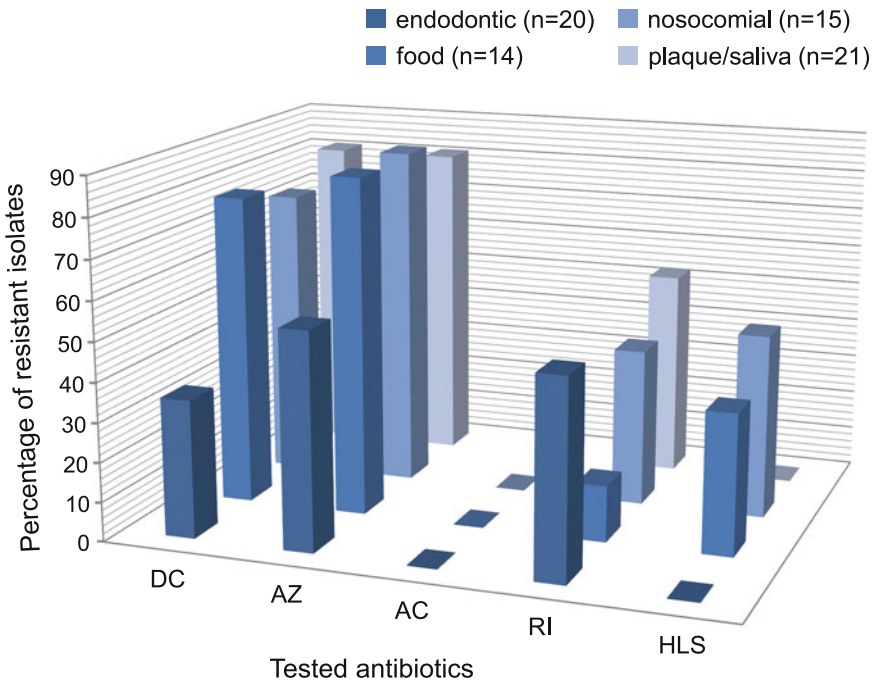

Fig. 1 Phenotypic resistance of *E. faecalis* isolates from food, secondary endodontic infections, plaque/saliva and nosocomial infections analyzed by Etest (*DC* doxycycline, *AZ* azithromycin, *AC* amoxicillin, *RI* rifampicin, *HLS* high-level streptomycin)

against high-level gentamicin, high-level streptomycin, ciprofloxacin/levofloxacin, vancomycin, linezolid and doxycycline. According to these guidelines, several isolates of the nosocomial infections (sample nr. 110053, 109891, 512359, resistant to doxycycline, high-level streptomycin, high-level gentamicin and levofloxacin/ciprofloxacin as well as 109898 and 512176, resistant to high-level streptomycin, high-level gentamicin and levofloxacin/ciprofloxacin; this study and (Anderson et al. 2015)), can be classified as multidrug-resistant. Still, many of the other tested isolates showed resistance to more than one antibiotic. Several other nosocomial isolates showed resistance to doxycycline and high-level gentamicin or high-level gentamicin and levofloxacin/ciprofloxacin. Nine plaque/saliva isolates showed combined resistance against doxycycline and high-level gentamicin and one endodontic isolate showed resistance to doxycycline and linezolid.

5.3 Distribution of Antibiotic Resistance Genes in *E. faecalis* Isolates

Figure 2 shows the percentage of the detected resistance genes in the *E. faecalis* isolates. The *tetM* gene was present in isolates from all four origins in high percentages, i.e. 65.0%, 80.0%, 86.7% and 90.5% of the endodontic, nosocomial, food and plaque/saliva isolates respectively, while other tetracycline resistance genes (*tetO*) were not detected. The *ermB* gene was detected in many nosocomial isolates (60%) and in 47.6% and 26.6% of the plaque/saliva and food isolates respectively. Bla_{TEM} genes were found primarily in nosocomial infection isolates (53.3%), but also 30.0% of the endodontic isolates and 13.3% of the food isolates harbored these genes. *ErmC* genes and genes for the resistance to vancomycin were not present in any of the isolates.

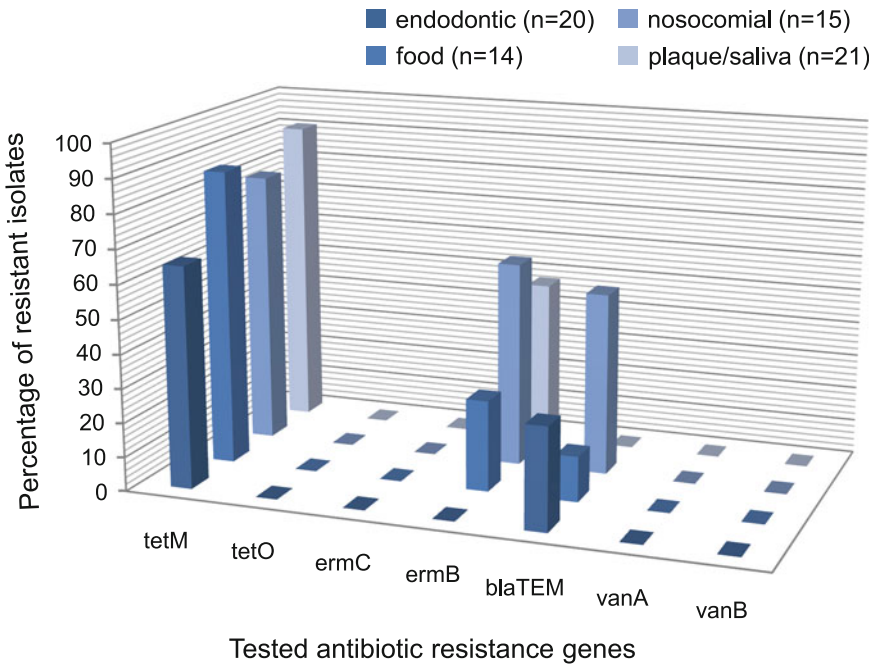

Fig. 2 Prevalence of resistance genes detected by PCR in *E. faecalis* isolates from food, secondary endodontic infections, plaque/saliva and nosocomial infections

5.4 Statistical Results – Significant Associations of Detected Resistance and the Origin of the *E. faecalis* Isolates

The analysis of possible correlations between the antibiotic resistance results and the origin of the isolates revealed that the presence of the *ermB* gene correlated with the origin ($p \leq 0.001$), 60% of the nosocomial isolates and 47% of the plaque/saliva isolates were found positive for this gene, whereas none of the endodontic and only 26.5% of the food isolates were positive for it. The bla_{TEM} gene also correlated with the origin ($p \leq 0.001$), 53.3% of the nosocomial isolates possessed this trait and 30% of the endodontic isolates, yet only 13.3% of the food and none of the plaque/saliva isolates. Phenotypic doxycycline resistance correlated with the origin ($p \leq 0.01$), it was widespread in food, nosocomial and plaque/saliva isolates (78.6%, 73.3%, 81.0%), yet only few endodontic isolates (35%) showed resistance. Similarly, high-level streptomycin resistance correlated with the origin of the isolates ($p \leq 0.001$), food and nosocomial isolates were frequently resistant (35.7% and 46.7% respectively), whereas all oral isolates were susceptible.

The pairwise comparison of selected traits showed significantly more doxycycline-resistant isolates from plaque/saliva and from food than from endodontic infections ($p \leq 0.01$ and $p = 0.036$ resp.). Also, significantly more food isolates and nosocomial isolates were resistant to high-level streptomycin than endodontic and plaque/saliva isolates ($p = 0.006$ / $p = 0.006$ and $p \leq 0.001$ / $p \leq 0.001$ resp.) In addition, significantly more isolates from nosocomial infections harbored the bla_{TEM} gene than isolates from endodontic infections ($p = 0.04$).

6 Discussion

Especially since the 1990s, *Enterococcus faecalis* has emerged as a leading nosocomial pathogen and has been shown to have the ability to acquire and spread resistance genes readily (Arias and Murray 2012). However, the role of the oral cavity as a potential reservoir for resistant *E. faecalis* has not been clarified yet. Therefore this study reports the antibiotic susceptibility and antibiotic resistance genes of isolates from supragingival plaque and saliva of healthy individuals and of endodontic infections in comparison to isolates from nosocomial infections and food. Notably the plaque/saliva isolates stand out regarding their equipment with tetracycline resistance genes (*tetM*) and erythromycin resistance genes (*ermB*) which are comparable to the nosocomial isolates (90.5% versus 80% and 47.6% versus 60.0% respectively). Phenotypic azithromycin resistance of the plaque/saliva isolates is similar to the nosocomial isolates and phenotypic resistance to doxycycline and rifampicin is shown in the same range in the plaque/saliva isolates as in the nosocomial isolates (81.0% versus 73.3% and 52.4% versus 40% respectively) which indicates a possible role of the oral cavity as a reservoir for these resistance traits. The doxycycline resistance in plaque/saliva was significantly higher than in the food and the endodontic isolates. Both, doxycycline and azithromycin as well as tetracycline are used for the treatment of periodontitis and other dental diseases (Poveda Roda et al. 2007; Preshaw et al. 2004; Roberts and Mullany 2010). Erythromycin, next to clindamycin, can be prescribed for patients allergic to penicillin e.g. in endodontic infections (Jacinto et al. 2003). Rifampicin is commonly used in the treatment of serious infections as well as for chemoprophylaxis in bacterial meningitis (Gaetti-Jardim et al. 2010).

Up to now, only the results of one other study analyzing plaque isolates from healthy individuals are available (Poeta et al. 2009), but the authors only detected 3 *E. faecalis* isolates among other enterococci, which were all resistant to streptomycin, erythromycin and tetracycline, possessing *ermB* and *tetM*/*tetL* genes. In contrast to our results, Gaetti-Jardim et al., who analyzed mixed samples of saliva and plaque from healthy individuals and those with different dental diseases found much lower resistance to doxycycline (12.9) and no resistance to rifampicin, yet 19.4% resistance to amoxicillin compared to 0% in our isolates (Gaetti-Jardim et al. 2010). This could be explained by geographical differences considering antibiotic use, since these isolates stemmed from Brazilian patients. Another very recent Brazilian study analyzed a large number of *E. faecalis* isolates from oral rinses of healthy individuals reporting a similar amoxicillin resistance (12.3%) corresponding with Gaetti-Jardims results (Komiyama et al. 2016). Fifty-three percent of the tested isolates in their study were resistant to tetracycline compared to over 90% of plaque isolates in the present study harboring the *tetM* gene, and 85.7% being phenotypically resistant in an earlier study of our group (Anderson et al. 2015).

As far as studying isolates from healthy individuals, most other authors have analyzed fecal isolates, e.g. Kuch et al. (Kuch et al. 2012), finding resistance to rifampicin (37%), to tetracycline (55.6%) and to high-level gentamicin (8.6%) mostly in the same range as our results, although slightly less than we found in plaque/saliva from healthy individuals (52.4%, 85.7% and 47.5% respectively). Lietzau *et al.* analyzed feces samples from healthy individuals in Germany and found only 29.8% of the *E. faecalis* isolates resistant to doxycycline compared to 81% of our plaque/saliva isolates (Lietzau et al. 2006). Another study that investigated a large number of nosocomial and a few commensal strains from various geographical regions detected tetracycline resistance (conferred through *tetM* and *tetL*), erythromycin resistance (conferred through *ermB*) and high level gentamicin as well as vancomycin

resistance in the nosocomial isolates (McBride et al. 2007). Yet the commensal strains only harbored the *tetM* gene in contrast to our plaque/saliva isolates of which nearly 50% possessed the ermB gene.

Endodontic isolates have been extensively analyzed for their virulence and antibiotic resistance traits, since *E. faecalis* is thought to contribute to persistent root canal infections (Anderson et al. 2013). Increasing resistances, e.g. against tetracycline, rifampicin, ciprofloxacin and erythromycin have been reported (Al-Ahmad et al. 2014). Pinheiro et al. detected 85.8% azithromycin 71.5% erythromycin and 14.3% tetracycline as well as doxycycline resistance in endodontic *E. faecalis* (Pinheiro et al. 2004). In comparison, the present study detected 55% azithromycin, 35% doxycycline and 65% tetracycline resistant isolates. A recent study by Barbosa-Ribeiro et al. detected isolates showing intermediate and full resistance to amoxicillin, azithromycin, rifampicin and doxycycline from endodontic retreatment cases, concurring with our results, although with lower percentages for the latter three antibiotics (Barbosa-Ribeiro et al. 2016). Regarding their equipment with resistance genes, our endodontic samples showed a fairly high percentage of isolates carrying the bla_{TEM} gene (30%) compared with our nosocomial isolates (53.3%). Although enterococci possess intrinsic resistance against beta-lactam antibiotics, this resistance varies and e.g. ampicillin still has a high effectiveness against *E. faecalis* (Kristich et al. 2014). Other authors (Rocas and Siqueira 2013; Jungermann et al. 2011) have found bla_{TEM} in DNA extracts from endodontic infections. The equipment with resistance genes as well as phenotypic resistance against other tested antibiotics was lower for the endodontic isolates in the present study than for isolates of other sources.

For the last two decades most studies of nosocomial *E. faecalis* isolates regarding their antibiotic resistance have focused specifically on vancomycin resistance, while data on other antibiotics is less frequent (Ruiz-Garbajosa et al. 2006). In our study, we did not detect any vancomycin resistance in the nosocomial isolates, nor the isolates from other origins, which for isolates from the oral cavity is a consistent and favorable finding, considering the function of vancomycin as reserve antibiotic (Barbosa-Ribeiro et al. 2016; Komiyama et al. 2016; Pinheiro et al. 2004; Rams et al. 2013; C. M. Sedgley et al. 2005a; Dahlen et al. 2012).

On the other hand, high percentages of the nosocomial isolates of the present study exhibited doxycycline and azithromycin resistance as well as the possession of bla_{TEM}, *tetM* and *ermB*. Azithromycin resistance has also been found in a high percentage of nosocomial isolates from German patients by Wenzler *et al.* (72% versus 86.6% percent in our study) (Wenzler et al. 2004) and frequent rifampicin resistance has been reported by Kuch *et al.* (36.7% versus 40% in our study) (Kuch et al. 2012). Kuch also reported high-level streptomycin resistance in 26.7% of the German nosocomial isolates (Kuch et al. 2012) which is somewhat higher in our study, with 46.7% of our nosocomial isolates showing resistance.

Regarding the food isolates tested in the present study, we found a high resistance to doxycycline and azithromycin, comparable to the plaque/ saliva and nosocomial isolates, as well as frequent possession of *tetM*, while they were susceptible to most other tested antibiotics. In contrast to the oral isolates those from food showed a measure of high-level streptomycin resistance (35.7%). *E. faecalis* is frequently detected not only in raw milk but also raw milk cheeses (Jamet et al. 2012) and isolates have been found to be resistant to various antibiotics, e.g. tetracycline, erythromycin, rifampicin and streptomycin in concordance with our results (Jamet et al. 2012; Schlegelova et al. 2002; Templer and Baumgartner 2007). Jamet et al. found *tetM* and *ermB* genes widespread in

multiple resistant cheese isolates, and their multilocus sequence typing (MLST) analysis revealed several isolates from clonal complexes that have been associated with periodontitis (Jamet et al. 2012). This finding is significant against the backdrop of the study of Al-Ahmad et al. reporting that *E. faecalis* isolates from cheese were able to integrate into the oral biofilm *in vivo* (Al-Ahmad et al. 2010). Thus, also food isolates, e.g. raw milk and products thereof could serve as a resistance reservoir and facilitate the spread of resistance through transfer of genes to oral *E. faecalis*. The possibility of a transfer of resistance genes from these cheese isolates to the oral isolates or among different oral *E. faecalis* isolates through conjugative transposons *in vivo* has not been studied yet. Nevertheless, oral *E. faecalis* has been proven to be a recipient of resistance genes from other species (Roberts et al. 2001) *in vitro* and transfer of plasmid coded erythromycin resistance has been shown in root canals *ex vivo* (C. M. Sedgley et al. 2008). The presence of the Tn916-like element, carrying e.g. *tetM*, and other transposons has been detected in *E. faecalis* from food as well as oral enterococci (Jamet et al. 2012; Roberts and Mullany 2011; Kristich et al. 2014).

In summary we can conclude that both the oral cavity, in particular dental plaque and saliva, as well as foods can present a reservoir of *E. faecalis* strains with multiple antibiotic resistances including the potential of resistance transfer to other strains or even other species. Consequently, continued monitoring of *E. faecalis* for antibiotic resistance should be performed not only for nosocomial, but also for oral strains.

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Conflicts of Interest The authors deny any conflicts of interest related to this study.

Ethical Statement All endodontic and clinical isolates were obtained after approval by the Ethics Committee (no. 140/09, University of Freiburg).

Supplementary Material

Table S1 PCR amplification and cycling conditions for the detected antibiotic resistance genes

Target	Primer Concentrations	$MgCl_2$[a]	Template DNA	Annealing
E. faecalis				
bla_{TEM}	1.0 µl (5 µM) each	1 µl (2 mM)	1 µl	60 °C 30 s
tet(M)	0.5 µl (5 µM) each	–	1 µl	58 °C 45 s
tet(O)	0.5 µl (5 µM) each	–	2 µl	53 °C 30 s
erm(B)	0.5 µl (5 µM) each	–	2 µl	52 °C 30 s
erm(C)	0.25 µl (5 µM) each	–	2 µl	54 °C 60 s
*van*A	1.0 µl (5 µM) each	–	1 µl	54 °C 60 s
*van*B	2.0 µl (5 µM) each	–	1 µl	54 °C 60 s

[a]Qiagen, Hilden, Germany

Table S2 Antibiotic susceptibility and presence of antibiotic resistance genes in 70 *Enterococcus faecalis* isolates from four different sources

Isolates	Antibiotic resistance genes[a]	MIC (μg/mL)[b]					Resistance phenotype	Resistance phenotype[d] VITEK (Anderson 2015)
		DC[c]	AZ[c]	AC[c]	RI[c]	HLS[c]		
Endodontic								
1aR1		0.5	3	0.75	1	64		ERY-*T*/*S*
1anR8	*blaTEM*	**16**	**12**	0.75	**6**	128	DC-AZ-RI	ERY-*T*/*S*
11aRSP		0.75	2	0.75	3	128		ERY-*T*/*S*
12aSP	*tetM*	**24**	**8**	0.5	> **32**	96	DC-AZ-RI	ERY-*T*/*S*-TET
17aSP	*tetM*	**16**	**24**	0.38	**4**	32	DC-AZ-RI	ERY- *T*/*S*-TET-LIN
21aSP	*tetM*	**24**	**12**	0.75	8	128	DC-AZ	ERY-*T*/*S*-TET
33aR8	*blaTEM*	**16**	**8**	0.75	2	64	DC-AZ	ERY-*T*/*S*
44aR6	bla_{TEM-1}, *tetM*	6	**8**	0.5	3	64	AZ	ERY-*T*/*S*-TET
44aREnA	*tetM*	3	6	0.75	3	64		ERY-*T*/*S*-TET
44aF6	bla_{TEM-1}, *tetM*	3	**8**	0.75	3	64	AZ	ERY-*T*/*S*-TET
44aFEnA	bla_{TEM-1}, *tetM*	3	6	0.75	3	64		ERY-*T*/*S*-TET
44anR7	*tetM*	3	6	0.5	**4**	64	RI	ERY-*T*/*S*-TET
44anR10	*tetM*	3	6	0.75	**4**	64	RI	ERY-*T*/*S*-TET
44anF7	*tetM*	4	**8**	0.5	**4**	64	AZ-RI	ERY-*T*/*S*-TET
45aSP7	bla_{TEM-1}	0.75	3	0.75	**6**	64	RI	ERY-*T*/*S*
RGFR-81G8		0.38	**48**	0.75	**32**	192	AZ-RI	n.d.
RG20R72C3	*tetM*	8	> **256**	0.38	1	96	AZ	n.d.
RG18F102F2	*tetM*	**16**	1.5	0.5	> **32**	128	DC-RI	ERY-*T*/*S*-TET
MFCT7501C6		1	6	0.5	0.5	48		ERY-*T*/*S*
MFCT23S01A1	*tetM*	**24**	**12**	0.5	**4**	96	DC-AZ-RI	ERY-*T*/*S*-TET
Plaque/Saliva								
90sp		1	**8**	1	> **32**	96	AZ-RI	ERY-*T*/*S*
223sp	*tetM*	1.5	1	0.5	0.38	128		ERY-*T*/*S*
254p	*tetM*, *ermB*	**32**	> **256**	1.5	3	96	DC-AZ	ERY-*T*/*S*-TET-HGEN
255p	*tetM*	**24**	6	0.5	**8**	96	DC-RI	ERY-*T*/*S*-TET
282sp	*tetM*, *ermB*	**24**	> **256**	1	3	96	DC-AZ	ERY-*T*/*S*-TET-HGEN
288p	*tetM*, *ermB*	**16**	> **256**	2	3	96	DC-AZ	ERY-*T*/*S*-TET-HGEN
291sp	*tetM*, *ermB*	**16**	> **256**	1.5	2	96	DC-AZ	ERY-*T*/*S*-TET-HGEN
294sp	*tetM*, *ermB*	**16**	> **256**	1	4	96	DC-AZ	ERY-*T*/*S*-TET-HGEN
319p	*tetM*, *ermB*	**24**	> **256**	1	3	96	DC-AZ	ERY-*T*/*S*-TET-HGEN
327p	*tetM*, *ermB*	**16**	> **256**	1.5	2	96	DC-AZ	ERY-*T*/*S*-TET-HGEN
351p	*tetM*	**16**	**6**	1	**6**	96	DC-AZ-RI	ERY-*T*/*S*-TET
353p	*tetM*	**24**	**6**	0.5	**6**	96	DC-AZ-RI	ERY-*T*/*S*-TET
354p	*tetM*	**16**	**6**	0.75	**16**	96	DC-AZ-RI	ERY-*T*/*S*-TET
357sp		0.5	1.5	0.75	2	128		ERY-*T*/*S*

(continued)

Table S2 (continued)

Isolates	Antibiotic resistance genes[a]	MIC (μg/mL)[b]					Resistance phenotype	Resistance phenotype[d] VITEK (Anderson 2015)
		DC[c]	AZ[c]	AC[c]	RI[c]	HLS[c]		
359sp	*tetM*	**16**	**8**	0.75	> **32**	96	DC-AZ-RI	ERY-*T*/*S*-TET
383sp	*tetM*	8	**8**	0.75	> **32**	128	AZ-RI	ERY-*T*/*S*-TET
446sp	*tetM*, *ermB*	**24**	> **256**	1.5	**4**	96	DC-AZ-RI	ERY-*T*/*S*-TET-HGEN
447sp	*tetM*, *ermB*	**16**	> **256**	0.75	**4**	96	DC-AZ-RI	ERY-*T*/*S*-TET-HGEN
452sp	*tetM*, *ermB*	**16**	> **256**	0.75	3	96	DC-AZ	ERY-*T*/*S*-TET-HGEN
478sp	*tetM*	**24**	**8**	0.75	**4**	48	DC-AZ-RI	ERY-*T*/*S*-TET
513sp	*tetM*	**16**	6	0.75	**12**	64	DC-RI	ERY-*T*/*S*-TET
Food								
F2/19	bla_{TEM-1}, *tetM*	12	6	1	1.5	> **1024**	HLS	ERY-*T*/*S*-TET
E392	*tetM*	**16**	**12**	1.5	0.75	96	DC-AZ	ERY-*T*/*S*-TET
C339	*tetM*, *ermB*	6	**96**	0.75	1.5	96	AZ	ERY-*T*/*S*-TET
C350	*tetM*, *ermB*	**32**	> **256**	0.75	1.5	> **1024**	DC-AZ-HLS	ERY-*T*/*S*-TET
C409	*tetM*	**64**	**16**	0.75	1.5	> **1024**	DC-AZ-HLS	ERY-*T*/*S*-TET
C528		0.5	3	0.75	1	128		ERY-*T*/*S*
C671	*tetM*	**16**	**16**	0.75	3	192	DC-AZ	ERY-*T*/*S*-TET
C686	*tetM*	**16**	**16**	0.75	**4**	96	DC-AZ-RI	ERY-*T*/*S*-TET
C725/3	*tetM*	**16**	**24**	0.75	2	96	DC-AZ	ERY-*T*/*S*-TET
C729	*tetM*, *ermB*	**24**	> **256**	1	0.75	> **1024**	DC-AZ-HLS	ERY-T/S-TET
C737/1	*tetM*, *ermB*	**64**	> **256**	0.75	0.75	64	DC-AZ	ERY-T/S-TET
C771	*tetM*	**16**	**8**	0.75	1	96	DC-AZ	ERY-*T*/*S*-TET
C890	bla_{TEM-1}, *tetM*	**16**	**16**	0.75	1	96	DC-AZ	ERY-*T*/*S*-TET
C906/1	*tetM*	**16**	**16**	1	**4**	> **1024**	DC-AZ-RI-HLS	ERY-*T*/*S*-TET
Nosocomial								
110028	bla_{TEM-1}, *tetM*	**24**	**8**	0.75	2	96	DC-AZ	ERY-*T*/*S*-TET
110035	*tetM*, *erm*B	**24**	> **256**	0.75	**8**	128	DC-AZ-RI	ERY-T/S-TET-HGEN
110047		0.38	4	0.75	**8**	96	DC-AZ-RI	ERY-*T*/*S*
110053[e]	bla_{TEM-1}, *tetM*, *ermB*	**16**	> **256**	1	1.5	> **1024**	DC-AZ-HLS	ERY-T/S-TET-HGEN-LEV-CIP
109891	bla_{TEM-1}, *tetM*, *ermB*	**24**	> **256**	1.5	0.75	> **1024**	DC-AZ-HLS	ERY-T/S-TET-HGEN-LEV-CIP
109898	bla_{TEM-1}, *tetM*, *ermB*	**24**	> **256**	0.75	**4**	> **1024**	DC-AZ-RI-HLS	ERY-*T*/*S*-TET-HGEN
229355	bla_{TEM-1}	4	**8**	0.5	2	128	AZ	ERY-*T*/*S*-TET
512106	*tetM*	**24**	**16**	0.5	**4**	96	DC-AZ-RI	ERY-*T*/*S*-TET
512118	bla_{TEM-1}, *tetM*, *ermB*	**24**	> **256**	1	0.75	> **1024**	DC-AZ-HLS	ERY-T/S-TET

(continued)

Table S2 (continued)

Isolates	Antibiotic resistance genes[a]	MIC (μg/mL)[b]					Resistance phenotype	Resistance phenotype[d] VITEK (Anderson 2015)
		DC[c]	AZ[c]	AC[c]	RI[c]	HLS[c]		
512129	*tetM*	**16**	3	1	> **32**	> **1024**	DC-RI-HLS	ERY-*T*/*S*-TET
512176	$bla_{TEM\text{-}1}$, *ermB*	0.75	> **256**	0.75	0.75	> **1024**	AZ-HLS	ERY-T/S-HGEN-LEV-CIP
512188	*tetM*, *ermB*	**24**	> **256**	0.5	> **32**	96	AZ-RI	ERY-T/S-TET-HGEN-LEV-CIP
512276	*tetM*, *ermB*	**16**	> **256**	1	1.5	96	DC-AZ	ERY-*T*/*S*-TET-HGEN
512298	*tetM*	6	**8**	0.75	2	96	DC-AZ	ERY-*T*/*S*-TET
512359	$bla_{TEM\text{-}1}$,*tetM*, *ermB*	**64**	> **256**	1	1.5	> **1024**	DC-AZ-HLS	ERY-T/S-TET-HGEN-LEV-CIP

[a]Genes detected: $bla_{TEM\text{-}1}$, *tetM*, *tetW*, *tetQ*, *ermC*, *ermB*, *vanA*, *vanB*, bla_{TEM}
[b]minimal inhibitory concentrations, resistant isolates are marked in bold
[c]doxycycline (DC); azithromycin (AZ); amoxicillin (AC); rifampicin (RI); high-level-streptomycin (HLS)
[d]Resistance phenotypes analyzed with the VITEK-System, results published in previous study (Anderson et al. 2015); Erythromycin (ERY); tetracycline (TET); high-level gentamicin (HGEN); trimethoprim/sulfamethoxazol (T/S), *(T/S)* = *intermediate*; linezolid (LIN); levofloxacin (LEV); ciprofloxacin (CIP)
[e]bold: multi-drug-resistant isolates

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