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Occurrence of *Enterococcus faecium* in pigs and pork from organised slaughterhouse and retail outlets from Thrissur, Kerala

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Abstract

*Enterococcus faecium is an opportunistic pathogen of major public health importance and a recognised indicator of antimicrobial resistance in food production systems. This study investigated the occurrence of E. faecium in pigs, pork, and associated environments of organised slaughterhouses and retail meat outlets in Thrissur district, Kerala, using cultural and molecular approaches. A total of 330 samples were collected from pigs, pork, meat-contact surface swabs, water, and slaughtering personnel hand-wash samples. Isolation was performed using conventional culture methods followed by biochemical characterisation, and molecular confirmation was carried out by multiplex PCR targeting the Enterococcus genus-specific 16SrRNA gene and the E. faecium-specific ddl gene. Phenotypic analysis detected E. faecium in 5.45*per cent *of samples, while multiplex PCR confirmed 4.84*per cent*. The highest occurrence was observed on meat-contact surfaces, particularly knife and carcass cutting surface swabs, whereas pigs, pork, water, and hand-wash samples showed low or no positivity. Retail outlets exhibited a significantly higher occurrence of E. faecium than organised slaughterhouses by both cultural and molecular methods. These findings indicate that retail meat handling environments act as key points for persistence and dissemination of E. faecium, highlighting the need for improved hygiene and sanitation of food-contact surfaces to reduce food-chain transmission risks.*

Keywords: *Enterococcus faecium*, pork production, retail meat outlets, organised slaughterhouses, meat-contact surfaces, multiplex PCR, food safety, antimicrobial resistance, One Health

Food safety is a critical component of public health, particularly at the human-animal-environment interface where microorganisms can circulate and persist across multiple reservoirs. In animal-based food production systems, the presence of bacterial contaminants in meat and associated environments represents an important route for the transmission of opportunistic pathogens to humans. Monitoring indicator organisms along the food chain is therefore essential for assessing hygienic practices, identifying contamination sources, and evaluating potential risks to consumers (Havelaar et al., 2010). Among such organisms, *Enterococcus faecium* is of considerable importance. This bacterium is commonly present as a commensal in the gastrointestinal tracts of humans and animals and is frequently detected in foods of animal origin. Its ubiquitous nature, ability to survive under diverse environmental conditions, and persistence

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in food processing environments make it a useful indicator of fecal contamination and hygienic quality (Franz et al., 2011).

Although generally harmless as a commensal, *E. faecium* has increasingly been associated with opportunistic infections in humans, particularly in healthcare settings (Arias and Murray, 2012). In addition, *E. faecium* possesses intrinsic and acquired resistance to multiple antimicrobial agents, which enhances its ability to persist in diverse environments, including food production systems. While antimicrobial resistance (AMR) is not the primary focus of the present study, its association with *E. faecium* underscores its broader public health significance (Arias and Murray, 2012).

Food-producing animals serve as important reservoirs of antimicrobial resistant *E. faecium*, particularly in intensive production systems where antimicrobial exposure is common. Slaughterhouses and retail meat outlets represent critical points within the farm to fork continuum, where contamination of carcasses, equipment, and contact surfaces may occur. Inadequate hygienic practices at these stages can facilitate the persistence and spread of *E. faecium*, increasing the potential for exposure of food handlers and consumers and posing a food safety risk (Franz et al., 2011). Ensuring food safety is therefore essential not only for preventing foodborne infections but also for limiting the dissemination of antimicrobial-resistant bacteria through the food chain. Surveillance of *E. faecium* in slaughterhouse environments and retail meat outlets provides valuable insight into contamination pathways, hygiene practices, and potential public health risks, and supports the development of targeted interventions aimed at reducing the burden of AMR. Despite the recognised importance of *E. faecium*, data on its occurrence in organised slaughterhouses and retail meat outlets in India remain limited, particularly from southern regions such as Kerala. Thrissur district, characterised by organised slaughter facilities and an extensive network of retail meat outlets, offers a relevant setting to examine the circulation of *E. faecium* at the food-human interface.

The present study was therefore undertaken to investigate the occurrence of *Enterococcus faecium* in retail outlets and organised slaughterhouses in Thrissur, with the objective of generating local evidence on its presence at critical food safety points. The findings of this study aim to contribute to improved food safety practices and to strengthen One Health oriented approaches for controlling the spread of *E. faecium* along the food chain.

Materials and methods

Study area and sample collection

The present study was conducted to determine the occurrence of *Enterococcus faecium* in pigs, pork, and associated environments within organised slaughterhouses and retail meat outlets of Thrissur district, Kerala. A total of 330 samples were collected over a 10-month period from September 2024 to June 2025, encompassing animal, food, environmental, water, and human contact points along the slaughter and retail meat handling continuum.

A total of 60 pigs were included in the study, comprising 30 from organised slaughterhouses and 30 from retail outlets. During ante-mortem inspection, 120 animal swab samples (60 oral and 60 rectal) were collected following the method described by Osemeke et al. (2023), with aseptic handling and processing as per ICMR (2019). All samples were processed within 3-4 hours of collection. Additionally, 60 pork samples (30 each from slaughterhouses and retail outlets) were aseptically collected (~100 g per carcass) following ICMR (2019) guidelines. Environmental samples included 30 carcass cutting surface swabs and 30 knife swabs, collected as described by Uzoigwe et al. (2021). A total of 30 water samples were collected from slaughterhouses and retail outlets following APHA (2023) guidelines. To assess occupational exposure, 60 hand-wash samples were collected from slaughterhouse personnel before and after slaughter operations (30 each), following the method described by Kaltenthaler and Pinfold (1995).

All samples were collected in appropriately labelled sterile containers or bags and immediately placed in insulated containers maintained at approximately 4 °C for transport to the laboratory. Samples were transported and processed as promptly as possible to ensure sample integrity. All laboratory procedures were performed in a Biosafety Level II (BSL-II) laboratory at the Department of Veterinary Public Health, College of Veterinary and Animal Sciences, Mannuthy, in accordance with standard biosafety guidelines.

Isolation and characterisation of Enterococcus faecium

A pre-enrichment step was employed to enhance the recovery of enterococci from all collected samples. Oral, rectal, and environmental surface swabs were directly inoculated into 15 mL of Brain Heart Infusion Broth (BHIB). For liquid samples, 5 mL of each water and hand-wash samples was aseptically transferred into 45 mL of BHIB. For meat samples, 25 g of aseptically weighed pork was homogenised with 225 mL of BHIB in a sterile stomacher bag using a laboratory homogeniser (AES Smasher, France) for 120 seconds. All inoculated broths were incubated at 37 °C for 24 hours to facilitate bacterial growth prior to selective isolation (ICMR, 2019).

Following pre-enrichment, selective isolation of enterococci was performed. A loopful of each enriched broth culture was streaked onto Enterococcal Agar Base (HiMedia, India) and incubated at 37 °C for 24 hours. Presumptive *Enterococcus faecium* isolates typically

appeared as white to pale pink colonies, which were selected for further characterisation.

Presumptive isolates were subjected to biochemical characterisation using freshly grown cultures, following standard taxonomic criteria for enterococci (Vos et al., 2009). Isolates presumptively identified as *Enterococcus faecium* based on cultural and biochemical characteristics were subsequently subjected to molecular confirmation.

Molecular confirmation of E. faecium

Genomic DNA was extracted from presumptive *Enterococcus faecium* isolates using the snap-chill (boiling) method, as described by Singh et al. (2022). Briefly, four to five well-isolated colonies from fresh pure cultures were suspended in 100 µL of nuclease-free water in sterile 1.5 mL microcentrifuge tubes. The suspension was boiled at 100 °C for 15 minutes, immediately chilled at -20 °C for 15 minutes, and centrifuged at 12,000 rpm for 10 minutes. The supernatant containing genomic DNA was transferred to a fresh tube and stored at -20 °C until further use as template DNA.

Molecular confirmation of *Enterococcus faecium* was carried out using multiplex PCR targeting the genus-specific *16SrRNA* gene and the *E. faecium*-specific *ddl* (D-alanine:D-alanineligase) gene, following ICMR (2019) and Masoumi Zavaryani et al. (2015). PCR amplification was performed in a 30 µL reaction volume using a T100™ thermal cycler (Bio-Rad, USA). The cycling conditions included an initial denaturation at 95 °C for 3 minutes, followed by 34 cycles of denaturation at 95 °C for 1 minute, annealing at 51 °C for 45 seconds, and extension at 72 °C for 1 minute, with a final extension at 72 °C for 5 minutes.

Amplified products were resolved by 1.5% agarose gel electrophoresis in 1× TBE buffer and visualised using SYBR™ Safe DNA stain. The presence of characteristic amplicons of 800 bp (*16SrRNA*) and 550 bp (*ddl* gene) confirmed the isolates as *E. faecium* (Table 1).

Statistical analysis

The data obtained were subjected to statistical analysis using statistical software SPSS version 24.0. Descriptive statistics (frequencies, percentages, and means ± standard error) were computed to summarize the findings. The category-wise occurrence was analysed using Z test for proportions. A *p*-value of less than 0.05 was considered statistically significant.

Results and discussion

Occurrence of E. faecium by culture techniques

In the present study, *Enterococcus faecium* was recovered by conventional culture methods from 5.45 per cent (18/330) of samples collected across organised slaughterhouses and retail outlets in Thrissur district, confirming its presence along the pork production and handling continuum (Table 2). The predominant recovery from meat-contact surfaces, particularly knife swabs (16.67%) and carcass cutting surface swabs (13.33%), highlights these interfaces as critical points for persistence and dissemination of *E. faecium*. Similar findings have been reported from slaughterhouse and meat-processing environments, where knives and cutting surfaces act as reservoirs due to repeated contact with carcasses and inadequate sanitation between processing events (Rahman et al., 2018).

In contrast, animal-associated samples showed lower isolation rates, with *E. faecium* detected more frequently in oral swabs (6.67%) than in rectal swabs (3.33%). This pattern suggests that contamination may arise not only from intestinal carriage but also from oral cavities during handling, transport, and slaughter, as previously observed in pigs and other food animals (Layton et al., 2010; Kwon et al., 2013). The relatively low prevalence in pork samples (3.33%) indicates limited transfer to the final meat product, although even low-level contamination is epidemiologically relevant given the organism's ability to survive adverse conditions and acquire antimicrobial resistance (Hammerum, 2012; García-Solache and Rice, 2019). Similar observations have been reported in pig production systems, where *E. faecium* is frequently detected in pigs and associated environments but occurs at comparatively lower levels in finished meat products, suggesting that contamination is largely influenced by processing and handling practices rather than primary animal carriage (Aarestrup et al*.,* 2000).

In the Indian context, studies on pigs and pork production chains have demonstrated the occurrence of *Enterococcus* species, including *E. faecium*, across pig farms, slaughter environments, and retail outlets with variable prevalence. For instance, a study from Andhra Pradesh reported the isolation of *E. faecium* among

Table 1. Primers used for the identification of isolates

Organism	Genes	Primer	Primer sequences	Size (bp)	Reference
Enterococcus spp.	*16SrRNA*	F	5'GACTACCNGGGTATCTAATCC 3'	800	ICMR, 2019
		R	5'AGAGTTTGATCCTGGCTNAG 3'		
E. faecium	$ddl_{E. faecium}$	F	5'TAGAGACATTGAATATGCC 3'	550	Masoumi Zavaryani et al., (2015)
		R	5'CTAACATCGTGTAAGCT 3'		

multiple *Enterococcus* species from both pig farms and retail pork samples, indicating its distribution along the pork supply chain rather than consistent presence in final meat products (Kumar et al*.,* 2024). Similarly, Indian investigations on vancomycin-resistant enterococci (VRE) have documented their occurrence in pigs, pork, handlers, and environmental samples, highlighting the interconnected nature of contamination across the pork production continuum and the role of slaughter and retail environments as key transmission interfaces (Kiranmayee, 2022). These findings collectively suggest that while direct contamination of pork may be relatively low, slaughter and retail environments serve as critical points for introduction and dissemination of *E. faecium*, emphasizing the importance of hygienic handling practices and environmental control measures in limiting its spread.

Environmental contamination appeared minimal, with *E. faecium* detected in only one water sample (3.33%), consistent with reports showing lower enterococcal recovery from treated or intermittently used water sources compared with contact surfaces (Byappanahalli et al., 2012; Sidhu et al., 2014). Importantly, the absence of *E. faecium* in hand-wash samples collected before and after slaughter operations suggests limited direct human carriage at the time of sampling or effective hand hygiene practices, as also reported in some occupational exposure studies (Getachew et al., 2012).

Overall, the cultural isolation results indicate that equipment and environmental surfaces act as the primary reservoirs of *Enterococcus faecium* in slaughter and retail settings rather than animals or personnel. This observation is consistent with previous studies showing that post-slaughter environments play a key role in the persistence and dissemination of enterococci (Aarestrup et al., 2000). Contamination likely occurs through multiple pathways, including direct transfer from contaminated carcasses to knives and cutting surfaces, followed by subsequent cross-contamination of meat during repeated handling. The frequent reuse of inadequately sanitized equipment further amplifies this risk, enabling continuous spread across different batches of meat.

A major factor contributing to the persistence of *E. faecium* on such surfaces is its ability to form biofilms. Biofilm formation enhances bacterial adhesion to food-contact surfaces such as stainless steel and plastic and provides protection against environmental stress and cleaning procedures. Once established, biofilms can act as persistent reservoirs, allowing bacteria to survive routine sanitation and be intermittently released onto meat products during processing (Franz et al., 2011). Additionally, the presence of organic matter and surface irregularities further promotes bacterial attachment and biofilm development. Together, these factors create a cycle of contamination, persistence, and recontamination, emphasizing the importance of equipment hygiene as a critical control point in limiting the spread of *E. faecium* in meat processing environments.

Category-wise occurrence of E. faecium by culture techniques

Phenotypic analysis revealed a significantly higher occurrence of *Enterococcus faecium* in retail outlets compared with organised slaughterhouses, demonstrating the influence of market type on contamination dynamics. Retail markets recorded a prevalence of 8.48 per cent (14/165), whereas organised slaughterhouses showed a markedly lower occurrence of 2.42 per cent (4/165), with an overall prevalence of 5.45 per cent (18/330). The observed difference was statistically significant ($Z = 2.446$, $p = 0.014$), indicating that *E. faecium* contamination is substantially greater in retail environments (Table 3).

Similar observations have been consistently reported in previous studies, where retail meat outlets exhibited higher enterococcal recovery than slaughter facilities due to increased manual handling, repeated

Table 2. Occurrence of *E. faecium* by culture techniques

Sl. No.	Sample	Retail outlets			Organised slaughterhouse			Overall	
		No. of samples	Samples-Positive	Per cent	No. of samples	Samples-Positive	Per cent	Samples-Positive	Per cent
1.	Water	15	1	6.67	15	0	0.00	1	3.33
2.	Knife	15	3	20.00	15	2	13.33	5	16.67
3.	Cutting surfaces	15	4	26.67	15	0	0.00	4	13.33
4.	Oral	30	4	13.33	30	0	0.00	3	5.00
5.	Rectal	30	0	0.00	30	2	6.67	2	3.33
6.	Pork	30	2	6.67	30	0	0.00	1	1.67
7.	Handwash before slaughter	15	0	0.00	15	0	0.00	0	0.00
8.	Handwash after slaughter	15	0	0.00	15	0	0.00	0	0.00
Total		**165**	**14**	**8.48**	**165**	**4**	**2.42**	**18**	**5.45**

Table 3. Category-wise occurrence of *E. faecium* by culture techniques

Type of market	Samples analysed	Positive cases		Z-value	P-value
		Number	Per cent		
Retail outlet	165	14	8.48	2.446*	0.014
Organised slaughterhouse	165	4	2.42		
Total	330	18	5.45		

** Significant at 0.05 level*

use of equipment, prolonged exposure to ambient conditions, and variable sanitation practices (Rahman et al*.,* 2018). Enterococci are well adapted to survive desiccation, temperature fluctuations, and nutrient-limited environments, enabling their persistence on food-contact surfaces commonly encountered in retail settings (Hammerum, 2012; García-Solache and Rice, 2019). In contrast, organised slaughterhouses generally operate under regulated hygiene protocols and controlled processing conditions, which likely account for the significantly lower prevalence observed.

These findings underscore the critical role of retail meat handling environments as points of amplification for *E. faecium* and highlight the need for strengthened hygiene interventions, particularly targeting knives, cutting surfaces, and handling practices, to mitigate the dissemination of this opportunistic and antimicrobial-resistant pathogen along the food chain.

Molecular confirmation of E. faecium by multiplex PCR

Molecular confirmation using multiplex PCR identified *E. faecium* in 16 of 330 samples (4.85%), with a distribution largely consistent with phenotypic findings, though slightly lower in some categories due to the higher specificity of molecular detection (Jackson et al., 2004; Jurkovic et al., 2006). The presence of characteristic amplicons of 800 bp (*16SrRNA*) and 550 bp (*ddl*gene) confirmed the isolates as *E. faecium* (Fig. 1). The highest prevalence, as detected by molecular methods, was

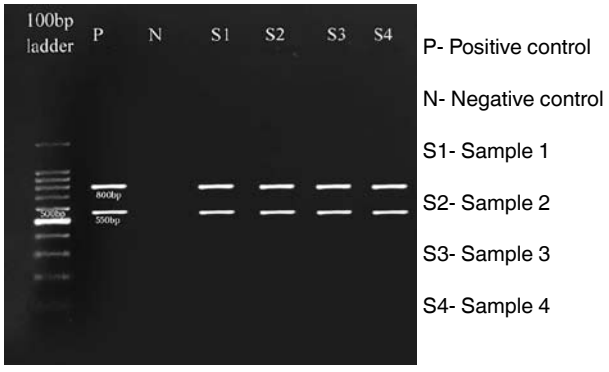

Fig. 1 Detection of *16SrRNA* gene and *ddl* $_{E.faecium}$ gene in *E.faecium*

observed in knife swabs (16.67%) and carcass cutting surface swabs (13.33%), confirming food-contact surfaces as primary reservoirs of *E. faecium*. Similar PCR-based studies have highlighted the role of contaminated equipment and biofilm formation in the persistence of enterococci within slaughter and retail environments (Cook et al., 2017; Uzoigwe et al., 2021).

Animal-associated samples showed lower prevalence using molecular detection, with *E. faecium* detected in oral (5%) and rectal (3.33%) swabs, consistent with reports indicating limited intestinal abundance of *E. faecium* in pigs compared with environmental persistence (Layton et al., 2010; Hammerum, 2012). Pork samples exhibited minimal contamination (1.67%), suggesting restricted transfer to the final product or partial reduction during processing (Table 4). Associated water samples

Table 4. Molecular confirmation of *E. faecium* by multiplex PCR

Sl. No.	Sample	Retail outlets			Organised slaughterhouse			Overall	
		No. of samples	Samples-Positive	Per cent	No. of samples	Samples-Positive	Per cent	Samples-Positive	Per cent
1.	Water	15	1	6.67	15	0	0.00	1	3.33
2.	Knife	15	3	20.00	15	2	13.33	5	16.67
3.	Cutting surfaces	15	4	26.67	15	0	0.00	4	13.33
4.	Oral	30	3	10.00	30	0	0.00	3	5.00
5.	Rectal	30	0	0.00	30	2	6.67	2	3.33
6.	Pork	30	1	3.33	30	0	0.00	1	1.67
7.	Handwash before slaughter	15	0	0.00	15	0	0.00	0	0.00
8.	Handwash after slaughter	15	0	0.00	15	0	0.00	0	0.00
Total		**165**	**12**	**7.27**	**165**	**4**	**2.42**	**16**	**4.84**

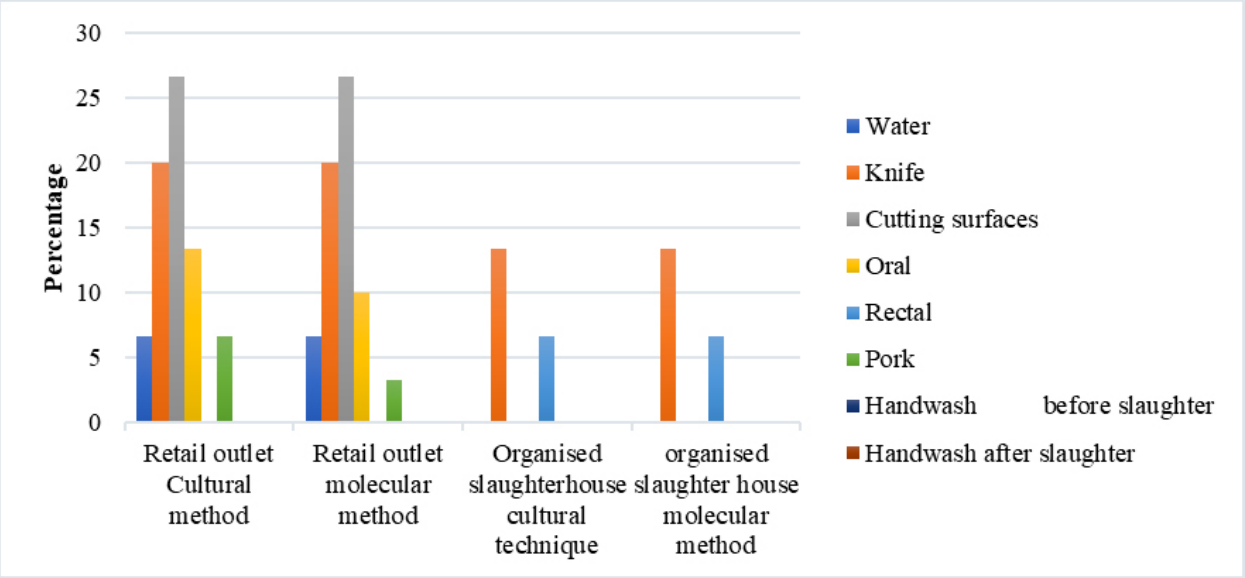

Fig. 2. Distribution of *E. faecium* by culture techniques and molecular confirmation

were positive only in retail outlets (6.67%), while all handwash samples were PCR-negative, indicating limited human carriage at the time of sampling. Overall, multiplex PCR confirms that *E. faecium* occurrence is predominantly associated with retail environments and food-contact surfaces, underscoring knives and cutting surfaces as critical control points for intervention (Fig. 2).

Category-wise occurrence of E. faecium by molecular confirmation

Based on molecular confirmation using PCR, a Z-test for proportions revealed a significantly higher occurrence of *E. faecium* in retail outlets compared to organised slaughterhouses. PCR positivity was 7.27 per cent (12/165) in retail markets and 2.42 per cent (4/165) in organised slaughterhouses, with an overall prevalence of 4.84 per cent (16/330). The difference was statistically significant (Z = 2.064, p = 0.039), suggesting that retail meat environments present a higher risk for *E. faecium* contamination.

This finding is consistent with previous PCR-based studies reporting higher enterococcal prevalence in retail settings due to increased handling, repeated use of equipment (Table 5), and less stringent hygiene controls compared with regulated slaughter facilities (Layton et al., 2010). The results further support the role of retail environments as important points for persistence and dissemination of *E. faecium* along the food chain.

Conclusion

The present study demonstrates that *Enterococcus faecium* occurs along the pork production and retail continuum in Thrissur district, with a significantly higher prevalence in retail meat environments compared to organised slaughterhouses. Knives and carcass cutting surfaces were identified as the primary reservoirs, highlighting the critical role of meat-contact surfaces in the persistence and dissemination of this organism. Minimal occurrence in pigs, pork, water, and personnel suggests that post-slaughter handling and retail practices are the major contributors to contamination. The strong agreement between cultural and molecular methods further confirms the reliability of the findings and supports the use of multiplex PCR for surveillance. These findings underscore the need for targeted hygiene interventions at the retail level. Routine sanitation of knives and cutting surfaces, implementation of standard cleaning procedures, and proper segregation of clean and contaminated areas are essential to reduce cross-contamination. Reinforcing personal hygiene among meat handlers, ensuring clean water use, proper waste management, and maintaining temperature control during storage are also critical. Additionally, regular microbiological monitoring and

Table 5. Category-wise occurrence of *E. faecium* by molecular confirmation

Type of market	Samples analysed	Positive cases		Z-value	P-value
		Number	Per cent		
Retail oulet	165	12	7.27	2.064*	0.039
Organised slaughterhouse	165	4	2.42		
Total	330	16	4.84		

** Significant at 0.05 level*

strengthened regulatory inspections are necessary to improve compliance. Enhancing hygiene practices at retail outlets is therefore crucial to limit the spread of *E. faecium*, an opportunistic and antimicrobial-resistant organism of public health importance, and to support food safety within a One Health framework.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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