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META-ANALYSIS OF THE GLOBAL PREVALENCE OF *ENTEROCOCCUS* SPP. IN FOODS: ANTIBIOTIC RESISTANCE PROFILE OF *ENTEROCOCCUS FAECALIS* AND *ENTEROCOCCUS FAECIUM*

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Abstract

The objective of this study was to evaluate the prevalence and diversity of *Enterococcus* spp. and antibiotic-resistant *Enterococcus faecalis* and *Enterococcus faecium* isolates in different foods worldwide. This study used meta-analytical methods. Besides, Web of Science (n= 705), Medline (n= 6), and Scopus (n= 1338) were searched for studies in the years 1995–2021 using related keywords. Results showed that the pooled prevalence for *Enterococcus* spp. and antibiotic-resistance of *E. faecalis* and *E. faecium* isolates were 0.41 (95% C.I. 0.34–0.47), 0.25 (95% C.I. 0.13–0.38), respectively. According to the results of the subgroup analysis, the lowest and highest prevalence of *Enterococcus* spp. in food types were calculated for red meat (0.56), and fermented foods (0.29). Also, as a result of subgroup analyses by country the highest prevalence of *Enterococcus* spp. was calculated in studies conducted in Slovakia (0.74). In contrast, the lowest prevalence was calculated in studies conducted in Georgia (0.07). The meta-analyses improved our understanding of the prevalence of *Enterococcus* spp. and the antibiotic resistance of *E. faecalis* and *E. faecium* isolates in different foods and provided results that can be useful as input for quantitative microbiological risk evaluation modeling.

Key words: antimicrobial resistance, *E. faecalis*, *E. faecium*, food, meta-analysis, prevalence

Enterococci are part of the natural microbiota of the gastrointestinal tract of warm-blooded animals. They can be released into the environs by feces and contaminate the soil, water, and plants (Byappanahalli et al., 2012; Chajęcka-Wierzchowska et al., 2021). *Enterococci* are a large genus of Gram-positive bacteria, non-spore-forming ovoid in shape, catalase-negative, oxidase-negative, and bile salts tolerant. They can hydrolyze esculin and generate lactic acid as its major product of glucose fermentation, facultative anaerobic *Enterococci. Enterococcus* spp. can be found in many kinds of food, especially raw products of animal origin, and in environments associated with inadequate hygienic conditions and it is also accepted as an indicator of fecal contamination in the food production chain (Hanchi et al., 2018; García-Solache and Rice, 2019; Atiyah and Alkhafaji, 2020; Ducková et al., 2021; Kim et al., 2021).

The ubiquitous nature of the *Enterococci* and the resistance to adverse environmental conditions account for their ability to colonize different habitats and their easy spread potential through the food chain. These commensal *Enterococci* of the human microbiome are not typically pathogenic in healthy hosts while they can cause severe infections in susceptible individuals and/or in some conditions (Higuita and Huycke, 2014; Espíndola et al., 2021; Kim et al., 2021). The pathogenesis of *Enterococci* is related to a variety of virulence factors. The adhesion factors that play a role in biofilm formation are *Asa* (aggregation substance), *esp* (extracellular surface protein), *efaA* (*E. faecalis* antigen A), *ace* (adhesin of collagen from *E. faecalis*), and *ebp* (endocarditis and biofilm-associated). *Enterococci* secrete the pathogenic factors *cylA* (cytolysin) and *gelE* (gelatinase), which are responsible for the exacerbation of infection. *Enterococcus* spp. can accumulate multiple genetic elements encoding virulence traits and antibiotic resistance genes and develop biofilm that has a prominent role in *Enterococcus* spp. infections. The biofilm of *Enterococcus* spp. promotes tolerance to harsh environmental conditions and contributes significantly to persistence during infection and food processing environment, causing ecological contamination (El-Zamkan and Mohamed, 2021; Alzahrani et al., 2022). Therefore, the existence of antimicrobial-resistant *Enterococci* in foods has been an issue of increasing concern (Ducková et al., 2021; Kim et al., 2021). In this regard, many earlier studies focused on *E. faecalis* and *E. faecium* which are known as the most prevalent species recovered (Anderson et al., 2016; Kim et al., 2021).

Therefore, this study aimed to assess the prevalence of *Enterococcus* spp. and antibiotic-resistant *E. faecalis* and *E. faecium* isolate in different foods worldwide through a systematic literature review and meta-analysis of reviewed publications between 1995 and 2021.

Material and methods

Data Selection

In the study, meta-analyses of the prevalence of *Enterococcus* spp. and antibiotic-resistant *E. faecalis* and *E. faecium* isolates in different foods were carried out. The search strategy was created with the keywords "*Enterococcus* spp. in the food" and "*Enterococcus* spp.". Inclusion criteria in the meta-analysis were determined as "Studying *Enterococcus* spp. in foods" and "Providing prevalence data". The flow diagram of the meta-analysis is given in Figure 1 (Page et al., 2021). As a result of the literature search, 2049 studies were obtained. Among those which meet the inclusion criteria, 56 out of 82 prevalence data on *Enterococcus* spp. and 35 of 78 prevalence data of antibiotic resistance of *Enterococcus faecalis* and *Enterococcus faecium* isolates were evaluated. Also, the data on the prevalence of *Enterococcus* spp. and antibiotic resistance of *E. faecalis* and *E. faecium* are given in Tables 1 and 2, respectively.

Statistical Analysis

Effect sizes were taken as prevalence and the random-effects model was used due to the high heterogeneity between studies. After the studies meeting the criteria for inclusion in the meta-analysis were determined, publication bias was evaluated. Funnel Plot Asymmetry Regression test and funnel plots were drawn to detect publication biases in the study samples. The random-effect model was used to determine the heterogeneity between the studies. The DerSimonian Laird method (τ^2) was used to determine the study variance. In meta-analyses for the prevalence of *Enterococcus* spp. sample types (fermented foods, milk and milk product, red meat and meat product, white meat and meat product, other foods), countries (Brazil, Egypt, Georgia, Germany, Italy, Japan, Korea, Portugal, Serbia, Slovakia, Spain, Thailand, Turkey, USA), and years (1995–2004, 2005–2014, 2015–2021) subgroups were established to identify the source of heterogeneity between studies. Subgroups for the prevalence of antibiotic resistance were established as antibiotic (ampicillin, gentamycin, penicillin, streptomycin, and vancomycin) and years (2000–2009, 2010–2021) subgroups. Subgroup analyses for prevalence of *Enterococcus* spp. and antibiotic resistance of *E. faecalis* and *E. faecium* were performed with these covariates. Statistical analyses were performed using the "meta", "tidyverse" and "metaphor" packages in R (www.r-project.org). Within the scope of the study, the meta-analyses that were applied regarding the PRISMA 2020 checklist are given in Figure 1 (Borenstein and Higgins, 2013; Page et al., 2021).

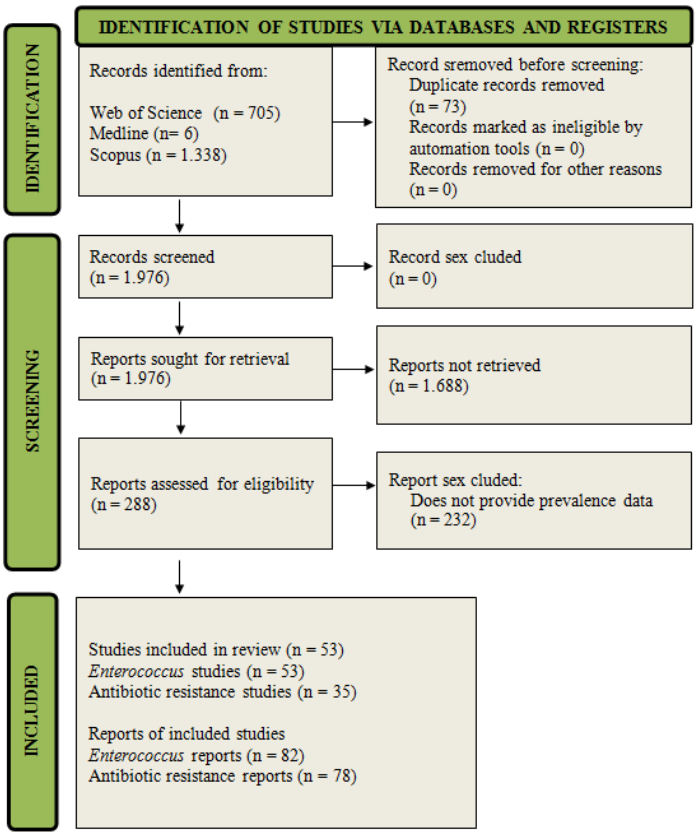


Figure 1. Flow diagram of the study's selection process (Prisma, 2020)

Table 1. Characteristics of studies on the prevalence of *Enterococcus* spp.

Study No.	Authors	Publication Year	Country	Samples	N	M
1	2	3	4	5	6	7
1	Klein et al., 1998	1998	Germany	Red Meat and Meat Product	555	48
	Klein et al., 1998	1998	Germany	Milk and Milk Product	555	48
2	Lemcke and Bulte, 2000	2000	Germany	Milk and Milk Product	1643	205
3	Pavia et al., 2000	2000	Italy	Red Meat and Meat Product	100	34
	Pavia et al., 2000	2000	Italy	White Meat and Meat Product	100	65
	Pavia et al., 2000	2000	Italy	Milk and Milk Product	100	34
4	Robredo et al., 2000	2000	Spain	White Meat and Meat Product	92	25
5	Simjee et al., 2002	2002	USA	White Meat and Meat Product	32	30
	Simjee et al., 2002	2002	USA	White Meat and Meat Product	16	5
6	Petersen et al., 2003	2003	Thailand	White Meat and Meat Product	410	374
7	Frahm and Obst, 2003	2003	Germany	Other Foods	55	8
8	Harada et al., 2004	2004	Japan	Red Meat and Meat Product	137	18
	Harada et al., 2004	2004	Japan	White Meat and Meat Product	137	18
9	Drahovska et al., 2004	2004	Slovakia	Milk and Milk Product	117	109
10	Novais et al., 2005	2005	Portugal	White Meat and Meat Product	92	82
11	Mcgowan et al., 2006	2006	Georgia	Red Meat and Meat Product	396	16
	Mcgowan et al., 2006	2006	Georgia	Milk and Milk Product	396	16
	Mcgowan et al., 2006	2006	Georgia	White Meat and Meat Product	396	56
12	Messi et al., 2006	2006	Italy	Red Meat and Meat Product	59	20
	Messi et al., 2006	2006	Italy	Milk and Milk Product	59	20
13	Poeta et al., 2007	2007	Portugal	White Meat and Meat Product	66	20
14	Jurkovic et al., 2005	2005	Slovakia	Milk and Milk Product	400	308
15	Fracalanzza et al., 2007	2007	Brazil	White Meat and Meat Product	294	158
16	Hajikhani et al., 2007	2007	Turkey	Milk and Milk Product	84	12
	Hajikhani et al., 2007	2007	Turkey	Milk and Milk Product	84	12
17	Mcgowan et al., 2007	2008	USA	Milk and Milk Product	619	77
	Mcgowan et al., 2007	2008	USA	Red Meat and Meat Product	619	77
18	Abriouel et al., 2008	2008	Spain	Milk and Milk Product	150	59
	Abriouel et al., 2008	2008	Spain	Red Meat and Meat Product	150	59
19	Mcgowanet al., 2008	2008	USA	White Meat and Meat Product	80	41
	Mcgowan et al., 2008	2008	USA	White Meat and Meat Product	80	4
	Mcgowan et al., 2008	2008	USA	Milk and Milk Product	80	14
20	Keeratipibul et al., 2009	2009	Thailand	White Meat and Meat Product	300	28
21	Oktay et al., 2009	2009	Turkey	White Meat and Meat Product	150	48
22	Riboldi et al., 2009	2009	Brazil	Other Foods	189	56
23	Veljovic et al., 2009	2009	Serbia	White Meat and Meat Product	26	1
	Veljovic et al., 2009	2009	Serbia	Milk and Milk Product	26	2
	Veljovic et al., 2009	2009	Serbia	Red Meat and Meat Product	26	2
	Veljovic et al., 2009	2009	Serbia	Milk and Milk Product	26	22
24	Kročkoet al., 2011	2011	Slovakia	White Meat and Meat Product	138	128
25	Kročkoet al., 2007	2011	Slovakia	White Meat and Meat Product	110	29
26	Macedo et al., 2011	2011	Portugal	Other Foods	76	45
27	Schwaiger, 2011	2011	Germany	Other Foods	1001	459
28	Ananchaipattana et al., 2012	2012	Thailand	Other Foods	133	103
29	Cetinkaya et al., 2013	2013	Turkey	Milk and Milk Product	170	1
	Cetinkaya et al., 2013	2013	Turkey	White Meat and Meat Product	70	6
30	Koo et al., 2013	2013	Korea	White Meat and Meat Product	124	111
31	Yurdakul et al., 2013	2013	Turkey	White Meat and Meat Product	50	4

Table 1 – contd.

1	2	3	4	5	6	7
32	García-Cano et al., 2014	2014	Spain	Milk and Milk Product	22	18
33	Acurcio et al., 2014	2014	Brazil	Milk and Milk Product	20	16
34	Camargo, 2014	2014	Brazil	White Meat and Product	74	35
35	Carminati et al., 2014	2014	Italy	Milk and Milk Product	7	4
36	Pesavento et al., 2014	2014	Italy	White Meat and Meat Product	214	142
	Pesavento et al., 2014	2014	Italy	Milk and Milk Product	278	208
	Pesavento et al., 2014	2014	Italy	Red Meat and Meat Product	404	25
37	Terkuran et al., 2014	2014	Turkey	White Meat and Meat Product	5	1
38	Başbülbül et al., 2015	2015	Turkey	Fermented Foods	83	10
39	Hammad et al., 2015	2015	Egypt	Milk and Milk Product	175	155
40	Hidano et al., 2015	2015	Japan	White Meat and Meat Product	156	138
41	Rodriguez-López et al., 2015	2015	Spain	Milk and Milk Product	270	1
42	Guerrero-Ramos et al., 2016	2016	Spain	Milk and Milk Product	215	90
	Guerrero-Ramos et al., 2016	2016	Spain	Red Meat and Meat Product	215	70
43	Güldas et al., 2016	2016	Turkey	Milk and Milk Product	150	84
44	Kim et al., 2016	2016	Korea	Fermented Foods	14	3
45	Laukova et al., 2016	2016	Slovakia	White Meat and Meat Product	42	34
46	Osman et al., 2017	2017	Egypt	White Meat and Meat Product	80	11
47	Elmali et al., 2018	2018	Turkey	Red Meat and Meat Product	40	37
48	Jeon et al., 2018	2018	Korea	Fermented Foods	60	55
49	Sanlibaba and Senturk, 2018	2018	Turkey	White Meat and Meat Product	122	97
50	Dogan et al., 2017	2017	Turkey	Fermented Foods	130	127
51	El-Tawab, 2019	2019	Egypt	Milk and Milk Product	20	2
	El-Tawab, 2019	2019	Egypt	Red Meat and Meat Product	20	2
52	Kim et al., 2020	2020	Korea	White Meat and Meat Product	300	222
	Kim et al., 2020	2020	Korea	Milk and Milk Product	100	53
	Kim et al., 2020	2020	Korea	Red Meat and Meat Product	100	53
53	Klaharn et al., 2021	2021	Thailand	White Meat and Meat Product	3066	558

N: Number of samples, M: Number of *Enterococcus* spp. isolates.

Table 2. Characteristics of studies on the prevalence of antibiotic resistances of *E. faecalis* and *E. faecium*

Study No.	Authors	Years	Antibiotic	N	M	Method
1	2	3	4	5	6	7
1	Wilson et al., 2002	2002	Streptomycin	76	18	Disc diffusion
	Wilson et al., 2002	2002	Vancomycin	178	9	Disc diffusion
2	Chingwaru et al., 2003	2003	Ampicillin	1101	96	Disc diffusion
3	Getachew et al., 2013	2003	Vancomycin	103	27	Disc diffusion
4	Harada et al., 2004	2003	Gentamycin	137	9	Disc diffusion
5	Peters et al., 2003	2003	Vancomycin	253	128	Disc diffusion
	Peters et al., 2003	2003	Penicillin	253	118	Disc diffusion
	Peters et al., 2003	2003	Ampicillin	253	118	Disc diffusion
	Peters et al., 2003	2003	Gentamycin	253	118	Disc diffusion
6	Citak et al., 2005	2005	Vancomycin	147	48	Disc diffusion
	Citak et al., 2005	2005	Streptomycin	147	95	Disc diffusion
7	Citak et al., 2005	2006	Streptomycin	177	128	Disc diffusion
8	Ducková, 2007	2007	Vancomycin	164	41	Disc diffusion
	Ducková, 2007	2007	Ampicillin	164	60	Disc diffusion
	Ducková, 2007	2007	Gentamycin	164	45	Disc diffusion

Table 2 – contd.

1	2	3	4	5	6	7
9	Mcgowan-Spicer et al., 2008	2007	Penicillin	80	3	Disc diffusion
10	Abriouel et al., 2008	2008	Gentamycin	39	4	Disc diffusion
	Abriouel et al., 2008	2008	Streptomycin	39	2	Disc diffusion
11	Moore et al., 2008	2008	Penicillin	536	532	Disc diffusion
	Moore et al., 2008	2008	Streptomycin	536	532	Disc diffusion
12	Valenzuela et al., 2008	2008	Vancomycin	32	26	Disc diffusion
	Valenzuela et al., 2008	2008	Vancomycin	38	3	Disc diffusion
13	Ciftci et al., 2009	2009	Gentamycin	67	41	Disc diffusion
	Ciftci et al., 2009	2009	Penicillin	67	50	Disc diffusion
14	Riboldi et al., 2009	2009	Vancomycin	50	6	Disc diffusion
	Riboldi et al., 2009	2009	Ampicillin	50	2	Disc diffusion
	Riboldi et al., 2009	2009	Gentamycin	50	24	Disc diffusion
15	Kročkoet al., 2011	2011	Vancomycin	260	43	Disc diffusion
	Kročkoet al., 2011	2011	Ampicillin	260	84	Disc diffusion
16	Macedo et al., 2011	2011	Gentamycin	32	6	Disc diffusion
	Macedo et al., 2011	2011	Streptomycin	32	2	Disc diffusion
17	Terkuran et al., 2014	2011	Streptomycin	97	1	Disc diffusion
18	Chajęcka-Wierzchowska et al., 2016	2012	Penicillin	76	1	Disc diffusion
	Chajęcka-Wierzchowska et al., 2016	2012	Ampicillin	76	1	Disc diffusion
	Chajęcka-Wierzchowska et al., 2016	2012	Gentamycin	76	5	Disc diffusion
	Chajęcka-Wierzchowska et al., 2016	2012	Streptomycin	76	5	Disc diffusion
19	Chajęcka-Wierzchowska et al., 2012	2012	Streptomycin	38	6	Disc diffusion
	Chajęcka-Wierzchowska et al., 2012	2012	Gentamycin	76	7	Disc diffusion
20	Kročkoet al., 2012	2012	Gentamycin	260	112	Disc diffusion
	Kročkoet al., 2012	2012	Streptomycin	134	6	Disc diffusion
21	Cervinkova et al., 2013	2013	Ampicillin	50	14	Disc diffusion
22	Cetinkaya et al., 2013	2013	Vancomycin	12	6	Disc diffusion
	Cetinkaya et al., 2013	2013	Penicillin	12	1	Disc diffusion
23	Pesavento et al., 2014	2014	Gentamycin	429	10	Disc diffusion
	Pesavento et al., 2014	2014	Penicillin	254	15	Disc diffusion
	Pesavento et al., 2014	2014	Ampicillin	254	5	Disc diffusion
	Pesavento et al., 2014	2014	Gentamycin	254	44	Disc diffusion
24	Pesavento et al., 2014	2014	Vancomycin	254	7	Disc diffusion
	Pesavento et al., 2014	2014	Penicillin	254	34	Disc diffusion
25	Hammad et al., 2015	2015	Vancomycin	27	2	Disc diffusion
	Hammad et al., 2015	2015	Ampicillin	14	6	Disc diffusion
26	Olawale et al., 2015	2015	Vancomycin	226	26	Disc diffusion
	Olawale et al., 2015	2015	Gentamycin	226	115	Disc diffusion
27	Boss et al., 2016	2016	Penicillin	61	34	Disc diffusion
28	Ramos et al., 2016	2016	Vancomycin	31	15	Disc diffusion
	Ramos et al., 2016	2016	Ampicillin	31	8	Disc diffusion
	Ramos et al., 2016	2016	Streptomycin	31	1	Disc diffusion
29	Stępień-Pyśniak et al., 2016	2016	Vancomycin	1926	21	Disc diffusion
	Stępień-Pyśniak et al., 2016	2016	Gentamycin	1926	1920	Disc diffusion
30	Raafat, 2016	2016	Vancomycin	114	10	Disc diffusion
	Raafat, 2016	2016	Penicillin	12	1	Disc diffusion
	Raafat, 2016	2016	Vancomycin	114	6	Disc diffusion

Table 2 – contd.

1	2	3	4	5	6	7
31	Ramos, 2016	2016	Vancomycin	55	26	Disc diffusion
	Ramos, 2016	2016	Penicillin	55	11	Disc diffusion
	Ramos, 2016	2016	Ampicillin	55	5	Disc diffusion
	Ramos, 2016	2016	Streptomycin	55	5	Disc diffusion
32	Yılmaz et al., 2016	2016	Streptomycin	8	1	Disc diffusion
	Yılmaz et al., 2016	2016	Vancomycin	104	3	Disc diffusion
	Yılmaz et al., 2016	2016	Penicillin	104	8	Disc diffusion
	Yılmaz et al., 2016	2016	Ampicillin	104	5	Disc diffusion
33	Elmali et al., 2018	2018	Vancomycin	38	16	Disc diffusion
34	Sanlibaba and Senturk, 2018	2018	Penicillin	97	8	Disc diffusion
	Sanlibaba and Senturk, 2018	2018	Ampicillin	97	59	Disc diffusion
	Sanlibaba and Senturk, 2018	2018	Gentamycin	97	2	Disc diffusion
35	Różańska et al., 2019	2019	Vancomycin	365	3	Disc diffusion
	Różańska et al., 2019	2019	Penicillin	365	14	Disc diffusion
	Różańska et al., 2019	2019	Gentamycin	365	33	Disc diffusion
	Różańska et al., 2019	2019	Streptomycin	365	177	Disc diffusion

N: Number of *E. faecalis* and *E. faecium*, M: Number of antibiotic resistances of *E. faecalis* and *E. faecium.*

Results

As a result of the meta-analysis, a high level of heterogeneity was found between both *Enterococcus* spp. and the studies of antibiotic resistance of *E. faecalis* and *E. faecium* (Cochran's Q=21386.07, P<0.001, I^2=99.62; Cochran's Q = 235309.63, P<0.001, I^2=99.97, respectively). Prevalence of *Enterococcus* spp. and prevalence of antibiotic resistance of *E. faecalis* and *E. faecium* 0.41 (95% C.I. 0.34–0.47), 0.25 (95% C.I. 0.13–0.38) were calculated, respectively (Table 3). The forest plots created as a result of the meta-analysis of the prevalence of *Enterococcus* spp. and antibiotic resistance studies of *E. faecalis* and *E. faecium* are given in Figure 2 A and Figure 2 B. In addition, the heterogeneities among studies can be observed in the forest plots drawn. According to the results of the Regression test for Funnel Plot Asymmetry, no publication bias was detected in the prevalence of *Enterococcus* spp. and antibiotic resistance studies (Table 4, Figure 3 A and Figure 3 B). As a result of subgroup analyses based on samples, the highest prevalence of *Enterococcus* spp. was in fermented foods at 0.56 (95% CI: 0.1; 1.00), while the lowest prevalence was calculated in red meat and red meat products at 0.29 (95% CI: 0.18; 0.40). There is no significant difference in the prevalence of *Enterococcus* spp. between food types (Cochran's Q = 5.38, df = 4, P = 0.250). Concerning subgroup analyses by country, the highest prevalence of *Enterococcus* spp. was calculated in studies conducted in Slovakia at 0.74 (95% CI: 0.56; 0.93), while the lowest prevalence was calculated in studies conducted in Georgia at 0.07 (95% CI: 0.02; 0.12). The prevalence of *Enterococcus* spp. showed significant variability by country (Cochran's Q = 125.70, df=13, P<0,001) (Table 5). It was observed that the prevalence of *Enterococcus* spp. tends to decrease over the years. However, no significant difference was found between antibiotics and between years (Cochran's Q = 1.21, df = 4, P = 0.877; Cochran's Q = 2.24, df=1, P = 0.135, respectively) (Table 6). According to the subgroup analyses made by the country, the highest antibiotic resistance was seen in studies conducted in Germany (0.48 (95% CI: 0.45–0.51), and the lowest antibiotic resistance was seen in studies conducted in Vietnam (0.03 (95% CI: 0.03–0.06)). In the subgroup analyses made according to years, it was seen that antibiotic resistance was higher in the years 2000–2009 compared to the years 2010–2021 (0.39 and 0.17, respectively). There were significant differences in antibiotic resistance between countries and years (Cochran's Q = 551.16, df = 10, P<0.00; Cochran's Q = 12.77, df = 1, P<0.001, respectively) (Table 7).

Table 3. The pooled prevalence of *Enterococcus* spp. and antibiotic resistances of *E. faecalis* and *E. faecium*

	N	Prevalence (95% CI)	Cochran's Q	I^2	τ^2	P (Cochran's Q)
Enterococcus spp.	82	0.41 (0.34–0.47)	21386.07	99.62	0.09	<0.001
Antibiotic resistances of *E. faecalis* and *E. faecium*	78	0.25 (0.13–0.38)	235309.63	99.97	0.30	<0.001

N: Number of study, 95% CI: 95% Confidence interval, I^2: Ratio of variance in observed effects to variance in true effects rather than sampling error, τ^2: Variance in true effect sizes.

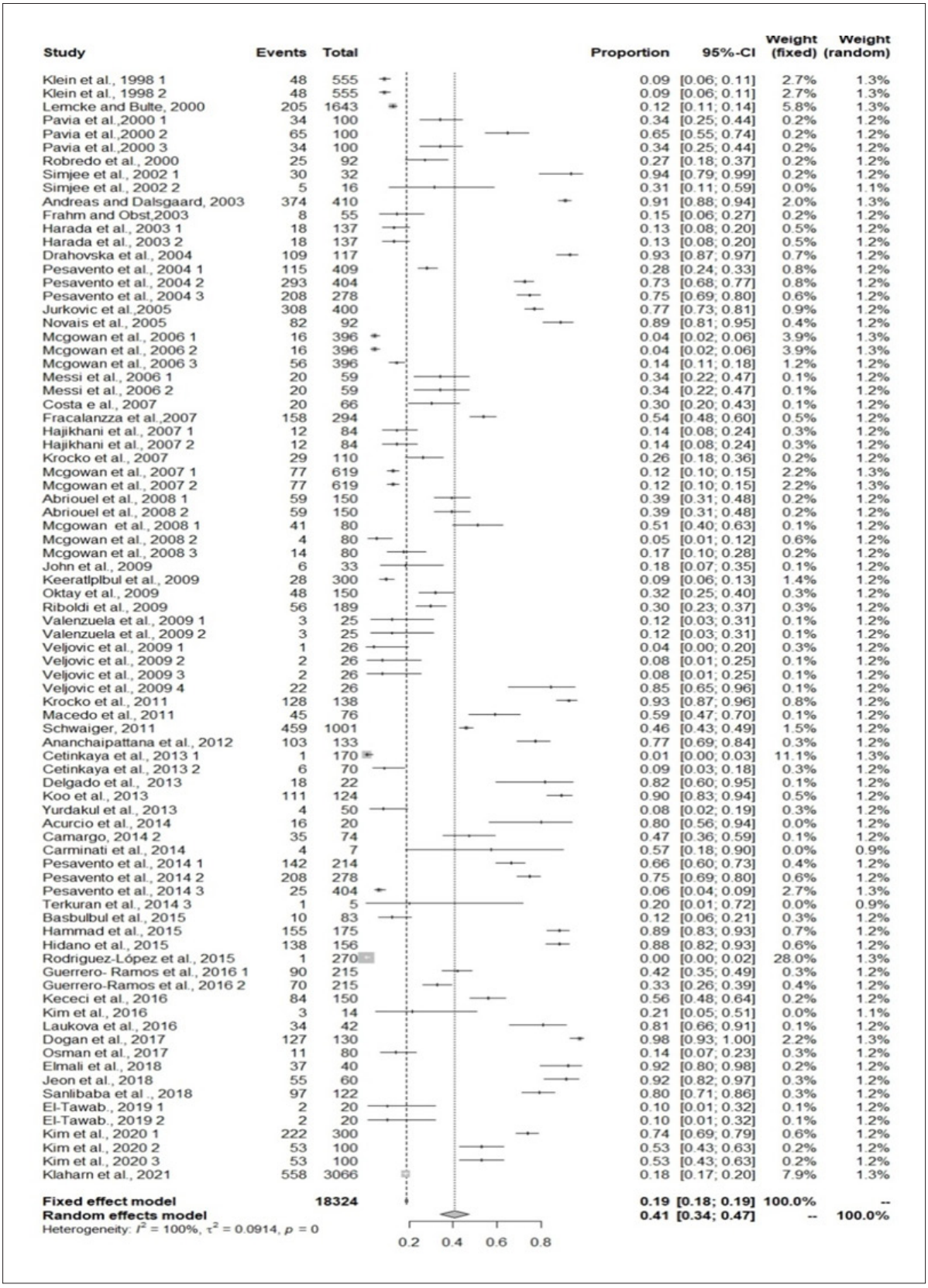


Figure 2 A. Forest plots of meta-analysis of *Enterococcus* spp. studies

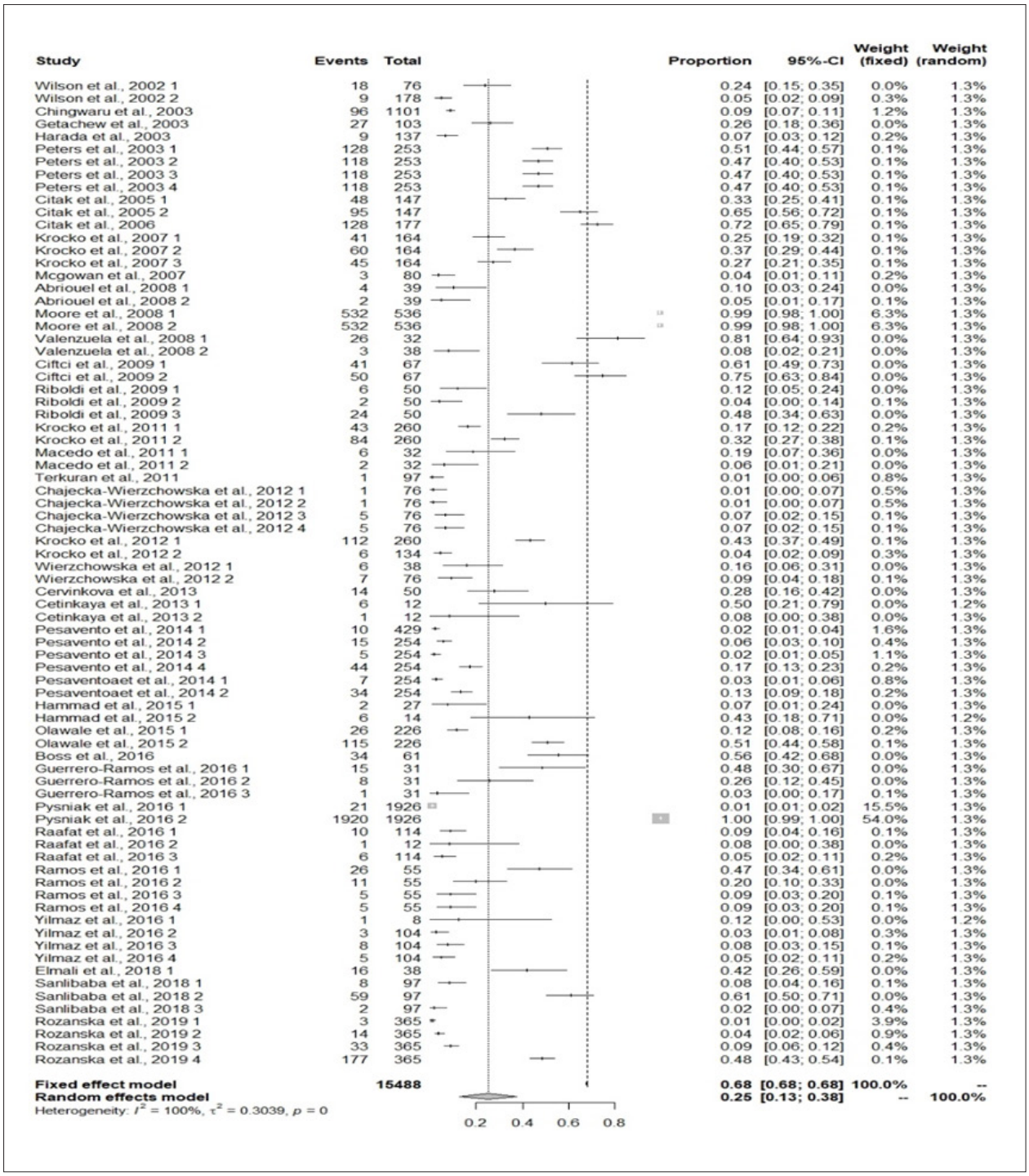


Figure 2 B. Antibiotic resistances of *E. faecalis* and *E. faecium* studies

Table 4. Publication bias of study samples included in the meta-analysis

Regression test for funnel plot asymmetry			
	Coefficient (95% CI)	z Statistic	P-value
Enterococcus spp.	0.388 (0.286–0.490)	0.448	0.654
Antibiotic resistance of *E. faecalis* and *E. faecium*	0.178 (–0.020–0.376)	0.918	0.359

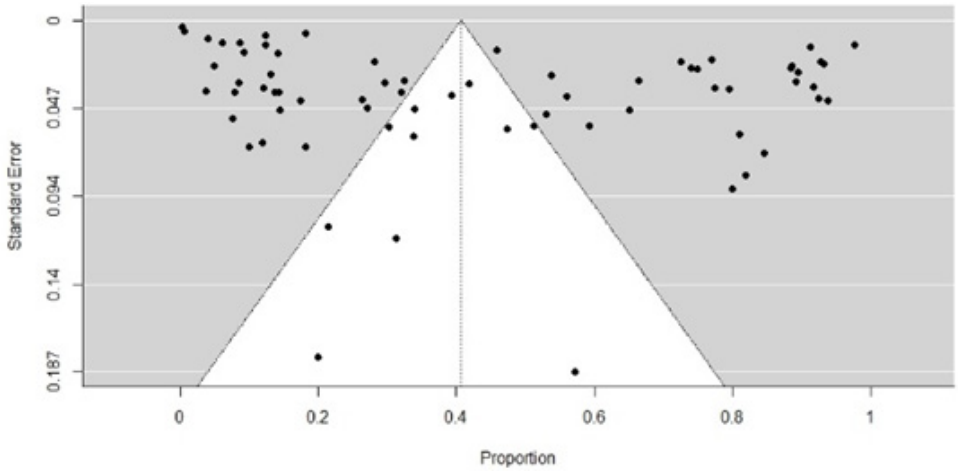


Figure 3 A. Funnel plots of *Enterococcus* spp. studies

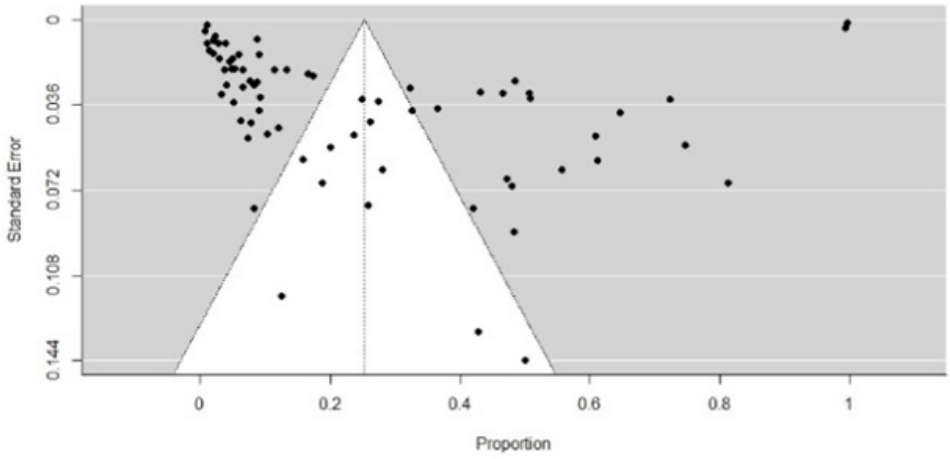


Figure 3 B. Funnel plots of antibiotic resistances of *E. faecalis* and *E. faecium* studies

Table 5. Result of subgroup analysis of *Enterococcus* spp. prevalence

Subgroup	N	Prevalence (95% CI)	Cochran's Q	I^2	τ^2
1	2	3	4	5	6
Samples					
Fermented Foods	4	0.56 (0.13–1.00)	543.39	99.4	0.195
Milk and Milk Product	28	0.39 (0.30–0.48)	6199.24	99.6	0.055
Other Foods	5	0.45 (0.28–0.63)	148.82	97.3	0.039
Red Meat and Meat Product	15	0.29 (0.18–0.40)	1379.26	99.0	0.044
White Meat and Meat Product	30	0.45 (0.33–0.58)	5590.66	99.5	0.122
Between-Groups	Cochran's Q = 5.38, df = 4, P = 0.250				
Countries					
Brazil	4	0.51 (0.34–0.68)	45.84	93.5	0.027
Egypt	4	0.31 (0.0–0.80)	395.97	99.2	0.245
Georgia	3	0.07 (0.02–0.12)	28.70	93.0	0.002
Germany	5	0.18 (0.06–0.30)	450.04	99.1	0.019
Italy	12	0.48 (0.29–0.68)	1473.33	99.3	0.112
Japan	3	0.38 (0.0–0.89)	529.83	99.6	0.203
Korea	6	0.66 (0.51–0.81)	110.92	95.5	0.031

1	2	3	4	5	6
Portugal	3	0.60 (0.24–0.95)	87.31	97.7	0.097
Serbia	4	0.26 (0.0–0.56)	109.42	97.3	0.093
Slovakia	5	0.74 (0.56–0.93)	227.69	98.2	0.044
Spain	9	0.32 (0.14–0.49)	554.30	98.6	0.067
Thailand	4	0.49 (0.08–0.90)	2524.38	99.9	0.173
Turkey	12	0.36 (0.07–0.66)	5261.52	99.8	0.265
USA	8	0.30 (0.16–0.44)	406.63	98.3	0.039
Between-Groups	Cochran's Q = 125.70, df=13, P<0,001				
Years					
1995–2004	11	0.51 (0.31–0.71)	889.52	98.9	0.108
2005–2014	55	0.43 (0.34–0.51)	12630.32	99.6	0.107
2015–2021	16	0.27 (0.15–0.40)	4820.78	99.7	0.064
Between-Groups	Cochran's Q = 5.30, df=2, P = 0.071				

N: Number of study, 95% CI: 95% Confidence Interval, df: Degree of freedom, I^2: Ratio of variance in observed effects to variance in true effects rather than sampling error, τ^2: Variance in true effect sizes.

Table 6. Subgroup analysis of antibiotic resistance prevalence of *E. faecalis* and *E. faecium*

Subgroup	N	Prevalence (95% CI)	Cochran's Q	I^2	τ^2
Antibiotics					
Ampicillin	13	0.22 (0.15–0.29)	494.61	97.6	0.015
Gentamycin	16	0.29 (0.0–0.60)	30971.31	100	0.411
Penicillin	14	0.26 (0.0–0.57)	18094.80	99.9	0.368
Streptomycin	14	0.27 (0.0–0.59)	13180.74	99.9	0.370
Vancomycin	21	0.19 (0.15–0.22)	720.85	97.2	0.006
Between-Groups	Cochran's Q = 1.21, df = 4, P = 0.877				
Years					
2000–2009	29	0.36 (0.21–0.51)	20398.29	99.9	0.167
2010–2021	49	0.19 (0.01–0.36)	209946.34	100.0	0.393
Between-Groups	Cochran's Q = 2.24, df=1, P = 0.135				

N: Number of study, 95% CI: 95% Confidence interval, df: Degree of freedom.

Table 7. Results of subgroup analysis of antibiotic resistance prevalence of *E. faecalis* and *E. faecium*

Country	N	Prevalence (95% CI)	P-value	Cochran's Q	I^2
Brazil	3	0.21 (0.01–0.47)	0.050	33.77	94.1
Czech Republic	3	0.43 (0.01–1.00)	0.261	134860.58	100.0
Egypt	5	0.07 (0.04–0.11)	<0.001	8.50	52.9
Germany	4	0.48 (0.45–0.51)	<0.001	1.19	0.0
Italy	6	0.07 (0.02–0.12)	0.004	64.00	92.2
Poland	6	0.14 (0.03–0.29)	0.030	348.46	98.6
Portugal	6	0.18 (0.06–0.29)	0.019	32.62	84.7
Slovakia	7	0.26 (0.17–0.36)	0.016	178.01	96.6
Spain	5	0.17 (0.02–0.33)	0.028	28.39	85.9
Turkey	16	0.31 (0.17–0.46)	0.080	1053.05	98.6
Vietnam	4	0.03 (0.03–0.06)	<0.001	5.66	47.0
Cochran's Q = 551.16, df = 10, P < 0.001					
Years					
2000–2009	17	0.39 (0.28–0.50)	0.050	579.94	97.2
2010–2021	48	0.17 (0.11–0.23)	0.039	209380.13	100.0
Cochran's Q = 12.77, df = 1, P < 0.001					

N: Number of study, 95% CI: 95% Confidence interval, df: Degree of freedom, I^2: Ratio of variance in observed effects to variance in true effects rather than sampling error, τ^2: Variance in true effect sizes.

Discussion

Enterococci constitute the most predominant bacteria group in foods, due to their resistance to challenging conditions in the environment during production technology, coupled with their high adaptability and food storage situation (Igbinosa and Beshiru, 2019; Dapkevicius et al., 2021). Even though it is known that *Enterococci* are ubiquitous organisms in the gut, it is one of the emerging organisms causing infections in humans. Recent studies reported that *Enterococci* contamination occurs in a wide range of foods due to poor hygienic conditions during their production, and it may pose a threat to human health (Yılmaz et al., 2016; Hanchi et al., 2018). *Enterococcus* spp. is considered a potential resistance gene reservoir that can be transferred horizontally to other bacteria via the food chain (Pesavento et al., 2014). *E. faecium* and *E. faecalis* have become increasingly important pathogens worldwide because they are related to life-threatening hospital-acquired infections. *E. faecalis* is the most pathogenic species, while *E. faecium* is generally involved in the acquisition and transfer of antimicrobial resistance. The high percentage of multidrug-resistant *E. faecium* and *E. faecalis* in food animals is worrying, especially given that very few antimicrobial agents can be used to control Enterococcal infection. Such resistance is likely to be passed from food animals to humans through the food chain. Determining the prevalence of antibiotic resistance of *Enterococci* is a significant step for interventions to control the emergence and transmission of resistant pathogens (Melese et al., 2020; Kim et al., 2021).

There was a statistically significant difference in the prevalence of *Enterococcus* spp. between year subgroups. The main reason for this may be derived from many species of *Enterococcus* spp. and poor laboratory capacity to identify species of *Enterococci*. Notably, due to subgroup analysis, the highest prevalence of *Enterococcus* spp. was calculated in fermented foods (0.56), while the lowest prevalence was calculated in red meat and red meat products (0.29). This might result from some *Enterococci* strains as natural in fermented foods.

Notably, the unthinking use of antibiotics in poultry and livestock can cause the spread of multiple-drug-resistant *Enterococci* which would get transmitted to humans via consuming or handling their products contaminated with the pathogen. The linezolid- (LRE) and vancomycin-resistant *Enterococci* represent a significant threat to clinical treatment. Vancomycin-resistant *Enterococcus* spp. (VRE) has been identified as the leading multidrug-resistant *Enterococcus* spp. in healthcare environments. Due to its clinical and public health significance, the World Health Organization (WHO) and the U.S. Centers for Diseases Control and Prevention listed VRE as a high-priority pathogen in urgent need of drug research and development (Ayobami et al., 2020; Yi et al., 2022). Emaneini et al. (2016) reported that the prevalence of vancomycin-resistant *Enterococci* infections among *Enterococcus* species was 9.4% (95% C.I. 7.3–12) in a meta-analysis of clinical cases in Iran. In the meta-analysis studies conducted by Melese et al. (2020), the prevalence of vancomycin-resistant *Enterococcus* spp. was found to be rated at 20% (95% C.I. 14%–26%) and 14.8% (95% C.I. 8.7–24.3), respectively. In this study, the highest antibiotic rates for *E. faecium* and *E. faecalis* were calculated as gentamycin at 0.29% (95% C.I 0.60) and vancomycin at 0.27% (95% C.I. 0.59). However, in the earlier study conducted by Shiadeh et al. (2019), the highest antibiotic resistance rated for *E. faecalis* and *E. faecium* were calculated as quinupristin/dalfopristin and penicillin at 97% (95% C.I.89–99.2) and 85% (95% C.I. 81–89), respectively. In a study by Tang et al. (2017), the pooled antibiotic resistance was reported as 0.24 (95% C.I. 0.42 to 0.06). The result obtained in the study was similar to that of the current study.

In this study according to the subgroup analyses made by the country, the highest antibiotic resistance was seen in studies conducted in Germany (0.48; 95% CI: 0.45–0.51), and the lowest antibiotic resistance was seen in studies conducted in Vietnam (0.03; 95% CI: 0.03–0.06). However, Lawpidet et al. (2021) conducted studies on continents, and they found the prevalence of antibiotic resistance was highest in Africa (18.5%; 95% CI = 12.8 to 26.1) and lowest in North America (0.3%; 95% CI = 0.1 to 1.19). In other study by Wada et al. (2022) the pooled prevalence of antibiotic resistance in Malaysia was estimated at 24.0% (95% CI; 16.7–33.1%). Unlike other regions, probably antibiotics have been used as a feed additive for animal production in Germany.

Conclusion

According to the subgroup analysis in our study, antibiotic resistance increased between 2000 and 2009. The reason for antibiotic resistance in food-originated isolates between 2000 and 2009 can also be associated with high and unconscious use of antibiotics to treat diseases, such as prophylaxis, and growth promotion in animal production. For many of the antibiotics examined in our study, the resistance was found to be decreased worldwide from 2010 to 2021. Probably the most crucial reason for the decline in antibiotic resistance in most countries is the decrease in unconscious consumption of antibiotics in livestock and poultry. This meta-analysis study provided pooled prevalence data for *E. faecalis* and *E. faecium,* other *Enterococci* in various food types. In conclusion, the estimate of antibiotic-resistant *E. faecalis* and *E. faecium* prevalence worldwide in foods was calculated as 0.25, indicating the load of these agent contaminations in foods. We demonstrated the antibiotic resistance of *E. faecalis* and *E. faecium* in foods and gaps that could be addressed in the future. With the emergence of drug-resistant bacterial strains, including vancomycin, there is a need to investigate newer antimicrobials in veterinary medicine. Based on our study, regular monitoring of VRE in foods is required in developing effective control measures. Further, livestock farmers should be educated

on antibiotic resistance and trained on the responsible utilization of antibiotics. Awareness of antimicrobial resistance should be raised by all stakeholders and lastly, reduction of contamination by encouraging proper hygiene in animal husbandry, food production, and processing.

Conflict of interest

The authors declare no conflict of interest.

Authors contributions

MB contributed to the conception, design, interpretation, and data analysis. CG contributed to the acquisition of data. All authors have read and approved the final version of the manuscript and agree to be accountable for their contribution to the manuscript.

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