International Journal of Food Studies | IJFS October 2021 | Volume 10 | pages 359–370

Tetracycline Resistance in Enterococci and *Escherichia coli* Isolated from Fresh Produce and Why it Matters

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Received: 25 June 2020; Published online: 18 Octover 2021

Abstract

The contamination of fresh produce with antibiotic-resistant bacteria is of particular concern as they are often eaten raw and can be a source for foodborne diseases. Tetracyclines have been largely used in humans, animals and plants which might have accelerated microbial resistance to them. Enterococci and *Escherichia coli* can be used as indicators to monitor contamination of the fresh produce with tetracycline-resistant bacteria. The investigation related to this issue is very scarce in Oman.This study aimed at identifying tetracycline-resistant enterococci and *E. coli* in fresh produce at the market place. Thirty-one enterococci and ten *E. coli* were isolated from local (Oman) and imported fruits and vegetables (N= 105). Using the standard Kirby-Bauer disc diffusion method, resistance to tetracycline was found in 6 (19 %) enterococci, isolated from cucumber, lettuce and radish, and 5 (50 %) *E. coli*, obtained from cabbage, lettuce and radish. Genetic analysis revealed the presence of tetracycline resistance genes, *tet*(A) and *tet*(K), in *E. coli* and *tet*(K), *tet*(L) and *tet*(M) in enterococci, including *Enterococcus sulfureus, Enterococcus mundtii, Enterococcus casseliflavus* and *Enterococcus faecalis*. The integron integrase *IntI 1* gene, which is known to facilitate the dissemination of antibiotic resistance genes among bacteria, was detected in 2 isolates of *E. coli* . These results demonstrated the capability of fresh produce to act as a potential source for disseminating tetracycline or possibly other antibiotic-resistant bacteria through the food chain. Thus, control strategies are needed to reduce exposure of the public to such microorganisms.

Keywords: Antibiotic resistance; *E. coli* ; Enterococcus; Integron; Vegetables; Tetracycline

1 Introduction

Tetracyclines (TEs) are amongst the most important antibiotic groups used in medicine (Hernandez et al., 2003). They exhibit a broad spectrum of activity against Gram-positive and Gram-negative bacteria, chlamydiae, rickettsiae, nematodes, mycoplasmas and protozoans. They inhibit protein synthesis in bacterial cells (Hernandez et al., 2003; Markley & Wencewicz, 2018) by preventing attachment of aminoacyl-tRNA to the acceptor (A) site of the ribosome. However, this attachment is reversible rendering TEs to be bacteriostatic (Kohanski et al., 2010; Roberts, 2005).TEs are used to treat infections in humans and animals, preserve harvested fruits and veg-

 10.7455/ijfs/10.2.2021.a7

Nomenclature

ARB	antibiotic-resistant bacteria	HGT	horizontal gene transfer
ARG	antibiotic resistance genes	TE(s)	tetracycline(s)

etables, kill insect pests, and as growth promoters for animals (Hernandez et al., 2003). As resistance to TEs occurred shortly after their first clinical use in the 1940s, new semi-synthetic TEs were generated resulting in the evolution of the TEs scaffold and expansion of their use (Markley & Wencewicz, 2018).

Genetic acquisition of *tet* genes is the major cause of resistance to TEs. The *tet* efflux genes code for membrane-associated proteins that export TE from the cell. Examples include *tet*(A) , *tet*(B), *tet*(C), *tet*(D), *tet*(K), *tet*(L) and *tet*A(P). Ribosomal protection proteins are cytoplasmic proteins that protect bacterial ribosomes from the effect of TEs. The *tet*(M), *tet*(O) and *Otr*A are among these proteins. TEs can also be altered enzymatically, resulting in their inactivation by the cytoplasmic protein product of the *tet*(X) gene (Roberts, 2005). Reduced permeability through alteration or reduced expression of porins or morphological changes and mutation in the ribosome can also lead to tetracycline (TE) resistance (Markley & Wencewicz, 2018).

Horizontal gene transfer (HGT) among bacteria plays an important role in the dissemination of multidrug resistance, especially when antibiotic resistance genes (ARG) are located on mobile genetic elements such as plasmids and transposons. Transposons contain special gene sequences known as integrons. The gene sequence of an integron serves as a site-specific recombination system allowing it to capture or excise novel genetic elements known as gene cassettes that code for various ARG (Roe & Pillai, 2003). The integrase enzyme of the integron can insert gene cassettes at a specific site known as the attI site and then express them (Gillings et al., 2009). Romaine lettuce, alfalfa sprouts and Savoy spinach in Canada were demonstrated to harbor antibiotic-resistant bacteria (ARB) that carried integron DNA (Bezanson et al., 2008). Use of the class 1 integrase-encoding gene (*IntI 1* gene) has been proposed as a generic marker for anthropogenic contaminants because of its common association with antibiotic resistance in pathogenic and commensal bacteria (Jones-Dias et al., 2016).

Enterococcus bacteria have emerged as potential pathogens due to their multiple drug resistance as a result of their capabilities to acquire antibiotic resistance through mutation and HGT (Johnston & Jaykus, 2004). Therefore, antibiotic resistance of enterococci should be monitored to identify foods that can impose a real risk to people (Pesavento et al., 2014). *E. faecalis, E. faecium E. hirea* (Ben Said et al., 2016) and *E. coli* that were isolated from different produce types were found to be resistant to TE (Ben Said et al., 2016; Campos et al., 2013). Many outbreaks have been linked to specific strains of *E. coli* such as *E. coli* O157:H7 in ready-to-eat salads in 2013 (CDC, 2014a) and *E. coli* O121 in raw clover sprouts in 2014 (CDC, 2014b). Treating infections becomes more complicated when the causative agent of an outbreak is resistant to antibiotics as well (O'Flahertya et al., 2019).

The prevalence of antibiotic-resistant bacteria was found to vary among countries due to various factors such as the type of antibiotics used (Chewapreecha, 2014). In Oman, TE-resistant *E. coli* was previously isolated from the oviductal fluid of green turtles (Al-Bahry et al., 2012) and chicken (Al-Bahry et al., 2012) but TE-resistance has not been previously investigated in produce-associated bacteria. The aim of this study was to identify phenotypic and genotypic TE resistance in enterococci and *E. coli* that we previously isolated from different types of locally pro-

duced and imported fresh produce. The *E. coli* isolates were also screened for the presence of the *IntI 1* gene. The results of this study can help in understanding one of the routes of human exposure to TE-resistant bacteria through consumption of fresh produce. This may be used to develop control strategies to reduce exposure of the public to ARB through food consumption.

2 Materials and Methods

2.1 Sample collection and bacterial isolation and identification

Isolation and identification of enterococci and *E. coli* from different local (39 samples) and imported (66 samples) fresh fruits and vegetables were performed as previously reported (Al-Kharousi et al., 2016). About 93 enterococci isolates and 15 *E. coli* isolates were collected. To avoid analyzing duplicate or clonal bacteria, one typical colony (details can be found by searching the manufacturer's website; http://www.oxoid.com/UK/blue/index.asp?c=UK&lang=EN) of the bacterial isolate from each species was selected from each sample (31 enterococci and 10 *E. coli* isolates) for subsequent analysis.

2.2 Phenotypic screening of enterococci and *E. coli* for resistance to TE

The standard Kirby-Bauer disc diffusion method described by the Clinical and Laboratory Standards Institute (CLSI, 2015) was used to test the resistance of enterococci and *E. coli* to TE. Mueller-Hinton agar and TE discs were supplied by Oxoid, UK. *E. coli* ATCC 25922 (TE-susceptible), *Enterococcus faecalis* ATCC 51299 (TE-susceptible) and *E. faecalis* ATCC 29212 (TE-resistant) were used as reference control strains. Enterococci were considered to be resistant if the inhibition zone was $\leq$ 14 mm while *E. coli* was considered resistant if the inhibition zone was $\leq$ 11 mm (CLSI, 2015).

2.3 Identification of TE resistance genes

According to the method developed by Ng et al. (2001), multiplex PCR was used to screen for the presence of 14 *tet* genes in the isolates that showed phenotypic resistance to TE. The sequences of primers are presented in Table 1. In brief, the PCR reaction mix contained template DNA at a concentration of 10 ng/μl and sterile Milli-Q water was added to make a total volume of 25 μl. Primers were supplied by Macrogen, South Korea for *tet* genes as follows: group I; *tet*(B) (0.25 μM), *tet*(C) (0.25 μM) and *tet*(D) (2.0 μM), group II; *tet(A)* (1.0 μM), *tet*(E) (1.0 μM) and *tet*(G) (1.0 μM), group III; *tet*(K) (1.25 μM), *tet*(L) (1.0 μM), *tet*(M) (0.5 μM), *tet*(O) (1.25 μM) and *tet*(S) (0.5 μM), and group IV; *tet*A(P) (1.25 μM), *tet*(Q) (1.25 μM) and *tet*(X) (1.25 μM). PCR beads (puReTaq Ready-To-Go PCR beads) were supplied by GE Healthcare, UK. The thermal profile (Veriti 96-well Thermal cycler, Applied Biosystems, Singapore) for the PCR reaction was as follows: stage 1; denaturation at 94^{o}C for 5 min, stage 2; denaturation at 94^{o}C for 30 sec, annealing at 55^{o}C for 1 min, extension at 72^{o}C for 1.5 min (35 cycles), stage 3; final extension at 72^{o}C for 10 min and then kept at 4^{o}C.

Enterococci and *E. coli* isolates were screened for the *IntI 1* gene according to the previously reported methods (Gaze et al., 2005; Rosser & Young, 1999). The PCR mixtures were as previously described for the type of primer pairs (described in Table 1). The thermal profile for the PCR reaction of the *IntI 1* gene (1 pmole/μl of each primer) was as follows: stage 1; denaturation at 96^{o}C for 5 min, 55 for 1 min, 70^{o}C for 3 min (one cycle), stage 2; denaturation at 96^{o}C for 15 sec, annealing at 55^{o}C for 30 sec, extension at 70^{o}C for 3 min (24 cycles), stage 3; final extension at 70^{o}C for 5 min and then kept at 4^{o}C. Five-microliter aliquots of PCR products of *tet* and *IntI 1* genes were analyzed by gel electrophoresis with 2 % agarose (Thermo Scientific, TopVision, USA) and 0.5 μg/ml ethidium bromide (Sigma-Aldrich, USA). Gels were visualized by UV using GelDoc (GeneFlash, Syngene, USA). A 100-bp ladder (Fermetas,

O'RangeRuler, Thermo Fisher Scientific) was run on each gel as a molecular size marker. The PCR products of *tet* and *IntI 1* genes were sequenced abroad (Macrogen, South Korea). DNA sequences were aligned and analyzed through the ChromasPro program (Version 1.41, Technelysium Pty Ltd) and compared online with those found at the NCBI using the BLAST program. The DNA sequences were submitted to the European Nucleotide Archive (ENA) to be assigned accession numbers.

2.4 Statistical analysis

Statistical tests were performed using JMP® SAS 14.3, USA to identify significant differences that were considered as $P<0.05$. Chi-square analysis was used to test if TE-resistance differed significantly according to the species and the source of enterococci (local or imported) and the source of *E. coli* (local or imported).

3 Results and Discussion

3.1 Prevalence of enterococci and *E. coli*

Enterococci were recovered from 31 out of 105 samples (30 %: 12 local out of 39; 31 % and 19 imported out of 66; 29 %), including cabbage, cucumber, dates, mango, lettuce, papaya, radish, tomato and watermelon but not banana, pomegranate, carrot or capsicum. *E. coli* was recovered from 10 samples (10 %: 6 local out of 39; 15 % and 4 imported out of 66; 6 %) of cabbage, lettuce and radish. More detailed information on the prevalence and identity of enterococci and the prevalence of *E. coli* can be found in our previous publication (Al-Kharousi et al., 2016).

3.2 TE-resistance of enterococci

Nineteen percent (6 isolates out of 31, from imported samples only) of enterococci were resistant to TE (Fig. 1). These were *Enterococcus casseliflavus, E. faecalis, Enterococcus mundtii* and *Enterococcus sulfureus* (Table 2), isolated from cucumber, lettuce and radish. *E. casseliflavus* had *tet*(L) and *tet*(M). The latter was also found in *E. faecalis*. *E. mundtii* possessed *tet*(L) while *E. sulfureus* harbored *tet*(K) (Table 3).

Enterococci are involved in food intoxication and in spreading antibiotic resistance through the food chain (Abriouel et al., 2008), and they are a leading cause of nosocomial infections (Abriouel et al., 2008; Tian et al., 2019). TE-resistant enterococci were previously isolated from different produce types (Ben Said et al., 2016; Campos et al., 2013). Twenty-nine percent of *Enterococcus faecium* isolated from fresh produce grown in the United States of America were found to be resistant to TE while all *E. faecalis* isolates were susceptible to TE (Johnston & Jaykus, 2004). In this study, resistance to TE was exhibited by six enterococci (19 %) isolated from the imported samples only. These were *E. casseliflavus, E. faecalis, E. mundtii* and *E. sulfureus*, isolated from cucumber (source: United Arab Emirates; UAE), lettuce (source: Iran and Jordan) and radish (source: China). *tet*(K) was harbored by *E. sulfureus* and *tet*(L) by *E. casseliflavus* and *E. mundtii*, whilst *tet*(M) was found in *E. casseliflavus* and *E. faecalis*. The *tet*(K) and *tet*(L) genes code for efflux proteins while *tet*(M) codes for ribosomal-protection proteins (Roberts, 2005).

Statistical tests showed that the type of species of enterococci had no significant effect on their resistance to TE; χ^2 (7, N= 31) = 3.00, P= 0.8853. However, the source (local or imported) of enterococci significantly affected the number of TE-resistant enterococci χ^2 (1, N= 31) = 4.699, P= 0.0302. Actually, all enterococci originated from local samples were susceptible to TE. This may indicate the absence or low levels of TE-resistant enterococci in locally produced fresh produce as compared to those imported from different countries. In particular, TE-resistance was found in enterococci isolated from imported produce from Iran, Jordan, China and the UAE. Information regarding TE-resistance of enterococci associated with fresh produce in these mentioned countries is very limited. The resistance of *Enterococcus* sp. to most antimicrobials was reported to be more prevalent in China than in European or other Asian countries (Liu et al., 2013). The re-

Table 1: Primers used for multiplex PCR for screening tetracycline resistance genes (Ng et al., 2001) and the *IntI 1* gene (Gaze et al., 2005; Rosser & Young, 1999)

Targeted gene	Primers sequence 5'-3'	Amplicon size
Tet(A)	GCT ACA TCC TGC TTG CCT TC CAT AGA TCG CCG TGA AGA GG	210
Tet(B)	TTG GTT AGG GGC AAG TTT TG GTA ATG GGC CAA TAA CAC CG	659
Tet(C)	CTT GAG AGC CTT CAA CCC AG ATG GTC GTC ATC TAC CTG CC	418
Tet(D)	AAA CCA TTA CGG CAT TCT GC GAC CGG ATA CAC CAT CCA TC	787
Tet(E)	AAA CCA CAT CCT CCA TAC GC AAA TAG GCC ACA ACC GTC AG	278
Tet(G)	GCT CGG TGG TAT CTC TGC TC AGC AAC AGA ATC GGG AAC AC	468
Tet(K)	TCG ATA GGA ACA GCA GTA CAG CAG ATC CTA CTC CTT	169
Tet(L)	TCG TTA GCG TGC TGT CAT TC GTA TCC CAC CAA TGT AGC CG	267
Tet(M)	GTG GAC AAA GGT ACA ACG AG CGG TAA AGT TCG TCA CAC AC	406
Tet(O)	AAC TTA GGC ATT CTG GCT CAC TCC CAC TGT TCC ATA TCG TCA	515
Tet(S)	CAT AGA CAA GCC GTT GAC C ATG TTT TTG GAA CGC CAG AG	667
TetA(P)	CTT GGA TTG CGG AAG AAG AG ATA TGC CCA TTT AAC CAC GC	676
Tet(Q)	TTA TAC TTC CTC CGG CAT CG ATC GGT TCG AGA ATG TCC AC	904
Tet(X)	CAA TAA TTG GTG GTG GAC CC TTC TTA CCT TGG ACA TCC CG	468
IntI 1	ATCATCGTCGTAGAGACGTCGG GTCAAGGTTCTGGACCAGTTGC	892

Table 2: Levels of antibiotic resistance in enterococci (n= 31) and *Escherichia coli* (n= 10) isolated from fresh fruits and vegetablese

Bacteria species	Total #	Susceptible		Resistant	
		Local	Imported	Local	Imported
Enterococcus casseliflavus	13	1	10	0	2
Enterococcus faecalis	7	5	0	0	2
Enterococcus faecium	2	1	1	0	0
Enterococcus gilvus	1	0	1	0	0
Enterococcus hirae	1	1	0	0	0
Enterococcus mundtii	4	2	1	0	1
Enterococcus raffinosus	1	1	0	0	0
Enterococcus sulfureus	2	1	0	0	1
Escherichia coli	10	4	1	2	3

Table 3: Levels of antibiotic resistance in enterococci (n= 31) and *Escherichia coli* (n= 10) isolated from fresh fruits and vegetables

Bacteria No.	Identity (PCR)	Source	Gene	Accession #
1	*E. coli*	Cabbage 1*, Oman	*tet*(A)	LT548573
2	*E. coli*	Cabbage 2, Oman	*tet*(A)	LT548574
4	*E. coli*	Lettuce 2, Jordan	*tet*(A)	LT548575
7	*E. coli*	Radish 1, China	*tet*(A)	LT548576
15	*E. coli*	Radish 2, China	*tet*(A)	LT548579
4	*E. coli*	Lettuce 2, Jordan	*tet*(K)	-
27	*E. sulfureus*	Lettuce 2, Jordan	*tet*(K)	-
35	*E. casseliflavus*	Radish 1, China	*tet*(L)	LT548584
36	*E. mundtii*	Radish 2, China	*tet*(L)	LT548585
25	*E. casseliflavus*	Cucumber 3, UAE	*tet*(M)	LT548581
26	*E. faecalis*	Lettuce 1, Jordan	*tet*(M)	LT548582
29	*E. faecalis*	Lettuce 1, Iran	*tet*(M)	LT548583
1	*E. coli*	Cabbage 1, Oman	*IntI 1*	LT548588
7	*E. coli*	Radish 1, China	*IntI 1*	LT548589

*: Sample number

-: Not available

sistance rate of enterococci isolated from clinical samples, in China, to TE was found to be 49.3 %, and *tet*(M) was detected in all TE-resistant isolates, including 22 *E. faecalis* and 14 *E. faecium* . Likewise, high resistance rates to TE (93 %) were reported in enterococci (*E. faecalis*, *E. faecium*, *E. hirae*, *E. gallinarum* and *E. casseliflavus*) isolated from chickens and pigs in China (Liu et al., 2013). Enterococci isolated from clinical samples in Iran were also found to be resistant to various antibiotics including TE (Asadollahi et al., 2018). Very limited information is available regarding antibiotic resistance of enterococci (clinical or environmental) in the UAE and Jordan.
Potential sources of contamination of fresh produce with ARB include animals, feces, soil, irrigation water, insecticides, fungicides, inadequately composted manure and human (Olaimat & Holley, 2012). In China, residues of TE and various *tet* genes, including *tet(A)* , *tet*(B), *tet*(C), *tet*(E), *tet*(M), *tet*(O), *tet*(S) and *tet*(X), were found in the irrigation wastewater and soil. Longterm irrigation with wastewater increased the abundance of *tet* genes in soil (Pan & Chu, 2018). Thus, the irrigation of vegetables with wastewater in China (Khan et al., 2008) can be a route for transferring ARB to vegetables. Also, the introduced *tet* genes into soils, if present on the transferable plasmids, may persist for a long time due to HGT and may be transferred to other bacteria or human pathogens (Pan & Chu, 2018). Likewise, in the UAE, irrigation with wastewater was reported to be a possible route for vegetable microbial contamination (Hussain & Qureshi, 2020). In Iran, fresh produce was found to have inadequate microbiological quality. Thus, improvements in vegetable production, assessment of contamination of irrigation water and fertilizers, and methods used for the identification of microbial contaminants will be necessary for a safer supply of fresh produce (Soltan Dallal et al., 2015).

3.3 TE-resistance of *E. coli*

Resistance to TE (Fig. 1) was found in 5 (50 %) *E. coli* isolates (two from local samples; cabbage and three from imported samples; lettuce and radish) (Tables 2 and 3). All of the TE-resistant *E. coli* harbored the *tet(A)* resistance gene. In addition, one *E. coli* that was isolated from lettuce harbored *tet*(K) (Table 3). The *IntI 1* gene was not detected in enterococci but in 2 isolates of *E. coli* ; one originated from local cabbage and the other from radish imported from China (Table 3). The accession numbers that were given to sequences of *tet* and *IntI 1* genes are available at http://www.ebi.ac.uk/ena/data/view/LT548573-LT548593 accessed June 24, 2020). Sequences of the *IntI 1* genes identified in the 2 isolates of *E. coli* were also recorded in the 'INTEGRALL' platform which is dedicated to integrons and can be reached on http://integrall.bio.ua.pt/?acc=LT548588 and http://integrall.bio.ua.pt/?acc=LT548589 (both accessed June 24, 2020).
E. coli can be used as an indicator species to determine the antibiotic resistance state of enteric microorganisms (Teuber, 1999). Resistance to TE by *E. coli* isolated from vegetables was previously reported in other countries (Hassan et al., 2011). All of the TE-resistant *E. coli* in the current study harbored *tet(A)* . One *E. coli* that was isolated from lettuce harbored *tet*(K). Both of these genes are efflux genes that code for membrane-associated proteins that export TE out of the cell (Ng et al., 2001; Roberts, 2005). There was no significant difference in the frequency of TE-resistant *E. coli* isolated from local and imported samples; χ^2 (1, N = 10) = 1.67, P= 0.1967 and this may indicate similar levels of contamination with TE-resistant *E. coli* . Investigation of TE-resistance in produce-associated *E. coli* was not previously studied in Oman. TE-resistance of animal-associated *E. coli* was previously studied by Al-Bahry et al. (2013) who found high levels of TE resistance (97.9 %) in *E. coli* isolated from the colon of chickens which were collected from three poultry farms in Oman, where the most common TE-resistant determinants were *tet(A)* followed by *tet*(B). In this study, the *IntI 1* gene for the class 1 integron was detected in 2 *E. coli* isolates; one obtained from local cabbage and the other from radish imported from China. Jones-Dias et al. (2016) detected the class 1 intergron in Gram-negative bacteria recovered from fresh produce grown in Portugal. The class 1 integron is widespread in Enterobacteriaceae bacteria and often harbors ARG (Jaglic

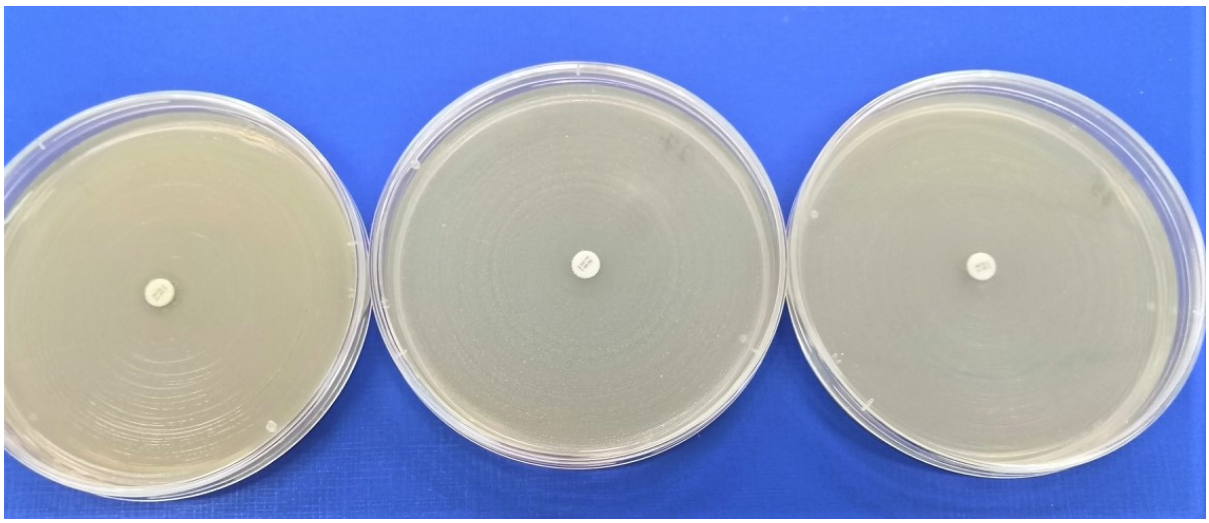

Figure 1: Disc diffusion method showing, from left to right, the resistance of *E. coli*, *E. sulfureus* and *E. casseliflavus* to tetracycline (diameters of growth inhibition zones: 7, 6 and 6 mm, respectively).

& Cervinkova, 2012). It would be interesting to investigate if TE or other ARG are located on the integrons of the 2 isolates of *E. coli* . Ingestion of foods contaminated with ARG, located on integrons, can facilitate transferof these determinants by HGT and thus influence the pool of antibiotic resistance in humans (Bezanson et al., 2008).

3.4 Significance of TE resistance in fresh produce-associated enterococci and *E. coli*

This study demonstrated phenotypic and genotypic TE resistance in enterococci and *E. coli* isolated from fresh produce ready for consumption. As ARB were also reported to be prevalent in humans and animals, as well as in natural (Gasparrini et al., 2020) and food environments, this may suggest that both pathogenic and commensal microbes have played an important role in spreading ARG, and that the food chain may act as a non-negligible route for bridging the antibiotic resistance between the environment and humans (Li & Wang, 2010). Different strains of enterococci and *E. coli* which originate from fresh produce may act as commensals and opportunistic or primary pathogens. Both of them can colonize the human gastrointestinal tract and may cause an opportunistic infection that might happen even years after the ingestion of the contaminated food (Hoelzel et al., 2018). As the results of this study demonstrated the presence of different types of *tet* genes in enterococci and *E. coli* isolated from fresh produce at the market place, these bacteria may colonize the intestine and affect the *tet* resistance pool especially if the *tet* genes can persist in the gut for a long time (Forslund et al., 2013). They also can serve as a vehicle for transfer of *tet* resistance genes in the intestine (Karami et al., 2006) or in the fresh produce environment before ingestion as was demonstrated to occur in lettuce (Jung & Matthews, 2016). In fact, in this study, *tet*(K) was detected in *E. sulfureus* and *E. coli* originated from the same lettuce sample (Table 3). It would be interesting to determine if any genetic event occurred between these 2 isolates in lettuce.

Although previous studies reported high levels of TE-resistance in enterococci (Cauwerts et al., 2007) and *E. coli* (Al-Bahry et al., 2013) from animal sources, DNA is partially degraded by heat, and thus, consumption of raw fresh produce is more likely to deliver a higher concentration of ARG into the gastrointestinal tract

(Hoelzel et al., 2018). Multidrug-resistant bacteria were previously isolated from various ready-to-eat foods (Vincenti et al., 2018), including fresh produce that contained multidrug-resistant enterococci (Johnston & Jaykus, 2004) and *E. coli* (Al-Kharousi et al., 2016). Thus, these food commodities, which usually receive no or minimal heat treatment before consumption, may act as a vehicle for transfer of multidrug-resistant enterococci or *E. coli* to humans (Johnston & Jaykus, 2004). This transfer may pose a risk to public health as drug resistance can decrease drug efficiency, increase the cost of the treatment of infectious diseases and increase the morbidity and mortality rates (Zhang et al., 2006).

4 Conclusions

Half of the isolated *E. coli* (sources: cabbage, lettuce and radish) were resistant to TE and possessed *tet(A)* and *tet*(K) genes. The integron integrase *IntI 1* gene was detected in 2 isolates of *E. coli* . This can potentiate the capability of fresh produce to disseminate antibiotic resistance among bacteria especially when these genetic elements are equipped with ARG. About a fifth of the tested enterococci (sources: cucumber, lettuce and radish) were resistant to TE and possessed *tet*(K), *tet*(L) or *tet*(M) genes. These results demonstrate the contamination of fresh produce with TE-resistant enterococci and *E. coli* . Effective measures are needed to prevent antibiotic-resistant foodborne bacteria from reaching humans.

Acknowledgements

We thank Sultan Qaboos University (SQU), Oman, for providing the funding for this study.

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