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Screening of Antibiotic Resistance and Virulence Genes of *Enterococcus* spp. Strains Isolated from Urfa Cheese

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ABSTRACT

Enterococcus faecium, *E. durans* and *E. faecalis* species were isolated and identified from traditional Urfa cheese samples which were produced from unpasteurized raw milk. The enterococcal load of the cheese samples was between 4.4-5.6 log cfu g^{-1}. High-level tetracycline, streptomycin, erythromycin, gentamycin, and penicillin resistance was determined in the enterococcal isolates. Multiple antibiotic resistance was also determined in *E. faecalis* (20.4%) and *E. faecium* (16.3%) strains. 36.7% of the enterococcal isolates were greater than 0.2 MAR index ratio in this study. The *gel*E and *agg*2 genes were found in 40 (81.63%) of the enterococcal isolates, whereas the *van*B gene was found in 3 (6.12%) of the enterococcal isolates. The results indicate that the consumption of Urfa cheese, which is produced using raw milk, may have public health risk because of its antibiotic resistance characteristics and virulence genes of enterococcal biota.

Keywords: Urfa cheese, Traditional Turkish cheese, Enterococci, Antibiotic resistance, Virulence genes, Public health

1. Introduction

Urfa cheese, which is traditionally produced mainly from sheep, goat or bovine unpasteurized raw milk, is a semi-hard and brined cheese variety in Turkey. Starters are not added to the production of this cheese variety (Kırmacı 2016, Atasoy et al. 2021). The use of raw milk in the production of this cheese may cause serious public health risks. Conversely, it was revealed that the pasteurization process may negatively affect cheese flavor quality because of killing lactic microflora in raw milk. In a study, the lactic acid bacteria (LAB) load in Urfa cheese was reported to be between 4.78-9.68 log cfu g^{1}, while the distribution of LAB members was found to include *Enterococcus* spp. (48.95%), *Lactococcus* spp. (40.55%), *Lactobacillus* spp. (9.10%), *Streptococcus* spp. (0.69%) and *Leuconostoc* spp. (0.69%). In this study, the genus *Enterococcus* was found to be a dominant LAB member with *E. faecium* (40%), *E. durans* (32.85%), *E. faecalis* (18.57%), *E. lactis* (5.71%) and *E. hirae* (2.85%) species (Kırmacı et al. 2016).

Enterococci is a member of the gastrointestinal microbiota of humans and animals. They are also presented especially in fermented foods such as sausages and cheeses and may contribute to cheese flavor with their proteolytic and lipolytic enzyme activities. They have an ability to survive in unsuitable conditions in the food environment such as pasteurization temperature, 6.5% NaCl concentration, etc. (İspirli et al. 2017).

Enterococci may have beneficial characteristics; however, some strains are still an important issue for food industry and public health. Enterococci are also considered as a nosocomial pathogen and they have ability to develop a wide range of antibiotic resistance and to have a potential to carry some virulence determinants (Terkuran et al. 2014, Calonico et al. 2018).

Although antibiotic-resistant enterococcal species may be found in cheeses made by using raw and pasteurized milk, their presence in food chain may cause a serious public health risk of spreading antibiotic resistance from food to humans, animals and the environment (Çitak et al. 2004, Camara et al. 2020). Academic studies reinforce the argument that *Enterococcus* species isolated from European cheeses may be resistant to one or more antibiotics including chloramphenicol, tetracycline, erythromycin, penicillin, gentamycin, rifampicin, lincomycin, vancomycin and fusidic acid (Citak et al. 2004; Kürekçi et al. 2016; İspirli et al. 2017; Mrkonjic Fuka et al. 2017; Sanlibaba & Senturk 2018; Silvetti et al. 2019; Camara et al. 2020).

Virulence factors determined in enterococci, such as cytolysin (*cyl*A, *cyl*B, *cyl*M genes), aggregation substance (*agg*2 gene), and gelatinase (*gel*E gene), should be evaluated for the pathogenicity of enterococcal isolates. Cytolysin may cause the

deformation of cell membranes such as erythrocytes and other mammalian cells. An aggregation substance is a protein which is surface-localized and efficiently allows conjugal transfer in a fluid environment. Gelatinase is an enzyme that hydrolyzes bioactive compounds such as collagen, gelatin, hemoglobin (Templer & Baumgartner 2007). There were some studies about a screening of virulence genes isolated from traditional raw milk cheeses. It was reported that enterococcal strains isolated from raw milk cheese may carry at least one of the virulence genes such as *gel*E (Templer & Baumgartner 2007, Hammad et al. 2015).

The aim of this study was to determine antibiotic resistance characteristics and virulence genes of enterococcal strains isolated from traditional white-brined Urfa cheese samples.

2. Material and Methods

2.1. Isolation and identification of enterococcal strains

In the study, the cheese samples (n=20) were obtained from local producers in Urfa, Turkey. The cheese samples (10 g) were homogenized with 90 milliliters of buffered peptone water (BPW; Merck, Germany) using a laboratory stomacher for 1 min. The decimal dilutions of the samples were made in sterile BPW and spread on Kanamycin Aesculin Azide (Merck, Germany) agar and then incubated at 37 °C for 48 h. Three suspicious black colonies were taken from each cheese sample plates and purified on Trypticase Soy agar (Merck, Germany).

For the identification of the pure strains, Gram staining, catalase reaction, growth in 10 °C, 45 °C, pH 9.6, 6.5% NaCl were applied, and API 20 Strep (bioMérieux, France) biochemical test kit was used. The strains were stored at −20 °C in Brain Heart Infusion (Merck) broth with 30% glycerol (Citak et al. 2004; Jurkovic et al. 2006).

2.2. Screening of antibiotic resistant enterococcal strains

The antibiotic-resistance characteristics of the enterococcal strains were detected for some antibiotics, including erythromycin (15 µg), vancomycin (30 µg), ampicillin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), penicillin G (10 µg), and gentamycin (10 µg) on Muller Hinton agar (Merck), using a disc diffusion method as described by the Clinical and Laboratory Standards Institute (2017). The tested antibiotic discs were obtained from Oxoid (UK). The results were evaluated according to the cut-off levels in CLSI (2017) standard for the antibiotics.

The multiple antibiotic resistance (MAR) index of each isolate was also calculated (Krumperman 1983). The MAR index is the ratio of the total number of antibiotics to which the isolate was resistant to the number of antibiotics to which the isolate was exposed (Krumperman 1983). If the calculated MAR index is greater than 0.2, it means that the isolate was heavily exposed to human- or animal-sourced antibiotics; if the MAR index is equal to or smaller than 0.2, it means that the antibiotics were used very rarely or were not used at all.

2.3 *Screening of enterococcal strains for virulence and antibiotic resistance genes*

The vancomycin (*van*A and *van*B) and erythromycin (*erm*B), resistance genes, and virulence determinants (*agg*2, *gel*E, *cyl*M, *cyl*B, *cyl*A) of the enterococcal strains isolated from the Urfa cheese samples were determined by polymerase chain reaction (PCR). The genomic DNAs of the enterococcal strains were extracted by using a commercial DNA isolation kit (Qiagen). PCR primers for antibiotic resistance and virulence genes (Table 1) were selected according to Eaton & Gasson (2001), Reviriego et al. (2005); Pasquaroli et al. (2014).

PCR amplifications were performed in 25 µL reaction mixtures using 1 mM dNTP mix (Promega, Sunnyvale, CA, USA), 1 U Go Taq Flexi DNA polymerase (Promega), 1 µL of DNA and 10 pmol of each primer obtained from IDT (Integrated DNA Technologies, Coralville, IA, USA). The samples were exposed to an initial cycle of denaturation at 95 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 53 °C for 30 s and elongation at 72 °C for 45 s (Eaton and Gasson 2001; Reviriego et al. 2005).

E. faecalis NCIMB 700584 and *Enterococcus hirae* FM 2.16 were used as positive control strains for virulence genes and *erm*B gene, respectively (Eaton & Gasson 2001; Pasquaroli et al. 2014).

Table 1- The primers for virulence and antibiotic resistance genes

Genes	*Primer sequence (5'-3')*	*Product size (bp)*
***agg*2**	F-5' GTT GTT TTA GCA ATG GGG TAT R-5' TCC TGT CAC TCC TCT TCT CAG	1210
***gel*E**	F-5' ACC CCG TAT CAT TGG TTT R-5' ACG CAT TGC TTT TCC ATC	419
***cyl*M**	F-5' TGC TTC TCC ACT GTG ACC T R-5' ATC TAG TAA ATG TTA AGA AAT ACA	742
cylB	F-5' TGG AAG CAT TAC TTC CAG CT R-5' AAC TGC AAC CTC AAG ATT GG	843
***cyl*A**	F-5' AAT CCT ATC GGT TAC TGC TTA R-5' AGC ATC ACA ACC ATC CTA AC	517
***van*A**	F-5' GTA CAA TGC GGC CGT TA R-5' GGG ACA GTT ACA ATT GC	732
***van*B**	F-5' GTG CTG CGA GAT ACC ACA GA R-5' CGA ACA CCA TGC AAC ATT TC	1145
***erm*B**	F-5' CAT TTA ACG ACG AAA CTG GC R-5' GGA ACA TCT GTG GTA TGG CG	425

3. Results and Discussion

3.1. Distribution of enterococcal strains

Urfa cheese is a traditional white cheese variety which is produced in the southeastern part of Turkey. This cheese type is produced using raw ovine or bovine milk or a mixture of these milk types. It is kept in a high-dense brine solution or boiled for 2-3 minutes to obtain the microbiological quality. However, these methods not enough for providing hygienic quality. It was mentioned that Urfa cheese was produced by raw milk heated up to 30-35 °C and that the microbial load of the cheese was, therefore, quite high (Uraz et al. 2008).

The enterococcal counts of the Urfa cheese samples were found to be between 4.4-5.6 log cfu g^{-1} in the study. A total of 54 isolates were picked from the cheese samples and 49 of them were identified as *Enterococcus* spp. The isolates were also defined as *E. faecalis* (24), *E. faecium* (22) and *E. durans* (3) using the API 20 Strep biochemical test kit (Table 2).

There were limited data about the enterococcal load and distribution of Urfa cheese samples in different studies. Uraz et al. (2008) analyzed 11 Urfa cheese samples and found out that *E. faecalis* (33%) and *E. faecium* (13%) were the dominant flora member of lactic acid bacteria in Urfa cheese samples. In another study (Kırmacı et al. 2016), the indigenous enterococcal load was determined to be between 4.78-7.04 log cfu g^{-1} in 20 Urfa cheese samples. Besides, *Enterococcus* spp. (48.95%) was a dominant part of the lactic flora of Urfa cheese with the distribution of *E. faecium* (40%), *E. durans* (32.85%), *E. faecalis* (18.57%), *E. lactis* (5.71%) and *E. hirae* (2.85%) species. Because of the resistance of enterococci to high salt content and low pH, this bacterial genus becomes a dominant member of lactic acid bacteria in Urfa cheese. Not only in Urfa cheese but also in many different traditional kinds of cheese produced from raw or pasteurized milk samples in the Mediterranean region, *Enterococcus* spp. may be found widespread (Kırmacı et al. 2016; Sanlibaba & Senturk 2018; Silvetti et al. 2019; Camara et al. 2020).

Enterococci is a part of natural gastrointestinal tract of humans and farm animals; so, these bacteria may contaminate milk directly during milking through animal faeces or indirectly through production equipment (Mrkonjic Fuka et al. 2017; Camara et al. 2020).

3.2. Antibiotic resistance characteristics and virulence genes of strains

Most of the enterococcal isolates were found to be resistant in high or intermediate level against at least one of the tested antibiotics (Table 2). Certain strains were highly resistant to tetracycline (51%), streptomycin (26.5%), erythromycin (10.2%), gentamycin (2%), and penicillin (2%), while some strains were intermediate-level resistant to erythromycin (30.6%) tetracycline (18.3%) and vancomycin (2%). The number of the antibiotic-resistant strains of E. faecalis was higher than that of the E. faecium strains. Ten (20.4%) of the E. faecalis strains and eight (16.3%) of the E. faecium strains showed multidrug resistance. In this study, it was also found that the MAR index was greater than 0.2 in 18 (36.7%) enterococcal isolates (Figure 1). Although most of the tested antibiotic resistance genes (vanA, ermB) were not detected in the strains, only the vanB gene was found in 3 (6.12%) of the enterococcal isolates (Table 2).

Table 2- Distribution, virulence genes, and antibiotic resistance characteristics of enterococci in Urfa cheese samples

No	*Isolate No*	*Cell Morphology*	*Gram staining*	*Catalase*	*Growth at*				*Species*	*Resistance* Characteristics*	*Virulence* Genes*
					15 ˚C	*45 ˚C*	*pH 9.6*	*6.5% NaCl*			
1	1.1	Coccus	+	-	+	+	+	+	*E. faecalis*	S_R, E_R, T_R	*gel*E, *agg2*
2	1.2	Coccus	+	-	+	+	+	+	*E. faecium*	T_I	*gel*E, *agg2*
3	1.3	Coccus	+	-	+	+	+	+	*E. faecium*	T_I	*gel*E, *agg2*
4	2.1	Coccus	+	-	+	+	+	+	*E. faecalis*	T_R	*gel*E, *agg2*
5	2.2	Coccus	+	-	+	+	+	+	*E. faecium*		*gel*E, *agg2*
6	2.3	Coccus	+	-	+	+	+	+	*E. faecalis*	E_R, T_R	*agg2*
7	3.1	Coccus	+	-	+	+	+	+	*E. faecalis*	P_R, T_I	*gel*E, *agg2*
8	3.2	Coccus	+	-	+	+	+	+	*E. faecium*	E_R, T_I	*gel*E, *agg2*
9	3.3	Coccus	+	-	+	+	+	+	*E. faecium*		*gel*E
10	5.2	Coccus	+	-	+	+	+	+	*E. faecalis*	T_R	*gel*E, *agg2*
11	10.1	Coccus	+	-	+	+	+	+	*E. faecalis*	S_R, T_R, E_I	*gel*E, *agg2*
12	10.2	Coccus	+	-	+	+	+	+	*E. faecalis*	T_I	*gel*E, *agg2*
13	10.3	Coccus	+	-	+	+	+	+	*E. faecalis*	T_R	*gel*E, *agg2*
14	11.1	Coccus	+	-	+	+	+	+	*E. faecalis*	T_I	*gel*E, *agg2*
15	11.2	Coccus	+	-	+	+	+	+	*E. faecalis*	T_R	*gel*E, *agg2*
16	11.3	Coccus	+	-	+	+	+	+	*E. faecium*	E_I	*gel*E, *agg2*
17	12.1	Coccus	+	-	+	+	+	+	*E. faecalis*	T_R	*agg2*
18	12.2	Coccus	+	-	+	+	+	+	*E. faecium*	S_R, T_I	
19	12.3	Coccus	+	-	+	+	+	+	*E. faecium*	T_R	*gel*E
20	13.1	Coccus	+	-	+	+	+	+	*E. faecium*	E_I	*agg2*
21	13.2	Coccus	+	-	+	+	+	+	*E. faecium*	E_I	*agg2*
22	13.3	Coccus	+	-	+	+	+	+	*E. faecalis*	T_R, *van*B	*agg2*
23	14.2	Coccus	+	-	+	+	+	+	*E. faecalis*	S_R, T_R, *van*B	*gel*E, *agg2*
24	14.3	Coccus	+	-	+	+	+	+	*E. faecalis*	T_R	*gel*E, *agg2*
25	15.1	Coccus	+	-	+	+	+	+	*E. faecium*		*gel*E, *agg2*
26	15.2	Coccus	+	-	+	+	+	+	*E. faecium*	S_R, T_R, E_I	*gel*E, *agg2*
27	15.3	Coccus	+	-	+	+	+	+	*E. faecalis*	T_R	*gel*E, *agg2*
28	16.1	Coccus	+	-	+	+	+	+	*E. durans*	G_R, *van*B	*gel*E, *agg2*
29	16.2	Coccus	+	-	+	+	+	+	*E. faecalis*	S_R, T_R, E_I	*gel*E, *agg2*
30	16.3	Coccus	+	-	+	+	+	+	*E. faecalis*	S_R, E_R, T_R	*gel*E, *agg2*
31	17.1	Coccus	+	-	+	+	+	+	*E. faecium*	T_R, E_I	

Table 2 (Continue)- Distribution, virulence genes, and antibiotic resistance characteristics of enterococci in Urfa cheese samples

No	*Isolate No*	*Cell Morphology*	*Gram staining*	*Catalase*	*Growth at*				*Species*	*Resistance* Characteristics*	*Virulence* Genes*
					15 ˚C	*45 ˚C*	*pH 9.6*	*6.5% NaCl*			
32	17.2	Coccus	+	-	+	+	+	+	*E. faecium*	S_R, T_R, E_I	
33	17.3	Coccus	+	-	+	+	+	+	*E. faecium*	V_I, E_I, T_I	*gel*E, *agg2*
34	18.1	Coccus	+	-	+	+	+	+	*E. durans*		*gel*E, *agg2*
35	18.2	Coccus	+	-	+	+	+	+	*E. faecium*	T_R	*gel*E
36	18.3	Coccus	+	-	+	+	+	+	*E. faecalis*	S_R, T_R, E_I	*gel*E
37	19.2	Coccus	+	-	+	+	+	+	*E. faecalis*	S_R, E_I	*gel*E, *agg2*
38	19.3	Coccus	+	-	+	+	+	+	*E. faecalis*	E_I	*agg2*
39	20.1	Coccus	+	-	+	+	+	+	*E. faecium*	T_R	*gel*E, *agg2*
40	20.2	Coccus	+	-	+	+	+	+	*E. faecium*	T_R, E_I	*gel*E, *agg2*
41	20.3	Coccus	+	-	+	+	+	+	*E. faecalis*	T_R	*gel*E, *agg2*
42	22.1	Coccus	+	-	+	+	+	+	*E. faecalis*	T_R	*gel*E, *agg2*
43	22.3	Coccus	+	-	+	+	+	+	*E. faecium*		*gel*E, *agg2*
44	23.1	Coccus	+	-	+	+	+	+	*E. faecium*	E_I	*gel*E, *agg2*
45	23.2	Coccus	+	-	+	+	+	+	*E. durans*		*gel*E, *agg2*
46	23.3	Coccus	+	-	+	+	+	+	*E. faecalis*	S_R, E_R, T_I	*gel*E
47	24.1	Coccus	+	-	+	+	+	+	*E. faecium*	S_R	*gel*E, *agg2*
48	24.2	Coccus	+	-	+	+	+	+	*E. faecium*		*gel*E
49	24.3	Coccus	+	-	+	+	+	+	*E. faecalis*	S_R, T_R, E_I	*gel*E, *agg2*

* S_R: Streptomycin resistance; E_R: Erythromycin resistance; E_I: Intermediate level erythromycin resistance; T_R: Tetracycline resistance; T_I: Intermediate level tetracycline resistance; P_R: Penicillin resistance; G_R: Gentamycin resistance; V_I: Intermediate level vancomycin resistance; *gel*E: gelatinase encoded gene; *agg*2: aggregation substance encoded gene; *van*B: vancomycin resistance gene

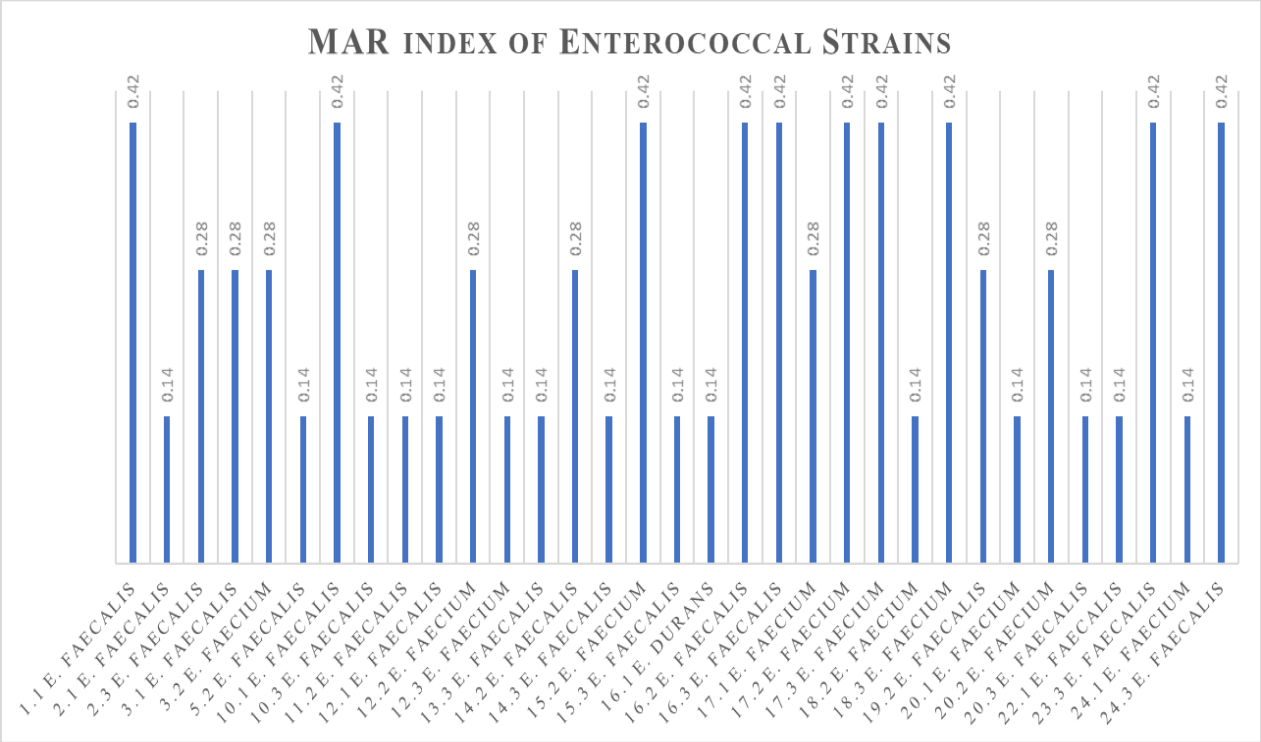

Figure 1- Multiple antibiotic resistance (MAR) index of enterococcal strains isolated from Urfa cheese

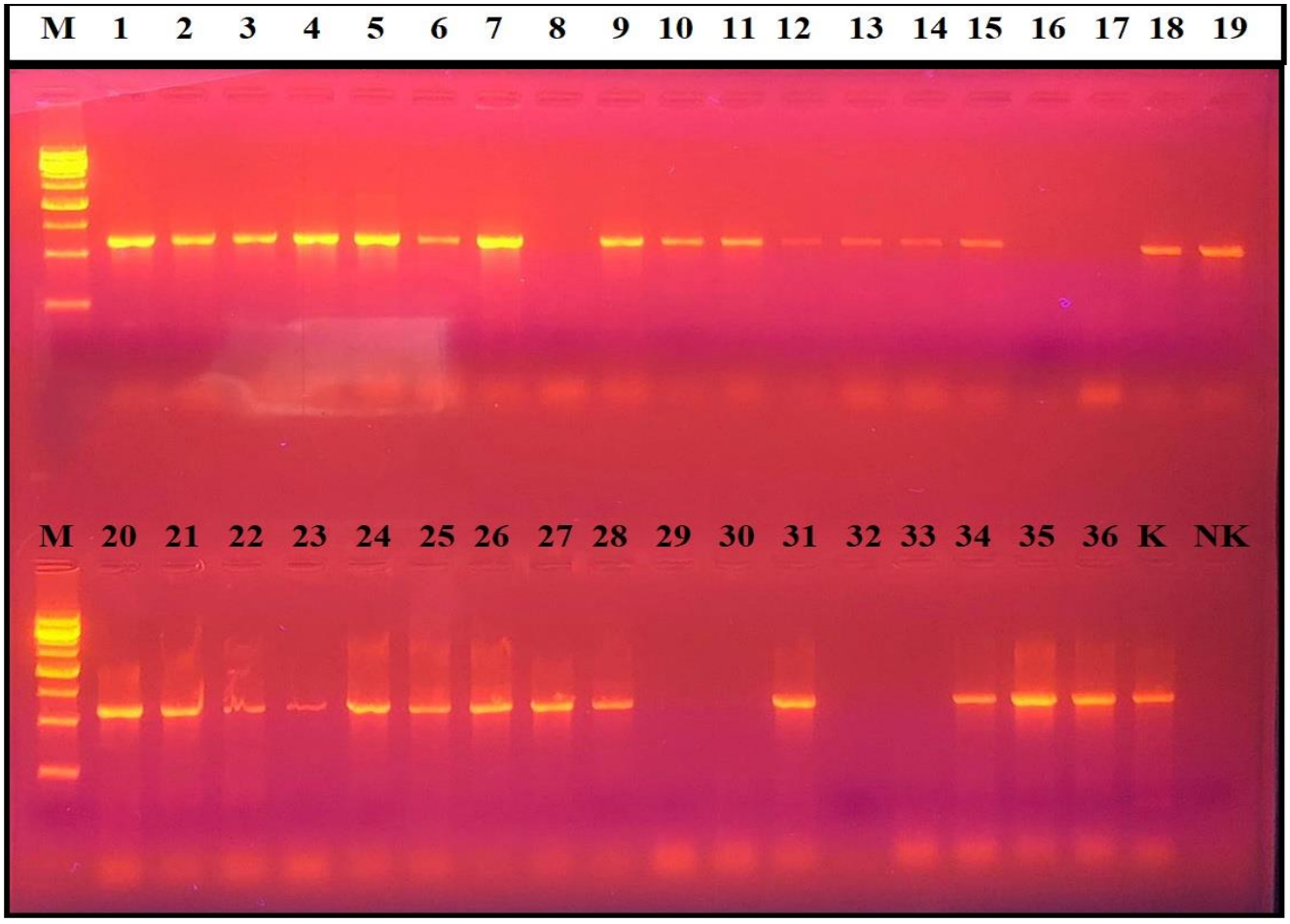

Figure 2- The agarose gel screen of *agg*2 gene positive enterococcal strains isolated from Urfa cheese samples (M; Marker, K; positive control, NK; negative control, *agg*2 positive strains; 1-7, 9-15, 18-19, 20-28, 31, 34-36)

Although many enterococci are the endogenous and beneficial microbial part of fermented foods, certain strains are recognized as a nosocomial pathogen and may carry virulence genes and antibiotic resistance characteristics. Misused and uncontrolled antibiotic treatment in human health and animal husbandry result in an increase in the development of antibiotic resistance among commensal bacteria of animal-sourced foods, especially in raw milk and dairy products (Hammad et al. 2015; Mrkonjic Fuka et al. 2017, Özdemir & Tuncer 2020).

In a study conducted by Zdolec et al. (2016), it was reported that enterococcal strains isolated from the milk samples of drug-treated udders and healthy cow udders were found to be resistant to tetracycline, chloramphenicol, and erythromycin with equal distribution. It was thought that this could be the result of animal cohabitation and cross-contamination. Although food-borne enterococci are not a direct cause of resistant enterococci in humans, they may transfer resistance determinants to bacteria in human microbiota; therefore, raw milk consumption without thermal process is considered to be a potential health risk for the public.

In another study carried out by Bouymajane et al. (2019), 150 raw cow's milk samples obtained from street traders in Meknes city, Morocco were analyzed for the identification of *Enterococcus* spp. and the antimicrobial susceptibility of the isolates was determined. It was found that ampicillin, streptomycin, and tetracycline resistance in *Enterococcus* spp. strains and as well as the multiple antibiotic resistance (MAR) index were higher than 0.5 in most of *Enterococcus* spp. The researchers emphasized that these findings may be indicated as a risk for public health.

Sanlibaba & Senturk (2018) isolated and identified *E. faecalis* (n=125) and *E. faecium* (n=88) strains from 215 traditional Turkish cheese samples such as White, Kasar, Tulum, Ezine, Lor, Orgu, and Civil. The isolates were found to be resistant against nalidixic acid (100%), kanamycin (98.6%), rifampicin (78.4%), ampicillin (48.8%), ciprofloxacin (45.5%), erythromycin (18.8%), tetracycline (11.7%), penicillin G (5.6%), chloramphenicol (4.2%), gentamycin (3.8%) and streptomycin (1.4%) in phenotype, and none of them were resistant to vancomycin. The antibiotic resistance levels of *E. faecium* strains were higher than those of *E. faecalis* strains. Moreover, it was determined that *E. faecium* (100%) and of *E. faecalis* (88.8%) strains were resistant to multiple drugs.

In another study about the antibiotic resistance of enterococci in traditional Turkish cheese varieties such as Tulum, Ezine, Antep, Civil, White, Sülk, Lor, Dil, Van Otlu, Kasar, and Orgu, it was determined that there was a resistance against lincomycin (88.5%), kanamycin (84.2%), gentamycin (51.1%), rifampin (46.8%), tetracycline (33.8%), high levels of gentamycin (2.2%) and streptomycin (5.8%), and low levels of ciprofloxacin, erythromycin and chloramphenicol. It was suggested to establish and monitor a quality control system for dairy products from farm to retail in antimicrobial resistance among emerging food-borne pathogens (Kürekçi et al. 2016).

Silvetti et al. (2019) investigated the antibiotic resistance incidence of 40 *E. faecalis* isolated from 10 Italian raw milk cheeses. While tetracycline, rifampicin, chloramphenicol, and erythromycin resistance were determined in the isolates, vancomycin resistance was not observed. It was concluded that *E. faecalis* strains from raw milk cheese may be a source for transferring antimicrobial resistance and other pathogenic characteristics to humans.

Calonico et al. (2018) reported drug resistance against vancomycin, chloramphenicol, ampicillin, tetracycline, linezolid and teicoplanin with a higher prevalence of *E. faecalis* than *E. faecium* in cheese samples. It was also reported that the patterns of resistance feature varied over the years for both *E. faecalis* and *E. faecium* and that the number of antibiotic-resistant and multidrug-resistant strains increased from 2002 to 2015.

The study conducted by Camara et al. (2020) analyzed the antibiotic resistance characteristics of 28 autochthonous *Enterococcus* isolates from Pico cheese and reported that tetracycline, rifampicin, erythromycin, and chloramphenicol resistance was found in the isolates. They emphasized the importance of evaluating the safety of enterococcal isolates from artisanal cheeses.

It was reported that enterococcal strains isolated from artisan Istrian raw milk cheese showed multidrug resistance (83.72%). They were found to be resistant against clindamycin (63.07%), streptomycin (82.00%), rifampicin (72.35%), chloramphenicol (28.41%), tetracycline (17.99%), erythromycin (29.35%), and vancomycin (23.48%) (Mrkonjic Fuka et al. 2017).

It was reported that there was very few knowledge about the passage of virulent and/or multidrug enterococcal strains from fresh raw milk cheese to the human gastrointestinal tract (Hammad et al. 2015). They isolated enterococci from Egyptian raw milk cheese and karish cheese, and detected some *E. faecalis and E. faecium* strains that carried one or more virulence genes, including *gel*E, *asa*1, *cyl*A, *esp*, and *hyl*. It was concluded that the potential reservoir of virulent and antibiotic-resistant enterococci may have a risk for public health.

Templer & Baumgartner (2007) tested the virulence genes (*gel*E, *agg*, *esp*, *cyl*, *efaAfs*, *efaAfm*, *cpd*, *ccf* and *cob*) in enterococci isolated from artisanal raw milk cheese (Schabziger and Appenzeller) produced in Switzerland. They reported that all tested strains contained at least 2 of the 9 virulence genes analyzed and that Schabziger and Appenzeller cheeses may be the

source of some antibiotic resistance and virulence determinants. All virulence genes found in the strains analyzed were also present in human clinical isolates.

4. Conclusions

Traditional Urfa cheese, which is produced using raw milk, may carry enterococci as a dominant part of the autochthonous microbiota. Different studies showed that *Enterococcus faecium*, *E. faecalis* and *E. durans* were the most frequently isolated species from Urfa cheese samples. The antibiotic resistance, which is a rising concern nowadays, has become a serious public health issue even for the commensal bacterial member of foods. A high-level tetracycline, streptomycin and erythromycin resistance, and multiple antibiotic resistance were found in most of the enterococcal strains isolated from Urfa cheese. Besides, *agg*2 and *gel*E genes, which were related to pathogenicity, were detected from the isolates in high rate. These results indicated that consuming unpasteurized milk and milk products may cause important health risk for humans and environment.

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