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The prevalence, antibiotic resistance and biofilm formation ability of *Enterococcus* spp. isolated from food products in Algeria.

Yacine Titouche[1*], Madjid Akkou[2], Fawzi Rostane Meklati[3], Amel Kaced[3], Mohand Teffane[1], Abdelmadjid Tahi[1] and Karim Houali[1].

[1]Laboratory for Analytical Biochemistry and Biotechnology, Mouloud Mammeri University of Tizi Ouzou, Tizi-Ouzou, Algeria
[2]Institute of Veterinary Sciences, Saad Dahleb University, Blida, Algeria
[3]Center for Scientific and Technical Research in Physico-Chemical Analysis (CRAPC), Bou-Ismail, Tipaza, Algeria

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ABSTRACT

Enterococci are ubiquitous bacteria in the normal intestinal microbiota of both humans and animals. They can be used in the food industry as starter or probiotic cultures. However, some species have emerged as an important nosocomial pathogens, and have been implicated in severe multi-resistant infections. In this study, a total of 235 food products were analyzed for the presence of *Enterococcus* spp. Overall, 54 (22.9%) out of the 235 samples were contaminated and 54 strains were isolated. The latter were identified by matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and tested for antibiotic susceptibility against seven antibiotic molecules, in addition to their ability to form a biofilm. The predominant species was *Enterococcus faecalis* (70.4%), followed by *Enterococcus hirae* (12.9%), *Enterococcus faecium* (11.1%) and *Enterococcus durans* (5.5%). The highest resistance profile was ascribed to tetracycline (66.7%) and penicillin G (33.3%). Six enterococcal isolates (11.1%) were resistant to at least three antibiotic families. All isolated strains were able to form a biofilm and exhibited gelatinase activity. However, only 4 (7.4%) were β-hemolytic. This study revealed that food products might play a role in the spread of enterococci through the food chain to humans with these virulence and resistance characteristics. As a result, continuous investigations are necessary to assess the health hazards associated with the consumption of contaminated food products.

Key words: *Enterococcus*; food products; antibiotic susceptibility; biofilm formation; MALDI-TOF MS.

Introduction

Enterococci are Gram-positive, facultative, anaerobic bacteria, that live as part of the natural flora in the intestinal tract of humans and animals (RANOTKAR et al., 2014), including birds, insects and reptiles, and can also found in the soil, water and foods (GHOSH and ZUREK, 2015). To date, over 50 different enterococcal species have been described (AHMED and BAPTISTE,

*Corresponding author:
Dr Yacine Titouche. Laboratoire de Biochimie Analytique et Biotechnologie. Département de Biochimie-Microbiologie. Faculté des Sciences Biologiques et des Sciences Agronomiques. Université Mouloud Mammeri, Tizi Ouzou. Algérie, phone number: +213 554242911, e-mail: yacinetitouche@yahoo.fr

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2018), of which *E. faecium* and *E. faecalis* are the most common in the human gastrointestinal tract, whereas, among farm animals *E. faecium* together with *E. cecorum*, *E. faecalis* and, to some extent *E. hirae* predominate, while *E.* munditii and *E. casseliflavus* are commonly found in plant sources (RAMOS et al., 2020). Moreover, enterococci occur in a large number of foods, especially those of animal origin, such as fermented sausages and cheeses (GIRAFFA, 2002). As they produce bacteriocins, enterococci have been used widely over recent decades in the food and feed industries as probiotics and also, due to their role in flavor development and fermentation, as starter cultures in the production of fermented salami and several types of ripened cheese (GHOSH and ZUREK, 2015). Several enterococcal species from raw milk have demonstrated important technological properties with an impact upon the sensorial characteristics of dairy products, such as diacetyl production, autolytic activity, proteolytic activity and lipolytic activity, as well as probiotic potential (DAPKEVICIUS et al., 2021).

Over the past three decades, enterococci have become the most common nosocomial human pathogens (SELLECK et al., 2019). They have emerged as an important nosocomial pathogen, second only to staphylococci, which are the leading cause of nosocomial infections worldwide (SAKKA et al., 2008). They are associated with serious and life-threatening infections in humans, including urinary tract infections, blood stream infections, and endocarditis (FISHER and PHILLIPS, 2009). *E. faecalis* and *E. faecium* account for the majority of human enterococcal infections, and are a leading cause of hospital-acquired and multidrug-resistant infections (AHMED and BAPTISTE, 2018).

Studies on the antimicrobial susceptibility patterns of enterococci have affirmed the worldwide emergence of multi-resistant strains, with a high proportion resistant to vancomycin and tetracyclines (REHAIEM et al., 2016). As is well-known, enterococci show intrinsic resistance to many antimicrobial agents, including cephalosporins, lincosamides, most β-lactams, and low levels of aminoglycosides, suggesting that treatment of enterococcal infections could be difficult (GIRAFFA, 2002). Glycopeptides, such as vancomycin, are the best alternative, if the causative strain is resistant to one or both of these drugs, or in cases where aminoglycoside use is contraindicated (BORTOLAIA and GUARDABASSI, 2015). The emergence of vancomycin-resistant enterococci (VRE) poses a major therapeutic challenge due to their intrinsic resistance to commonly used antibiotics and their ability to acquire resistance to many available antibiotics, either by mutation or by acceptance of foreign genetic materials (GHOSH and ZUREK, 2015). In addition, enterococci can rapidly develop resistance against last resort antimicrobials used to treat glycopeptide and multidrug resistance, such as quinupristin-dalfopristin, linezolid, daptomycin and tigecycline (AHMED and BAPTISTE, 2018). Furthermore, enterococci are able to transfer resistance determinants to their own species, to other pathogens (BORTOLAIA and GUARDABASSI, 2015), or to non-pathogenic bacteria, in humans or animals, in the environment, or even in food, contributing to the dissemination and persistence of antimicrobial resistance (PESAVENTO et al., 2014).

Beside antibiotic resistance, enterococci are able to produce potential virulence factors. These enhance their pathogenicity by allowing the colonization and invasion of host tissue, translocation through epithelial cells, and evading the host's immune response. These putative virulence factors, such as aggregation substances, adhesins, hemolysin, hyaluronidase and gelatinase, play an important role in establishing infection (RAMOS et al., 2020). Although virulence factors are less frequent in isolates from foods than in isolates of clinical origin, they can make it possible for them to be transferred to human microbiota in the food chain (CHAJECKA-WIERZCHOWSKA et al., 2017).

In Algeria, the prevalence and the pheno/genotypic characteristics of enterococci isolated from humans have been described. However, there is little knowledges on the antibiotic resistance of enterococci strains existing in communities and in foods. This study aimed to provide the first data on the prevalence, antibiotic resistance and virulence factors of enterococci strains of food origin.

Materials and methods

*Sample collection, isolation and biochemical identification of Enterococcus spp***.** In order to isolate *Enterococcus* spp, 235 samples of different food items, including minced meat (80), pastries (79), butter (30), raw milk (4), raw milk cheeses (4), l'ben (2), rayeb (3), pizza (10) and sandwiches (23), were obtained from retail markets, butchers, dairy shops and fast food outlets in the Tizi Ouzou area, Algeria, over a period of 5 months (from February 2018 to June 2018). All samples were purchased from several randomly selected market points, as part of the research, and collected in sterile plastic bags, stored in with ice packs and transported immediately to the laboratory within 2 h for microbiological analysis.

A 10 g portion of each food sample was taken aseptically, placed in 90 ml of buffered peptone water (Conda Pronadisa, Madrid, Spain) and homogenized. One milliliter of this homogenate was placed on 10 ml of Rothe broth (Conda Pronadisa, Madrid, Spain), followed by incubation at 37°C for 24h. From each Rothe Broth with bacterial growth, 0.1 ml was placed on EVA broth (Ethyl, Violet, Azide, Litsky) (Conda Pronadisa, Madrid, Spain). After incubation at 37°C for 24 h, a loopful of EVA broth culture showing turbidity was then streaked onto bile esculin azide agar (Biokar, Paris, France). Following 24 h incubation at 37°C, one colony per sample with typical enterococci morphology (a dark brown or black colony) was then transferred onto tryptone soy agar plates (Conda Pronadisa, Madrid, Spain) in order to obtain pure cultures. These isolates were then subjected to Gram staining to confirm coccus morphology. The identification of isolates was completed using the following biochemical tests : catalase test, oxidase production, growth in brain-heart infusion broth with 6.5% NaCl at 45°C for 24h, and hydrolysis of esculin. *E. faecalis* WDCM 00009 was used as the positive control. After identification, all strains were stored in the brain heart infusion broth (Conda Pronadisa, Madrid, Spain) with glycerol (30% vol/vol) at -20°C for further analysis.

Identification of isolates by MALDI-TOF MS. All isolates obtained from the media culture were identified using MALDI-TOF MS (Bruker Microflex LT, Germany) as described by JAHAN et al. (2021). Briefly, one pure colony was randomly selected from the freshly overnight growth in bile esculin azide agar (Biokar, Paris, France) plates, and transferred onto a steel MALDI target plate containing inoculation spots. Two spots were assigned to each isolate. The inoculated spots were then overlaid with 1µl of 70% formic acid (Sigma-Aldrich) and air dried. Finally, the dried spots were overlaid with 1µl of matrix (α-cyano-4-hydroxycinnamic acid). The protein mass spectra were analyzed using Flex Control software and MALDI-Biotyper RTC 4.0 software (Bruker Daltonics). The MALDI-TOF MS results were obtained according to the manufacturer's technical specifications, as follows: correct genus and species identification (≥2), correct genus identification (1.7-2.0), and no reliable identification (<1.7).

Antibiotic susceptibility of isolates. All enterococcal strains were tested for their susceptibility to seven antimicrobial agents using the disc diffusion method according to the Clinical Standards Institute (CLSI, 2018) recommendations. The antibiotic disks from Liofilchem (Roseto, Italy) are listed as follow (antibiotic concentration in µg, unless otherwise specified): penicillin G (10 IU), ampicillin (10), tetracycline (30), erythromycin (15), chloramphenicol (30), vancomycine (30), and teicoplanin (30). The results were interpreted following the CLSI breakpoint tables (CLSI, 2018).

Production of gelatinase and hemolysin. For the detection of gelatinase, enterococci isolates were inoculated onto brain heart infusion agar plates (Biokar, Paris, France) containing 3% gelatin (Biochem Chemopharma, Nièvre, France). The appearance of a clear halo around the colonies after incubation at 37 °C for 24 h in aerobic conditions followed by refrigeration at 4 °C for 30 min was considered to be a positive indication of gelatinase production. *E. faecalis* WDCM 00009) was used as a positive control.

Production of hemolysin was determined by streaking enterococcal isolates on fresh human blood agar plates, then grown overnight at 37°C. A clear zone of β-hemolysis around the streak was considered to be a positive reaction for hemolysin production.

Biofilm formation. The ability of enterococcal isolates to form a biofilm was tested, as described by ACHEK et al. (2020), with some modifications. Briefly, isolates were cultivated in brain heart agar (BHA) (Conda Pronadisa, Madrid, Spain) at 37°C for 24h under aerobic conditions. After verifying the purity of the strain, two colonies were inoculated into 5ml of Trypticase Soy Broth (TSB) (Conda Pronadisa, Madrid, Spain), supplemented with 1% of Glucose (Sigma-Aldrich, Isère, France) and incubated overnight at 37°C. Overnight cultures were diluted to 1:50 with TSB-1% glucose, and 200µl of cell suspensions were transferred to individual wells of a flat bottom 96-well polystyrene microtiter plate (ProLab Scientific Co Ltd, Zhejiang, China). TSB-1% glucose served as a negative control and *E. faecalis* WDCM 00009 as a positive control. After incubation at 37°C for 24 hours, detached cells were gently rinsed three times with phosphate-buffered saline (PBS; 7mM Na_2HPO_4, 3 mM NaH_2PO_4, and 130 mM NaCl, pH 7.4). Adherent bacteria were fixed with methanol (Honeywell, Seelze, Germany) for 15mn and stained with 150µl of aqueous solution of crystal violet 0.5% (Biochem Chemopharma, Nièvre, France) for 15min. Following staining, the plates were rinsed to remove the excess stain. The dye bound to the cells was solubilized by addition of 150µl of ethanol (Honeywell, Seelze, Germany). The optical density (OD) of each well was measured at 560nm using a microtiter-plate reader (Gentaur, Paris, France). Each assay was performed in triplicate. Wells without bacterial inoculation were considered as the negative controls. The average OD value of all tested strains (ODs) and negative controls was calculated. The cut-off OD (ODc) was defined as three standard deviations above the mean OD of the negative control. The strains were classified as: no biofilm produced if ODs≤ODc, weak biofilm producer if ODc<ODs≤2×ODc, moderate biofilm producer if 2×ODc<ODs≤4×ODc and strong biofilm producer if ODs>4×ODc.

Statistical Analysis. Pearson's chi-square (χ^2) test was used to compare between the percentages of Enterococcus isolation in raw products and ready to eat products and between percentages of antimicrobial resistance profiles in Enterococcus species, using XLSTAT 2019. 2.2.59614.

Results

In this study, 22.9% of the analyzed food samples were positive for enterococci, with a higher prevalence in the raw products (45.2%) than in the ready-to-eat products (10.6%) (P<0.0001) (Table 1). In the ready-to-eat products, l'ben (50%) and rayeb (33.3) were more contaminated than other products (P<0,0001) (Table 1). A total of 54 enterococcal isolates were obtained from the culture media. MALDI-TOF MS showed that *E. faecalis* was the most prevalent species detected (70.4%), followed by *E. hirae* (12.9%), *E. faecium* (11.1%) and *E. durans* (5.5%) (Table 1). The percentages of *E. faecalis* and *E. hirae* detected were higher in raw products than in ready-to-eat products (P<0,05) (Table 1). However, *E. faecium* and *E. durans* were more frequent in ready-to-eat products than in raw products (P<0,05) (Table 1).

High percentages of tetracycline, penicillin and erythromycin resistance were detected among our enterococcal isolates, with rates of 66.7%, 33.3% and 29.6%, respectively. However, low resistance was observed against chloramphenicol (5.5%) and ampicillin (3.7%). All isolates were susceptible to vancomycin and teicoplanin (Table 2). A significant difference (P<0,05) among the various species of enterococci analyzed was observed in relation to their antibiotic resistance against the antibiotic molecules tested (Table 2). Six enterococcal isolates (11.1%) were resistant to least 3 antibiotic families, and three phenotypes of multidrug resistance were observed (Table 3).

In our study, all the analyzed strains were gelatinase and strong biofilm producers. However, only four strains (7.4%) were hemolysin positive (3 *E. faecalis* and 1 *E. hirae*). *Enterococcus hirae* (14.3%) was the producer of most hemolysin in comparison with the other species (P<0,0001) (Table 4).

Table 1. The prevalence and distribution of *Enterococcus* spp species in various analyzed food products

Type of products	No. (%) of samples			No. (%) of *Enterococcus.* spp isolates			
	Total (n)	With *Enterococcus.* spp	No. of strains	*E. faecalis* No = 38 (70.4)	*E. faecium* No = 6 (11.1)	*E. hirae* No = 7 (12.9)	*E. durans* No = 3 (5.5)
Raw products							
Minced meat	80	36 (45)	36	27 (75)	2 (5.5)	6 (16.7)	1 (2.8)
Raw milk	4	2 (50)	2	1 (50)	0 (0)	0 (0)	1 (50)
Total	84	38 (45.2)	38	28 (73.7)	2 (5.3)	6 (15.8)	2 (5.3)
χ^2 P=	< 0,0001		< 0,0001	< 0,0001	0,0002	< 0,0001	< 0,0001
Ready to eat products							
Pastries	79	6 (7.6)	6	5 (83.3)	1 (16.7)	0 (0)	0 (0)
Butter	30	6 (20)	6	4 (66.7)	0 (0)	1 (16.7)	1 (16.77)
Raw milk cheese	4	0 (0)	0	0 (0)	0 (0)	0 (0)	0 (0)
L'ben	2	1 (50)	1	0 (0)	1 (100)	0 (0)	0 (0)
Rayeb	3	1 (33.3)	1	1 (100)	0 (0)	0 (0)	0 (0)
Pizza	10	2 (20)	2	0 (0)	2 (100)	0 (0)	0 (0)
Sandwichs	23	0 (0)	0	0 (0)	0 (0)	0 (0)	0 (0)
Total	151	16 (10.6)	16	10 (62.5)	4 (25)	1 (6.25)	1 (6.25)
χ^2 P=	< 0,0001		< 0,0001	< 0,0001	< 0,0001	< 0,0001	< 0,0001
χ^2 (between raw products and ready to eat products) P=	< 0,0001		0,0073	0,0073	0,0073	0,01430	0,0073

Table 2. The number and percentage of isolated strains resistant to antimicrobials

	No. (%) of *Enterococcus*. spp isolates						
	Penicillin G	Ampicillin	Tetracycline	Erythromycin	Chloramphenicol	Vancomycin	Teicoplanin
E. faecalis	12 (31.6)	2 (5.3)	30 (78.9)	13 (34.2)	2 (5.3)	0 (0)	0 (0)
E. faecium	3 (50)	0 (0)	3 (50)	1 (16.7)	0 (0)	0 (0)	0 (0)
E. hirae	3 (42.8)	0 (0)	3 (42.8)	2 (28.6)	1 (14.3)	0 (0)	0 (0)
E. durans	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	18 (33.3)	2 (3.7)	36 (66.7)	16 (29.6)	3 (5.5)	0 (0)	0 (0)
χ^2 P=	< 0,0001	0,0002	< 0,0001	< 0,0001	< 0,0001		

Table 3. Phenotypic resistance patterns among multidrug-resistant enterococcal isolates

Antimicrobial resistance phenotype	Number of isolates
P-TE-E	3
TE-E-C	1
P-TE-E-C	2
Total	6

P: penicillin; TE: tetracycline ; E: erythromycin ; C: chloramphenicol

Table 4. Distribution of virulence factors in enterococcal isolates

	Phenotypic tests No (%)		
	Gelatinase	Hemolysin	Biofilm formation
E. faecalis	38 (100)	3 (7.9)	38 (100)
E. faecium	6 (100)	0 (0)	6 (100)
E. hirae	7 (100)	1 (14.3)	7 (100)
E. durans	3 (100)	0 (0)	3 (100)
Total	54 (100)	4 (7.4)	54 (100)
χ^2 P=		< 0,0001	

Discussion

In this study, the enterococcal contamination rate was lower than previously observed by many authors, who reported high incidences in various food products (KLIBI et al., 2013; REHAIEM et al., 2016; SANLIBABA and SENTURK, 2018). However, the prevalence obtained in this study was identical to that observed by PESAVENTO et al. (2014), who reported a rate of 23.6%. As reported by ABRIOUEL et al. (2008), enterococci can be detected not only in raw materials but also in ready-to-eat foods, of animal and plant origin. The presence of enterococci in meat products can be considered an indicator of fecal contamination during evisceration in slaughterhouses (KLIBI et al., 2013). However, other sources of enterococcal contamination in milk and dairy products were identified, including mastitis, milking machines and the milking environment (DAPKEVICIUS et al., 2021). In addition, previous studies stressed that the cheese maker and the cheese making equipment were the main sources of enterococci in the cheeses, and that contamination of milk by enterococci of bovine fecal origin was low (HAMMAD et al., 2015). Besides their thermoduric character, post-treatment recontamination also explains the presence of enterococci in pasteurized milk products, such as cheeses (GIRAFFA, 2002). The differences between the results obtained from various studies about the prevalence rates of enterococci may be related to geographical discrepancies, or to different isolation methodologies (KLIBI et al., 2013).

Our results corroborate with those of many authors who indicated that *E. faecalis* was the most frequently isolated species in foods of animal origin (BARBOSA et al., 2010; KLIBI et al., 2013; SANLIBABA and SENTURK, 2018). In contrast to our findings, *E. faecium* was the most frequently detected species in other studies (PESAVENTO et al., 2014; HAMAD et al., 2015; REHAIEM et al., 2016). As reported, ecology and epidemiological studies have found that *E. faecalis* and *E. faecium* were the most frequently species isolated from various food products and the environment (TORRES et al., 2018).

High percentages of tetracycline, penicillin and erythromycin resistance were detected. These results are consistent with those of many other authors (KLIBI et al., 2013; PESAVENTO et al., 2014; REHAIEM et al., 2016). As is well-known, tetracycline, penicillin and macrolide are antibiotics frequently used in veterinary medicine, which might explain the levels of resistance detected in enterococcal isolates against these antibiotic molecules. However, it should be noted that resistance to tetracycline has little clinical importance as it is not a drug of choice for the treatment of enterococcal infections. Of serious concern is the dissemination of tetracycline resistance from enterococci to a large diversity of bacterial genera through an unusual gene transfer element (HAMMAD et al., 2015). Slight resistances were observed to ampicillin and chloramphenicol. These results corroborate with those observed by PESAVENTO et al. (2014) in strains isolated from retail cheeses, ready-to-eat salads, ham and raw meat. In contrast, SANLIBABA and SENTURK (2018) have reported a higher resistance against ampicillin. BORTOLAIA et al. (2015) reported in a study concerning a comparison of the antimicrobial susceptibility of *E. faecium* isolates from poultry meat and human origin, that human isolates showed very high rates of ampicillin resistance in all European countries, but resistance in food isolates was significantly lower than in humans. The detection of chloramphenicol resistance in our isolates may suggest its use in veterinary practice.

Several studies have implied that antibiotic resistance enterococci (AREs), and especially vancomycin-resistant enterococci (VREs), which have entered the food chain have gained importance with the increasing significance of VREs in hospital infections (TERKURAN et al., 2019). In this study, no vancomycine resistance strain was identified among our enterococcal isolates. This is reassuring from a medical point of view since vancomycin is an important reserve antibiotic for the treatment of enterococcal infections. These results agree with other studies that have noted the absence of acquired-vancomycin resistance enterococci in food products (REHAIEM et al., 2016; BEN SAID et al., 2017; SILVETTI et al., 2019), although some others authors detected VREs in various food products, including chicken meat (HAMMAD et

al., 2015), beef meat (PESAVENTO et al., 2014; GUERRERO-RAMOS et al., 2016), cheeses (PESAVENTO et al., 2014), raw milk (CITAK et al., 2005), pork meat (GUERRERO-RAMOS et al., 2016) and ready made salads (PESAVANTO et al., 2014). Differences in the rates of VREs detection in different countries could be due to the different politics of antibiotic use in animals (KLIBI et al., 2013). In Europe, it has been suggested that the massive use of avoparcin in animal husbandry was associated with the high prevalence rates of VRE strains detected in food-producing animals (RAMOS et al., 2020). In the current study, resistance to three or more classes of antibiotics (multidrug resistance) was found to be at the level of 11% for enterococci isolates, which is not in agreement with the results found by KLIBI et al. (2013), who reported a high incidence of multidrug-resistance strains, amounting to 24%. Although, the relationship of foodborne enterococci with clinical infections has not yet been clearly elucidated, it has been argued that foodborne enterococci, with horizontal gene transfer, may play a role in the expansion of virulence genes (TERKURAN et al., 2019).

To cause infection, enterococci must be able to colonize the host tissue, resist host immune defense mechanisms, and cause pathological changes. Therefore, they have to possess virulence factors (HAMMAD et al., 2015). In our study, all strains were gelatinase producers, which is in agreement with results obtained by KLIBI et al. (2013). However, only 38.6% of strains were gelatinase producers, in the study conducted by BEN SAID et al. (2017). As is well-known, gelatinase is an extracellular zinc endopeptidase that hydrolyzes collagen, gelatin, hemoglobin and bioactive compounds, and is found both in clinical strains and in those isolated from food (CHAJECKA-WIERZCHOWSKA et al., 2017). A β-haemolytic was demonstrated in four enterococci (three *E. faecalis* and one *E. hirae*). These findings agree with those of other authors, who reported the presence of haemolytic producer enterococci of food origin (KLIBI et al., 2013; BEN SAID et al., 2017; SANLIBABA and SENTURK, 2018). Due to their easy transferability by means of conjugative plasmids, the presence of genetic determinants encoding for β-hemolysis is considered undesirable in food strains, especially those used as starters in food fermentations (SILVETTI et al., 2019). In the same way, *in vitro* biofilm formation capacity was analyzed in this study, showing that all strains exhibited a strong capacity for biofilm formation. Our results corroborate those of many authors, who indicated a high incidence of biofilm producer strains among food isolates (POPOVIĆ et al., 2018; IGBINOSA and BESHIRU, 2019). In food processing environments, biofilm formation by enterococci, which could carry virulence factors and even resistance to several antibiotics, is a matter of concern. As a result, producers need to clean and sanitize their instruments and materials in order to prevent this adherence and also product contamination with virulent strains during food processing (BARBOSA et al., 2010).

Conclusions

Although enterococcal strains from clinical specimens have been widely studied, there is little informations on the antibiotic resistance of enterococci strains originating from food in Algeria. The aim of this study was to provide data on the prevalence, antibiotic resistance and virulence factors of enterococci strains from food items, as they can be a vehicle for the spread of antibiotic-resistance and virulence genes. The results of this study revealed that enterococci are common contaminants in food products. The significant usage of tetracycline and penicillin antibiotics in veterinary medicine has probably led to the emergence of tetracycline and penicillin-resistant *Enterococcus* strains, as shown in our study. The absence of vancomycin-resistant enterococci in this study is reassuring from a medical point of view, as vancomycin is a clinically relevant antibiotic for the treatment of enterococcal infections. However, the presence of virulence traits in the analyzed strains revealed the health hazards associated with the spread of these enterococci strains through the food chain to humans, and transfer of virulence genes to other pathogenic bacteria. Further genotyping characterization is needed to generate more information about these *Enterococcus* spp. isolates.

Conflicts of Interest:

The authors declare no conflicts of interest.

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SAŽETAK

Enterokoki su ubikvitarne bakterije u fiziološkoj crijevnoj mikrobioti ljudi i životinja. Mogu se upotrijebiti u prehrambenoj industriji kao starterna ili probiotička kultura, međutim neke su se vrste pojavile kao važni nozokomijalni patogeni povezani s teškim multirezistentnim infekcijama. U ovom je istraživanju ukupno 235 prehrambenih proizvoda analizirano na prisutnost enterokoka. Od navedenog broja, 54 uzorka (22,9 %) bila su kontaminirana te su izolirana 54 soja. Potonji su identificirani masenom spektofotomerijom temeljenoj na MALDI-TOF (engl. *Matrix Assisted Laser Desorption/Ionization Time-of-Flight*) tehnologiji te je analizirana osjetljivost na sedam molekula antibiotika kako bi se potvrdila sposobnost stvaranja biofilma. Prevladavajuće su vrste bile *Enterococcus faecalis* (70,4 %), zatim *Enterococcus hirae* (12,9 %), *Enterococcus faecium* (11,1 %) i *Enterococcus durans* (5,5 %). Te su vrste najveću rezistenciju pokazale na tetraciklin (66,7 %) i penicilin G (33,3 %). Šest izolata enterokoka (11,1 %) bilo je rezistentno na najmanje tri porodice antibiotika. Svi su izolirani sojevi bili sposobni stvoriti biofilm i pokazali su aktivnost želatinaze, no samo su četiri soja (7,4 %) bila β-hemolitička. Ovo je istraživanje pokazalo da se enterokoki s ovakvom virulencijom i svojstvima rezistencije na antibiotike mogu širiti putem prehrambenih proizvoda. Potrebna su stoga kontinuirana istraživanja kako bi se procijenili zdravstveni rizici povezani s uzimanjem hrane kontaminirane bakterijama roda *Enterococcus* spp.

Ključne riječi: enterokoki; prehrambeni proizvod; osjetljivost na antibiotike; stvaranje biofilma; MALDI-TOF MS