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Antibiotic susceptibility and biofilm-forming capacity of enterococci isolated from food of animal origin*)

VIERA DUCKOVÁ, MARGITA ČANIGOVÁ, MIROSLAV KROČKO, MONIKA LAVOVÁ

Department of Evaluation and Processing Animal Products, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia

Ducková V., Čanigová M., Kročko M., Lavová M.

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Summary

Enterococci are found in the gastrointestinal tract of humans and animals, in soil, and in water, but they also have a long history of use in the production of traditional fermented food. Some strains of enterococci are considered as emerging pathogens of humans. The intrinsic and acquired resistance of enterococci to antibiotics has special significance. Food of animal origin may be a source of resistant enterococci. The main cause of food contamination with enterococci is improper cleaning and disinfection of equipment. The aim of this study was to assess the antibiotic susceptibility of enterococci isolated from different types of food of animal origin and the ability of these microorganisms to form biofilm. Out of a total of 63 isolates, relatively few enterococci were resistant to gentamicin (1.59%). The highest level of resistance was noted for vancomycin (7.94%) and ampicillin (9.52%). However as many as 19.05% and 44.44% of enterococci were resistant to erythromycin and tetracycline, respectively. The ability to form biofilm was detected in 14.29% of the strains of enterococci tested.

Keywords: enterococci, antibiotic susceptibility, biofilm, food of animal origin

Enterococci belong to the lactic acid bacteria and are of importance for food fermentation and food spoilage. They can be responsible for the spoilage of cooked meat products, but they can also contribute to the ripening and aroma development of certain cheeses or fermented sausages. Certain enterococcal strains are also successfully used as probiotics to improve human or animal health (3). However, several studies have shown that enterococci possess virulence determinants, such as the enterococcal surface protein gene (esp), aggregation substances (agg), cell wall adhesions (efaAfm and efaAfs), gelatinase (gelE), and cytolysin (cyl). The presence of such virulence factors, intrinsic and acquired antibiotic resistance of enterococci, and their association with human disease may explain their potential pathogenic activity (1). The increasing antibiotic resistance of enterococci (especially to vancomycin) creates serious problems concerning the effective therapy of enterococcal infections in humans (11). Animals and food of animal origin can be sources of resistant enterococci (8, 13, 15).

The ability of enterococci to form biofilm is a further important virulence property. Biofilm production can increase resistance to antibiotics (14). Biofilms constitute a protected mode of growth that allows microorganisms to survive in a hostile environment, since their physiology and behaviour are significantly different from those of their planctonic counterparts. In food industry, biofilms can be a source of contamination, causing food spoilage, and are possible causes of public health problems such as the outbreaks of foodborne pathogens (12).

The aim of this study was to assess the antibiotic susceptibility and biofilm-forming capacity of enterococci isolated from different types of food of animal origin.

Material and methods

Sixty-three strains of enterococci were isolated from food of animal origin (poultry n = 30, pork n = 5, bryndza cheese n = 2, sheep curd cheese n = 5, sheep's milk n = 6, cow's milk n = 15). Isolated strains were identified by a commercial En-coccus test (Pliva-Lachema, Czech Republic). Susceptibility to antibiotics was tested by the disc diffusion method according to the recommendations of CLSI (2). The following antimicrobial drugs (HiMedia, India) were used: 10 µg ampicillin (A), 15 µg erythromycin (E), 120 µg gentamicin (G), 30 µg tetracycline (T), and 30 µg vancomycin (Va). The method with crystal violet staining (7) was used for the assessment of the ability of enterococci to form biofilm. Strains of enterococci were cultured in tryptic soya broth (HiMedia, India), in a 96-well polystyrene microtitre plate, at 37°C for 18-20 hours. After appropriate washing and staining with crystal violet, the absorbance at 630 nm of dye solutions was measured in a ELx808IU microtitre plate spectrophotometer (BioTek, USA).

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Results and discussion

The results of the evaluation of antibiotic susceptibility and biofilm-forming capacity are shown in tab. 1. Out of a total of 63 isolates, a relatively small number of enterococci was resistant to gentamicin (1.59%). The highest level of resistance was noted for vancomycin (7.94%) and ampicillin (9.52%). However, as many as 19.05% and 44.44% of enterococci were resistant to erythromycin and tetracycline, respectively. Similarly to our results, Różańska (11) found that among 138 enterococci isolated from food of animal origin 2.2% were resistant to ampicillin, 6.5% to vancomycin, 23.2% to erythromycin, and 39.1% to tetracycline. In a study by Koluman et al. (5), 2% of 100 enterococcal isolates were resistant to ampicillin, 14% to tetracycline, 22% to vancomycin, and 24% to erythromycin. These authors recorded the highest resistance in cream cheese samples (resistance to 12 types of antibiotics) and the lowest in chicken samples (resistance to 2 types of antibiotics), whereas the enterococci isolated from poultry in our study showed the highest multiresistance to antibiotics. Jung et al. (4) isolated vancomycin-resistant enterococci from animal sources, mostly from samples of meat (77% of positive poultry samples, 38% of pork, and only 0.4% of raw milk).

Tab. 1. Antibiotic resistance and biofilm-forming capacity of enterococci tested (n = 63)

Enterococci	Number of strains	% of resistant strains					% of strains able to form biofilm
		A 10	E 15	G 120	T 30	Va 30	
E. faecalis	41	7.32	19. 51	2.44	58.54	7.32	17.07
E. faecium	13	7.69	23.08	0	23.08	15.38	15.38
E. casseliflavus	1	100	0	0	0	0	0
E. galinarum	1	0	0	0	0	0	0
E. mundtii	1	0	0	0	0	0	0
E. group III	5	20	20	0	0	0	0
E. sp.	1	0	0	0	100	0	0

Explanations: A – 10 µg ampicillin, E – 15 µg erythromycin, G – 120 µg gentamicin, T – 30 µg tetracycline, Va – 30 µg vancomycin

In our study, 14.29% of all enterococci strains tested (n = 63) were classified as forming biofilm. Out of these biofilm-forming enterococci, 22.22% were resistant to tetracycline, 11.11% to ampicillin, and 11.11% to vancomycin. By growing enterococci in glass tubes, Necidová et al. (10) found that 28% of *Enterococcus* spp. strains had the ability to form biofilm. In their study a higher number of biofilm-forming strains of *Enterococcus faecium* (33%) and *Enterococcus faecalis* (28%) was found in comparison with our results (15.38% and 17.07% respectively). Koreňová et al. (6) evaluated biofilm-forming bacteria isolated from small and medium-sized enterprises processing ewe's milk and meat. Out of 81 enterococcal isolates, only 4 strains (4.94%) formed biofilm. Barbosa et al. (1) evaluated the biofilm-forming capacity of enterococci isolated from traditional fermented meat products in batch and fed-batch mode measurement. They reported that in the batch mode, only 28.0% and 3.9% of isolates were classified as moderate and strong biofilm producers, respectively, whereas in the fed-batch mode, the corresponding figures were 35.7% and 63.2%. Macovel et al. (9) reported that 30.5% of 396 enterococci from animal faeces had the ability to form biofilm. Tsikrikonis et al. (14) compared biofilm-forming capacities of animal and human enterococcal isolates and found that animal isolates exhibited a significantly lower capacity for biofilm formation than isolates from human samples ($P < 0.0001$). In their study, 29.5% of *Enterococcus faecalis* and 34.4% of *Enterococcus faecium* from animals formed biofilm.

It can be concluded that food of animal origin contains enterococci resistant to antibiotics, including vancomycin, and some strains are also able to form biofilm. Therefore it is important to protect food from contamination with enterococci. Biofilm formation and the spread of enterococci should be prevented through hygiene and sanitation.

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Author's address: Ing. Viera Ducková, PhD., Department of Evaluation and Processing Animal Products, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia; e-mail: viera.duckova@uniag.sk