

# Antibiotic susceptibility of *Enterococcus* spp. isolated from food of animal origin

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### Summary

During the last two decades antibiotic-resistant strains of *Enterococcus* spp. have emerged as important pathogens responsible for serious nosocomial infections in humans. From the medical point of view the resistance to vancomycin and aminoglycosides has special significance. Several studies suggest that resistant strains of enterococci could be transmitted from animals to humans through the food chain. In the Polish literature there is no data about antibiotic sensitivity of enterococci isolated from food of animal origin. The aim of the study was the evaluation of antibiotic susceptibility of enterococci recovered from different types of such food. One hundred and thirty eight strains of *Enterococcus* spp. obtained from regional veterinary laboratories were tested using the disk diffusion method. All strains were isolated from food of animal origin (milk and milk products, raw meat, minced meat). The percentage of resistant strains ranged from 2.2 for ampicillin to 84.1 for streptomycin. Most of the strains (133 isolates) were resistant for two or more antibacterial substances. The obtained results confirmed a relatively high level of resistance of enterococci for antibiotics used in animals and humans.

**Keywords:** enterococci, food, antimicrobial susceptibility

Enterococci are the part of the normal intestinal flora of humans and animals. However, they may also be important pathogens responsible for serious infections, such as endocarditis, septicaemia, meningitis, urinary tract infections (3, 4, 15). These organisms are able to survive in different conditions of the environment, such as a temperature range of 10-45°C, pH 2.7-10, NaCl contents up to 6.5%. These features of enterococci and their ability to acquire, accumulate and transfer genetic elements such as plasmids and transposons are reasons for their increased importance as human and animal pathogens. The increasing antibiotic resistance of enterococci creates serious problems concerning the effective therapy of enterococcal infections in humans. WHO included enterococci into groups of alarm pathogens. The animals and food of animal origin may be a source of resistant enterococci pathogenic for humans (4, 5, 7, 12, 14, 16, 17).

The aim of the study was the evaluation of antibiotic susceptibility of enterococci isolated from different types of food of animal origin.

### Material and methods

One hundred and thirty eight strains of *Enterococcus* spp. obtained from regional veterinary laboratories were tested using the disk diffusion method. All strains were isolated

from food of animal origin (milk and milk products, raw meat, minced meat). The following antimicrobial drugs (BioMerieux) were used: penicillin G (10 i.u.), ampicillin (10 µg), vancomycin (30 µg), erythromycin (15 µg), tetracycline (30 µg), doxycycline (30 µg), ciprofloxacin (5 µg), nitrofurantoin (300 µg), rifampicin (30 µg), chloramphenicol (30 µg), gentamicin (10 µg), and streptomycin (10 µg). The reference strain *Enterococcus faecalis* ATCC 29212 was used as a control. Interpretation of the diameter of the inhibition zones was performed according to recommendations of CLSI (2).

The tested strains of enterococci were cultivated overnight in BHI broth (pH 7.0) at 37 ± 1°C. These cultures were suspended in 7.5% NaCl solution to obtain the optical density 0.5 on the McFarland standard. Two to 3 ml of these suspension were transferred onto the surface of Mueller-Hinton Agar (pH 7.3) and spread out. The excess of the fluid was removed using a pipette. The antibiotic disks were placed on the surface of agar. The plates were incubated for 24 h at 37 ± 1°C. The zones of inhibition were measured using an electronic caliper, with 1 mm precision. On the base of diameters of inhibition zones the strains of enterococci were evaluated as sensitive or resistant.

### Results and discussion

The results are showed in tab. 1. The obtained data indicated a different level of resistance of enterococci

**Tab. 1. Antibiotic susceptibility of enterococci isolated from food of animal origin (n = 138)**

Antibiotics	Number of resistant strains	%
Penicillin G	7	5.1
Ampicillin	3	2.2
Vancomycin	9	6.5
Erythromycin	32	23.2
Tetracycline	54	39.1
Doxycycline	23	16.7
Ciprofloxacin	15	10.9
Nitrofurantoina	13	9.4
Rifampicin	28	20.3
Chloramphenicol	11	8.0
Gentamycin	65	47.1
Streptomycin	116	84.1

isolated from food of animal origin depending on antibiotics used. A relatively small number of isolates was resistant to  $\beta$ -lactams (penicillin G and ampicillin – 5.1 and 2.2%, respectively), nitrofurantoina (9.4%) and chloramphenicol (8.0%). The highest level of resistance was noted for tetracycline (39.1%), gentamicin (47.1%) and streptomycin (84.1%). Nine strains were evaluated as vancomycin-resistant (6.5%). One hundred thirty three of the tested strains of enterococci were resistant to two or more antibiotics.

There are no data in the Polish literature about antibiotic susceptibility of enterococci isolated from food of animal origin. However, data from other countries confirmed a high level of antimicrobial resistance of these microorganisms. In Lithuania 84.5% of the enterococci isolated from poultry products were resistant to tetracycline, 63.8% – to erythromycin and 36.2% – to ciprofloxacin (13). A relatively high level of resistance to tetracycline and ciprofloxacin was reported by other authors (7). The percentage of strains resistant to penicillin was similar to the present data. Another source (6) reported a high level of resistance to ciprofloxacin, penicillin and erythromycin of the strains isolated from different species of animals, but the level of resistance varied, depending on the species.

Many authors reported resistance of enterococci isolated from food to vancomycin (VRE – vancomycin resistant enterococci) and aminoglycosides (HLAR – high level aminoglycoside resistant). According to Kročko et al. (8) 15% of enterococci isolated from raw meats were resistant to vancomycin (10, 11). Citak et al. (1) noted that 48% of the strains of *Enterococcus faecalis* and 26% *Enterococcus faecium* from raw milk were resistant to this antibiotic. In Spain 27.2% of the enterococci from chicken were resistant to vancomycin, but VRE were not isolated from turkeys and pork (12). Enterococci are naturally resistant to low levels of aminoglycosides. This is confirmed in the present

study, in which 84.1% of the tested strains were resistant to streptomycin in a concentration 10  $\mu$ g per disk.

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