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Antimicrobial resistance of *Enterococcus faecalis* isolated from meat

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Abstract

The aim of the study was the evaluation of the antimicrobial resistance of *Enterococcus faecalis* strains isolated from cattle, pig, and poultry meat. A test was performed on 111 strains using the minimum inhibitory concentration technique. The highest number of isolates (94 strains) were resistant to lincomycin, the second-highest resistance was to quinupristin/dalfopristin (88 strains), tetracycline followed (65 strains), and erythromycin resistance was also notable (40 strains). All isolates tested were sensitive to daptomycin, nitrofurantoine, and tigecycline, whereas only few strains were resistant to ciprofloxacin, gentamicin, penicillin, and vancomycin. The obtained results showed that meat may be a source of antimicrobial resistant enterococci which may be transferred to humans.

Keywords: meat, *Enterococcus faecalis*, antimicrobial resistance.

Introduction

Antimicrobial resistance seems to be the main problem in human and veterinary medicine. It is estimated that in the European Union, Iceland, and Norway alone resistant bacteria are responsible for about 400 000 infections and 25 000 deaths yearly (7). One of the most important bacterial species causing nosocomial infections, such as endocarditis, septicaemia, meningitis, and urinary tract infections, are enterococci. Their ability to survive in different environmental conditions, innate resistance to many antibiotics, and ability to acquire, accumulate, and transfer genetic determinants of resistance are reasons for their increased significance as human and animal pathogens (6, 18, 22, 24, 27). Many authors have indicated a possible transfer of the resistant enterococci from animals to humans *via* the food chain; however, opinions on this matter are divided (1, 3, 4, 10, 12, 14, 15, 18-23, 25). In Poland, there are no available data about the antimicrobial resistance of enterococci isolated from meat; therefore, the aim of the study was the evaluation of this phenotypic feature among strains of *Enterococcus faecalis*.

Material and Methods

Enterococci were isolated from cattle, pig, and poultry meat. The samples with mean weight of about 200 g were taken by veterinary inspectors in slaughterhouses in different parts of Poland, and were sent to the laboratory under cooling conditions. The breakdown of the 224 tested samples was: 118 from pigs, 57 from cattle, and 49 from poultry. To isolate the bacteria, 10 g of the sample was incubated in Brain-Heart Infusion (BHI) broth (Oxoid, UK) overnight at 37 ± 1ºC. Then, 50 µL of the culture was transferred into a broth with sodium azide and crystal violet (azide dextrose broth supplemented with 1.6% bromocresol purple and 1.3 mL/L crystal violet (Merck, Germany). After incubation for 24 h at 37± 1ºC, one loop of the culture was spread on the surface of Slanetz and Bartley agar (Oxoid, UK) and incubated overnight at 37± 1ºC. The suspected enterococci colonies were further analysed using the API rapid ID 32 STREP kit (bioMérieux, France) according to the manufacturer's instructions. Antimicrobial resistance of the confirmed *Enterococcus* isolates was evaluated using the Minimum Inhibitory Concentration (MIC) technique (CMV3AGPF Sensititre plates, Trek Diagnostic

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Systems, UK) with decreasing concentrations of tigecycline (TGC), tetracycline (TET), chloramphenicol (CHL), daptomycin (DAP), streptomycin (STR), tylosin tartrate (TYLT), quinupristin/dalfopristin (SYN), linezolid (LZD), nitrofurantoin (NIT), penicillin (PEN), kanamycin (KAN), erythromycin (ERY), ciprofloxacin (CIP), vancomycin (VAN), lincomycin (LIN), and gentamicin (GEN). All investigations were performed with a Sensititre autoreader device (Trek). The reference *E. faecalis* ATCC 29212 strain was used as a control.

Results

Results were obtained from 111 *E. faecalis* strains isolated from 224 (49.5%) tested meat samples: 52 (44.1%) pork, 35 (61.4%) cattle, and 24 (49.0%) poultry. The resistance analyses showed that most of the *E. faecalis* isolates (Table 1) were resistant to lincomycin (84.7%), quinupristin/dalfopristin (79.3%), and tetracycline (58.6%). Fewer isolates, numbering 40 (36.0%), demonstrated resistance to erythromycin and 20 (18.0%) did so to kanamycin, streptomycin, and tylosin. Isolates resistant to linezolid totalled 8 (7.2%). Only a few strains were resistant to gentamicin and vancomycin (2.7%), as well as to ciprofloxacin and penicillin (1.8%). All tested strains were susceptible to daptomycin, nitrofurantoine, and tigecycline. Significant differences in the susceptibility of the strains isolated from beef, pork, and poultry meat were observed (Table 1 and Fig. 1). Resistance to chloramphenicol was noted in 10 strains isolated from beef (28.6%) and 5 from poultry meat (20.8%), whereas all isolates from pork were susceptible to this antibiotic. Poultry meat yielded 2 isolates with resistance to ciprofloxacin. The percentage of strains resistant to erythromycin varied from 28.6 and 29.2 for beef and poultry meat respectively to 44.2 for pork. Very significant differences were observed for kanamycin and streptomycin. Only 2 isolates from beef and 2 from pork were resistant to these antibiotics, whereas the percentage of resistant strains from poultry meat was established as 66.7. Similar differences were observed for tylosin. Generally, the percentage of isolates resistant to the majority of antimicrobials tested was higher in the case of poultry meat. Based on obtained results, the resistance profiles of the isolates were established (Table 2). As is shown, simultaneous resistance to lincomycin and quinupristin/dalfopristin or lincomycin, quinupristin/dalfopristin and tetracycline was most often observed. The total isolates resistant to 3 or more antimicrobials were 63 (56.8%) and the strains susceptible to all substances numbered only 4.

Table 1. Antimicrobial resistance of *Enterococcus faecalis* isolated from meat

Antimicrobials	Number/% of resistant strains			
	Total (n = 111)	Cattle (n = 35)	Pigs (n = 52)	Poultry (n = 24)
Chloramphenicol (CHL)	15/13.5	10/28.6	0	5/20.8
Ciprofloxacin (CIP)	2/1.8	0	0	2/8.3
Daptomycin (DAP)	0	0	0	0
Erythromycin (ERY)	40/36.0	10/28.6	23/44.2	7/29.2
Gentamicin (GEN)	3/2.7	0	0	2/8.3
Kanamycin (KAN)	20/18.0	2/5.7	2/3.8	16/66.7
Lincomycin (LIN)	94/84.7	30/85.7	40/76.9	24/100.0
Linezolid (LZD)	8/7.2	4/11.4	2/3.8	2/8.3
Nitrofurantoin (NIT)	0	0	0	0
Penicillin (PEN)	2/1.8	1/2.9	1/1.9	0
Quinupristin/dalfopristin (SYN)	88/79.3	34/97.1	31/59.6	23/95.8
Streptomycin (STR)	20/18.0	2/5.7	2/3.8	16/66.7
Tetracycline (TET)	65/58.6	26/74.3	18/34.6	21/87.5
Tigecycline (TGC)	0	0	0	0
Tylosin (TYLT)	20/18.0	3/8.6	1/1.9	16/66.7
Vancomycin (VAN)	3/2.7	1/2.9	1/1.9	1/4.2

Table 2. Resistance proflies of *E. faecalis* strains isolated from cattle, pig, and poultry meat

Number of strains	Resistance to
24	LIN, SYN
15	LIN, SYN, TET
9	LIN
8	SYN
8	ERY, LIN, SYN, TET
4	LIN, SYN, STR, TET
4	LIN, SYN, STR, TET, TYLT
4	CHL, ERY, KAN, LIN, SYN, TET, TYLT
3	CHL, ERY, GEN, KAN, LIN, SYN, TET, TYLT
3	CHL, ERY, KAN, LIN, SYN, STR, TET, TYLT
2	ERY, LIN, LZD, PEN, SYN, TET, VAN
2	ERY, LIN, LZD, SYN, TET
2	ERY, KAN, LIN, SYN, STR, TET, TYLT
2	ERY, LIN, TET
1	CHL, KAN, LIN, LZD, SYN, TET
1	CHL, ERY, LIN, SYN, TET, TYLT
1	CHL, ERY, KAN, LIN, STR, TET, TYLT, VAN
1	CHL, ERY, LIN, SYN, TET
1	CHL, LIN, SYN, TET
1	CHL, LIN, TET
1	ERY, KAN, LIN, LZD, SYN, TET, TYLT
1	CIP, LIN, SYN, TET
1	ERY, KAN, LIN, SYN, STR, TET
1	CIP, ERY, KAN, LIN, SYN, STR, TET
1	ERY, LIN, STR, TET, TYLT
1	ERY, LIN, STR, TET
1	LIN, STR
1	ERY, LIN, SYN, TET
1	LIN, TET
1	TET
1	ERY, KAN, SYN, TET, TYLT
4	Sensitive to all

For antibiotic abbreviations see Table 1

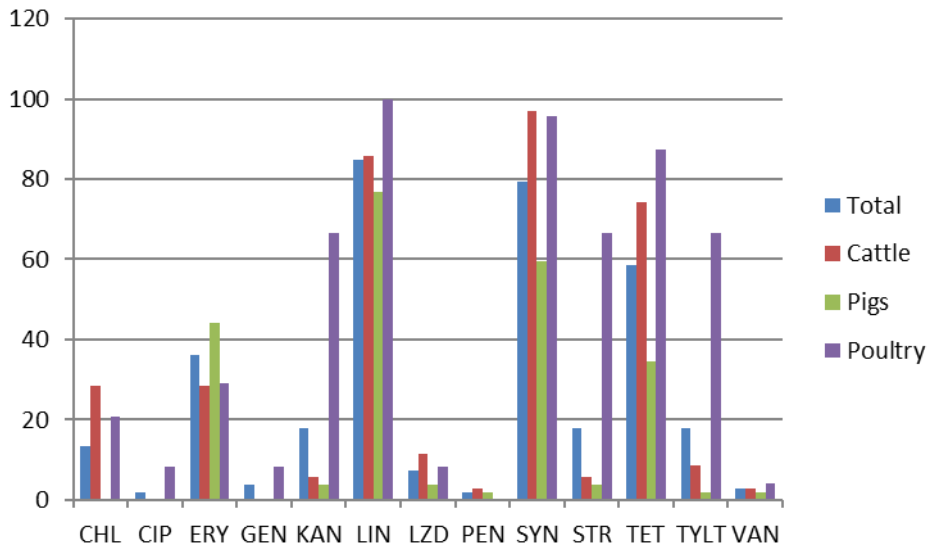


Fig. 1. Comparison of antimicrobial resistance of *E. faecalis* isolated from beef, pork, and poultry meat. For antibiotic abbreviations see Table 1

Discussion

The obtained results confirmed the frequent resistance of enterococci isolated from beef, pork, and poultry meat, especially to quinupristin/dalfopristin, lincomycin, and erythromycin. There are no sufficient data from other results from Poland for any meaningful comparison, but many authors from other countries have indicated the high percentage of these microorganisms resistant to different antimicrobials. The innate resistance of *E. faecalis* to streptogramins is typical for these bacteria (10) and was confirmed by the high number of isolates resistant to quinupristin/dalfopristin. The high percentage of strains resistant to lincomycin (66.3%) was noted by McGowan *et al.* (16) for *E. faecalis* isolated from meats. In the presented study, a relatively high percentage of strains resistant to aminoglycosides (streptomycin and kanamycin) was observed, especially of those isolated from poultry meat. Imperviousness to this extent is in accordance with the data presented by Jackson *et al.* (11), Kolár *et al.* (13), and Ford *et al.* (8). The strains resistant to high concentrations of aminoglycosides usually exhibit resistance to 3 or more groups of antibiotics. This was confirmed by the data presented in Table 2. Among 28 strains of *E. faecalis* resistant to one or two aminoglycosides, almost all isolates were resistant to 4 or more substances from different groups of antimicrobials. According to Kročko *et al.* (15) 91% of enterococci isolated from poultry products were resistant to tetracycline. A similar percentage was observed in the presented study (87.5%). Šustáčková *et al.* (26) found that 44% of *E. faecalis* and 72% of *E. faecium* strains isolated from meat and meat products were resistant to erythromycin and 34.5% and 17.4% respectively to tetracycline. Ducková *et al.* (5) also indicated the high percentage of strains resistant to tetracyclines. In the presented study only 3 strains were resistant to vancomycin. Similar results (4.8%) were noted by Kolář *et al.* (13), whereas Gambarotto *et al.* (9) found 100% of vancomycin resistant isolates from poultry and 60% from swine tissues. Chadwick *et al.* (3) also noted a high number of enterococci isolated from meat with resistance to glycopeptides (52%). Hayes *et al.* (10) observed a relatively high percentage of isolates from raw meat resistant to ciprofloxacin, whereas in the present study only 2 strains resistant to this antibiotic were identified. The cited data indicated the differences in antimicrobial resistance of enterococci isolated from meat in different countries. Probably it depends on the breeding structure and antibiotic usage in veterinary medicine country by country. However, most authors agree that resistant enterococci are found in meat and may be transferred to humans via the food chain, a fact seemingly partially confirmed by the presented results.

Conflict of Interests Statement: The authors declare that there is no conflict of interest regarding the publication of this article.

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