Occurrence, isolation and antibiotic resistance of *Enterococcus* species isolated from raw pork, beef and poultry

MIROSLAV KROČKO – MARGITA ČANIGOVÁ – VIERA DUCKOVÁ

Summary

The aim of this work was to investigate the prevalence of enterococci and antimicrobial resistance of *Enterococcus* spp. in 110 samples of raw pork (64%), raw beef (23%) and poultry (13%). The samples for enumeration of total bacterial counts were cultured on diagnostic Plate count agar and the samples for enumeration of enterococci count were cultured on selective diagnostic Slanetz–Bartley agar. The counts of enterococci were in the range of 0.60–6.47 log CFU.cm^{-2} in pork, 0–2.14 log CFU.cm^{-2} in beef and 1.45-4.00 log CFU.cm^{-2} in poultry. Susceptibilities of isolated enterococci from pork and poultry to antimicrobial agents were tested using the disk diffusion method. *Enterococcus faecium* was the dominant species recovered from pork (72%) and poultry (39%), followed by *E. faecalis* 10% (pork) and 23% (poultry). Of 75 isolates of enterococci, 15% were resistant to vancomycin and 15% were resistant to erythromycin, 27% to ampicillin, 25% to gentamicin and 56% to tetracycline. We found a higher prevalence of intermediate resistant isolates of pork and poultry to ampicillin (70 and 40%), gentamicin (66 and 40%), tetracycline (only pork 54%) and erythromycin (only pork 64%). Our study suggests that raw meat plays a potential role as a reservoir of enterococci resistant to antibiotics.

Keywords

Enterococcus; raw meat; antibiotic resistance; vancomycin resistant enterococci

Meat is one of the most perishable foods, and its composition is ideal for the growth of a wide range of spoilage bacteria [1]. The bacterial spoilage of meat is not only influenced by temperature, oxygen availability and water activity, but also determined by quantities and type of microorganisms growing on meats [2, 3]. The part of spoilage bacteria are enterococci which usually contaminate raw meats in the range of 10^2–10^4 CFU.g^{-1}. These are obviously resistant to temperature, pH and salinity effects, may multiply to high numbers and cause spoilage of processed meats [4]. Some enterococci are potentially virulent and can exhibit pathogenic traits [5]. It is well known that antibiotic-resistant bacteria that have been identified in animals may contaminate also meat being obtained from those animals [6], and it is possible that insufficient thermal or culinary procedures can bring about infection of the human gastrointestinal tract.

The present study was designed to determine the prevalence and antibiotic resistance of enterococci isolated from raw beef, pork and poultry.

MATERIALS AND METHODS

Samples for microbiological examination (110 samples) of pork (n = 70), beef (n = 25) and poultry (n = 15) were taken from the animals originated from several farms. The swine were processed in three different slaughterhouses with different sanitary and pre-slaughtering conditions. The cattle were processed in one slaughterhouse. Broilers were processed in our private processing plant. For the microbiological analysis, sampling of pork and beef was carried out with sterile cotton swabs (area 25 cm^2) after 24 h post mortem and sampling of poultry was carried out 1 h post mortem (area 25 cm^2).

Total aerobic mesophilic bacterial counts and counts of enterococci

The samples for enumeration of total bacterial counts were cultured on diagnostic Plate count agar (HiMedia, Mumbai, India). Samples were incubated at temperature (30 ± 1) °C for (72 ± 2) h.

Miroslav Kročko, Margita Čanigová, Viera Ducková, Slovak University of Agriculture, Tr. A. Hlinku 2, SK - 949 76 Nitra, Slovakia.

Correspondence author:
Miroslav Kročko, e-mail: mirokrocko@yahoo.com

The samples for enumeration of enterococci counts were cultured on selective diagnostic Slanetz–Bartley agar at temperature (37 ± 1) °C for (48 ± 2) h (Biokar Diagnostic, Pantin, France).

Isolation and species identification of enterococci

Suspect colonies of *Enterococcus* spp. derived from pork and poultry were transferred to a selective medium containing bile, esculin and azide (Biokar Diagnostic) and to blood agar. Samples were incubated at (37 ± 1) °C for (48± 2) h. All isolated species were Gram-positive and hemolytic negative cocci. Based on native microscopical preparation (conformation, motility, cleanness of cultures), negative catalase and positive PYR test (PYRAtest, Lachema, Brno, Czech Republic), the species of *Enterococcus* spp. were then determined by means of EN-COCCUS test (Lachema).

Antimicrobial susceptibility tests

Before resistance tests, the isolates were resuscitated on Plate count agar for 24 h (HiMedia). Inoculum was prepared by suspending of colonies from Plate count agar and the suspension was adjusted to equal a 0.5 McFarland standard according to the recommendations of National Committee for Clinical Laboratory Standards (NCCLS) [7]. Susceptibilities to antimicrobial agents were tested using the disk diffusion method according to the NCCLS requirements, using the following antimicrobial disks: vancomycin (VAN) 30 μg/disk, gentamicin (GEN) 10 μg/disk, erythromycin (ERY) 15 μg/disk, tetracycline (TET) 30 μg/disk, ampicillin (AMP) 10 μg/disk (HiMedia). The isolates were classified as susceptible, intermediate resistant or resistant.

Statistical analysis

Microbiological data were transformed into logarithms of number of colony forming units ($CFU.cm^{-2}$) and were subjected to analysis of variance (ANOVA). Means and standard deviations were calculated, and, when *F*-values were significant at the $P < 0.05$ level, mean differences were significant by the Least Significant Differences (LSD) procedure [8].

RESULTS AND DISCUSSION

Microbiological analysis

The total bacterial counts (TBC) and counts of enterococci, presented by plotting the $\log_{10}$ values of $CFU.cm^{-2}$ in pork, beef and poultry, are shown in Table 1. Pork and poultry had significantly ($P < 0.05$) higher numbers of TBC than beef. It is probably related with different sampling areas of beef, pork and poultry. The differences among TBC in pork from three abattoirs were statistically significant ($P < 0.05$), which could be explained by pre-slaughter factors (stabling conditions, shower, transport distance), scalding and evisceration technique, sanitary and chilling conditions of abattoirs. Also LABADIE [9] presented, that the composition of the meat spoilage flora is greatly influenced by the slaughtering process, storage conditions, such as temperature and type of packaging. On the other hand, HANSSON [10] does not find significant differences in the amount of aerobic microorganisms between pork carcasses from low- and high-capacity slaughterhouses in Sweden.

The recommendations of Slovak government statute No. 281/2003 [11] about placing on the market of fresh meat permits acceptable amount of contamination for pork TBC $< 4 \log CFU.cm^{-2}$ and bacteria of *Enterobacteriaceae* family $< 2 \log CFU.cm^{-2}$. The acceptable amount of contamination for beef TBC $< 3.5 \log CFU.cm^{-2}$ and for bacteria of the *Enterobacteriaceae* family $< 1.5 \log CFU.cm^{-2}$. The counts of other microorganisms including *Enterococcus* spp. are not assessed by statutes. Twelve percent of beef samples and thirty-one percent of pork samples did not conform with the Slovak gov-

Tab. 1. The total bacterial count and count of enterococci in different samples.

	Count [log $CFU.cm^{-2}$]					
	Total bacterial count			*Enterococcus* spp.		
	Pork	Beef	Poultry	Pork	Beef	Poultry
x	3.61	2.59	4.11	1.98	1.16	2.40
min.	2.38	1.78	3.14	0.60	0	1.45
max.	5.86	4.11	5.42	6.47	2.14	4.00
s	0.78	0.63	0.68	1.29	0.52	0.72

x - values are geomeans, *s* - standard deviation.

Tab. 2. The occurrence of enterococci [%] in pork and poultry.

	Count of *Enterococcus* spp. [%]					
	E. faecium	*E. faecalis*	*E. casseliflavus*	*E. mundtti*	*E. durans*	*Enterococcus* spp.
Pork	72	10	–	8	–	10
Poultry	39	23	15	–	4	19

ernment statutes. In comparison, MAYR et al. [12] found higher counts of aerobic mesophilic bacteria on the beef surface (5.13 log CFU.g^{-1}) and on the pork surface (4.74 log CFU.g^{-1}). Furthermore, they found higher counts of enterococci in both beef and pork (3.81 log CFU.g^{-1}, 3.29 log CFU.g^{-1} respectively). KNUDTSON AND HARTMAN [13] reported that pig carcasses from three different slaughtering plants contained mean counts of 10^4–10^8 enterococci per 100 cm^2 of carcass surface.

The average of the total bacterial counts in poultry was 4.11 log CFU.cm^{-2}. The enterococci constituted 2.17% of TBC. Comparable results of TBC (4.98 log CFU.cm^{-2}) presented VAIDYA et. al [14], however they found a higher percentage of enterococci (24.21%).

Among the 75 enterococci isolates, the following species were identified: *Enterococcus faecium* (60.53%), *E. faecalis* (14.47%), *E. casseliflavus*, *E. mundtti* (both 5.26%), *E. durans* (1.31%) and 13.16% *Enterococcus* spp. All isolated species grew in the 6.5% NaCl broth, at pH 9.6 and bile-esculin azide agar. All species produced pyrrolidonyl arylamidase. *E. faecium* was the predominant species recovered from pork (72%) and poultry (39%), followed by *E. faecalis* (10% pork and 23% poultry), *E. casseliflavus* (15% poultry). Other enterococcal isolates were obtained sporadically (Table 2). In study of KNUDTSON AND HARTMAN [13]. *E. faecium* and *E. faecalis* were the predominant species isolated from pork in three different slaughterhouses. FRANZ et. al. [15] found that *E. faecalis* predominated in the Gram-positive cocci isolated from chicken samples collected at poultry abattoirs. On the contrary, the predominant species isolated from poultry abdomen in our results was *E. faecium*.

Antimicrobial susceptibility

Resistance to antibiotics of isolated enterococci performed by agar disc diffusion method is shown in Fig. 1 (pork) and Fig. 2 (poultry). Isolates from both pork and poultry showed resistance to two or more antibiotics. Of 75 isolates of enterococci, 15% were resistant to vancomycin and 15% were resistant to erythromycin, 27% to ampicillin, 25% to gentamicin and 56% to tetracycline. Intermediate resistance to ampicillin (60%), gentamicin (57%), erythromycin (48%) and tetracycline (56%) were more prevalent. *E. faecium* was the main species of 12% vancomycin resistant enterococci (VRE) isolated from pork. On the contrary, the main species of 20% VRE isolated from poultry was *E. faecalis*.

Isolates from pork resistant to AMP (18%), GEN (24%), TET (34%) and ERY (10%) were de-

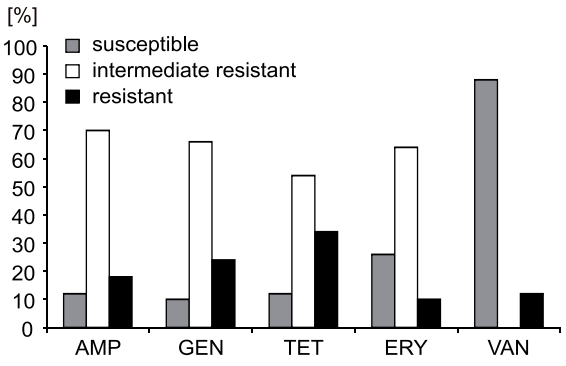

Fig. 1. Antimicrobial resistance profiles of *Enterococcus* species isolated from pork.
AMP - ampicillin, GEN -gentamicin, TET - tetracycline, ERY - erythromycin, VAN - vancomycin.

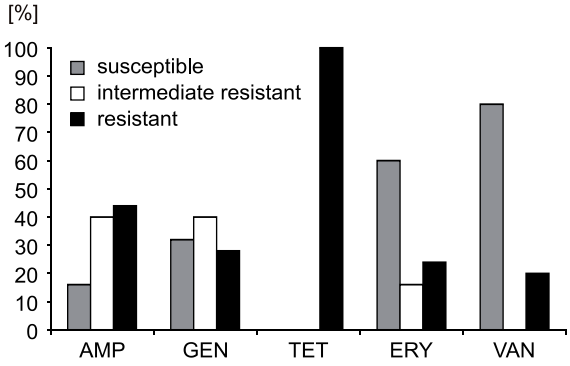

Fig. 2. Antimicrobial resistance profiles of *Enterococcus* species isolated from poultry.
AMP - ampicillin, GEN -gentamicin, TET - tetracycline, ERY - erythromycin, VAN - vancomycin.

tected. Resistance of isolates from poultry to AMP (44%) and TET (100%) were significantly higher ($P < 0.05$) than in pork. The high level of resistance to tetracycline in isolates of pork and poultry is likely related to the wide use of this class of antibiotics in husbandry activities [16]. We found a higher prevalence of intermediate resistant isolates in pork than in poultry. Among the isolates, this type of resistance was detected mainly in *E. faecium*. Significantly higher ($P < 0.05$) prevalence of intermediate resistant isolates from pork to AMP (70%), GEN (66%), TET (54%) and ERY (64%) were detected (Fig. 1). Multi-resistance phenotype TET + ERY + VAN was detected only in the *E. faecalis* isolated from pork and *E. casseliflavus* isolated from poultry.

Resistance to TET and ERY demonstrates a link between the administration of antibiotics to farm animals and occurrence of resistance in bacteria isolated from food of animal origin [17]. Results of recent studies indicate that *Enterococcus* spp. commonly contaminates retail meats and that dissimilarities in antimicrobial resistance patterns among enterococci recovered from different meat types may reflect the use of approved antimicrobial agents in each food animal production class [2, 18]. An interesting point of present study is that 15% of VRE were not resistant to erythromycin. Several investigations have indicated that strong associations exist between the genetic determinants of vancomycin and erythromycin resistance. This is consistent with the observation that erythromycin and vancomycin resistance genes can be localized in the same mobile element [19]. According to MATEU AND MARTIN [20], foodstuffs contaminated with enterococci become - after consumption - source of resistance genes for bacteria present in the intestinal contents of humans.

CONCLUSION

Our study suggests that raw meat plays a potential role as a reservoir of resistance determinants. Finally, with regard to the consequence for the consumer, it must be stressed that the microbiological risk represented by meat preparations can only be dramatically reduced when these products are properly cooked.

Acknowledgments

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