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ANTIBIOTIC RESISTANCE OF *ENTEROBACTERIACEAE* SPECIES ISOLATED FROM PORK MEAT

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ABSTRACT

The aim of this study was monitoring of antibiotic resistance of *Enterobacteriaceae* genera isolated from *musculus longissimus* at *thoracis* and *musculus semimembranosus* of pork. The pigs had been fed in two groups (control group and experimental wit selenium application). For the antibiotic susceptibility testing disk diffusion method was used. *Enterobacteriaceae* genera were tested against four antibiotics: gentamycine, tygecycline, ofloxacin, levofloxacin. For the detection and identification of each species, Chromogenic coliform agar, UriSelect agar, Triple Sugar Iron agar and biochemical test (ENTEROtest 24) were used. The highest resistance was determined in isolates of *Enterobacteriaceae* genera to gentamycin (64.70%) in both groups. From pig's meat following species were isolated: *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and two non-enterobacteriaceae species *Staphylococcus aureus* and *Enterococcus faecium*.

INTRODUCTION

Conventional and non-conventional meat products are now readily available for retail in developed countries, to satisfy consumers' demand for high-quality products that meet the following requirements: guaranteed animal welfare during production; absence of chemical agents during animal feeding; environmental-friendliness, and better taste than conventional products (Dransfield et al., 2005; Miranda et al., 2008). However, little is known about the microbiological status of animal products and the potential microbiological risks linked to meat production. Thus, raising of animals outdoors, use of slow-growing breeds, strict restrictions in the therapeutic use of antimicrobial agents and use of small slaughtering facilities may not guarantee strict microbiological control of animals destined for human consumption (Dransfield et al., 2005; Soonthornchaikul et al., 2006).

The *Enterobacteriaceae* family is commonly used as an indicator of faecal contamination during food microbiological analyses, and includes important zoonotic bacteria such as *Salmonella*

spp., *Yersinia* spp. and *Escherichia coli*. *Enterobacteriaceae* are the significant causes of serious infection, and many of the most important members of this family are becoming increasingly resistant to currently available antimicrobials (Paterson, 2006). This is an important phenomenon that requires vigilance and finds measures to control the further spread of resistance by pathogens included in this family.

The objective of our study was to determine the antibiotic resistance in ubiquitous bacterial cenose isolated from *musculus longissimus at thoracis* and *musculus semimembranosus* of pork, as well as to determine species spectrum of ubiquitous bacteria from meat of pigs.

MATERIAL AND METHODS

Antibiotic resistance study was done in *Enterobacteriaceae* genera isolated from *musculus longissimus at thoracis* MLT and *musculus semimembranosus* MSM of pigs from conventional breeding from Slovakia. 30 animals were obtained from pigs breeding farm (15 animals - control group and 15 animals from group with selenium application). In our study, we identified strains which were resistant and susceptible to antibiotics. Bacterial strains were isolated from meat and collected with a kit containing the swab (Copan Inovation, Brescia) and transported in medium to laboratory Department of Microbiology, Faculty of Biotechnology and Food Science in Slovak University of Agriculture in Nitra. Samples were suspended in physiological solution. For cultivation of *Enterobacteriaceae* genera MacConkey agar (Biomark, Pune) was used. After incubation on the MacConkey agar, we used Chromogenic coliform agar (Biomark, Pune), UriSelect agar (Biomark, Pune) and Triple Sugar Iron agar (Biomark, Pune) and we choose the method of linear insulating. Incubation was conducted for 24 hours at 37°C. We repeated this step until we obtained completely cleaned culture of strains from *Enterobacteriaceae* genera. The pure inoculum of strains of *Enterobacteriaceae* genera was prepared by suspending of colonies from the agar plates and suspension was adjusted to equal a 0.5 McFarland standard. The sensitivity of all strains of *Enterobacteriaceae* genera was tested against: gentamycin (CN 30) 30 µg.disc⁻¹, tygecycline (TGC 15) 15 µg.disc⁻¹, ofloxacin (OFX 5) 5 µg.disc⁻¹ and levofloxacin (LEV 5) 5 µg.disc⁻¹. We used disc diffusion methods according EUCAST (European committee on antimicrobial susceptibility testing). The incubation of strains was done at the temperature 37 °C. The interpretation of inhibition zones around the disc was according to EUCAST. The inhibition zones were controlled with the reference *Escherichia coli* ATCC 25922. Initial identification of strains of *Enterobacteriaceae* genera were done on the Iso Sensitive agar (Biolife, Italiana). Biochemical identification of strains of *Enterobacteriaceae* genera was done by ENTEROtest 24 (Pliva, Lachema). Evaluation of biochemical tests was done in identifying computer program TNW Lite 7.0 software (Pliva, Lachema).

RESULTS AND DISCUSSION

European consumers generally have positive attitude to pork production that may or may not (Ngapo et al., 2003) be linked to higher consumption of pork. Pork is seen as suitable for different dishes although not a meat for special occasions (Bryhni et al., 2002) and may be perceived by some as being relatively fatty and unhealthy compared to beef and poultry (Verbeke et al., 1999).

Enterobacteriaceae genera are very important group of bacteria for clinical microbiology, because this group includes facultative and obligate pathogens. Many scientists consider *Enterobacteriaceae* genera being a reservoir of resistant genes and frequent contaminant of food and water. Finally, resistant bacteria can be transferred to animal and human intestinal tract where bacteria can receive resistant genes. In our experiment in control group 17 species of bacteria and in experimental group 34 species were isolated. Percentages of *Enterobacteriaceae* species of pork meat showed table 1. The higher resistance of isolated bacteria were found on gentamycin (64.70%) in control and experimental group, too. The lower resistance of isolated bacteria were found on levofloxacin (8.82%) in experimental group. The higher sensitivity of isolated bacteria were found on levofloxacin in control group (35.29%) and in experimental group (58.82%). The most resistance bacteria of *Enterobacteriaceae* genera was *Escherichia coli*.

Escherichia coli is the main representative of the *Enterobacteriaceae* genera. The high values of resistance to ampicillin (100 %) of all tested strains of *E. coli* O 157 Solomakos et al. (2009) reported. Farzana et al. (2009) reported the similar results, but samples were isolated from Indian milk and products there of. They determined 100 % resistance to ampicillin in *Escherichia coli* mainly. Also Farzana et al. [29] determined high values of resistance to chloramphenicol in more bacterial strains like *Klebsiella* (more than 60 %), *Enterobacter* (more than 50 %), but mainly in *E. coli* (100 %).

Table 1 Percentages of *Enterobacteriaceae* isolated from pork meat susceptible (S), intermediate (I) and resistant (R) to antimicrobial agents by disk diffusion methods

Antimicrobial agent (μg)	Control group			Experimental group		
	S	I	R	S	I	R
gentamycin	17.65	17.65	64.70	23.53	11.76	64.70
tygecycline	17.65	41.18	41.18	32.35	29.41	38.24
ofloxacin	23.53	29.41	47.06	41.18	29.41	29.41
levofloxacin	35.29	23.53	41.18	58.82	32.35	8.82

From MLT and MSM pork meat *Enterobacteriaceae* species *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and non-enterobacteriaceae species *Staphylococcus aureus*, *Enterococcus faecalis* were isolated (tab. 2). The higher isolated species from *Enterobacteriaceae*

genera was *Escherichia coli* in control group with 70.59 % and in experimental group with 73.53 %.

During recent years, several studies have reported the antimicrobial resistance of some *Enterobacteriaceae* genera isolated from poultry, such as *Escherichia* and *Salmonella* (Antunes et al., 2003; Cornican et al., 2001; Guerra et al., 2003; Kijima-Tanaka et al., 2003; Sáenz et al., 2001; Van den Bogaard et al., 2001). The antimicrobial resistance rates obtained in our study are in agreement with those described by these authors, and also provide evidence supporting the fact that antimicrobial resistance of *Enterobacteriaceae* isolates can change.

Table 2 Genera distribution of *Enterobacteriaceae* and non-enterobacteriaceae genera isolated from pork meat

Microorganisms	Control group %	Experimental group%
<i>Escherichia coli</i>	70.59	73.53
<i>Klebsiella pneumoniae</i>	23.53	11.76
<i>Proteus mirabilis</i>	0.00	2.94
<i>Staphylococcus aureus</i>	0.00	2.94
<i>Enterococcus faecalis</i>	5.88	8.82

Quantitatively, the detection rates and the bacterial cell counts of *E. coli* and *S. aureus* among the symplex were decreased along the processing. In *Enterococcus* spp., on the other hand, the bacterial cell counts were not changed along the processing chain while the detection rates were decreased as other bacterial spp. Generally, *E. coli* strains were highly resistant against beta-lactams and tetracycline. *S. aureus* strains were more resistant to penicillins, tetracycline and erythromycin than to other antibiotics and *Enterococcus* spp. strains were more resistant to tetracycline, erythromycin and ciprofloxacin than to others. The detection rates of antimicrobial resistant *E. coli* and *S. aureus* were decreased while that of *Enterococcus* spp. was not changed along the processing. On the other hand, the multidrug resistance rates of the *E. coli* were not changed while those of *S. aureus* and *Enterococcus* spp. Were decreased along the processing (Moon et al., 2011).

CONCLUSION

Generally, the contamination of pork meat by pathogenic bacteria was tending to be decreased quantitatively. The antimicrobial resistance showed diverse patterns depending on the bacteria. This study provides scientific database to control pathogenic and antimicrobial resistant bacteria in pork meat processing chain from slaughterhouses to retail shops.

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References

1. ANTUNES, P., RÉU, C., SOUSA, J.C., PEIXE, L., PESTANA, N. 2003. Incidence of *Salmonella* from poultry products and their susceptibility to antimicrobial agents. In *International Journal of Food Microbiology*, vol. 82, 2003, p. 97-103.
2. BRYHNI, E. A., BYRNE, D. V., RØDBOTTEN, M., CLAUDI-MAGNUSSEN, C., AGERHEM, H., JOHANSSON, M., LEA, P., MARTENS, M. 2002. Consumer perceptions of pork in Denmark, Norway and Sweden. In *Food Quality and Preference*, vol. 13, 2002, p. 257-266.
3. CORNICAN, M., BUCKLEY, V., CORBETT-FEENEY, G., SHERIDAN, F. 2001. Antimicrobial resistance in *Escherichia coli* isolates from turkey and hens in Ireland. In *Journal of Antimicrobial Chemotherapy*, vol. 48, 2001, p. 587-588.
4. DRANSFIELD, E., NGAPO, T.M., NIELSEN, N.A., BREDAHL, L., SJÖDÉN, P.O., MAGNUSSON, M. 2005. Consumer choice and suggested price for pork as influenced by its appearance, taste and information concerning country of origin and organic pig production. In *Meat Science*, vol. 69, 2005, p. 61-70.
5. EUCAST., Antimicrobial susceptibility testing – EUCAST disk diffusion method. Version 1.0, december 18, 2009. European Society of Clinical Microbiology and Infectious Diseases.
6. FARZANA, K., AKHTAR, S., JABEEN, F. 2009. Prevalence and antibiotic resistance of bacteria in two ethnic milk based products. In *Pakistan Journal of Botany*, vol. 41, 2009, no. 2, p. 935-943.
7. GUERRA, B., JUNKER, E., SCHOETER, A., MALORNY, B., LEHMANN, S., HELMUTH, R. 2003. Phenotypic and genotypic characterization of antimicrobial resistance in German *Escherichia coli* isolates from cattle, swine and poultry. In *Journal of Antimicrobial Chemotherapy*, vol. 52, 2003, p. 489-492.
8. KIJIMA-TANAKA, M., ISHIHARA, K., MORIOKA, A., KOJIMA, A., OZONO, T., OGIKUBO, K. 2003. A national surveillance of antimicrobial resistance in *Escherichia coli* isolated from food-producing animals in Japan. In *Journal of Antimicrobial Chemotherapy*, vol. 51, 2003, p. 447-451.
9. MIRANDA, J.M., GUARDDON, M., VÁZQUEZ, B.I., FENTE, C.A., BARROS-VELÁZQUEZ, J., CELERA, A., FRANCO, C.M. 2008. Antimicrobial resistance in *Enterobacteriaceae* strains isolated from organic chicken, conventional chicken and

- conventional turkey meat: A comparative survey. In *Food Kontrol*, vol. 19, 2008, no. 4, p. 412-416.
10. MOONA, B.Y., KWONA, K.H., KIMA, K.Y., HONGA, M.K., AHNA, K.J., HWANGA, S.J., PARKA, K.Y., SHINA, S., KOOA, H.CH., PARKA, Y.H. 2011. Changes of antimicrobial resistant and pathogenic bacteria along the pork meat processing Chain. In *Proceedings of the 5th Asian Pig Veterinary Society Congress 7-9 March 2011, Pattaya, Thailand*, p. 119.
 11. NGAPO, T. M., DRANSFIELD, E., MARTIN, J. F., MAGNUSSON, M., BREDAHL, L., NUTE, G. R. 2003. Consumer perceptions: pork and pig production. Insights from France, England, Sweden and Denmark. In *Meat Science*, vol. 66, 2003, p. 125-134.
 12. PATERSON, D.L. 2006. Resistance in Gram-negative bacteria: *Enterobacteriaceae*. In *American Journal of Medicine*, vol. 119, 2006, p. 520-528.
 13. SÁENZ, Y., ZARAZAGA, M., BRIÑAS, L., LANTERO, M., RUIZ-LARREA, F., TORRES, C. 2001. Antibiotic resistance in *Escherichia coli* isolates obtained from animals, foods and humans in Spain. In *International Journal of Antimicrobial Agents*, vol. 18, 2001, p. 353-358.
 14. SOLOMAKOS, N., GOVARIS, A., ANGELIDIS, A. S., POURNARAS, S., BURRIEL, A.R., KRITAS, S. K., PAPAGEORGIOU, D. K. 2009. Occurrence, virulence genes and antibiotic resistance of *Escherichia coli* O157 isolated from raw bovine, caprine and ovine milk in Greece. In *Food Microbiology*, vol. 26, 2009, p. 865-871.
 15. SOONTHORNCHAIKUL, N., GARELICK, H., JONES, H., JACOBS, J., BALL, D., CHOUDHURY, M. 2006. Resistance to three antimicrobial agents of *Campylobacter* isolated from organically- and intensively-reared chickens purchased from retail outlets. In *International Journal of Antimicrobial Agents*, vol. 27, 2006, p. 125-130.
 16. VAN DEN BOGAARD, A.E., LONDON, N., DRIESSEN, C., STOBBERINGH, E.E. 2001. Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers and poultry slaughterers, In *Journal of Antimicrobial Chemotherapy*, vol. 47, 2001, p. 763-771.
 17. VERBEKE, W., VAN OECKEL, M. J., WARNANTS, N., VIAENE, J., BOUCQUE, CH. V. 1999. Consumer perceptions, facts and possibilities to improve acceptability of health and sensory characteristics of pork. In *Meat Science*, vol. 53, 1999, p. 77-99.