Comparison of Antibiotic Resistance Profile between *Salmonella* Spp., *Salmonella Enterica* Ser. Typhimurium *a*nd Enteritidis and *Escherichia Coli* Isolated from Rectal Swabs of Chicken

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Abstract

The aim of this experiment was comparing of antibiotic resistance profile between *Salmonella* spp. and *Escherichia coli* isolated from rectal swabs of chicken from conventional breeding. For the antibiotic susceptibility testing disk diffusion method was used. The both tested bacteria were exposed against thirteen antibiotics: ampicillin, piperacillin, cefotaxime, ceftriaxone, doripenem, meropenem, levofloxacin, ofloxacin, amikacin, gentamycin, tygecycline, tetracycline and chloramphenicol. For the identification of these strains, we used Chromogenic coliform agar, Triple sugar iron agar and biochemical test (ENTEROtest 24). We identified *Salmonella* spp. by used MicroSEQ® *Salmonella* spp. Detection Kit for identification of this strain in Step ONE Real Time PCR. In this study, we determined that *Salmonella* spp. was more resistant like *Escherichia coli*. The highest resistance had isolates of *Salmonella* spp. to levofloxacin (100%) and to ofloxacin (100%). Also to ampicillin was resistance in *Salmonella* spp. isolates about 83%. Only in case of piperacillin was resistance in *Salmonella* spp. isolates lower (50%) like in *Escherichia coli* isolates (66.6%). The both strains were 100 % sensitive to doripenem, meropenem, amikacin, gentamycin and tygecycline. Antibiotic resistance is a biological danger. Bacteria, which we study, are considered to reservoirs of resistant genes and they are facultative and obligate pathogens. If these pathogen bacteria cause diseases those these diseases are difficult to treat.

Keywords: Antibiotic resistance, chicken, *Escherichia coli*, rectal, *Salmonella* spp

1. Introduction

Antibiotic resistance is significant health, social and economic problem at this time. Antibiotic resistance of bacteria is biological risk, which increases morbidity and mortality of animals and humans [1]. Keyser et al. [2] note that in recent years accumulating problems with bacteria that are resistant to antibiotics occur. It is leading them to predictions that we return to the time before the discovery of antibiotics. One of the possibility could be the introducing of different antibacterial preparation, which used Buňková et al. [3, 4] in their experiments. Most technologies in the production and food processing reduced the incidence of pathogens including resistant bacteria to antibiotics. Experimental monitoring confirmed that the treatment of food technology based on damage to cell membranes and enzymes may help to generate and transfer of antibiotic resistance [5-7]. The health safety of foods [8, 9], including meat is an integral part of consumers policy and health [10]. The use of antimicrobial agents in any venue, including therapeutically in human and veterinary medicine, or as prophylaxis for growth promotion in animal husbandry, ultimately exerts selective pressure favorable for the propagation of antimicrobial-resistant bacteria [11]. Resistant bacteria from the intestines of food animals may

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be transferred to retail meat products resulting from fecal contamination during various stages of the slaughter process (e.g. evisceration) and subsequent handling of animal tissue [12]. Endogenous bacterial flora may play an important role as acceptor and donor of transmissible drug resistance genes [13, 14]. *Escherichia coli* is commonly found in the intestinal tract of humans and animals [14, 15] and can also be implicated in human and animal infectious diseases [16]. Animal food products are an important and frequent source of *E. coli* as fecal contamination of carcasses at the slaughterhouse. These microorganisms and their possible resistance determinants may be transmitted to humans if these foods are improperly cooked or otherwise mishandled. The level of antibiotic resistance in *E. coli* represents a useful indicator of the resistance dissemination in bacterial populations. There are some reports in which antibiotic susceptibility of *E. coli* isolates from healthy humans [17-19] or animals [20-23,14] have been studied, but in few cases comparative results have been shown [14,24] or isolates from foods analyzed. *Salmonella* spp. that includes more than 2500 different serotypes represents a leading cause of foodborne infections worldwide [24-26]. Nearly 1.4 million cases of salmonellosis occur each year in the United States, of which 95% are foodborne cases [27]. A variety of foods have been implicated as vehicles transmitting salmonellosis to humans, including poultry, beef, pork, eggs, milk, cheese, fish, shellfish, fresh fruits and juice, and vegetables [28]. *Salmonella* gastroenteritis is generally self-limiting illness, but severe cases in immuno-compromised individuals, elderly persons or neonates, and systemic infections may require effective chemotherapy [29]. Currently the increasing prevalence of multidrug resistance among salmonella and resistance to the clinically important antimicrobial agents such as fluoroquinolones and third-generation of cephalosporins has also been an emerging problem in China and other countries [30-32]. One of the ways to speed up the process of detection is polymerase chain reaction (PCR). PCR technique is assumed to have the potential sensitivity and specificity [33-38] required to achieve the necessary detection limits for bacterial pathogens in food. PCR methods suitable for identification of *Salmonella* have been reported using a variety of primers [39-43]. The aim of this study was to determine and compare antibiotic resistance of *Escherichia coli* and *Salmonella* spp., *Salmonella enterica* ser. typhimurium and enteritidis (*Salmonella* spp.) isolated from rectal swabs of chicken from conventional breeding in Slovakia.

2. Materials and methods

Collection of the samples and isolation of *Salmonella* spp. and *Escherichia coli*

The samples were obtained from rectal swabs of chicken from one conventional farm in Slovakia. From this conventional chicken-farm it was obtained twelve samples of rectal swabs of chicken. The samples were collected by sterile cotton swabs (Copan Inovation, Brescia) and transported to the laboratory (SUA in Nitra, Department of Microbiology). *Escherichia coli* and *Salmonella* spp. isolations were performed by a conventional plating method. The first step was done on the MacConkey agar (Biomark, Pune). Incubation was performing for 24 hours at 37°C. After incubation on the MacConkey agar, we used Chromogenic coliform agar (Biolife, Italiana), XLD agar (Biolife, Italiana) and SS agar (MkB test, Rosina) and we chose the streak plate (four-ways) method for obtaining the pure colonies. Incubation was conducted for 24 hours at 37°C. This step was repeating until we had completely cleaned culture of *Escherichia coli* and *Salmonella* spp. After the incubation and identification it was isolated six pure colonies of *Salmonella* spp. and six pure colonies of *Escherichia coli*.

The biochemical identification of *Escherichia coli* and *Salmonella* spp.

Method on the Triple sugar iron agar (Biolife, Italiana) for the basic biochemical identification of *Escherichia coli* and *Salmonella* spp. and ENTEROtest 24 (Pliva-Lachema, Brno), including TNW Lite 7.0 identification software (Pliva-Lachema, Brno) for more detailed biochemical identification was used. Preparation of indentification plates of ENTEROtest 24 was done inside the Laminaire box (ADS Laminaire, Le Pre-Saint Gervais) to ensure the high sterility, less risk of contaminations from air and for precise results. Working procedure of ENTEROtest 24 is described in the competent manual.

The isolation of DNA from *Escherichia coli* and *Salmonella* spp

The pure colonies of *Escherichia coli* and *Salmonella* spp. were subjected to DNA isolation using PrepSEQ™ Rapid Spin Sample Preparation Kit (Applied Biosystem, USA). Complete working procedure is described in the kit manual.

General Sample Preparation Protocol

Sample of 750 µL was loaded onto the spin column and microcentrifuged for 3 minutes at maximum speed (12000 rpm). Supernatant was discarded and 50 µL of Lysis Buffer was added to the pellet. Samples were incubated for 10 minutes at 95°C. The samples after incubation were added to cool for 2 min at room temperature. Then were added 250 µl of water to samples. After the samples were centrifuged one minute at maximum speed (12000 rpm).

Identification of *Escherichia coli* and *Salmonella* spp. by Real time PCR

Step ONE® Real time PCR (Applied Biosystem, USA) for a genetic confirmation of belonging to the genus *Salmonella* spp. MicroSEQ® *Salmonella* spp. Detection Kit (Applied Biosystem, USA) was used for the actual PCR reaction. Complete information is described in the kit manual. PCR reaction in Step ONE® Real time PCR for a genetic identification of *Escherichia coli* was used. In PCR reaction specific primer was used, which was designed by Shu-Chen Hsu and Hau-Yang Tsen [44]. Also, their PCR protocol was followed.

Primers:

Emdh1: 5´- ACTGAAAGGCAAACAGCCAAG - 3´ (1123–1144),

Emdh2: 5´- CGTTCTGTTCAAATGGCCTCAGG - 3´ (1514–1492).

Molecular weight of the expected PCR product is 392 bp. The correct lenght of PCR product was evaluated by electrophoresis gel and it was visualized.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done by disk diffusion method (according EUCAST [45] – European committee on antimicrobial susceptibility testing). Antibiotic disks were used (Oxoid, England). The pure inoculum of strain of *Escherichia coli* and *Salmonella* spp. were prepared by suspending of colonies from the agar plates in physiological solution and the suspension was adjusted to equal a 0.5 McFarland standard. We streaked 100 µl suspensions to plates surface and we spreaded over surface of agar thoroughly. Antimicrobial susceptibility testing was performed according to the manufacturer's instructions. The following antimicrobials were tested: ampicillin (AMP 10) 10 µg.disk^{-1}, piperacillin (PRL 30) 30 µg.disk^{-1}, cefotaxime (CTX 5) 5 µg.disk^{-1}, ceftriaxone (CRO 30) 30 µg.disk^{-1}, doripenem (DOR 10) 10 µg.disk^{-1}, meropenem (MEM 10) 10 µg.disk^{-1}, levofloxacin (LEV 5) 5 µg.disk^{-1}, ofloxacin (OFX 5) 5 µg.disk^{-1}, amikacin (AK 30) 30 µg.disk^{-1}, gentamycin (CN 10) 10 µg.disk^{-1}, tygecycline (TGC 15) 15 µg.disk^{-1}, tetracycline (TE 30) 30 µg.disk^{-1} and chloramphenicol (C 30 30 µg.disk^{-1}. The incubation of strains was performing for 24 hours at the temperature 37 °C. The interpretation of inhibition zones around the disk was done according to EUCAST [45]. The inhibition zones were controlled with the reference of *Escherichia coli* ATCC 25922.

3. Results and discussion

We studied antibiotic resistance in strains of *Enterobacteriaceae* genera and in *Salmonella* spp., which are considered to be potential reservoirs for resistant genes in animal farm. Farm reservoirs of resistant bacteria provide potential sources for resistant genes transfer between bacteria as well as an environment for dissemination to new animals, environment and food products. Finally, pathogenic bacteria can get into the human body and cause diseases, which is difficult to treat. Therefore, identifying these reservoirs and mechanisms of persistence could be a key to reducing the load of resistant bacteria everywhere.

Antibiotic resistance profile of studied strains

In our study was studied antibiotic resistance profile and comparison between *Escherichia coli* and *Salmonella* spp. isolated from rectal swabs of chicken from conventional farm from Slovakia. We determined that antibiotic resistance profile in *Salmonella* spp. was a higher like in *Escherichia coli*. We found resistant cases in *Escherichia coli* isolates and in *Salmonella* spp. too. The highest resistance was found in *Salmonella* spp. isolates to levofloxacin (100 %) and to ofloxacin (100 %). Also, the higher resistance was determined in *Salmonella* spp. isolates to ampicillin (83.3 %). The higher resistance was found in *Salmonella* spp. isolates to chloramphenicol (66.6 %) and to tetracycline (66.6 %). In other cases of antibiotics

was antibiotic resistance similar in both studied strains. Similarly, in the isolates of *Salmonella* spp. and *Escherichia coli* was found 100% susceptibility to doripenem, meropenem, amikacin, gentamycin and tygecycline. Complete results with the size of inhibition zones are shown in the table 1. Also Miranda et al. in 2008 [46] determined high resistance in *Enterobacteriaceae* genera to ampicillin (48.3%). However, Miranda et al. [46] found resistant *Enterobacteriaceae* genera to gentamycin and to chloramphenicol only 6.7%.

During recent years, several studies have reported the antimicrobial resistance of some *Enterobacteriaceae* genera isolated from poultry, such as *Escherichia* and *Salmonella* [47-52]. The several researches like Lira *et al*. [53], Picozzi *et al*. [54], Caro *et al*. [55] and Čížek *et al*. [56], who researched antibiotic resistance in *E. coli* or *Salmonella* spp., respectively in *Enterobacteriaceae* genera isolated from different products have argued, that results of antibiotic resistance vary from study to study.

Table 1 Comparison antibiotic resistance between *Escherichia coli* and *Salmonella* spp. and sizes of the inhibition zones around the discs

	Escherichia coli							*Salmonella* spp.						
ATB / samples	**37**	**38**	**39**	**40**	**41a**	**42a**	**R %**	**1**	**2**	**3**	**4**	**5**	**6**	**R %**
AMP10	R/7	S/18	S/22	R/7	R/7	R/7	**66,6**	S/15	R/7	R/14	R/7	R/7	R/7	**83.3**
PRL30	R/7	S/24	S/26	R/10	R/7	R/9	**66,6**	S/21	I/16	S/20	R/13	R/14	R/12,5	**50.0**
CTX5	R/10	S/29	S/30,5	R/8,5	R/7	R/10	**66,6**	S/24	R/13	S/24	R/11	R/10	R/10	**66.6**
CRO30	R/15	S/33	S/32	R/14	R/11	R/13	**66,6**	S/27	R/19	S/24	R/18	R/18	R/17	**66.6**
DOR10	S/31	S/32	S/31	S/26	S/32	S/33	**0**	S/27	S/27,5	S/27	S/28	S/27	S/29	**0.0**
MEM10	S/30	S/32	S/30	S/33	S/28	S/30	**0**	S/30	S/30	S/27,5	S/27	S/29	S/30	**0.0**
LEV5	I/21	I/21	R/18	R/10	R/10,5	R/11	**66,6**	R/7	R/8,5	R/8,5	R/9	R/8	R/8	**100**
OFX5	S/18	I/19	R/16	R/7	R/8	R/7,5	**66,6**	R/8	R/8	R/7	R/7	R/8	R/8	**100**
AK30	S/22	S/22	S/23	S/22	S/20,5	S/20	**0**	S/23	S/19	S/21	S/19	S/20	S/21	**0.0**
CN10	S/24	S/27	S/23	S/21	S/20	S/20,5	**0**	S/17	I/15	I/15	I/15	I/16	S/17	**0.0**
TGC15	S/25	S/25	S/24	S/22	S/23	S/24,5	**0**	S/22	S/21,5	S/21	S/21	S/22	S/23	**0.0**
C30	S/27	S/29	S/25	R/7	R/7	R/7	**50**	S/19	R/7	S/17	R/7	R/9	R/7	**66.6**
TE30	S/27	S/27	S/26	R/7	R/7	R/7	**50**	S/25	R/7	S/23,5	R/7	R/7	R/7	**66.6**

Legend: R-resistance, S-susceptibility, I-intermediate, ATB-antibiotics

Identification of *Salmonella* spp.

For the complete identification of *Salmonella* spp. we used several methods of identification. Relevant identification agar (Triple sugar iron agar, XLD) showed that *Salmonella* spp. was present in samples. XLD agar turned black because of the presence of H_2S. With Triple sugar iron agar we detected the presence of *Salmonella* spp. as well. Also with use of a biochemical test ENTEROtest 24 we determined the presence of *Salmonella* spp. and TNW 7.0 Lite software was used to calculate that the identification was conducted on 100%. The same test for identification of *Enterobacteriaceae* genera Kmeť et al. [57, 58] used. Similar test for identification of *Salmonella* spp. (ENTEROtest 16) Špánová et al. [59] recorded. The most sensitive detection of *Salmonella* spp. was obtained using PrepSEQ™ Rapid Spin Sample Preparation Kit and MicroSEQ® Salmonella spp. Detection Kit compatible with StepOne™ Systems was less time-consuming than the other methods and was relatively easy to use. Thus, the PCR-based detection of bacteria depends on the efficiency of the DNA extraction procedure used to prepare the template DNA. In the investigated samples with incubation we could detect strain of *Salmonella* spp. in six out of twenty samples, as well as the internal positive control (IPC), which was positive in all samples (Figure 1).

Identification of *Escherichia coli*

For the complete identification of *Escherichia coli* we used several methods of identification. Relevant identification agar (Chromogenic coliform agar, Triple sugar iron agar and XLD) showed that *Escherichia coli* was present in samples. On the Chromogenic coliform agar *Escherichia coli* made blue colonies. On the XLD agar made *E. coli* yellow colonies. With triple sugar iron agar we detected the presence of *E. coli* as well. Also with use of a biochemical test ENTEROtest 24 we determined the presence of *E. coli* and TNW 7.0 Lite software was used to

calculate that the identification was conducted on 100%. Also, we used PCR method for detection of *E. coli*. PCR method showed that E. coli was present in samples (figure 2).

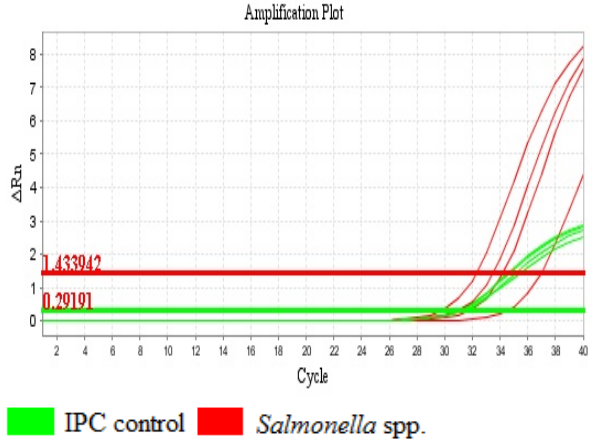

Figure 1. Process of Real Time PCR for *Salmonella*

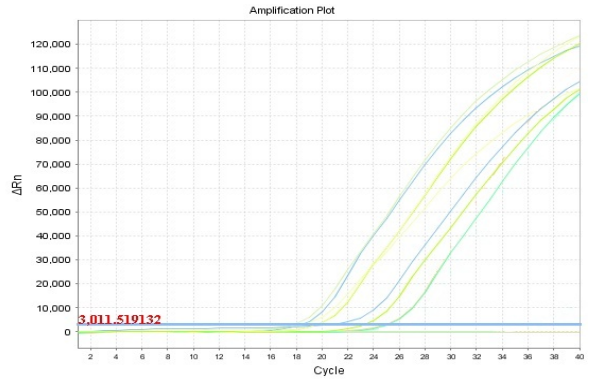

Figure 2. Process of Real time PCR for *E. coli*

For determination of primer products size, we used agarose electrophoresis and we visualised gel (figure 3).

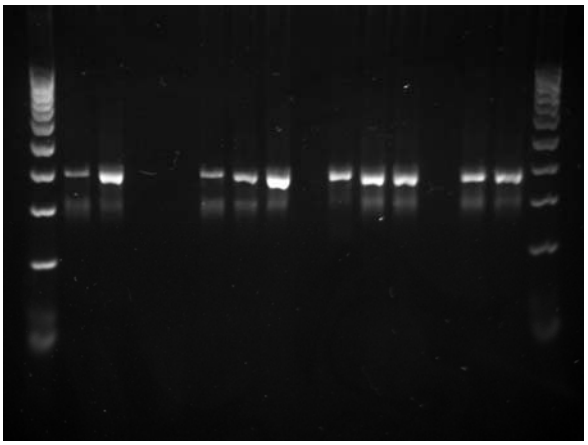

Figure 3. Visualisation of primer products from PCR

4. Conclusions

Using of antibiotics in livestock farming cause that more and more obligatory and facultative pathogens are resistant to various antibiotics used commercially. Our experiment results show that antibiotics used in this breeding or rearing were introduced into the external environment. Results confirm that antibiotic resistance was higher in *Salmonella* spp. against *Escherichia coli*. It is very important in commercial breeding to observe of sanitation and hygiene conditions. Meats and eggs are end products, which are also used in human food chain. If coliforms bacteria including *Salmonella* spp. and *Escherichia coli* are resistant to undesirable reproducing it may cause consumers infections and diseases, which are then difficult to treat. For diseases caused by resistant bacteria are antibiotic unnecessary and useless. Therefore, the monitoring of resistant bacteria is needed to reduce or eradicate this global problem.

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References

1. EFSA, Foodborne antimicrobial resistance as a biological hazard, Draft Scientific Opinion of the Panel on Biological Hazards (Question No EFSA– Q-2007-089), Draft endorsed on 6 March, 2008.
2. Keyser, P., Elofson, M., Rosell, S., Wolf-Watz, H., Virulence blockers as alternatives to antibiotics: type III secretion inhibitors against Gram-negative bacteria, J. of Inter. Med. 264, 2008, pp. 17-29.
3. Buňková, L., Buňka, F., Doležálková, I., Kráčmar, S., Antibacterial effect of monocaprin, undecanoylglycerol and undecenoylglycerol, in: Proceding of the work of the International Scientific Conference of Food Safety and control in Nitra: SUA, 2009, pp. 45-48.
4. Bunková, L., Pleva, P., Buňka, F., Valášek, P., Kračmár, S., Antibacterial effect of commercially available phosphates on selected microorganisms, Acta univ. agric. et silvic. Mendel. Brun. 2008, 56, pp.1-6
5. Lado, B., Yousef, A., Alternative foodpreservation technologies: efficacy a mechanisms, Microb. Infect. 2002, 4, 433-440
6. Kharazmi, Y., Hammes, W.P., Hertell, C., Construction of a marker rescue system in Bacillus subtilis for detection of horizontal gene transfer in food, Syst. Appl. Microbiol., 2002, 25, 471-477

7. McMahon, M. A. S., Blair, I. S., Moore, J. E., Mc Dowell, D. E., The rate of horizontal transmission of antibiotic resistence plasmids is increased in food preservation-stressed bacteria, J. Appl. Microbiol. 2007, 10, 1883-1888
8. Mareček, J., Fikselová, M., Frančáková, H., Nutritional and technological value of selected edible potatoes during storage. Zeszyty problemowe postepow nauk rolniczych. Warszawa: Polska akademia nauk, 2008, 530,293-299
9. Fikselová, M., Šilhár, S., Mareček, J., Frančáková, H., Extraction of carrot (*Daucus carota* L.) carotenes inder different conditions. Czech J. Food Sci., 26, 2008, 4, 268-274
10. Bíreš, J., Current legislation in the field of milk hygiene. The Dairy, 2004, 35, 1, 33-35
11. Witte, W., Medical consequences of antibiotic use in agriculture. Science, 1998, 279, 996-997
12. Jackson, T. C., Marshall, D. L., Acuff, G. R., Dickson, J. S., Meat, poultry, and seafood. In Doyle, M.P., Beuchat, L.R., Montville, T.J. (Eds.), Food Microbiology: Fundamentals and Frontiers, 2nd ed. ASM Press, Washington, DC, p. 91-109.
13. Davies, J., Inactivation of antibiotics and the dissemination of resistance genes. Science, 1994, 264, 375-382
14. Sunde, M., Fossum, K., Solberg, A., Sørum, H., Antibiotic resistance in *Escherichia coli* of the normal intestinal flora of swine. Microbial Drug Resistance, 1998, 4, 289-299
15. Tannock, G. W., Normal Microflora. An Introduction to Microbes Inhabiting the Human Body, London: Chapman and Hall, 1995, pp. 37-47.
16. Threlfall, E. J., Cheasty, T., Graham, A., Rowe, B., Antibiotic resistance in *Escherichia coli* isolated from blood and cerebrospinal fluid: a 6-year study of isolates from patients in England and Wales. International Journal of Antimicrobial Agents, 1998, 9,201-205
17. Bongers, J. H., Franssen, F., Elbers, A. R. W., Tielen, M. J. M., Antimicrobial resistance of *Escherichia coli* isolates from the faecal flora of veterinarians with different professional specialites. Veterinary Quarterly, 1995, 17, 146-149
18. London, N., Nijsten, R., Van Den Bogaard, A., Stobberingh, E., Carriage of antibiotic-resistant *Escherichia coli* healthy volunteers during a 15-week period. Infection, 1994, 22, 187-192
19. Nijsten, R., London, N., Van Den Bogaard, A., Stobberingh, E., Antibiotic resistance among *Escherichia coli* isolated from faecal samples of pig farmers and pigs. Journal Antimicrobial Chemotherapy, 1996, 37, 1131-1140
20. Adesiyun, A. A., Campbell, M., Kaminjolo, J. S., Prevalence of bacterial enteropathogens in pet dogs in Trinidad. Journal of Veterinary Medicine, 1997, B44, 19-27
21. Blanco, J. E., Blanco, M., Mora, A., Blanco, J., Prevalence of bacterial resistance to quinolones and other antimicrobials among avian *Escherichia coli* strains isolated from septicemic and healthy chickens in Spain. Clinical microbiology, 1997, 35, 2184-2185
22. Mathew, A. G., Saxton, A. M., Upchurch, W. G., Chattin, S. E. Multiple antibiotic resistance patterns of *Escherichia coli* isolated from swine farms. Applied and Environmental Microbiology, 1999, 65, 2270-2272
23. Nijsten, R., London, N., Van Den Bogaard, A., Stobberingh, E., Antibiotic resistance of *Enterobacteriaceae* isolated from the faecal flora of fattening pigs. Veterinary Quarterly, 1993, 15, 152-156
24. Van Den Bogaard, A. E., Antimicrobial resistance. Relation to human and animal exposure to antibiotics. Journal of Antimicrobial Chemotherapy, 1997, 40, 453-461
25. Chen, S., Zhao, S.H., White, D.G., Schroeder, C.M., Lu, R., Yang, H.C., McDermott, P.F., Ayers, S., Meng, J.H., Characterization of multiple-antimicrobial-resistant Salmonella serovars isolated from retail meats, Appl. Environ. Microbiol., 2004, 70, 1-7
26. Magistrali, C., Dionisi, A.M., Curtis, P.D., Cucco, L., Vischi, O., Scuota, S., Zicavo, A., Pezzotti, G., Contamination of *Salmonella* spp. in a pig finishing herd, from the arrival of the animals to the slaughterhouse, Res. Vet. Sci., 2008, 85, 204-207
27. White, D. G., Zhao, S. H., Simjee, S., Wagner, D. D., McDermott, PF., Antimicrobial resistance of foodborne pathogens, Microb. Infect., 2002, 4, 405-412
28. Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., Griffin, P. M., Tauxe, R. V., Food-related illnessand death in the United States, Emerg. Infect. Dis., 1999, 5, 607-625
29. Gomez, T. M., Motarjemi, Y., Miyagawa, S., Kaferstein, F. K., Stohr, K., Foodborne salmonellosis, World Health Stat., 1997, 50, 81-89
30. Lee, L. A., Puhr, N. D., Maloney, E. K., Bean, N. H., Tauxe, R. V., Increase in antimicrobial-resistant Salmonella infections in the United States, 1989–1990, J. Infect. Dis., 1994, 170, 128-134
31. Brands, D. A., Inman, A. E., Gerba, C. P., Maré, C. J., Billington, S. J., Saif, L. A., Levine, J. F., Joens, L. A., Prevalence of *Salmonella* spp. in oysters in the United States. Appl. Environ. Microb., 2005, 71, 893-897
32. Chao, G. X., Zhou, X. H., Jiao, X. N., Qian, X. Q., Xu, L., Prevalence and antimicrobial resistance of foodborne pathogens isolated from food products in China, Foodborne Pathog. Dis., 2007, 4, 277-284
33. Gabor, M., Trakovicka, A., Miluchova, M., Analysis of polymorphism of CAST gene and CLPG gene in sheep by PCR-RFLP Metod. Lucřari stiintifice Zootehnie si Biotehnologii, 2009, 42, 470-476
34. Gabor, M., Trakovicka, A., Miluchova, M., Polymorphism of stearoyl-coenzyme A desaturase gene

in Slovak Pinzgau cattle. Lucřari stiintifice Zootehnie si Biotehnologii, 2009, 42, 249-254
35. Miluchova, M., Trakovicka, A., Gabor, M., Analysis of polymorphism of Alpha S1 casein of Slovak Pinzgau cattle by PCR-RFLP. Lucřari stiintifice Zootehnie si Biotehnologii, 2009, 42, 284-287
36. Miluchova, M., Trakovicka, A., Gabor, M., Analysis of polymorphism of beta casein of Slovak Pinzgau cattle by PCR-RFLP for allels A1 and A2. Lucřari stiintifice Zootehnie si Biotehnologii, 2009, 42, 288-292
37. Rafayova, A., Lieskovská, Z., Trakovická, A., Kováčik, A., Detection of MSTN polymorphism in rabbit. Lucřari stiintifice Zootehnie si Biotehnologii, 2009, 42, 637-641
38. Rafayova, A., Trakovická, A., Parkányi, V.,. Kováčik, A., Detection of SRY of newborn rabbits ment for xenoimplantates Lucřari stiintifice Zootehnie si Biotehnologii, 2009, 42,366-369
39. Gebreyes, W.A., Thakur, S., Multidrug-resistant *Salmonella enterica* serovar Muenchen from pigs and humans and potential interserovar transfer of antimicrobial resistance, Antimicrob. Agents Chemother., 2005, 49, 503-511
40. Aabo, S., Anderson, J. K., Olsen, J. E., Detection of *Salmonella* in minced meat by the polymerase chain reaction methods, Lett. Appl. Microbiol., 1995, 21, 180-182
41. Kwang, J., Littledike, E. T., Keen, J. E., Use of the polymerase chain reaction for *Salmonella* detection, Lett. Appl. Microbiol., 1996, 22, 46-51
42. Mahon, J., Murphy, C.K., Jones, P.W., Barrow, P.A., Comparison of multiplex PCR and standard bacteriological methods of detecting *Salmonella* on chicken skin, Lett. Appl. Microbiol., 1994, 19, 169-172
43. Soumet, C., Ermel, G., Salvat, G., Colin, P., Detection of *Salmonella* spp. in food products by polymerase chain reaction and hybridisation assay in microplate format, Lett. Appl. Microbiol.,1997, 24, 113-116
44. Hsu, S. C., Tsen, H. Y., PCR primers designed from malic dehydrogenase gene and their use for detection of *Escherichia coli* in water and milk samples. International Journal of Food Microbioogy, 2001, 64, 1-11
45. Eucast., Antimicrobial susceptibility testing – EUCAST disk diffusion method. Version 1.0, december 18, 2009. European Society of Clinical Microbiology and Infectious Diseases.
46. Miranda, J. M., Guarddon, M., Vázquez, B. I., Fente, C. A., Barros-Velázquez, J., Cepeda, A., Franco, C.M., Antimicrobial resistance in *Enterobacteriaceae* strains isolated from organic chicken, conventional chicken and conventional turkey meat: A comparative survey. Food Control, 2008, 19, 412-416
47. Antunes, P., Re´u, C., Sousa, J. C., Peixe, L., & Pestana, N., Incidence of *Salmonella* from poultry products and their susceptibility to antimicrobial agents. International Journal of Food Microbiology, 2003, 82, 97-103
48. Cornican, M., Buckley, V., Corbett-Feeney, G., & Sheridan, F., Antimicrobial resistance in *Escherichia coli* isolates from turkey and hens in Ireland. Journal of Antimicrobial Chemotherapy, 2001, 48, 587-588
49. Guerra, B., Junker, E., Schoeter, A., Malorny, B., Lehmann, S., & Helmuth, R., Phenotypic and genotypic characterization of antimicrobial resistance in German *Escherichia coli* isolates from cattle, swine and poultry. Journal of Antimicrobial Chemotherapy, 2001, 52, 489-492
50. Kijima-Tanaka, M., Ishihara, K., Morioka, A., Kojima, A., Ozono, T., et al, A national surveillance of antimicrobial resistance in *Escherichia coli* isolated from food-producing animals in Japan. Journal of Antimicrobial Chemotherapy, 2003, 51, 447-451
51. Sa´enz, Y., Zarazaga, M., Brin˜ as, L., Lantero, M., Ruiz-Larrea, F., & Torres, C., Antibiotic resistance in Escherichia coli isolates obtained from animals, foods and humans in Spain. International Journal of Antimicrobial Agents, 2001, 18, 353-358
52. Van den Bogaard, A. E., London, N., Driessen, C., & Stobberingh, E. E., Antibiotic resistance of faecal Escherichia coli in poultry, poultry farmers and poultry slaughterers. Journal of Antimicrobial Chemotherapy, 2001, 47, 763-771
53. Lira, W. M., Macedo, C., Marin, J. M., The incidence of Shiga toxin-producing *Escherichia coli* in cattle with mastitis in Brazil. Journal of Applied Microbiology, 2004, 97, 861-866
54. Picozzi, C., Foschino, R., Heuvelink, A., Beumer, R., Phenotypic and genotypic characterization of sorbitol-negative or slow-fermenting (suspected O157) *Escherichia coli* isolated from milk samples in Lombardy region. Letters in Applied Microbiology, 2004, 40, 491-496
55. Caro, I., Mateo, J., Garci´A-Armesto, M. R., Phenotypical characteristics of Shigalike toxin *Escherichia coli* isolated from sheep dairy products. Letters in Applied Microbiology, 45, 2007, p.295–300.
56. Čížek, A., Dolejská, M., Novotná, R., Haas, D., Vyskočil, M. Survey of Shiga toxigenic *Escherichia coli* O157 and drug-resistant coliform bacteria from in-line milk filters on dairy farms in the Czech Republic. Journal of Applied Microbiology, 2007, 104, 852–860
57. V. Kmeť, E., Piatnicová, Antibiotic resistance in commensal intestinal microflora, Folia Microbiol. 2010, 55, 332-335
58. V. Kmeť, M. Kmeťová, High level of quinolone resistance in *Escherichia coli* from healthy chicken broilers, Folia Microbiol, 2010, 55, 79-82
59. Špánová, A., Rittich, B., Karpíšková, R., Čechová , L., Škapová, D., PCR identification of *Salmonella* cells in food and stool samples after immunomagnetic separation, Bioseparation, 2001, 9, 379-384