Journal of Microbiology, Biotechnology and Food Sciences

Hleba et al. 2011 1 (1) 1-20

REGULAR ARTICLE

ANTIBIOTIC RESISTANCE OF *ENTEROBACTERIACEAE* GENERA AND *SALMONELLA* SPP., *SALMONELLA ENTERICA* SER. TYPHIMURIUM AND ENTERITIDIS ISOLATED FROM MILK, CHEESE AND OTHER DAIRY PRODUCTS FROM CONVENTIONAL FARM IN SLOVAKIA

L. Hleba, M. Kačániová, J. Pochop, J. Lejková, J. Čuboň, S. Kunová*

Address*: Ing. Lukáš Hleba, Department of Microbiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Tr. Andreja Hlinku 2, 94976, Nitra, Slovakia, E-mail: lukas.hleba@gmail.com, phone number: + 421 904 189 191

ABSTRACT

Antibiotic resistance was investigated in 67 samples of *Enterobacteriaceae* genera isolates from milk, cheese and other dairy products in this work. The samples were obtained from conventional farms in Slovakia. Four samples of isolates were positive for *Salmonella* spp., *Salmonella enterica* ser. typhimurium and enteritidis. A great proportion of resistant strain from *Enterobacteriaceae* genera was found. There was detected a high resistance in milk samples to ampicillin (57.14%), to streptomycin and tetracycline (14.28%), to chloramphenicol (9.52%). Bacteria from the cheese samples were resistant to ampicillin (84.0%), to tetracycline (24.0%). In the samples of other dairy products was found resistance to ampicillin (66.66%), to tetracycline (52.38%) and to streptomycin (14.28%). Among all samples observed, it was found resistance to ampicillin (69.26%), tetracycline (30.22%), streptomycin (9.52%) and to chloramphenicol (3.17%). Resistance to other antibiotics was not detected. From all observed samples we detected *Salmonella* spp. in 5.97 %, *Salmonella* spp. was found in dairy samples in 19.04 %. Antibiotic resistance was investigated in 13 colonies of *Salmonella* spp. as well. The highest resistance was to tetracycline (30.76 %), ampicillin and tigecycline (23.07 %), to piperacillin (15.38 %) and chloramphenicol (7.69 %). Resistance to the other monitored antibiotics was not detected. High number of antibiotic

resistant *Enterobacteriaceae* genera and *Salmonella* spp. was found in milk, cheese and other dairy products from conventional breeding and it is indicating the need for prudent drugs using to diminish development and to avoid spread of antibiotic resistance.

Keywords: Antibiotic resistance, *Enterobacteriaceae*, *Salmonella* spp., Real-time PCR

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 **(EFSA, 2008)**. **Keyser et al. (2008)** 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. (2009, 2008)** 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 **(Lado et al., 2002; Kharazmi et al., 2002; McMahon et al, 2007)**. The health safety of foods **(Mareček et al., 2008; Fikselová et al., 2008)**, including milk, is an integral part of consumers policy and health **(Bíreš, 2004)**. Milk is a suitable substrate for the growth of many pathogenic and toxicogenic microorganisms that may cause food-borne diseases which can threat the health of consumers **(Bobková et al., 2008)**. Coliforms bacteria could not survive during pasteurization. The survivance of coliforms bacteria during pasteurization indicates the lack of pasteurization **(Havlová et al., 1993)**. **Görner and Valík (2004)** distinguish the contamination of raw milk to primary and secondary. The primary source of bacteria contamination including tanks udder and teat channels, microorganisms from the surface of udder, from body and excrement of animal, from feed and dust, as well as hand and dairyman clothing, microorganisms which come to contact with milk, during milking, transport in the pipelines, filtering, cooling and storage of milk. A special case is the primary contamination, where microorganisms infect milk from the infected udder. Secondary contamination depends on the initiation of cooling after milking, milk temperature and time, which is needed for metabolism of microorganisms in milk. *Salmonella* spp. that includes

more than 2500 different serotypes represents a leading cause of foodborne infections worldwide **(Chen et al., 2004; Magistrali et al., 2008; White et al., 2002)**. Nearly 1.4 million cases of salmonellosis occur each year in the United States, of which 95% are foodborne cases **(Mead et al., 1999)**. 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 **(Gomez et al., 1997)**. *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 **(Lee et al., 1994)**. 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 **(Brands et al., 2005; Chao et al., 2007; Gebrezes et al., 2005)**. 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 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 **(Aabo et al., 1995; Kwang et al., 1996; Mahon et al., 1994; Soumet et al., 1997)**.

The aim of this study was to determine antibiotic resistance of *Enterobacteriaceae* genera and *Salmonella* spp., *Salmonella enterica* ser. typhimurium and enteritidis isolated from milk, cheese and other dairy products from conventional breeding in Slovakia.

MATERIAL AND METHODS

Collection of milk, cheese and other dairy samples and isolation of *Salmonella*

The samples were obtained from milk, cheese and other dairy products from one conventional sheepfarm in Slovakia. The Table 1 shows selected (67) food samples including

Table 1 Number of analyzed samples and *Salmonella* positive samples

Type of samples	*Enterobacteriaceae*	*Salmonella* spp.
	Analyzed samples	Positive samples
Milk	21	4/21
Cheese	25	0/25
Other dairy products	21	0/21
Total	67	4/67

milk (n = 21), cheese (n = 25) and other dairy products (whey, boiled whey, sheep cheese) (n = 21). The samples were collected by sterile cotton swabs (Copan Inovation, Brescia) and transported to the laboratory (SUA in Nitra, Department of Microbiology). *Enterobacteriaceae* genera and *Salmonella* spp. isolations were performed by a conventional plating method. The first step was done on the MacConkey agar (Biomark, Pune) for *Enterobacteriaceae* genera. 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 *Salmonella* spp. and other strains from *Enterobacteriaceae* genera. After the incubation and identification it was isolated 13 colonies of *Salmonella* spp. of 4 positive samples from milk.

The biochemical identification of *Salmonella* spp.

Method on the Triple sugar iron agar (Biolife, Italiana) for the basic biochemical identification of *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 *Salmonella* spp.

The pure colonies of *Salmonella* spp. were subjected to DNA isolation using PrepSEQ^{TM} 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 *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.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done by disk diffusion method (according **EUCAST (2009)** – European committee on antimicrobial susceptibility testing). Antibiotic disks were used (Oxoid, England). The pure inoculum of strain of *Salmonella* spp. and strains from *Enterobacteriaceae* genera was 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: see the Table 2.

Table 2 Tested antibiotics for *Enterobacteriaceae* genera

Antibiotics	**TE**	**S**	**AMP**	**C**	**AK**	**CN**	**NA**
Full name	tetracycline	streptomycin	ampicillin	chloramphenicol	amikacin	gentamycin	Nalixid acid
Disk content (µg.disk^{-1})	30	10	10	30	30	10	30

We used 469 antibiotic disk for tested *Enterobacteriaceae* genera. For *Salmonella* spp. we used antibiotics shown in the Table 3. We used 130 antibiotic disks for *Salmonella* spp testing. The incubation of strains was performing for 16-18 hours at the temperature 37 °C. The interpretation of inhibition zones around the disk was done according to **EUCAST (2009)**. The inhibition zones were controlled with the reference of *Escherichia coli* ATCC 25922.

Table 3 Tested antibiotics for *Salmonella* spp.

Antibiotics groups	Antibiotics	Content disk ($\mu g.disk^{-1}$)
Penicillins	Ampicillin – AMP	10
	Piperacilin – PRL	30
Cephalosporins	Cefotaxime – CTX	5
	Ceftriaxone – CRO	30
Carbapenems	Doripenem – DOR	10
	Meropenem – MEM	10
Flouroquinolones	Levofloxacin – LEV	5
	Ofloxacin – OFX	5
Aminoglycosides	Amikacin – AK	30
	Gentamycin – CN	10
Tetracyclines	Tetracycline – TE	30
	Tygecycline – TGC	15
Miscellaneous agents	Chloramphenicol - C	30

Statistical evaluation

From the obtained data we calculated basic statistical values using statistical program STATGRAPHICS and for better graphical representation of frequency of the size of inhibition zones was used STATISTICA.

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 of *Enterobacteriaceae* genera

In our study was studied antibiotic resistance of *Enterobacteriaceae* genera strains isolated from milk, cheese and other dairy products from conventional sheep farm from

Slovakia. We determined that *Enterobacteriaceae* genera were resistant against antibiotic and *Salmonella* spp. as well. Of 469 used antibiotics for susceptibility testing of *Enterobacteriaceae* genera, in 72 (15.35%) cases was found resistance. Of the samples of milk (n = 21) were resistant 14.28% to tetracycline, 14.28% to streptomycin, 57.14% to ampicillin, 9.52% to chloramphenicol and 0% to amikacin, gentamycin and nalixid acid. Among samples of cheese (n = 25) resistant were 24% to tetracycline, 84% to ampicillin and 0% to streptomycin, chloramphenicol, amikacin, gentamycin and nalixid acid. Among samples of other dairy products (n = 21) resistance was detected 52.38% to tetracycline, 14.28% to streptomycin, 66.66% to ampicillin and 0% to chloramphenicol, amikacin, gentamycin and nalixid acid. These results are shown in the Table 4.

Table 4 Antibiotic resistance profile of *Enterobacteriaceae* isolates

Antimicrobial agents	**% Resistance in animal products**			
	Total (n=67) (%)	Milk (n=21) (%)	Cheese (n=25) (%)	Other dairy products (n=21) (%)
Tetracycline, TE	30.22	14.28	24.00	52.38
Streptomycin, S	9.52	14.28	0.00	14.28
Ampicillin, AMP	69.26	57.14	84.00	66.66
Chloramphenicol, C	3.17	9.52	0.00	0.00
Amikacin, AK	0.00	0.00	0.00	0.00
Gentamycin, CN	0.00	0.00	0.00	0.00
Nalixid acid, NA	0.00	0.00	0.00	0.00

Escherichia coli is the main representative of the *Enterobacteriaceae* genera. 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 their samples were isolated from Indian milk and products thereof. They determined 100 % resistance to ampicillin mainly in *Escherichia coli*. Also **Farzana et al. (2009)** 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 %). High values of resistance to chloramphenicol (44.82 %) determined **Solomakos et al. (2009)**, because 13 of 29 strains (LFH1 – LFH29) of *E. coli* O 157 were resistant to chloramphenicol. Similar results determined **Dupont et al. (1978)**, they tested antibiotic resistance to chloramphenicol in *E. coli*.

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. (2010a,b)** used. Similar test for identification of *Salmonella* spp. (ENTEROtest 16) **Špánová et al. (2001)** 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 four out of sixty-seven samples, as well as the internal positive control (IPC), which was positive in all samples (Figure 1).

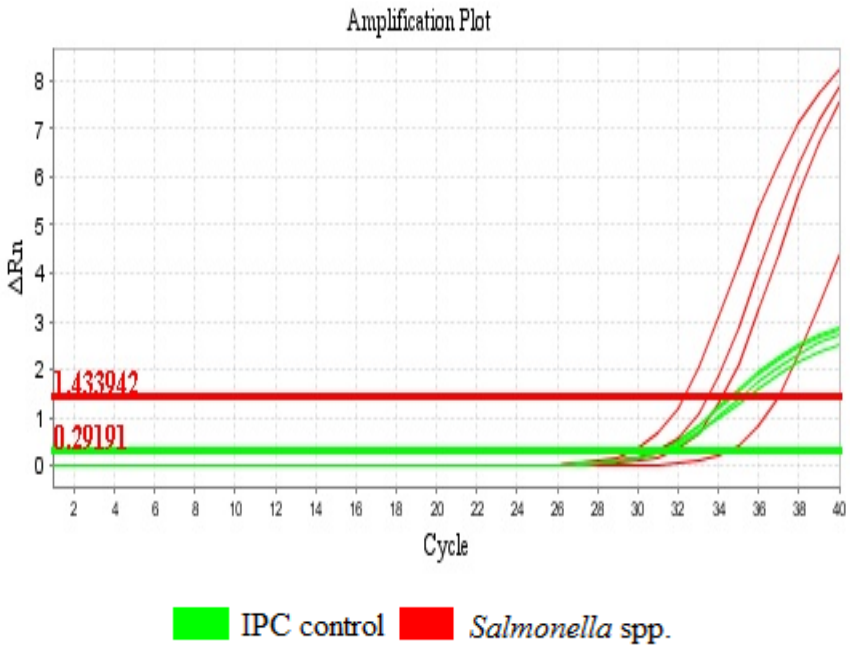

Fig 1 Process of Real Time PCR

The threshold value was 1.433942 by positive Salmonella samples and 0.29191 by internal positive control (IPC). The (Ct) value of positive Salmonella samples was on average 34.22 and IPC (Ct) value was on average 31.04, whereby the lowest value of positive Salmonella samples was reached at 32.27, the highest value was 36.96, the lowest IPC value was reached at 30.88 and the highest accomplish value was 31.27. **Higgins et al. (1998)** noted that Ct values are generally a good indicator of the contamination level of the target organism, as well as the efficiency of the PCR assay. The Ct values observed for the Instagel protocol (27.2±1.4) are in agreement with the notion that it provided cleaner templates for PCR than the Bax-lysis buffer (30.5±1.1). The IPC was correctly amplified in PCR reactions containing colonies from both *Salmonella* spp. and non-*Salmonella* strains, being overall TET ΔRn values of 1.33±0.35 and 0.49±0.12.

Presence of *Salmonella* spp. in the samples

Among *Enterobacteriaceae* genera we isolated *Salmonella* spp. from milk samples. Of all samples which were examined, 4 samples (5.97%) for *Salmonella* spp. was positive. In the milk samples (n = 21) was 19.04% *Salmonella* spp. positive. In the other samples of cheese (n = 25) and dairy products (n = 21) *Salmonella* spp. was not detected. Incidences of Salmonella spp. in different samples are shown in Table 5.

Table 5 Presence of *Salmonella* spp. in from milk and milk products samples

Evidence of *salmonella* spp.	The samples isolated from			
	Total (n=67) (%)	Milk (n=21) (%)	Cheese (n=25) (%)	Other dairy products (n=21) (%)
	5.97	19.04	0.00	0.00

Murphy et al. (2008) determined that 10 (1.8%) samples of 556 collections from in-line milk filters were *Salmonella* positive. Many different authors determined the presence of *Salmonella* spp. in dairy farms **(Fossler et al., 2005; Warnick et al., 2003; Veling et al., 2002; Graham et al., 2005)**.

Antibiotic resistance of *Salmonella* spp. isolated from milk

There were resistant twelve cases (9.23%) of 130 used antibiotics for susceptibility testing of *Salmonella* spp. From the milk samples (n = 13) *Salmonella* spp. was resistant to ampicillin (23.07%), to piperacillin (15.38%), to tetracycline (30.76%), to tigecycline (23.07%) and chloramphenicol (7.69%). Antibiotic resistance to antibiotics as cefotaxime, ceftriaxone, doripenem, meropenem, levofloxacin, ofloxacin, amikacin and gentamycin in samples of *Salmonella* spp. were not detected. Antibiotic resistance profile of *Salmonella* spp. isolated from milk samples is shown in the Table 6.

Table 6 Antibiotic resistance profile of *Salmonella* spp. isolates

Antibiotic	% Resistance to antimicrobial agent												
Resistance	**AMP**	**PRL**	**CTX**	**CRO**	**DOR**	**MEM**	**LEV**	**OFX**	**AK**	**CN**	**TE**	**TGC**	**C**
Profile	23.07	15.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	30.76	23.07	7.69

Also **Ray et al. (2007)** determined the antibiotic resistance in *Salmonella* spp. to ampicillin (10.1%), to tetracycline (13.0%) in samples isolated from dairy farm. 14 % resistance of *Salmonella* spp. to single agent and 22 % multiresistance of *Salmonella* spp. were reported by **Genovese et al. (2004**).

Summary statistics of inhibition zones

From obtained data about inhibition zones around the disks from *Enterobacteriaceae* isolates, were calculated the basic statistical values. The highest value of average inhibition zones was 21.16±0.93 mm to amikacin in the samples from milk and other dairy products. The lowest value was 21.10±0.88 mm in the samples from cheese. The highest average value to ampicillin was 14.52±6.29 mm from the milk samples. The lowest was 11.84±3.76 mm from the cheese samples. To chloramphenicol the highest value of average inhibition zone was 23.76±6.23 mm from the milk samples. The lowest value was 13.09±4.43 mm among all samples. The highest value of average inhibition zone to gentamycin was 23.36±0.88 mm from all samples and lowest value was 22.76±0.86 mm from the cheese samples. The value 22.76±1.84 mm was the highest average of inhibition zone from all samples and the lowest value 20.12±1.48 mm in the cheese samples was to nalixid acid. To streptomycin the highest value of average was 21.33±6.25 mm in the milk samples and 16.28±2.41 mm in the other dairy products samples. The highest average inhibition zone to tetracycline was 22.54±5.72 mm in the milk samples and lowest value was 14.66±3.49 mm in the other dairy samples. Other values and specifically results are shown in the Table 7 for *Enterobacteriaceae* genera.

Table 7 Summary statistic values for inhibition zones of *Enterobacteriaceae* genera

VALUES	PRODUCT	ANTIMICROBIAL AGENTS						
		AK	AMP	C	CN	NA	S	TE
Average (mm)	MILK	21.16	14.52	23.76	22.80	20.76	21.33	22.54
	CHEESE	21.10	11.84	23.20	22.76	20.12	20.64	17.64
	OTHERS	21.16	13.14	23.14	22.80	20.76	16.28	14.66
	TOTAL	21.14	13.08	13.09	23.36	22.79	20.52	19.49
Standard Deviation (mm)	MILK	0.93	6.29	6.23	0.91	2.02	6.52	5.72
	CHEESE	0.88	3.76	3.20	0.86	1.48	2.32	6.22
	OTHERS	0.93	5.97	3.63	0.91	2.02	2.41	3.49
	TOTAL	0.91	5.41	4.43	0.88	1.84	4.63	6.15
Coeff. of Variation (%)	MILK	4.44	43.36	26.24	4.01	9.77	30.57	25.39
	CHEESE	4.21	31.75	13.79	3.81	7.36	11.26	35.28
	OTHERS	4.40	45.47	15.71	4.01	9.77	14.80	23.84
	TOTAL	0.04	0.41	0.19	0.04	0.09	0.24	0.34
Min (mm)	MILK	20.00	7.00	7.00	21.00	18.50	12.00	9.00
	CHEESE	20.00	7.00	18.00	21.00	18.00	18.00	8.00
	OTHERS	20.00	7.00	18.00	21.00	18.50	12.00	7.00
	TOTAL	20.00	7.00	7.00	21.00	18.00	12.00	7.00
Max (mm)	MILK	22.50	28.00	32.00	24.00	27.00	29.00	29.00
	CHEESE	22.50	22.00	31.00	24.00	23.00	26.00	28.00
	OTHERS	22.50	32.00	30.00	24.00	27.00	19.00	19.00
	TOTAL	22.50	32.00	32.00	24.00	27.00	29.00	29.00
Range (mm)	MILK	2.50	21.00	25.00	3.00	8.50	17.00	20.00
	CHEESE	2.50	15.00	13.00	3.00	5.00	8.00	20.00
	OTHERS	2.50	25.00	12.00	3.00	8.50	7.00	12.00
	TOTAL	2.50	25.00	25.00	3.00	9.00	17.00	22.00

From obtained data of inhibition zones around the disks from *Salmonella* spp. samples, we calculated also the basic statistical values. The average of inhibition zones was 18.8±4.37 mm to ampicillin. To piperacyllin was average 25.7±6.95 mm, to cefotaxime 31.0±1.63 mm, to ceftriaxone 33.3±1.42 mm, to doripenem 33.4±1.51 mm, to meropenem 36.4±2.50 mm, to levofloxacin 29.0±1.15 mm, to ofloxacin 26.2±0.63 mm, to amikacin 23.9±2.28 mm, to gentamycin 23.6±1.96 mm, to tetracycline 18.8±7.35 mm, to tigecycline 19.3±5.21 mm and to chloramphenicol 24.6±3.78 mm. The other results are shown in the Table 8 for *Salmonella* spp. from the milk samples only.

Table 8 Summary statistic values for inhibition zones of *Salmonella* spp.

VALUES	ANTIMICROBIAL AGENTS												
	AMP	**PRL**	**CTX**	**CRO**	**DOR**	**MEM**	**LEV**	**OFX**	**AK**	**CN**	**TE**	**TGC**	**C**
Average (mm)	18.8	25.7	31.0	33.3	33.4	36.4	29.0	26.2	23.9	23.6	18.8	19.3	24.6
Standard Deviation (mm)	4.37	6.95	1.63	1.42	1.51	2.50	1.15	0.63	2.28	1.96	7.35	5.21	3.78
Coeff. of Variation (%)	0.23	0.27	0.05	0.04	0.05	0.07	0.04	0.02	0.10	0.08	0.39	0.27	0.15
Min (mm)	12.0	14.0	29.0	31.0	31.0	32.0	27.0	25.0	20.0	21.0	10.0	12.0	18.0
Max (mm)	23.0	31.0	33.0	35.0	35.0	39.0	31.0	27.0	26.0	26.0	29.0	28.0	30.0
Range (mm)	11.0	17.0	4.0	4.0	4.0	7.0	4.0	2.0	6.0	5.0	19.0	16.0	12.0

Frequency of the size of inhibition zones

Obtained data were processed by STATISTICA 7.0 software for frequency of the size of inhibition zones calculation. Frequency of the size of inhibition zone is value, where was the most frequently presence of inhibition zones. In our study were determined these frequentations for inhibition zone of *Salmonella* spp. for 13 types of antibiotics. From the group of penicillins was the most frequently size of inhibition zone about 22 mm, for ampicillin and for piperacillin about 30 mm. From the cephalosporins group was the most frequently 29 and 32 mm for cefotaxime and for ceftriaxone 33 and 35 mm. From the carbapenems group was 32, 33 and 35 mm for dormipenem and for meropenem 35 and 39 mm. From the fluoroquinolones group was the most frequently size of inhibition zone 26 mm for ofloxacine and for levofloxacine 29 mm. From the aminoglycosides group was found the most frequently 25 mm for amikacin and gentamycin as well. From the group of tetracyclines was detected the most frequently 10, 12 and 18 mm for tetracycline and 14 and 18 mm for tigecycline. For chloramphenicol from miscellaneous group was the most frequently size of inhibition zone 24 and 25 mm. The lines in the Figures 2-8 show averages of the size of inhibition zones for each antibiotic. The other results are shown in this graphs.

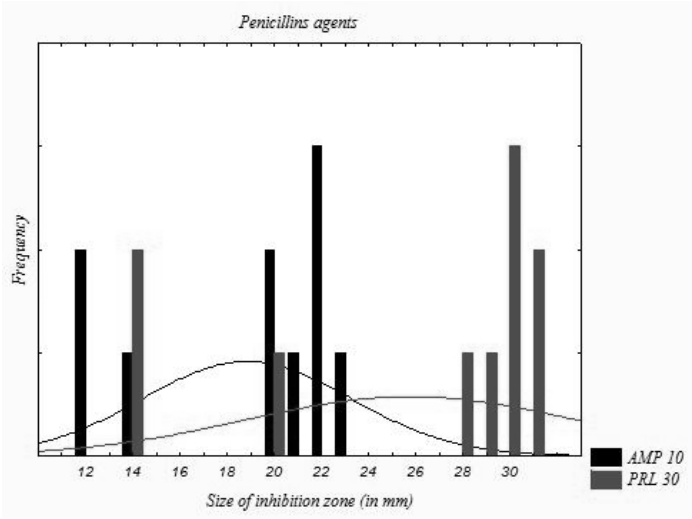

Fig 2 Frequency of the size of inhibition zone for penicillins agents

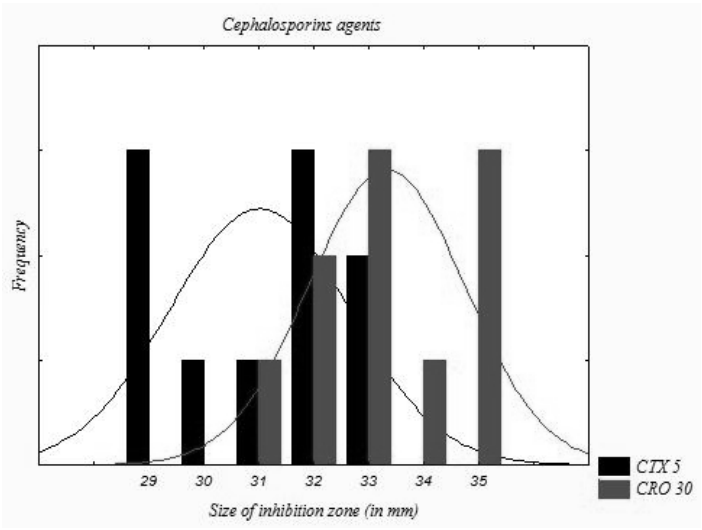

Fig 3 Frequency of the size of inhibition zone for cephalosporins agents

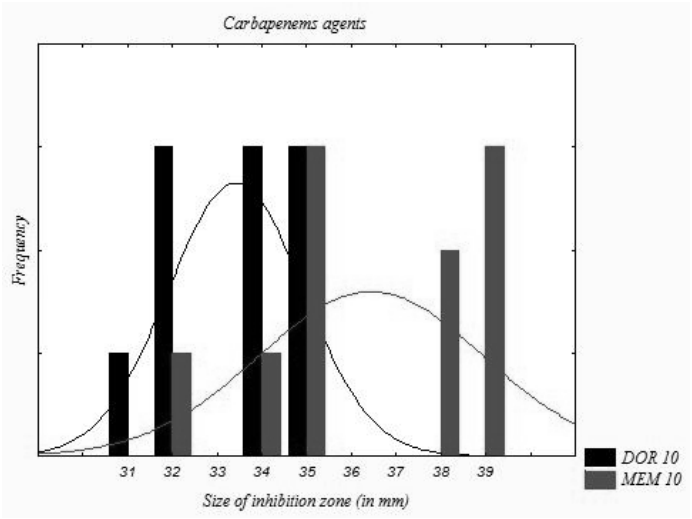

Fig 4 Frequency of the size of inhibition zone for carbapenems agents

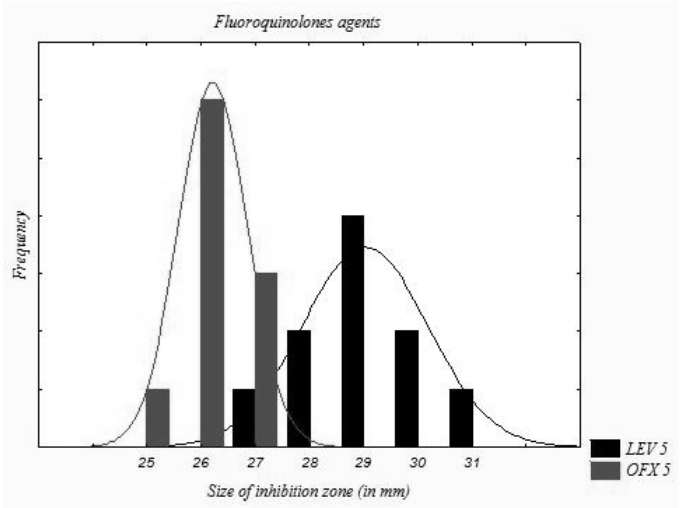

Fig 5 Frequency of the size of inhibition zone for fluoroquinolones agents

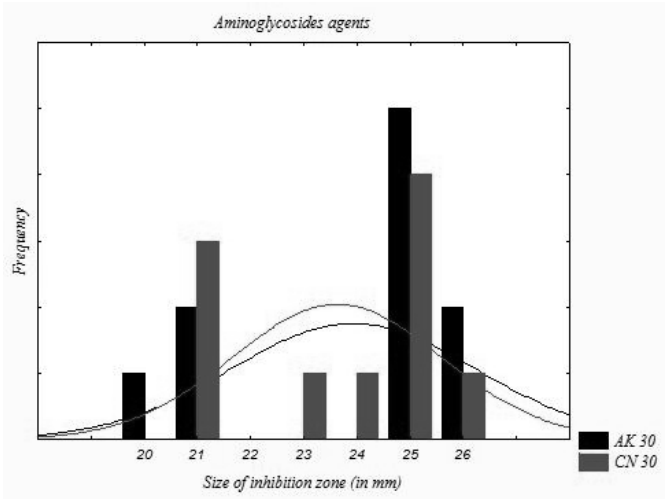

Fig 6 Frequency of the size of inhibition zone for aminoglycosides agents

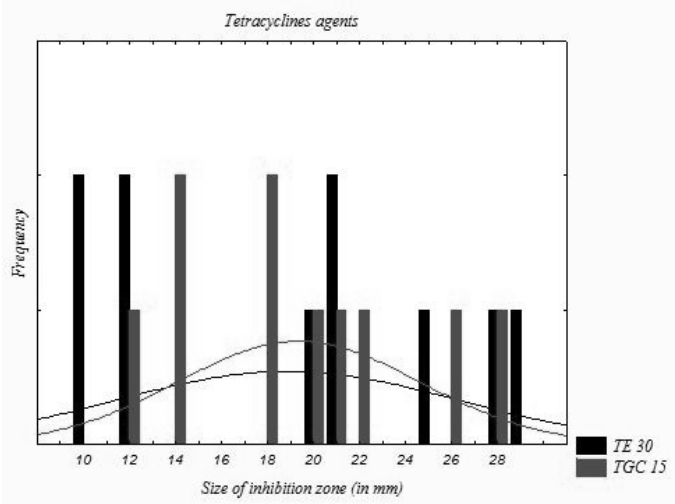

Fig 7 Frequency of the size of inhibition zone for tetracyclines agents

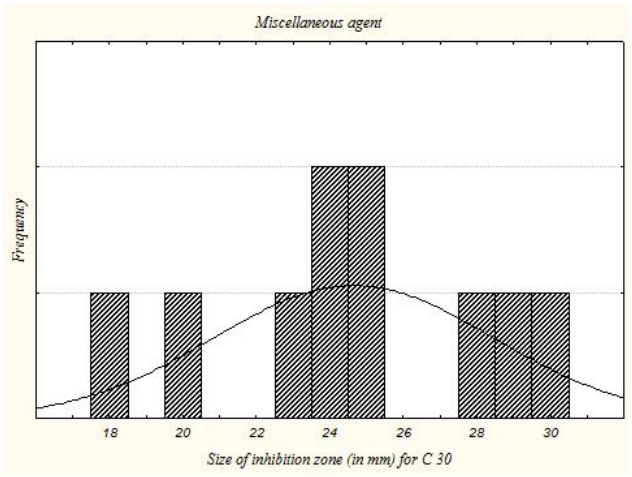

Fig 8 Frequency of the size of inhibition zone for miscellaneous agent

CONCLUSION

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 do not only through the digestive tract of animals but also in their final products such milk, cheese and other dairy products. It is very important in commercial breeding to observe of sanitation and hygiene conditions in the dairy products from sheep milk, because this milk is not pasteurize. Milk, cheese and other products are end products, which are also used in human food chain. If coliforms bacteria including *Salmonella* spp. 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.

Acknowledgments: This work has been supported by grant of VEGA 1/0372/09, KEGA 430-014SPU-4/2010 and VMSP-P-0111-09.

REFERENCES

AABO, S. - ANDERSON, J. K. - OLSEN, J. E. 1995. Detection of *Salmonella* in minced meat by the polymerase chain reaction methods. In *Letters of Applied Microbiology*, vol. 21, 1995, p. 180-182.

BÍREŠ, J. 2004. Current legislation in the field of milk hygiene. In *The Dairy*, vol. 35, 2004, p. 33-35.

BOBKOVÁ, A. - ZELEŇÁKOVÁ, L. - LOPAŠOVSKÝ, L. - PAVELKOVÁ, A. 2008. Evaluation of microbiological quality of yoghurt cream. In *Food Safety and control* : Proceding of th work of the International Scientific Conference in Nitra: SUA, 2008, p. 33-37.

BRANDS, D. A. - INMAN, A. E. - GERBA, C. P. - MARÉ, C. J. - BILLINGTON, S. J. - SAIF, L. A. - LEVINE, J. F. - JOENS, L. A. 2005. Prevalence of Salmonella spp. in oysters in the United States. In *Applied Environmental Microbiology*, vol. 71, 2005, p. 893-897.

BUŇKOVÁ, L. - BUŇKA, F. - DOLEŽÁLKOVÁ, I. - KRÁČMAR, S. 2009. Antibacterial effect of monocaprin, undecanoylglycerol and undecenoylglycerol. In *Food Safety and control*: Proceding of the work of the International Scientific Conference in Nitra: SUA, 2009, p. 45-48.

BUŇKOVÁ, L. - PLEVA, P. - BUŇKA, F. - VALÁŠEK, P. - KRAČMÁR, S. 2008. Antibacterial effect of commercially available phosphates on selected microorganisms. In *Acta universitatis agriculturae et silviculturae Mendelianae Brunensis*, vol. 56, 2008, p. 1-6.

CHAO, G. X. - ZHOU, X. H. - JIAO, X. N. - QIAN, X. Q. - XU, L. 2007. Prevalence and antimicrobial resistance of foodborne pathogens isolated from food products in China. In *Foodborne Pathogens and Disease*, vol. 4, 2007, p. 277-284.

CHEN, S. - ZHAO, S. H. - WHITE, D. G. - SCHROEDER, C. M. - LU, R. - YANG, H. C. - MCDERMOTT, P. F. - AYERS, S. - MENG, J. H. 2004. Characterization of multiple-antimicrobial-resistant Salmonella serovars isolated from retail meats. In *Applied Environmental Microbiology*, vol. 70, 2004, p. 1-7.

DUPONT, H. L. - WEST, H. - EVANS, D. G. - OLARTE, J. - EVANS, D. J. 1978. Antimicrobial susceptibility of enterotoxigenic *Escherichia coli*. In *Journal of Antimicrobial Chemotherapy*, vol. 4, 1978, p. 100-102.

EFSA. 2008. 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.

EUCAST. 2009. Antimicrobial susceptibility testing – EUCAST disk diffusion method. Version 1.0, december 18, 2009. European Society of Clinical Microbiology and Infectious Diseases.

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, p. 935-943.

FIKSELOVÁ, M. - ŠILHÁR, S. - MAREČEK, J. - FRANČÁKOVÁ, H. 2008. Extraction of carrot (*Daucus carota* L.) carotenes inder different conditions. In *Czech Journal of Food Science*, vol. 26, 2008, p. 268-274.

FOSSLER, C. P. - WELLS, S. J. - KANEENE, J. B. - RUEGG, P. L. - WARNICK, L. D. - EBERLY, L. E. - GODDEN, S. M. - HALBERT, L. W. - CAMPBELL, A. M. - BOLIN, C. A. - GEIGER ZWAL, A. M. 2005. Cattle and environmental sample-level factors associated with the presence of Salmonella in a multi-state study of conventional and organic dairy farms. In *Preventive Veterinary Medicine*, vol. 67, 2005, p. 39-53.

GEBREYES, W. A. - THAKUR, S. 2005. Multidrug-resistant Salmonella enterica serovar Muenchen from pigs and humans and potential interserovar transfer of antimicrobial resistance. In *Journal of Antimicrobial Agents and Chemotherapy*, vol. 49, 2005, p. 503-511.

GENOVESE, K. J. - JUNG, Y. S. - MCREYNOLDS, J. L. - ANDERSON, R. C. - NISBET, D. J. 2004. Antimicrobial resistance and serotype prevalence of Salmonella isolated from dairy cattle in the southwestern United States. In *Microbial Drug Resistance*, vol. 10, 2004, p. 51-56.

GOMEZ, T. M. - MOTARJEMI, Y. - MIYAGAWA, S. - KAFERSTEIN, F. K. - STOHR, K. 1997. Foodborne salmonellosis, In *World Health Statistics*, vol. 50, 1997, p. 81-89.

GRAHAM, S. L. - BARLING, K. S. - WAGHELA, S. - SCOTT, H. M. - THOMPSON, J. A. 2005. Spatial distribution of antibodies to Salmonella enterica serovar Thypimurium O antigens in bulk milk from Texas dairy herds. In *Preventive Veterinary Medicine*, vol. 69, 2005, p. 53-61.

GÖRNER, F. - VALÍK, L. 2004. Applied microbiology foodstuffs. Bratislava : Small center, 2004, 528 p. ISBN 80-967064-9-7.

HAVLOVÁ, J. - JIČINSKÁ, E. - HRABOVÁ, H. 1993. Microbiological methods in quality control of milk and milk products. Praha: ÚZPI, 1993, 243 p. ISBN – 80-85 120-37-2.

HIGGINS, J. A. - EZZEL, J. - HINNEBUSCH, B. J. - SHIPLEY, M. - HENCHAL, E. A. - IBRAHIM, S. S. 1998. 5´ nuclease PCR assay to detect Yersinia pestis. In *Journal of Clinical Microbiology*, vol. 36, 1998, p. 2284-2288.

KEYSER, P. - ELOFSON, M. - ROSELL, S. - WOLF-WATZ, H. 2008. Virulence blockers as alternatives to antibiotics: type III secretion inhibitors against Gram-negative bacteria. In *Journal of International Medicine*, vol. 264, 2008, p. 17-29.

KHARAZMI, Y. - HAMMES, W. P. - HERTELL, C. 2002. Construction of a marker rescue system in Bacilus subtilis for detection of horizontal gene transfer in food. In *Systematic Applied Microbiology*, vol. 25, 2002, p. 471-477.

KMEŤ, V. - PIATNICOVÁ, E. 2010. Antibiotic resistance in commensal intestinal microflora. In *Folia Microbiologica*, vol. 55, 2010, p. 332-335.

KMEŤ, V. - KMEŤOVÁ, M. 2010. High level of quinolone resistance in Escherichia coli from healthy chicken broilers. In *Folia Microbiologica*, vol. 55, 2010, p. 79-82.

KWANG, J. - LITTLEDIKE, E. T. - KEEN, J. E. 1996. Use of the polymerase chain reaction for *Salmonella* detection. In *Letters of Applied Microbiology*, vol. 22, 1996, p. 46-51.

LADO, B. - YOUSEF, A. 2002. Alternative foodpreservation technologies: efficacy a mechanisms. In *Microbial Infections*, vol. 4, 2002, p. 433-440.

LEE, L. A. - PUHR, N. D. - MALONEY, E. K. - BEAN, N. H. - TAUXE, R. V. 1994. Increase in antimicrobial-resistant Salmonella infections in the United States, 1989–1990. In *Internationa Journal of Infectious Diseases*, vol. 170, 1994, p. 128-134.

MAHON, J. - MURPHY, C. K. - JONES, P. W. - BARROW, P. A. 1994. Comparison of multiplex PCR and standard bacteriological methods of detecting *Salmonella* on chicken skin. In *Letters of Applied Microbiology*, vol. 19, 1994, p. 169-172.

MAGISTRALI, C. - DIONISI, A. M. - CURTIS, P. D. - CUCCO, L. - VISCHI, O. - SCUOTA, S. - ZICAVO, A. - PEZZOTTI, G. 2008. Contamination of Salmonella spp. in a pig finishing herd, from the arrival of the animals to the slaughterhouse. In *Research Veterinary Science*, vol. 85, 2008, p. 204-207.

MAREČEK, J. - FIKSELOVÁ, M. - FRANČÁKOVÁ, H. 2008. Nutritional and technological value of selected edible potatoes during storage. In *Zeszyty problemowe postepow nauk rolniczych*, Warszawa: Polska akademia nauk, 2008, p. 293-299.

MCMAHON, M. A. S. - BLAIR, I. S. - MOORE, J. E. - MC DOWELL, D. A. 2007. The rate of horizontal transmission of antibiotic resistence plasmids is increased in food preservation-stressed bacteria. In *Journal of Applied Microbiology*, vol. 10, 2007, p. 1883-1888.

MEAD, P. S. - SLUTSKER, L. - DIETZ, V. - MCCAIG, L. F. - BRESEE, J. S. - SHAPIRO, C. - GRIFFIN, P. M. - TAUXE, R. V. 1999. Food-related illnessand death in the United States. In *Emerging Infectious Diseases*, vol. 5, 1999, p. 607-625.

MURPHY, B. P. - BUCKLEY, J. F. - O´CONNOR, E. M. - GILROY, D. - FANNING, S. 2008. Comparison of *Salmonella* species recovered from Irish liquid milk production holdings

with temporal clinical veterinary isolates. In *International Journal of Hygiene and Environmental Health*, vol. 211, 2008, p. 283-289.

RAY, K. A. - WARNICK, L. D. - MITCHELL, R. M. - KANEENE, J. B. - RUEGG, P. L. - WELLS, S. J. - FOSSLER, C. P. - HALBERT, L. W. - MAY, K. 2007. Prevalence of antimicrobial resistance among *Salmonella* on midwest and northeast USA dairy farms. In *Preventive Veterinary Medicine*, vol. 79, 2007, p. 204-223.

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.

SOUMET, C. - ERMEL, G. - SALVAT, G. - COLIN, P. 1997. Detection of *Salmonella* spp. in food products by polymerase chain reaction and hybridisation assay in microplate format. In *Letters of Applied Microbiology*, vol. 24, 1997, p. 113-116.

ŠPÁNOVÁ, A. - RITTICH, B. - KARPÍŠKOVÁ, R. - ČECHOVÁ, L. - ŠKAPOVÁ, D. 2001. PCR identification of *Salmonella* cells in food and stool samples after immunomagnetic separation. In *Bioseparation*, vol. 9, 2001, p. 379-384.

VELING, J. - WILPSHAAR, H. - FRANKENA, K. - BARTELS, C. - BARKEMA, H. W. 2002. Risk factors for clinical Salmonella enterica subsp. Enterica serovar Typhimurium infection on Dutch dairy farms. In *Preventive Veterinary Medicine*, vol. 54, 2002, p. 157-168.

WARNICK, L. D. - KANISTANON, K. - MCDONOUGH, P. L. - POWER, L. 2003. Effect of previous antimicrobial treatment on fecal shedding of Salmonella enterica subsp. Enterica serogroup B in New York dairy herds with recent clinical salmonellosis. In *Preventive Veterinary Medicine*, vol. 56, 2003, p. 285-297.

WHITE, D. G. - ZHAO, S. H. - SIMJEE, S. - WAGNER, D. D. - MCDERMOTT, P. F. 2002. Antimicrobial resistance of foodborne pathogens. In *Microbial Infections*, vol. 4, 2002, p. 405-412.