COMPARISON OF ANTIBIOTIC RESISTANCE OF *ESCHERICHIA COLI* ISOLATED FROM RECTAL AND ABDOMINAL SWABS OF CALVES

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ABSTRACT

In this study was examined antibiotic resistance of *Escherichia coli* isolated from rectal and abdominal of calves. Eleven samples from rectal and eight samples from abdominal parts were analyzed. Nineteen isolates of *Escherichia coli* were isolated and each of isolates of *E. coli* were tested against to antibiotics. Four antibiotics were used – ampicillin, streptomycin, tetracycline, chloramphenicol and disk diffusion methods was used (according by EUCAST). From the samples of rectal isolates was determined the highest antibiotic resistance (54.54 %) to tetracycline and streptomycin. The highest resistance from the abdominal samples was (25 %) to tetracycline. To chloramphenicol resistance was not detected. For the identification of *Escherichia coli* was used a basic identification on the agar (MacConkey agar, Chromogenic coliform agar and Triple sugar iron agar). For the biochemical identification of *E. coli* was used ENTERO test 24 and TNW Lite 7.0 software. Results showed, that resistant genes can survive and spread in the environment. Importance of this study is in monitoring of antibiotic resistant genes, because it could be a key to reduce this world problem.

Keywords: antibiotic resistance, *Escherichia coli*, calves, abdominal, rectal

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 animal and human **(EFSA, 2008)**. **Keyser et al. (2008)** note that in recent years, accumulating problems with bacteria, which are resistant to antibiotics, leading to predictions that we return to the time before the discovery of antibiotics. One of the possibility could be the introduction of different antibacterial preparation, which used **Buňková et al. (2008, 2009)** in their experiments. The 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, Kaharzmi et al., 2002)**. The health safety of foods **(Mareček et al., 2008, Fikselová et al., 2008)**, including meat is an integral part of consumers policy and health **(Bíreš, 2004)**. 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 **(Witte, 1998)**. Resistant bacteria from the intestines of food animals may 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 **(Jackson et al., 2001)**. Endogenous bacterial flora may play an important role as acceptor and donor of transmissible drug resistance genes **(Davies, 1994 and Sunde et al., 1998)**. *Escherichia coli* is commonly found in the intestinal tract of humans and animals **(Sunde et al., 1998 and Tannock, 1995)** and can also be implicated in human and animal infectious diseases **(Threlfal et al., 1998)**. 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 **(Bongers et al., 1995; London et al., 1994; Nijsten et al., 1996)** or animals **(Adesiyun et al., 1997; Blanco et al., 1997; Mathew et al., 1996; Nijsten et al., 1993; Sunde et al., 1998)** have been studied, but in few cases comparative results have been shown **(Sunde et al., 1998; Van den Bogaard et al., 1997)** or isolates from foods analyzed.

The objective of our study was to compare antibiotic resistance in *E. coli* isolates obtained from rectal and abdominal samples of calves.

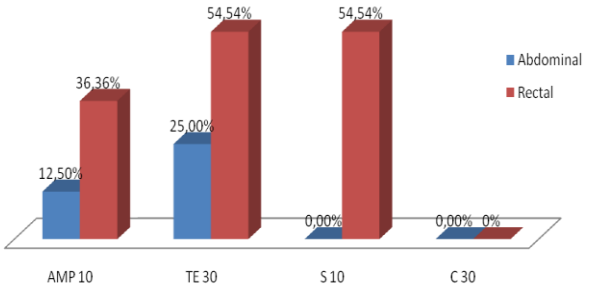
MATERIAL AND METHODOLOGY

Antibiotic resistance study was done on *Escherichia coli* isolated from six month years old calves from conventional farm of Slovakia. The bacterial strains were isolated from rectal (n = 11) and abdominal (n = 8) part of calves by the kit containing the swab (Copan Inovation, Brescia) and the transport in medium to laboratory (Department of Microbiology, SUA in Nitra). For cultivation of *Escherichia coli* Mac Conkey agar (Biomark, Pune) was used. Cultivation of *E. coli* was done during 24 hours at 37 °C. The pure colonies were recultivated at the same conditions. The inoculum of *E. coli* was prepared by suspending of colonies from agar plates and the suspension was adjusted to equal 0.5° McFarland standard. The sensitivity of all isolates was tested against: streptomycin (S 10) 10 $\mu g.disk^{-1}$, tetracycline (TE 30) 30 $\mu g.disk^{-1}$, ampicillin (AMP 10) 10 $\mu g.disk^{-1}$ and chloramphenicol (C 30) 30 $\mu g.disk^{-1}$. We used disk diffusion methods (according to EUCAST – European committee on antimicrobial susceptibility testing) **(EUCAST, 2009)**. The incubation of strains with antibiotic disks was done on ISO Sensitest agar (Biolife, Italiana) during 24 hours at 37 °C. The interpretation of inhibition zones around the disk was according to EUCAST –

European committee on antimicrobial susceptibility testing. The inhibition zones were controlled with reference strains *Escherichia coli* ATCC 25922. The basic differentiation of strains of *Escherichia coli* isolates was done on the identification agars like MacConkey agar (Biomark, Pune), Chromogenic coliform agar (Biolife, Italiana) and Triple Sugar Iron agar (Biolife, Italiana). Biochemical identification of strains of *E. coli* isolates was done by ENTERO test 24 (Pliva, Lachema). For the evaluation of biochemical test we used identification program TNW Lite 7.0 (Pliva, Lachema). For the calculating of the basic statistical values, we used statistical program STATGRAPHIC and for making the graph in higher quality, we used software STATISTICA.

RESULTS

The main role of this experiments is a monitoring of antibiotic resistance of bacteria, because it could be a key to reduce this problem with antibiotic resistant bacteria.
In this study, we found differences between samples from rectal and abdominal isolates of *E. coli*. In the samples from rectal isolates of *Escherichia coli*, we determined higher resistance to antibiotics compared to abdominal isolates. The highest resistance was determined in the samples from rectal isolates of *E. coli* to tetracycline (54.54 %) and streptomycin (54.54 %). Antibiotic resistance from rectal samples was 36.36 % to ampicillin and 0 % to chloramphenicol. The lower resistance to antibiotics, we found in the samples from abdominal isolates of *Escherichia coli*. The resistance of *E. coli* was 25 % to tetracycline and 12.5 % to ampicillin from abdominal samples. Resistance of *E. coli* to streptomycin and chloramphenicol was not detected. The results are shown on graph 1.



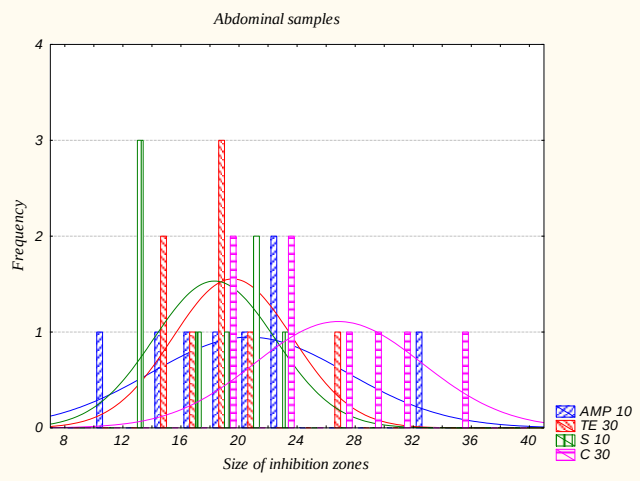
Graph 1: Antibiotic resistance profile of *Escherichia coli* isolated from abdominal and rectal parts

From the values of inhibition zones obtained from abdominal and rectal isolates of *E. coli*, we calculated the basic statistical values such as average, standard deviation, coefficient of variation, minimum, maximum, range and frequency of the size of inhibition zones. Average of inhibition zones from abdominal isolates were: 14.73±4.28 to ampicillin, 13.91±5.22 to tetracycline, 13.91±4.28 to streptomycin and 27.36±2.69 to chloramphenicol. Average of inhibition zones from rectal isolates of *E. coli* were: 20.63±6.74 to ampicillin, 19.50±4.11 to tetracycline, 18.25±4.17 to streptomycin and 26.75±5.75 to chloramphenicol. The other values are shown in Table 1.

Table 1: Values of inhibition zones from abdominal and rectal samples

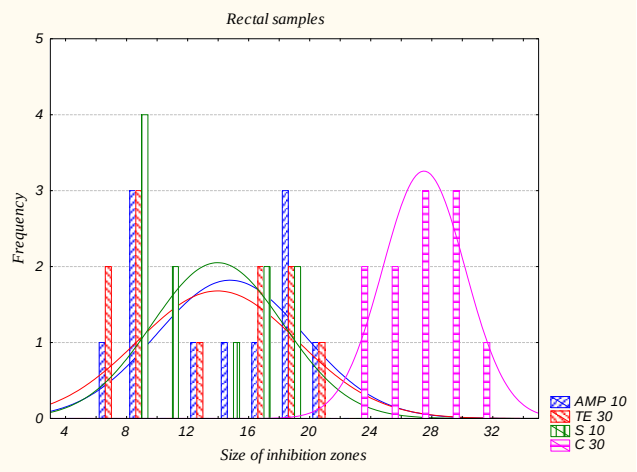
Summary statistical values								
	Abdominal samples				Rectal samples			
	AMP 10	TE 30	S 10	C 30	AMP 10	TE 30	S 10	C 30
Count (n)	11	11	11	11	8	8	8	8
Average (mm)	14.73	13.91	13.91	27.36	20.63	19.50	18.25	26.75
Standard deviation (mm)	4.82	5.22	4.28	2.69	6.74	4.11	4.17	5.75
Coeff. of variation (%)	32.72	37.56	30.75	9.84	32.67	21.06	22.83	21.50
Minimum (mm)	8	8	9	23	12	15	13	20
Maximum (mm)	21	21	20	31	34	28	24	36
Range (mm)	13	13	11	8	22	13	11	16

From the obtained data we calculated by STATISTICA frequency of the size of inhibition zones. It is value, where was the most frequently presence of inhibition zones. The lines in the graph (2,3) show averages of the size of inhibition zones for each antibiotic. In our study, we determined these frequentations of the sizes of inhibition zones of *Escherichia coli* isolated from abdominal and rectal samples for four types of antibiotics. From the abdominal samples was the most frequently size of inhibition zone about 22 mm for ampicillin. Tetracycline has the most frequently size of inhibition zones about 18 mm. Streptomycin has the most frequently of the size of inhibition zones about 13 mm. For chloramphenicol from miscellaneous antibiotics was the most frequently size of inhibition zone about 20 and 24 mm. The results are shown in following graphs (2,3).



Graph 2: Frequency of the size of inhibition zones from abdominal samples

From the rectal samples was the most frequently size of inhibition zone about 8 mm and 18 mm for ampicillin. Tetracycline has the most frequently size of inhibition zones about 8 mm. Streptomycin has the most frequently of the size of inhibition zones about 9 mm. For chloramphenicol from miscellaneous antibiotics was the most frequently size of inhibition zone about 28 and 29 mm.



Graph 3: Frequency of the size of inhibition zones from rectal samples

DISCUSSION

In **2003, Gunn et al.** compared antibiotic resistance of *Escherichia coli* isolated from groups of diarrhoeic and control calves and they determined, that resistance in *E. coli* isolated from health control calves was 70% to ampicillin. Also **Ahmed et al. (2009)** examined genetic analysis of antibiotic resistance in *E. coli* isolated from diarrhoeic neonatal calves and determined the presence of ampicillin, streptomycin, tetracycline and chloramphenicol resistant genes. They used the primers, which they designed by themselves **(Ahmed et al, 2007)**. **Berge et al. (2005)** examined antibiotic resistance in rectal *Escherichia coli* isolated from young calves, which were six week old and they determined 71.42% resistance to ampicillin, 42.85% to chloramphenicol, 85.71% to streptomycin and 92.85% resistance to tetracycline. The number of researchers, such as **Lira et al. (2004)**, **Picozzi et al. (2005)**, **Caro et al. (2007)**, **Čížek et al. (2007)**, which examined the antibiotic resistance of *E. coli*, respectively, *Enterobacteriaceae* genera isolated from different food samples have argued that the results of antibiotic resistance vary from study to study.

CONCLUSION

Escherichia coli is a model microorganism for many microbiological testing. It is considered as a reservoirs of resistant genes in the intestinal tract of animal. Resistant genes can be transfer from non-pathogen to pathogen microorganisms and can cause infections, which are difficult to treat. Our results showed, that antibiotics were used or rearing to animal from environment. Also, results showed that resistant genes survive in the environment as a results of recent use of antibiotics in animal livestock as growth hormones. The higher resistance in the rectal samples is cause a direct of contact with resistant genes in the intestinal tract. The most important fact of this experiments is a monitoring of antibiotic resistance for a reducing or full removing of this world problem.

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