Slovenská poľnohospodárska univerzita v Nitre
Fakulta biotechnológie a potravinárstva
Fakulta agrobiológie a potravinových zdrojov

# V. Vedecká konferencia doktorandov

**s medzinárodnou účasťou**

pri príležitosti Európskeho týždňa vedy

FAKULTA BIOTECHNOLÓGIE A POTRAVINÁRSTVA

FAKULTA AGROBIOLÓGIE A POTRAVINOVÝCH ZDROJOV

Nitra, 26. november 2010

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# SLEDOVANIE ANTIBIOTICKEJ REZISTENCIE BAKTÉRIÍ Z ČEĽADE *ENTEROBACTERIACEAE* IZOLOVANÝCH Z REKTÁLNYCH VÝTEROV OVIEC, CECKOV A MLIEKA

## MONITORING OF ANTIBIOTIC RESISTANCE OF BACTERIA OF *ENTEROBACTERIACEAE* GENERA ISOLATED FROM SHEEP RECTAL SWABS, TEAT AND MILK

Lukáš HLEBA, Miroslava KAČÁNIOVÁ, Jaroslav POCHOP

***Abstrakt:*** *Cieľom práce bolo zistiť antibiotickú rezistenciu čeľade Enterobacteriaceae izolovanej z rektálnych výterov oviec, ceckov a mlieka z konvenčného chovu zo Slovenska. Kultivácia prebiehala na selektívnom agare MacConkey určený pre čeľaď Enterobacteriaceae. Test antibiotickej citlivosti prebiehal na agare ISO sensitest agar s antibiotikami: Enrofloxacín (ENR 5), Tetracyklín (TE 30), Streptomycín (S 10) a Chloramphenicol (C 30). Pre identifikáciu druhov čeľade Enterobacteriaceae sme použili identifikačné agary (Chromogénny agar pre koliformné baktérie, Hajnov agar, XLD agar) a pre biochemickú identifikáciu ENTERO test 24. V našej štúdii sme zistili, že najvyššia rezistencia druhov čeľade Enterobacteriaceae bola (11,11 %) na Streptomycín a Chloramphenicol pri izolátoch z ceckov. Najvyššia rezistencia z izolátov mlieka bola v prípade Chloramphenicolu (5,88 %). V izolátoch z rektálnych výterov oviec bola najvyššia rezistencia čeľade Enterobacteriaceae na Tetracyklín (3,70 %). Vo všetkých izolátoch však prevládala vysoká citlivosť ku všetkých použitým antibiotikám. Najvyššiu citlivosť mala čeľaď Enterobacteriaceae na Enrofloxacín (100 %) zo všetkých izolátov. Pri Streptomycíne bola citlivosť 100 % v prípade izolátov z rektálnych výterov a mlieka. Pri Tetracyklíne bola rezistencia 100 % v prípade izolátov z ceckov a mlieka a pri Chloramphenicole bola citlivosť 100 % v prípade izolátov z rektálnych výterov. Z čeľade Enterobacteriaceae sme pomocou identifikačných agarov a biochemických testov zistili tieto druhy: Escherichia coli, ktorá bola rezistentná k Tetracyklínu a Chloramphenicolu, Serratia plymuthica, ktorá bola rezistentná k Chloramphenicolu, Citrobacter fruendii, ktorý bol rezistentný k Streptomycínu a Klebsiella pneumoniae , ktorá bola rezistentná k Chloramphenicolu. Z výsledkov vyplýva, že baktérie môžu prenášať svoje gény rezistencie nielen vertikálne ale aj horizontálne. Výsledky taktiež poukazujú na to, že gény pre antibiotickú rezistenciu neprechádzajú len tráviacim traktom, kde sa baktérie vyskytujú vo veľkej miere ale prechádzajú aj do finálnych produktov ako napr. mlieko. Na základe výsledkov môžeme konštatovať, že ľudský faktor zohráva významnú úlohu pri prenose génov rezistencie z človeka na zvieratá.*

***Kľúčové slová:*** *antibiotická rezistencia, Enterobacteriaceae, ovca, rektus, cecky, mlieko*

***Abstract:*** *The aim of this study was to determine antibiotic resistance of Enterobacteriaceae genera isolated from sheep rectal, teat and milk from conventional livestock from Slovakia. The cultivation was done on a selective agar for Enterobacteriaceae (MacConkey). Antibiotic susceptibility testing was done on the ISO sensitest agar with these antibiotics: Enrofloxacin (ENR 5), Tetracycline (TE 30), Streptomycin (S 10) and Chloramphenicol (C 30). For identification of strains of Enterobacteriaceae genera, we used identification agar (Chromogenic coliform agar, Triple Sugar Iron agar, XLD agar) and for biochemical identification ENTERO test 24. In our study, we determined that the highest resistance of Enterobacteriaceae strains was to Streptomycin and Chloramphenicol (11.11 %) in isolates from sheep teat. The highest resistance was to Chloramphenicol (5.88 %) from isolates of milk. In the isolates from rectal swabs of sheep was the highest resistance to Tetracycline (3.70 %). In the all isolates, however, there was a high susceptibility to all antibiotics used. The highest susceptibility has Enterobacteriaceae genera to Enrofloxacin (100 %) from to all of isolates. Susceptibility to Streptomycin was 100 % from the isolates of rectal swab and milk. To Tetracycline 100 % in the isolates from teat and milk and to Chloramphenicol was susceptibility 100 % from isolates of rectal swabs of sheep. From these genera, we identified by identifying agars and biochemical tests: Escherichia coli resistant to Tetracycline and Chloramphenicol, Serratia plymuthica resistant to Chloramphenicol, Citrobacter freundii resistant to Streptomycin and Klebsiella pneumonia resistant to Chloramphenicol. The results show that the bacteria can transfer resistance genes and their spread not only in vertical but also horizontal. Results also confirm that antibiotic resistance not only through the digestive tract of animals but also in their final product such as milk. It is also possible the results show that human factor plays important role in the transfer resistant genes from human to animal.*

***Key words:*** *antibiotic resistance, Enterobacteriaceae, sheep, rectal, teat, milk*

## INTRODUCTION

Antibiotic resistance is significant health, social and economic problem at this time. Antibiotic resistance of bacteria is biological risk, which it is increase morbidity and mortality of animal and human (EFSA, 2008). 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 a Yousef, 2002;





Kharazmi et al., 2002; Mc Mahon et al., 2007). The health safety of foods, including milk, it 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 which may be the cause of foodborne diseases that can endanger the health of the consumers (Bobková et al., 2008).

In our study, we followed antibiotic resistance of *Enterobacteriaceae* genera isolated from rectal of sheep, teat and milk from once conventional livestock from Slovakia.

## MATERIAL AND METHODS

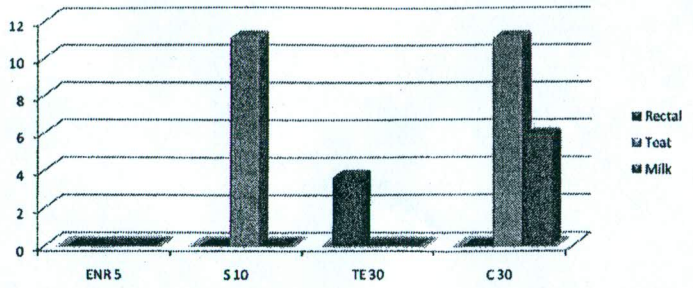
Antibiotics resistance study was done on *Enterobacteriaceae* genera isolated from rectal swabs of sheep, teat and milk. The bacterial strains were isolated from rectal swabs, teat, milk and collected with a kit containing the swab (Copan Inovation, Brescia) and the transport in medium to laboratory (Department of Microbiology, SUA in Nitra). For cultivation of *Enterobacteriaceae* Mac Conkey agar (Biomark, Pune) was used. Cultivation of these microorganisms was done during 24 hours for *Enterobacteriaceae* at 37 °C. The pure colonies were recultivation at the same conditions. The inoculum of *Enterobacteriaceae* was prepared by suspending of colonies from agar plates and the suspension was adjusted to equal a 0,5 McFarland standard. The sensitivity of all isolates was tested against: Streptomycin (S 10) 10 $\mu g.disk^{-1}$, Tetracycline (TE 30) 30 $\mu g.disk^{-1}$, Enrofloxacin (ENR 5) 5 $\mu g.disk^{-1}$ and Chloramphenicol (C 30) 30 $\mu g.disk^{-1}$. We used disk diffusion methods (according to EUCAST – European committee on antimicrobial susceptibility testing) (EUCAST). The incubation of strains with antibiotic disks was done on the ISO Sensitest agar (Biolife, Italiana) at the 37 °C. The interpretation of inhibition zones around the disk was according to BSAC version 8 January 2009 British Society for Antimicrobial Chemotherapy. The inhibition zones were controlled with reference strains *Escherichia coli* ATCC 25922. The basic differentiation of strains of *Enterobacteriaceae* genera was done on the identification agars like MacConkey agar (Biomark, Pune), Chromogenic coliform agar (Biolife, Italiana), Triple Sugar Iron agar (Biolife, Italiana) and LXD agar (Biolife, Italiana). Biochemical identification of strains of *Enterobacteriaceae* genera was done by ENTEROtest 24 (Pliva, Lachema). For the valuation of biochemical test we used identification program TNW Lite 7.0 (Pliva, Lachema).

## RESULTS AND DISCUSSION

We study antibiotic resistance in commensal *Enterobacteriaceae* genera, which are considered a potentional reservoirs of resistant genes in environment. This reservoirs of resistant bacteria provide a potentional source for resistant genes transfer between bacteria as well as an environment and food products. Therefore, identifying these reservoirs and mechanisms of persistance will be a key to reducing the load of resistant bacteria in everywhere.

In our study, we determined that the highest resistance of *Enterobacteriaceae* strains was to Streptomycin and Chloramphenicol (11.11%) from isolates of sheep teat. From the isolates of milk was the highest resistance to Chloramphenicol (5.88%). To the Tetracycline was (3.70%) the highest resistance of *Enterobacteriaceae* genera from isolates of rectal swabs of sheep. The susceptibility to Enrofloxacin was 100% to all of used antibiotics. To the Streptomycin was 100% susceptibility from isolates of rectal swabs and sheep milk. The susceptibility to Tetracycline was 100% from isolates of teat and milk and to Cloramphenicol was 100% only from rectal swabs of sheep. Results are shown in the fig. 1 and tab. 1.

Reported results Solomakos et al. (2009), which detected antibiotic resistant in the milk coming from the Greek. Their results show higher resistance of *E. coli* to Ampicillin and Chloramphenicol. Farzana et al. (2009) determined that in Indian milk resistance was 100% to Chloramphenicol. The similar results like Farzana et al. (2009) determined Dupont et al. (1978) in their work too. 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 milk have argued that the results of antibiotic resistance vary from study to study like our results.




**Legend:** ENR5 – Enrofloxacin, S 10 – Streptomycin, TE 30 – Tetracycline, C 30 – Cloramphenicol

**Figure 1** Antibiotic resistance profil isolates of *Enterobacteriaceae* from sheep rectal, teat and milk

**Table 1** Antibiotic resistance profil isolates of *Enterobacteriaceae* from sheep rectal, teat and milk

| Antibiotics/Isolates from | Sheep rectal | Sheep teat | Sheep milk |
|---|---|---|---|
| ENR 5 | 0,00 | 0,00 | 0,00 |
| S 10 | 0,00 | 11,11 | 0,00 |
| TE 30 | 3,70 | 0,00 | 0,00 |
| C 30 | 0,00 | 11,11 | 5,88 |

Of these samples isolated from rectal swabs, teat and sheep milk, we identified following strains: *Escherichia coli*, which was resistant to Tetracycline. *Serratia plymuthica*, which was resistant to Chloramphenicol. *Citrobacter freundii*, which was resistant to Streptomycin and *Klebsiella pneumoniae*, which was resistant to Chloramphenicol. In the tab. 2 are shown results of identification strains of *Enterobacteriaceae* genera in percentage.

**Table 2** Identified resistant strains and percentage of identification by ENTERO test 24

| Strains | Identification (%) | Resistance to |
|---|---|---|
| *Escherichia coli* | 100 | Tetracycline,Chloramphenicol |
| *Serratia plymuthica* | 100 | Chloramphenicol |
| *Citrobacter freundii* | 96,24 | Streptomycin |
| *Klebsiella pneumoniae* | 92,99 | Chloramphenicol |

## CONCLUSION

Use of antibiotics in livestock farming causes that more obligatory and facultative pathogens are resistant to various antibiotics used commercially. These pathogens microorganisms can causes many different infections or diseases, which are then difficult to treat. Our experiment results show that antibiotic were used in livestock farming or rearing were introduced into the external environment. The results show that the bacteria can transfer resistance genes and their spread not only in vertical but also horizontal. Results also confirm that antibiotic resistance not only through the digestive tract of animals but also in their final product such as milk. It is also possible the results show that human factor plays important role in the transfer resistant genes from human to animal.



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