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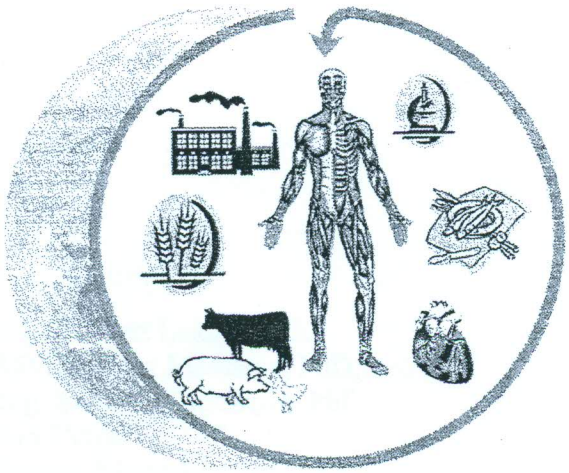

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ANTIBIOTIC RESISTANCE OF *ENTEROBACTERIACEAE* GENERA ISOLATED FROM UNPASTEURISED MILK

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Abstract

The aim of this study was to determine antibiotic resistance of *Enterobacteriaceae* genera isolated from milk from Slovakia. The cultivation was done on a selective agar for *Enterobacteriaceae* (MacConkey). Antibiotic susceptibility testing was done on the Muller-Hinton agar with these antibiotics: Ampicillin (AMP), Tetracycline (TE 30) and chloramphenicol (C 30). For identification of strains of *Enterobacteriaceae* genera, we used ENTERO test 24. In our study, we determined that the highest resistance of *Enterobacteriaceae* strains was to Tetracycline and Ampicillin (50 %). The lowest resistance was to Chloramphenicol (10 %). The highest susceptibility was to Chloramphenicol (90 %). The susceptibility to Tetracycline and Ampicillin was 50 %. From this genera, we identified *Serratia* spp. which was resitant to Ampicillin and Tetracycline. *Serratia plymuthica* which was resistant to Ampicillin. *Raoultella terrigena*, *Klebsiella pneumonae*, *Enterobacter aerogenes* which were 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. Milk is therefore necessary to pasteurize, because pasteurization is good for inactive of coliforms bacteria.

Key words: antibiotic resistance, *Enterobacteriaceae* genera, unpasteurised 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, 2008). Coliforms bacteria can not survive during pasteurization. If coliforms bacteria survive during pasteurization, it is indicate to the lack of pasteurization (Havlová, 1993).

In our study, we researched antibiotic resistance of *Enterobacteriaceae* genera isolated from unpasteurized milk from Slovakia.

Material and methods

Antibiotic resistance study was done on *Enterobacteriaceae* genera isolated from milk of Slovakia. The samples were taken from automat to fresh milk. The bacterial strains were isolated from milk swab and collected with a kit containing the swab (Copan Inovation, Brescia) and the transport in medium to laboratory. For cultivation of *Enterobacteriaceae* genera MacConkey agar was used. The pure bacterial strains of *Enterobacteriaceae* genera were prepared by suspending of colonies from agar plates and the suspension was adjusted to equal a 0.5 McFarland standard. Antibiotic susceptibility testing was done on Muller-Hinton agar. The sensitivity of all strains of *Enterobacteriaceae* genera was tested against: Ampicillin (AMP 10) 10 µg/disk, Tetracycline (TE 30) 30 µg/disk, and Chloramphenicol (C 30) 30 µg/disk. We used disk diffusion methods (according to CLSI – Clinical and Laboratory Standards Institute **(CLSI)**. The incubation of strains was done at the

temperature 37 °C per 24 hour. The interpretation of inhibition zones around the disk was according to EUCAST 2010 European Committee on Antimicrobial Susceptibility Testing (Table 1).

Table 1 Interpretation of inhibition zones for Enterobacteriaceae by EUCAST

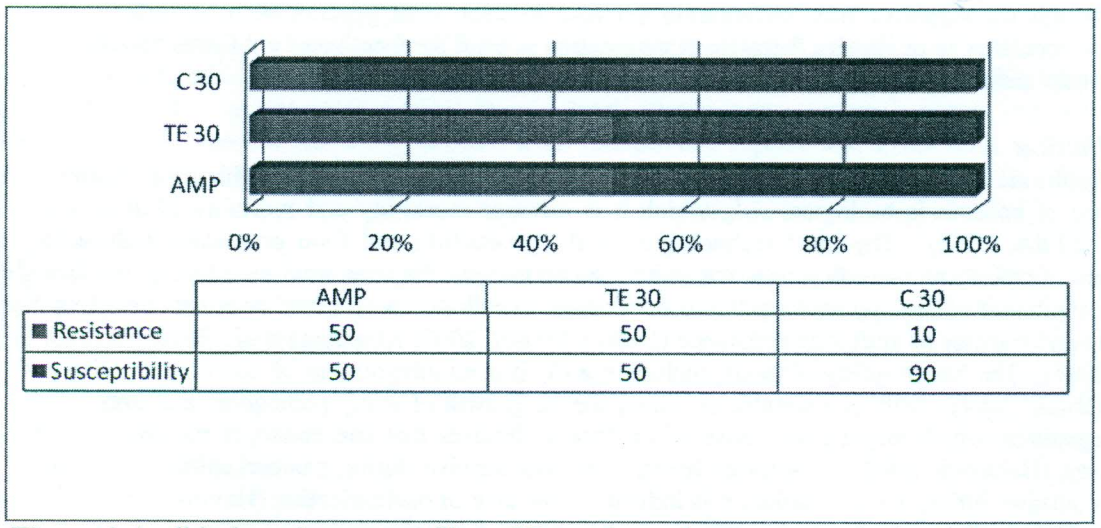
Enterobacteriaceae			
Antibiotics	**µg/disk**	**Susceptibility ≥**	**Resistance <**
Ampicillin	10	14	14
Tetracycline	30	19	14
Chloramphenicol	30	17	17

The inhibition zones were controlled with reference *Escherischia coli* ATCC 25922. Identification of strains from *Enterobacteriaceae* genera was done by ENTERO test 24 (Pliva, Lachema).

Results and discussion

We study antimicrobial drug resistance in commensal *Eneterobacteriaceae* genera, which are considered a potentional reservoir of resistant bacteria genes in environment. This reservoirs of resistant bacteria provide a potentional source for resistance gene 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 Tetracycline and Ampicillin (50 %). The lowest resistance was to Chloramphenicol (10 %). The highest susceptibility was to Chloramphenicol (90 %). The susceptibility to Tetracycline and Ampicillin was 50 %. The results can be show in Figure 1.



	AMP	TE 30	C 30
Resistance	50	50	10
Susceptibility	50	50	90

Figure 1 Antibiotic resistance profiles of Enterobacteriaceae genera isolated from milk

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.

In these samples isolated from milk, we identified following strains by ENTERO test 24: *Serratia plymuthica* (100 %), which was resitant to Ampicillin. *Serratia* spp. (100 %) which was multiresistant to Ampicillin and Tetracycline. *Raoultella terrigena* (82.79 %), *Klebsiella pneumonae* (10.91 %) and *Enterobacter aerogenes* (6.13 %), which were resistant to Chloramphenicol. Identified strains by ENTERO test 24 show Table 2.

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

Strains	Identification (%)	Resistance to
Serratia spp.	100	Ampicillin, Tetracycline
Serratia plymuthica	100	Ampicillin
Raoultella terrigena	82.79	Chloramphenicol
Klebsiella pneumonae	10.91	Chloramphenicol
Enterobacter aerogenes	6.13	Chloramphenicol

Conclusion

Use of antibiotics in livestock farming causes that more and more obligatory and facultative pathogens are resistant to various antibiotics used commercially. Our experiment results show that antibiotics were used in breeding or rearing were introduced into the external environment. Results confirm that antibiotic resistance not only through the digestive tract of animals but also in their final product such as milk. Unpasteurised milk contains coliforms bacteria. Milk is the end product, which is also used in human food chain. If coliforms bacteria are resistant undesirable reproduce, may cause consumers to infections and diseases, which are then difficult to treat. Milk is therefore necessary to pasteurize, because pasteurization is good for inactive of coliforms bacteria.

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