

**THE RESISTANCE TO ANTIBIOTICS IN STRAINS OF E. COLI AND ENTEROCOCCUS SP. ISOLATED FROM RECTAL SWABS OF LAMBS AND CALVES**

**REZISTENȚA LA ANTIBIOTICE A E. COLI ȘI ENTEROCOCCUS SP. IZOLAȚI DIN FECALILE DE LA MIEI ȘI VITEI**

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*The aim of this study was to determine the prevalence and antibiotic resistance of enterococci and E. coli strains isolated from dairy calves and lambs. Susceptibilities of isolated enterococci were tested using the disk diffusion method. The interpretation of inhibition zones around the disks was according to CLSI 2004 Performance standards for antimicrobial susceptibility testing. In our study, all isolates (E. coli and enterococci) were multiresistant (100%) to tetracycline, streptomycin and compound sulphonamides. Lower levels of resistance to enrofloxacin were noted. Antimicrobial resistance profiles of Enterococcus sp. isolated from lambs indicated that the highest percentage of susceptibility was exhibited to tetracycline (100%) and streptomycin (100%) and compound sulphonamides (100%). The intermediate resistance was exhibited against compound enrofloxacin (80%). The high frequencies of resistant isolates of Enterococcus sp. from calves were documented in tetracycline (100%), streptomycin (100%) and compound sulphonamides (100%) and enrofloxacin (50%). The high percentage (compound sulphonamides-100%, tetracycline-100% and streptomycin-100%) of multiresistant E. coli (isolates from dairy calves) was noticed. There were no significant correlations between groups.*

**Keywords** antibiotic resistance, E. coli, Enterococcus sp., lambs, calves

### **Introduction**

The resistance of microorganisms to antibiotics is considered to be the major international public health problem and involves the fields of both human and veterinary medicine. It has been widely demonstrated that the use of antibiotics in animals can lead to the selection of resistant strains that colonize the intestines and are subsequently excreted, which may lead to contamination of the environment and of meats destined for human consumption. The frequency of

multiresistant isolates from live animals or processed carcasses is closely related to the level of hygiene and potential previous antibiotic therapy of the slaughtered animals.

Enterococci and *Escherichia coli* are commensally organisms which are sometimes responsible for major infections. Enterococci belong to the endogenous flora of man and animals and are resistant to various antibiotics including cephalosporins, penicillinase-resistant penicilins and clinically available levels of lincosamides and aminoglykosides. Enterococci are important nosocomial pathogens; they have been implicated in infective endocarditis and urinary tract infections (van den Bogaard, 2002).

In the past few years, strains of *E. coli* have become increasingly resistant to most first-line antibiotics, including third-generation cephalosporins, aminoglycosides, and even fluoroquinolones. Infections caused by drug-resistant organisms are a major and costly problem in animal health. These infections prolong illness and, if not treated in time with more expensive, alternative antimicrobial agents, can cause loss of livestock (Witte, 2000).

The aim of this study was to determine the rates of antimicrobial resistance in strains of *E. coli* and *Enterococcus* sp. isolated from rectal swabs of dairy calves and lambs.

### Materials and Methods

The sensitivity study was done on enterococci and *E. coli* isolated from dairy calves and lambs from commercial farms. The examined animals had not been exposed to a direct influence of antibiotics. The strains were isolated from cloacal swabs collected with a kit containing the swab and the transport medium (Copan Innovation, Brescia). For cultivation of enterococci were used two selective media – Slanetz and Bartley medium and Pfizer Selective Enterococcus Agar (Biomark, Pune). For cultivation of *E. coli* McConkey Agar (Biomark, Pune) was used.

The inoculum of enterococcal and *E. coli* strains 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 enterococcal and *E. coli* isolates was tested against: tetracycline (TET) 300 µg/disk, enrofloxacin (ENR) 5 µg/disk, compound sulphonamides (S3) 300 µg/disk and streptomycin (S) 10 µg/disk. (according to the CLSI requirements) using the disk diffusion method (according to the Clinical and Laboratory Standards Institute requirements. The incubation of strains was done at the temperature 35°C. The interpretation of inhibition zones around the disks was according to CLSI 2004 Performance standards for antimicrobial susceptibility testing. The inhibition zones were controlled with the reference *Enterococcus faecalis* ATCC 29212 and *Escherichia coli* ATCC 25922.

## Results and Discussion

We studied antimicrobial drug resistance in commensal *E. coli* and enterococci isolates, which are considered a potential reservoir for resistance genes in farm animals. On-farm reservoirs of resistant bacteria provide a potential source for resistance gene transfer between bacteria as well as an environment for dissemination to new animals, environments, and food products. Therefore, identifying these reservoirs and mechanisms of persistence will be a key to reducing the load of resistant bacteria in commercial facilities.

In our study, all isolates (*E. coli* and *enterococci*) were multiresistant (100%) to tetracycline, streptomycin and compound sulphonamides. The lower levels of resistance to enrofloxacin were noted. There were no significant correlations between groups.

The resistance of *Enterococcus* sp. strains on chosen antibiotics is showed in Figure 1.

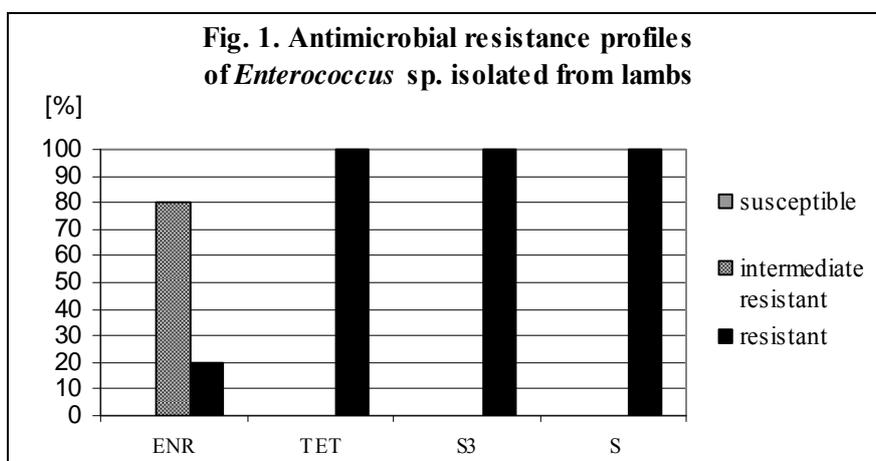


Fig.1. ENR – enrofloxacin, TET – tetracycline, S3 - compound sulphonamides, S – streptomycin

The data shown on the Fig. 1 (antimicrobial resistance profiles of *Enterococcus* sp. isolated from lambs) indicate that the highest percentage of susceptibility was exhibited to tetracycline (100%) and streptomycin (100%) and compound sulphonamides (100%). The intermediate resistance was exhibited against compound enrofloxacin (80%).

The resistance of the tested strains of *Enterococcus* sp. isolated from dairy calves is presented in Fig. 2. The higher frequencies of resistant isolates were documented in tetracycline (100%), streptomycin (100%) and compound sulphonamides (100%) and enrofloxacin (50%).

The high percentage of multiresistant bacteria isolated from young ruminants is unfavourable. The examined animals had not been exposed to a direct

influence of antibiotics but had been colonized after their birth with both bacterial microflora of their mothers and environmental microflora. We can see the threat of transmission of multiresistant strains on this example.

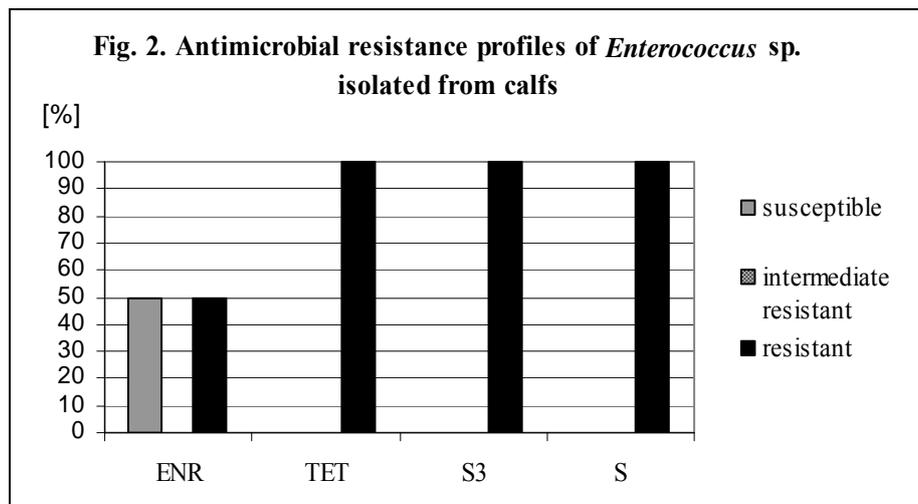


Fig.2. ENR – enrofloxacin, TET – tetracycline, S3 - compound sulphonamides, S – streptomycin

The data shown on the Fig. 3 (antimicrobial resistance profiles of *E. coli* isolated from dairy calves) indicate that the highest percentage of the resistance was exhibited against compound sulphonamides (100%), tetracycline (100%) and streptomycin (100%).

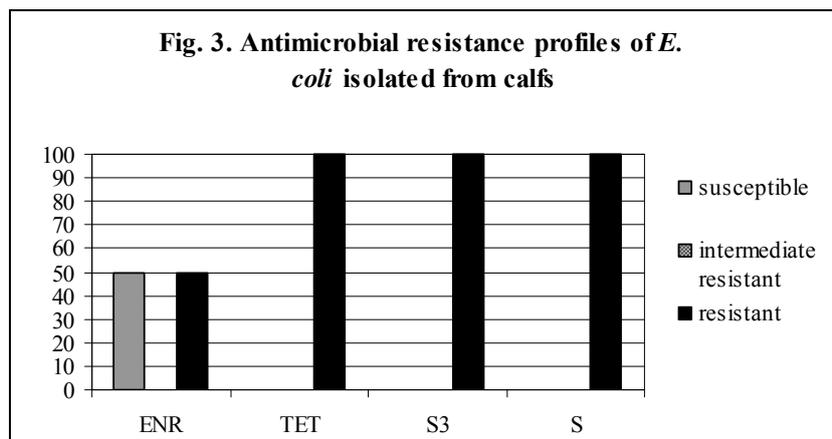


Fig.3. ENR – enrofloxacin, TET – tetracycline, S3 - compound sulphonamides, S – streptomycin

It has been reported that the selection and the maintenance of streptomycin-sulphonamides-tetracycline-resistant *Escherichia coli* may be due to environmental components independent of antibiotics selection (Khachatryan, 2006).

The importance of cattle and calves as potential sources of *E. coli* for man will depend on the load and frequency of excretion of these organisms. From the longitudinal studies described it would appear that *E. coli* is not regularly present in the faeces of adult cows and in the case of its occurrence is present in relatively low numbers. Long periods of time often occurred when no *E. coli* could be detected (tejto vete nerozumiem!). In contrast, the calves regularly excreted *E. coli* in high numbers. The importance of this observation, in the context of the potential sources of drug resistant strains for man, is enhanced by the additional evidence that antibiotic resistance was found frequently in *E. coli* from the calves (Hove, 2006).

### Conclusion

Antibiotic-resistant pathogens in animals are a problem not only with respect to the health of animals but because of possible transmission to humans as food-borne pathogens. The problem is compounded by the growing number of pathogens that are resistant to multiple, structurally unrelated drugs, leading to the concern that there are likely to be few effective antimicrobials available by the end of the decade. Accordingly, more attention is now being paid to the ease with which resistance to both single and multiple antimicrobials can develop among bacterial pathogens. If the current trends continue, we may see bacterial pathogens that are resistant to all currently available antimicrobials.

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