The Prevalence of Esbl-Producing Strains of *E.coli*, Isolated from Calves with Colibacillosis - Preliminary Remarks

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Abstract

The animals producing food have become an increasing reservoir of extended spectrum beta-lactamase producing *Enterobacteriaceae*. The calves and cows are exposed to a greater quantity of antibiotics, but the data concerning the prevalence of ESBL-producing *Enterobacteriaceae* are not enough, in comparison with other species of animals used for human consumption, such as birds (Hordijk *et al.*, 2013).

The aim of this study was to determine the prevalence of ESBL-producing *E. coli* involved in some episodes of colibacillosis in calves. Faeces samples were collected from 33 calves with the age ranging between 1-2 weeks and that presented clinical signs of colibacillosis. The samples were collected in a sterile medium for the taxonomic isolation and identification of the etiological agent involved, the ESBL screening being conducted subsequently using the ESBL Agar Oxoid Brilliance chromogenic medium. The phenotypic confirmation of the ESBL-producing strains was conducted in accordance with the CLSI (2014) standard through the combined disc method. Following the tests conducted, out of the 33 strains of isolated *E. coli*, 9 (27, 27%) were phenotypically confirmed as being ESBL strains.

The studies that were previously conducted on the dairy farms have pointed out that the young calves rapidly acquire bacterial strains resistant to antibiotics that are often ESBL strains (Hordijk *et al.*, 2013). The prevalence obtained by us, as well as an insufficient quantity of information concerning the antimicrobial resistance on this segment of species of animals used for the human consumption, support conducting a more thorough study, as well as the identification of ESBL resistance genes, but also of the plasmids that encode the transmission of these genes.

Keywords: calves, ESBL, *Escherichia coli*, antibiorezistance

INTRODUCTION

The prevalence of the bacterial strains which produce Beta – lactamases with a wide spectrum is continuously growing, data regarding the antimicrobial resistance through this mechanism being frequently reported worldwide (Ewers *et al.*, 2012). The presence of the ESBL positive *Enterobacteriaceae* in the bacterial infections encumber the treatment of these not only in the veterinary medicine but also in the human medicine. In the veterinary medicine,

Enterobacteriaceae that produce ESBL were isolated not only from the faeces of the pets (dogs and cats) but also from the commercial animals, these being considered a reservoir of bacteria that produce ESBL (Cozma *et al.*, 2015; Măciucă *et al.*, 2015). The calves and cows are exposed to the treatment with antibiotics either in a curative way, or sometimes in a prophylactic purpose. If in other countries data is known regarding the prevalence of the positive isolated ESBL strains from different animal species, Romania offers limited data from

Fig. 1

this point of view (Hordijk *et al.*, 2013; Haenni *et al.*, 2014; Duse *et al.*, 2015).

AIMS

The present study is a pilot study and has as a main purpose the establishing of the prevalence of the *Escherichia coli* ESBL of positive isolated strains from colibacillosis episodes appeared at a series of calves from within a farm in the North – Eastern part of Romania.

MATERIALS AND METHODS

In the period of time February – April 2016 there were collected 33 faeces samples from the calves aged between 1-2 weeks and which presented clinical signs of colibacillosis. The samples were collected in sterile recipients and processed in accordance with the work protocol considering the isolation and the identification of the etiological involved agent, the completion of the ESBL screening and finally considering the phenotypic confirmation of ESBL bacterial obtained strains.

After the collecting the samples they were inoculated on nutrient broth and incubated at 37°C for 24 h. 100 µl in a liquid medium and they were used for the execution of the ESBL screening on chromogen solid medium Oxoid Brilliance ESBL Agar supplemented with Cefpodoxime (Cozma *et al.*, 2015). The identification and taxonomic framing of the obtained colonies on the previously mentioned environment was achieved on the basis

of some minimal biochemical characters by using MIU and TSI medium.

The phenotypical confirmation of *E.coli* bacteria ESBL isolated strains was achieved through the method of combined disks recommended by the CLSI (2014) standard. There were used microcomprimates (Oxoid) of cefotaxime (30µg), cefotaxime/Clavulanic acid (30/10 µg), Cefotaxime (30µg), Cefotaxime/Clavulanic acid (30/10µg), Cefpodoxime (10ug) and Cefpodoxime/Clavulanic acid (10/10µg). As a quality control for the sensitivity test at antibiotics it was used the strain *E.coli* ATCC 25922.

RESULTS AND DISCUSSION

After the processing of data and of the results obtained it was determined the prevalence which is itself a statistic indicator.

From those 33 of *E.coli* obtained strains after the primary processing of the samples, 9 (27,27%) generated cultures on Oxoid Brilliance ESBL Agar environment. After the achievement of the method of the combined disks all those 9 strains were confirmed in a phenotypical way as being ESBL, by generating a prevalence of 27,27% of *E.coli* ESBL positive strains (Fig. 1).

The prevalence obtained by us is supported if we take into consideration the prophylactic or curative administration of the antibiotics in cows that conceived calves and their feeding with milk, without respecting the waiting period recommended by the antibiotics producer, this

being a favorable factor in transmission of these resistance genes from cows to calves.

Similar studies were also carried out in some other countries from the European Union. For example, in Germany, Schmid et al. in a study carried out in the year of 2013 they highlighted a prevalence of 56,2% of *E.coli* ESBL positive isolated strains in the faeces derived from calves, followed by the prevalence obtained for the cows of 41,1% and of 21,4% for the cows of beef (Schmid et al., 2013). In France, in the year of 2014, Haenni et al., signalled a prevalence of 29, 4% of the calves which produced *E.coli* ESBL positive strains in the faecal flora (Haenni et al, 2014).

CONCLUSION

The analysis of those 33 of the collected samples from the calves with colibacillosis, led to the identification of a prevalence of 27, 27% of *E.coli* strains that produce ESBL in the population taken into the study.

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