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Co-occurrence of extended spectrum beta- lactamase genes and mcr-1 gene in strains of Escherichia coli isolated from clinical cases of bovine mastitis in Greece

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Background: Clinical bovine mastitis triggered by *Escherichia coli* is costly disease with clinical signs that vary from mild mastitis to very severe or even fatal forms. The disease has also a potential threat to public health since cow's milk infected with enterobacteria may represent vector of antibiotic resistance genes such as extended spectrum β-lactamase genes and *mcr*-1. Isolates of *E. coli*, therefore, harvested from animals, foodstuff, and human beings that expose co-resistance to b-lactam antibiotics and to colistin are globally reported. The prevalence of such microorganisms is poorly studied in Greece. This research investigates the prevalence of extended spectrum β-lactamase genes and *mcr*-1 gene in *E.coli* isolates harvested from mastitic milk samples in dairy cows

Material/methods: Four hundred samples of mastitic milk from equal number of cows were enrolled during 2015-2016. Fifty μl of each sample was plated on TBX agar. After incubation at 44° C for 24–48 h, plates were inspected for colonies growth. One β-glucuronidase positive colony per plate was purified on nutrient agar and further identified as *E. coli* by API 20E identification strips. Species identification of the isolates was confirmed by polymerase chain reaction that detects *usp*(A) gene, which is characteristic for *E. coli* strains. Antimicrobial susceptibility tests were done on Mueller-Hinton agar using Kirby Bauer disk diffusion method. The antimicrobial agents tested were: nalidixic acid (30

μg), ciprofloxacin (5 μg), doxycycline (30 μg) tetracycline (30 μg), gentamicin (10 μg), trimethoprime & sulfomethoxazole (1.75/23.75 μg), streptomycin (10 μg), ceftriaxone (30 μg), cephotaxime (30 μg), ceftazidime (30 μg), amoxicillin-clavulanic acid (10/20 μg), aztreonam (30 μg), chloramphenicol (30 μg),) ampicillin (10 μg), and piperacillin (100 μg). Resistance data were interpreted according to CLSI guidelines (M100-21/2014). MICs of colistin were obtained on Mueller-Hinton agar by the agar dilution method in antibiotic concentrations ranging from 0.25 to 128 μg/ml and inoculum of 104 colony-forming units per spot. PCR/DNA sequencing was used to characterize *bla* genes and *mcr-1* gene

Results: The overall prevalence of *E.coli* isolated from cows' mastitic milk was 22% (89/400). From the 89 *E. coli* isolates that were tested 18 exposed co-resistance to ampicillin, piperacillin, amoxicillin-clavulanic acid, ceftriaxone, cefotaxime, ceftazidime, and aztreonam. All 18 isolates were further characterized by PCR as ESBL producers (16 CTX-M and 2 SHV). Interestingly MICs against colistin of 6 of the 18 ESBL producers ranged from 8 μg/ml to 32 μg/ml. Finally these 6 strains carried the *mcr*-1 resistance gene.

Conclusions: This the first report of the co-occurrence of extended spectrum β-lactamase genes and *mcr*-1 gene in strains of *E. coli* isolated from clinical cases of bovine mastitis in Greece. Finally these findings are also important in relation to the use of b-lactam antibiotics for the treatment of bovine clinical mastitis.