

P1364 Intestinal colonisation of Portuguese sheep with *Escherichia coli* producing CTX-M and MCR-1Josman Palmeira¹, Marisa Haenni², Jean-Yves Madec², Helena Maria Neto Ferreira^{*1,3}¹ University of Porto, Faculty of Pharmacy, Porto, Portugal, ² Anses Lyon, Lyon, France, ³ Faculty of Pharmacy, Microbiology, University of Porto, Porto, Portugal

Background: Food animal intestinal colonization with MDR threats including *mcr-1* and *bla*_{CTX-M} is an emerging "one health" problem around the world, including sheep production. This is the first report of *mcr-1*-positive and CTX-M-producing *Escherichia coli* isolated from sheep, worldwide.

Materials/methods: Fecal samples of sheep from a farm in the south of Portugal were screened for ESBL production and *mcr-1*. Selection of the isolates was performed on MacConkey agar with different antibiotics (oxymino-beta-lactams, tetracycline and ciprofloxacin). Twenty-one fecal samples were previously incubated in TSB. Susceptibility-testing was achieved by disk-diffusion method according to CLSI. Colistin MIC determination was accessed by broth-microdilution. Presumptive identification of isolates was performed with CHROMagar Orientation. Extended-spectrum beta-lactamase (ESBL) producers were screened by the double disk synergy test and PCR with specific primers *bla*_{TEM}, *bla*_{OXA} and *bla*_{SHV}, *bla*_{CTX-M} and *mcr-1*. Plasmid content was determined by PBRT kit (DIATHEVA). PFGE of ESBL-producers was performed using *Xba*I restriction enzyme.

Results: One hundred and sixty five antibiotic resistant *Enterobacteriaceae* isolates were obtained with different antibiotic selection. Showing resistance to amoxicillin (140/165), cefotaxime (108/165), ceftazidime (55/165), tetracycline (108/165), amoxicillin+clavulanic acid (100/165), ciprofloxacin (46/165), gentamicin (19/165), chloramphenicol (29/165) and sulfamethoxazole+trimethoprim (70/165). A total of 111 isolates showed an MDR phenotype. Sixty-two selected isolates were ESBL-producing *E. coli*, those showing a different beta-lactamase profile (*bla*_{CTX-M-1}, *bla*_{CTX-M-14}, *bla*_{CTX-M-15}, *bla*_{CTX-M-32}, *bla*_{CTX-M-98} and *bla*_{SHV-12}), and a different not beta-lactam antibiotic resistance background (including *aac6'lb-cr* positive). Most of the CTX-M-15 producers were positive for IncFIB. One CTX-M-1-producing *E. coli* isolate was positive for *mcr-1* gene, showing colistin MIC of 4mg/mL. PFGE profile of ESBL-producing *E. coli* showed different isolates with clonal relation, including a clone present in 14/21 animals.

Conclusions: This single farm showed different clones of ESBL-producing *E. coli* harboring CTX-M what highlights the dissemination of resistance genes in intestinal microbiota of sheep. Detection of *mcr-1* gene, alert us for the fast and quiet spread of this human-threat. Farm animals act as reservoirs of multi-resistant microorganisms relevant in terms of public-health that transcends animal health, it is a vital matter to therapeutic efficiency and it fits well in the concept of "One Health".