



Antibiotic Resistance in *Escherichia coli* Isolates from Poultry, Swine and Bovine Meat, Portugal (2016-2017)

Laura Moura^{1,2}, Vanessa Guerra^{1,3}, Ivone Correia¹, Teresa Albuquerque¹, Ana Botelho¹, Ana Amaro¹, Lurdes Clemente^{1,4}

¹National Reference Laboratory of Animal Health, Laboratory of Bacteriology and Mycology, National Institute of Agrarian and Veterinary Research; ²Lisbon University, Faculty of Pharmacy, Department of Toxicological and Bromatological Sciences; ³Lisbon University, Faculty of Science, Department of Plant Biology; ⁴CIISA- Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Science

INTRODUCTION

Antibiotic resistant bacteria are a public health concern worldwide. Food-producing animals, like bovine, swine and poultry, are considered large reservoirs of multidrug-resistant bacteria (MDR). *Escherichia coli* is a natural colonizer of the gut micro-flora of most warm-blooded species and has been associated with antibiotic resistance (ABR), namely to critically important antibiotics. One of the most important approaches to control the spread of ABR is to understand the molecular mechanisms behind this phenomenon.

AIM OF STUDY: To characterize the molecular mechanisms involved in resistance to third-generation cephalosporins and/or cephamycins, quinolones and colistin, in *E. coli* isolates from bovine, swine and poultry retail meat.

MATERIALS AND METHODS

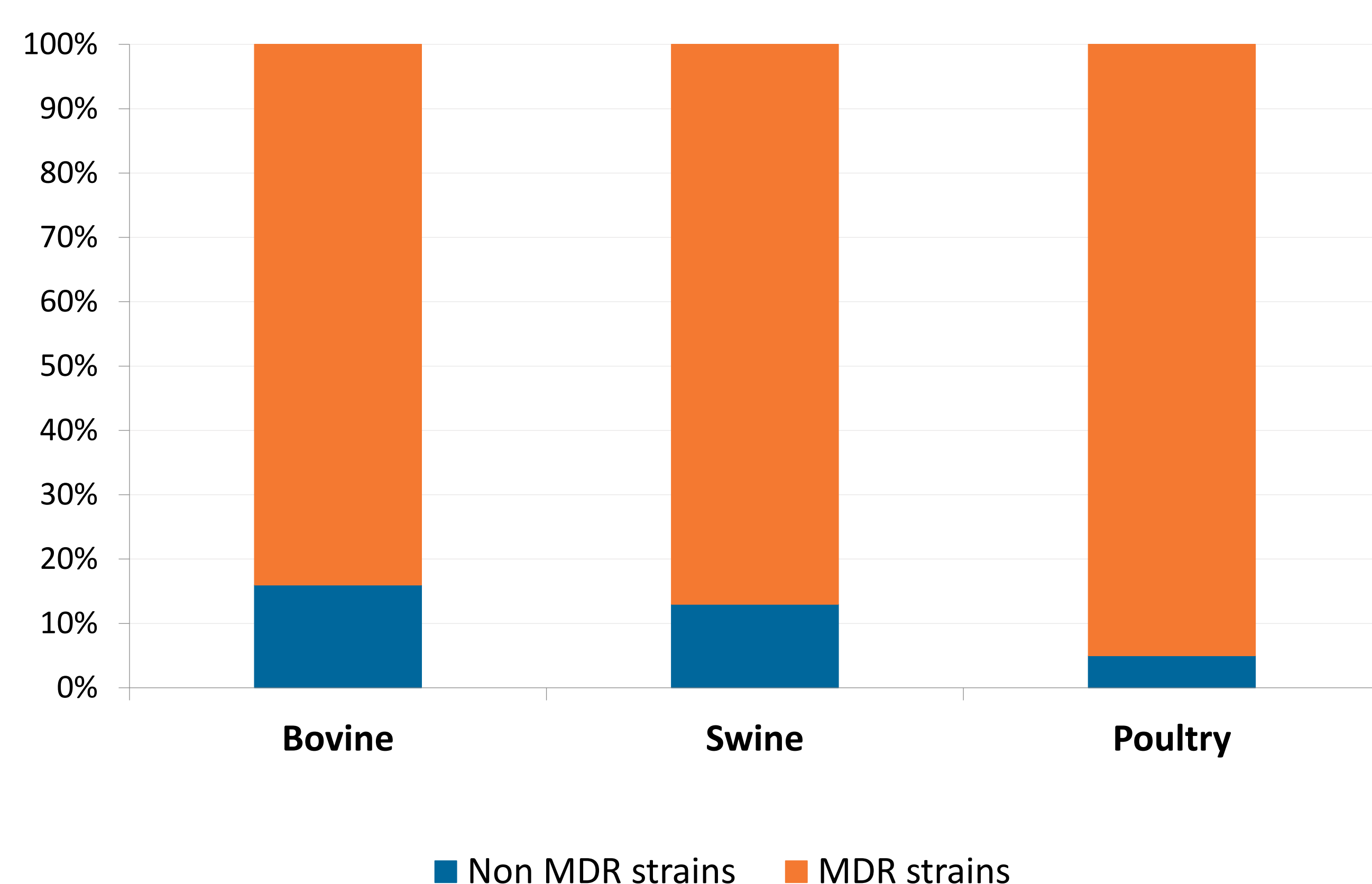
We analysed 109 *E. coli* strains with reduced susceptibility to cephalosporins and cephamycins isolated from bovine ($n=26$), swine ($n=23$) and poultry ($n=60$) retail meat in 2016 and 2017.

Antimicrobial susceptibility testing and Minimum Inhibitory Concentrations (MIC) were performed using EUVSEC[®] and EUVSEC2[®] microplates. Results were interpreted according to EUCAST epidemiological breakpoints¹.

Extended spectrum β -lactamases (ESBLs), plasmid-mediated AmpC β -lactamases (PMA β), plasmid-mediated quinolone and colistin resistance (PMQR; PMCR)-encoding genes and integrons, class 1, 2 and 3, were screened by multiplex polymerase chain reaction (mPCR)²⁻⁶.

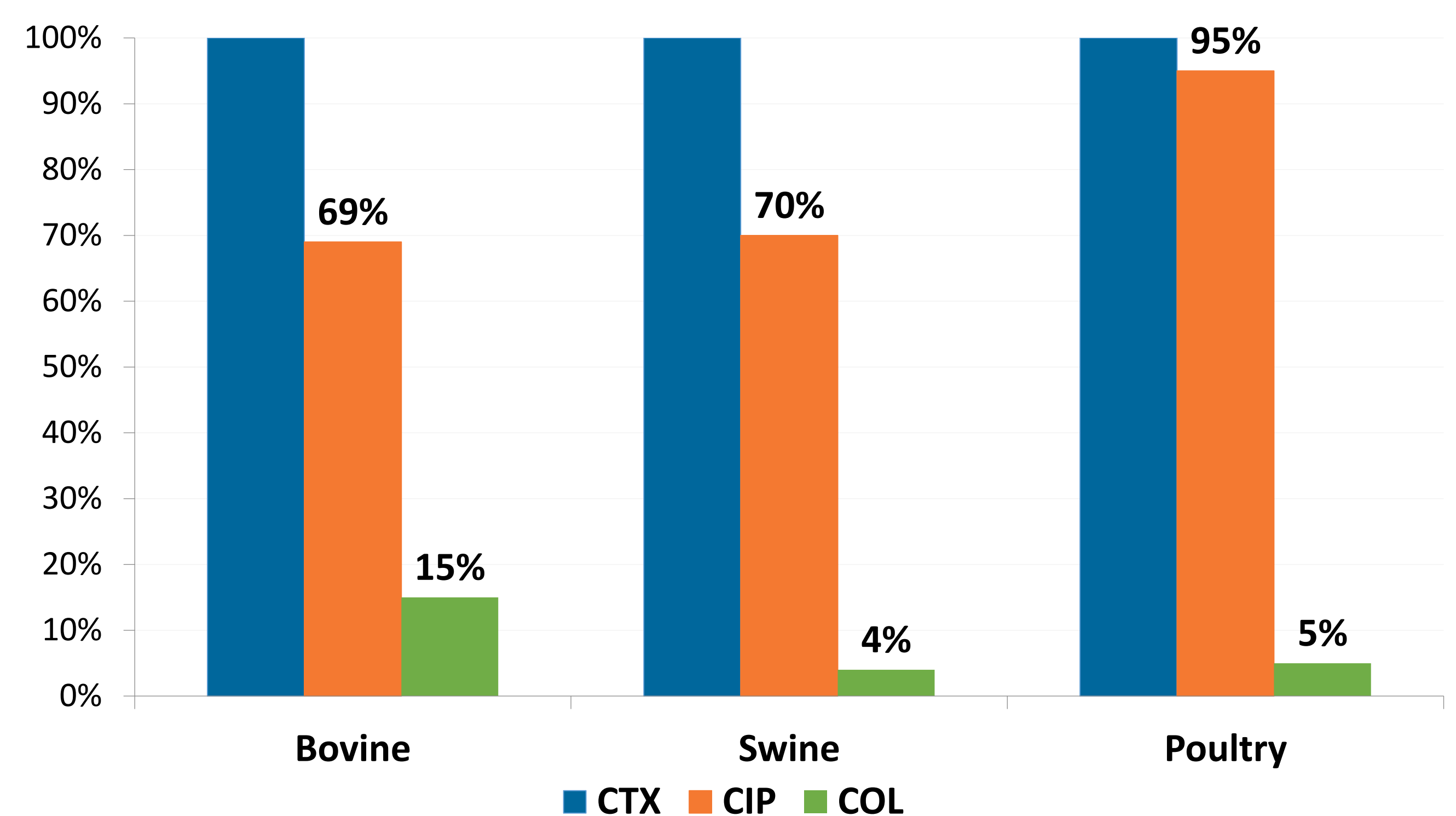
RESULTS

Results of antimicrobial susceptibility profiles revealed a high percentage of multidrug-resistant (MDR) strains in bovine (84%), swine (87%) and poultry (95%), as shown in Graph 1.



Graph 1 Prevalence of multidrug resistant (MDR) *E. coli* strains

Apart of cefotaxime (CTX) resistance, these strains also showed decreased susceptibility to other critically important antibiotics, namely ciprofloxacin (CIP) and colistin (COL) (Graph 2).



Graph 2 Prevalence of *E. coli* strains resistant to critically important antibiotics: cefotaxime (CTX); ciprofloxacin (CIP); and colistin (COL)

MDR strains detected phenotypically were further characterized at molecular level and the following determinants were detected: *bla*_{CTX-M-1-type}, *bla*_{CTX-M-9-type}, *bla*_{SHV-type}, *bla*_{TEM-type}, *bla*_{OXA-type}, *bla*_{CMY-type}, *qnrB*, *aac(6')-Ib* and *mcr-1* genes. Mobile genetic elements, Class 1 and Class 2 integrons were present alone or in association. Class 3 integron was not found. Results are shown in Table 1.

Table 1 Prevalence of ESBL, PMA β , PMQR and PMCR encoding-genes and integrons

Molecular mechanism	Resistance encoding-genes	Bovine (n)	Swine (n)	Poultry (n)	Total isolates (n)
ESBL	<i>bla</i> _{CTX-M-1-group}	11	12	21	44
	<i>bla</i> _{CTX-M-9-group}	8	6	4	18
	<i>bla</i> _{SHV-type}	3	0	17	20
	<i>bla</i> _{TEM-type}	3	0	17	20
	<i>bla</i> _{OXA-type}	0	0	1	1
PMA β	<i>bla</i> _{CMY-type}	4	4	1	9
PMQR	<i>qnrB</i>	1	2	12	15
	<i>aac(6')-Ib</i>	4	1	0	5
PMCR	<i>mcr-1</i>	3	1	3	7
Mobile genetic elements					
Integrons	Class 1	17	13	46	76
	Class 2	3	3	4	10

FINAL REMARKS

- ❑ High prevalence of multidrug resistant *E. coli* strains from retail meat particularly in poultry,
- ❑ The food chain is an important reservoir of isolates carrying ESBL/PMA β , PMQR and PMCR-encoding genes, which might be transmissible to humans, and a potential source for human pathogens to acquire these resistance genes,
- ❑ The spread of resistance encoding-genes is eminent and requires a multisectorial One Health approach to minimize its impact.

REFERENCES

1. EUCAST – website: www.eucast.org
2. Dallenne et al. (2010) *Journal Antimicrobial Chemotherapy*, 65(3), 490-95
3. Ciesielczuk et al. (2013) *Journal of Medical Microbiology*, 62(12), 1823-1827
4. DTU, National Food Institute – EURL-AR-2018
5. Kargar et al. (2014) *Osong Public Health Res Perspect*, 5(4), 193-198
6. Machado et al. (2005) *Antimicrobial Agents Chemotherapy*, 49, 1823–1829

ACKNOWLEDGEMENTS

This research was partly funded by the FCT grant number PTDC/CVT-CVT/28469/2017 – CIAinVET, and partly EU funded through the National Surveillance Program of Antimicrobial Resistance. Assistance to 1st ASM was made possible due to OHEJP consortium – WP1 funds.