OBSERVATIONS ON SENSITIVITY AND *AMR* OF *E.coli* IN PIGS AND HUMANS

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Abstract

Antimicrobial resistance of pathogenic *E. coli* impacts the swine industry due to the limited treatment options and growing public health concerns caused by potential transfer of antimicrobial resistance genes into the food chain. The study was carried out throughout the whole year; the samples were collected from the small intestine following necropsies performed in young pigs. From a laboratory database, 78 samples were positive for *E. coli* and were sampled and processed in order to describe the AMR following a classical microbiological exam. Each sample was cultured on selective media (Mac Conkey Agar) and antibiograms were performed using MicroScan Walk Away System. The antibiogram examinations were performed for 20 antibiotics and the results were presented in the form of the following categories: resistant, intermediate, susceptible. In 4 of the 20 antibiotics tested, the bacterial agent showed a sensitivity of over 85% and in 6 of the 20 antibiotics, it showed resistance of over 85%. On average, 47.8±37% of *E. coli* showed sensitivity to the 20 antibiotics tested and 48.5±38% resistance; 3.2±4% were classified as "intermediate". Similarities were noted in terms of sensitivity and resistance between the antibiograms from animal *E.coli* versus human *E.coli*. The same sensitivity was observed in 5 of the 20 antibiotics, and in 3 of the 20 antibiotics we noticed common resistance, but a future molecular biology analysis will be performed in order to identify the genes associated with AMR.

Key words: *antibiograms, E. coli, pig industry, AMR*

Introduction

Escherichia coli produces a series of diseases among pigs, generating serious losses both at the farm level and through possible transmission to humans. Swine colibacillosis is a multifactorial syndrome caused by *Escherichia coli* which exhibits three main disease conditions, namely, oedema disease, neonatal diarrhoea and post-weaning diarrhoea. Each of them can be differentiated through their pathogenesis, age-range of the affected animals, and the involved pathotype: Shiga toxin–producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), and atypical enteropathogenic *E. coli* (aEPEC). *(García-Meniñoet et a, 2020; Abubakar R. H. et al, 2017; Fairbrother J. M. et al, 2012, Moga et al. 2001)*

Antimicrobial resistance of pathogenic *E. coli* impacts the swine industry because of limited treatment options and growing public health concerns due to the potential transfer of antimicrobial resistance genes into the food chain. *(Fairbrother J. M. et al, 2012; MISUMI W. et al, 2021; Borow et al, 2022)*

The aim of the research was to describe in a transversal survey the sensitivity and resistance of *E. coli* to 20 types of antibiotics. The similarity between human and animal *E. coli* cases was also observed

Materials and methods

Animals sampling: The farm – a unit specialised in pig farming with a closed circuit. By clinical anamnesis of young pigs (aged between 4 and 11 weeks), the cadavers were selected for necropsy. Only cases with morphopathological signs were selected for microbiological exam. The samples were taken from the small intestine after the necropsy examination.

One sample was taken from an employee of the farm (who had direct contact with animals). The urine sample was taken because of their very frequent urinary infections, and then processed at

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an accredited laboratory. The result of the analysis was the infection with *E. coli*, which is the reason for some differences observed in the results of antibiograms (see table 1 - 77 vs. 78 results).

Microbiology analysis by *COPAN's ESwab™* system was conducted for all collected samples. The samples were sent and processed in the laboratory 12 hours after sampling. The germs were isolated by classical microbiological exam. Each sample was cultured on selective media (*Mac Conkey Agar - figure 1*), and after, placed in a thermostat for 24 hours for incubation. From the resulting culture, after identifying and confirming the existence of the bacteria by macroscopic examination, antibiograms were performed. The types of germs and antimicrobial sensitivity and resistance were analysed by *Walk Away System (figure 2)* using *MicroScan® Dried Panels – type NBC-42. (Cubin et al, 2022, Lungu, 2020)*

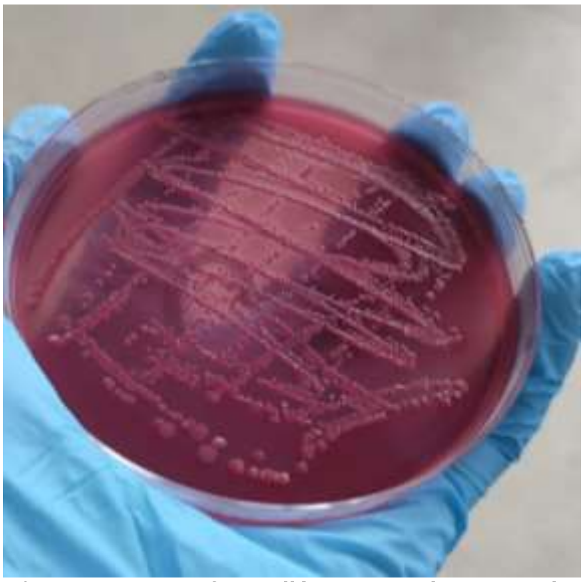

Figure 1 **Cuture of *E. coli* in *Mac Conkey Agar* plate**

Figure 2 **Entering data into the system and issuing the barcode for each sample**

Panels for human use were used because the transmission of the AMR phenomenon from animals to humans was assessed. Part of the bacterial culture obtained was stored in the freezer for subsequent genetic determinations. The information from the antibiograms was printed, recorded and processed. The antibiogram analyses were performed for 30 antibiotics, however only the results for 20 antibiotics were used in the present manuscript.

Statistical analysis - From a laboratory database, 78 samples positive to *E.coli* were chosen and processed in order to describe the AMR following a classical microbiological exam, and the microbiological cut-off of 20 from 30 antibiotics in following categories: resistant (R), intermediate (I), susceptible (S).

Results and discussion

The output was printed and analysed. After centralising the data obtained from *Walk Away System* (antibiograms) there results are shown in the table 1:

On average, 47.8±37% of *E. coli* showed sensitivity to the 20 antibiotics tested and 48.5±38% resistance; 3.2±4% was classified as "intermediate". Practically, for half of the antibiotics, resistance can be discussed and for the other half, sensitivity, with both classes having a rather large variability of 37-38%.

Considering the results obtained in the antibiograms (table 1), we can observe the following:

- the bacteria show sensitivity in 78/78 (100%) antibiograms to Amikacin and Meropenem, in 69/78 (88,5%) antibiograms to Pip/Tazo and in 74/77 (96,1%) antibiograms to Tigecycline.
- *E. coli* shows resistance in 76/78 (97,4%) antibiograms to Ampicillin, in 73/78 (93,5%) antibiograms to Ciprofloxacin, in 72/78 (92,3%) antibiograms to Levofloxacin, in 75/77 (97,4%) antibiograms to Mezlocillin, in 77/77 (100%) antibiograms to Moxifloxacin, in 67/78 (85,9%) antibiograms to Trimeth/Sulfa, in 58/78 (74,35%) antibiograms to Piperacillin and in 52/77 (67,5%) antibiograms to Tetracycline.

Table 1

Pharmacological and Microbiological cut-off of *E.coli* for 20 antibiotics

Antibiotic and pharmacological cut-off(*mg/l*)	**Microbiological classes**		
	Susceptible	**Intermediate**	**Resistant**
Amikacin (S≤16)	78	0	0
Amox/K Clav(S<=8/4;I 16/8; R>16/8)	53	14	11
Ampicillin(I 16; R>16)	0	2	76
Cefuroxime(S<=4; I 16; R>16)	33	2	43
Cefazolin(S<=8; I 16; R>16)	29	8	40
Cefepime(S<=8; R>16)	52	0	25
Cefoxitin(S<=8; R>8)	61	0	16
Ciprofloxacin(S<=2; I 2; R>2)	1	4	73
Fosfomycin(S<=32; R>32)	75	0	3
Gentamicin(S<=4; I 8; R>8)	43	4	31
Levofloxacin(S<=2; I 4; R>4)	3	3	72
Meropenem(S<=1)	78	0	0
Mezlocillin(I 64; R>64)	0	2	75
Moxifloxacin(R>1)	0	0	77
Pip/Tazo(S<=16; I 64; R>64)	69	5	4
Piperacillin(S<=16; I 64; R>64)	18	2	58
Tetracycline(S<=4; R>8)	25	0	52
Tigecycline(S<=1; I 2)	74	3	0
Tobramycin(S<=4; I 8; R>8)	42	1	34
Trimeth/Sulfa(S<=2/38; R>2/38)	11	0	67
Averages(n/78)	**47,8%**	**3,2%**	**48,5%**
Standard Deviation	**37%**	**4%**	**38%**

Similarities were noted in terms of sensitivity and resistance between the antibiograms from animal *E. coli* versus human *E. coli*. The same sensitivity was observed for Amikacin, Amox/K Clav, Meropenem, Pip/Tazo. There was also noted a common resistance to Ampicillin, Piperacillin and Tetracycline.

Antibiotics such as Florfenicol, Marbofloxacin, Enrofloxacin, Amoxicillin, Penicillin, Lincomycin and Tylosin are largely used in the pig industry. They are used both in individual and collective treatments, both *per os* and administered parenterally.

In 2021, Wang et al. studied the mechanisms of Tigecycline resistance in *Enterobacteriaceae* from a pig farm in China. The results of the study show that although tigecycline has not been approved in animals, tigecycline resistance mediated by the *tet*(A) variant and *tet*(X) via plasmid or ICE was observed on this pig farm. The use of tetracyclines such as doxycycline, one of the most used antimicrobial agents in food-producing animals in China, may be the reason for the emergence and transmission of tigecycline resistance. Appropriate measures should be taken to limit tigecycline resistance in animals. (Wang J. et al, 2021)

In 2021, Gruel et al. have tried to understand antimicrobial use in pigs, beef cattle, and poultry on farms on Guadeloupe, French West Indies, and to acquire data on AMR in *Escherichia coli* in these food-producing animals. This study provided the first baseline information on levels of antimicrobial use, on the dynamics of phenotypic and genotypic resistance to tetracyclines, and on ESBL- *E. coli* in small-scale pig, beef cattle, and poultry production on Guadeloupe. Despite rational use of antimicrobials, *E. coli* resistance to third-generation cephalosporins was found on these farms. Mechanisms other than selective pressure of these antimicrobials in the emergence of AMR remain to be elucidated. (Gruel G. et al, 2021)

In 2018, Lugsomyaet al. studied antimicrobial resistance (AMR) profiles in commensal *Escherichia coli* derived from healthy fattening pigs in Thai farms that used prophylactic antimicrobials (in-feed tiamulin fumarate and amoxicillin) [PAs], therapeutic antimicrobials (injectable enrofloxacin or gentamicin) [TAs], or no antimicrobials [NAs].The conclusions were that although *E. coli* isolates from all farms contained a core set of resistance to b-lactams and tetracyclines, the routine use of PA increased resistance rates to other important antimicrobials. (Lugsomya K. et al, 2018)

In 2019, Pirolo et al. studied the unidirectional animal-to-human transmission of methicillin-resistant *Staphylococcus aureus* ST398 in pig farming. Given the potential exchange of MRSA between animals and humans, and the infection risk associated with human colonisation by MDR LA-MRSA, the study was conducted to investigate: (i) the prevalence, genetic characteristics and antimicrobial resistance profile of MRSA isolated from swine farm workers in southern Italy, and (ii) the genome-based relatedness of human and animal MRSA isolates, for a better understanding of MRSA transmission to professionally-exposed farm workers. It was observed that workers employed in farms with an intensive type of breeding had higher MRSA carriage rates compared with workers employed in farms that adopted a non-intensive breeding system (27.1% vs. 0%). (Pirolo M. et al, 2019)

In 2022,Torres et al. found high similarity of macro-restriction patterns for isolates of *Yersinia enterocolitica* 4/O:3 obtained at a pork production chain from Minas Gerais, Brazil. They proposed to determine the clonality and the antibiotic resistance profiles of a subset of these isolates (n = 23) and human clinical isolates (n = 3). The close genetic relationship amongst *Y. enterocolitica* obtained from a pork production chain and human clinical isolates in Brazil was confirmed, and the role of swine in the potential transmission of an antibiotic-resistant clones of a pathogenic bio-serotype to humans, or the transmission of these resistant bacteria from people to animals, was highlighted. (Torres Furtado Martins B. et al, 2022)

CONCLUSIONS

- *E. coli* had sensitivity in 78/78 (100%) antibiograms to Amikacin, Meropenem, 69/78 (88.5%) antibiograms to Pip/Tazo and in 74/77 (96.1%) antibiograms to Tigecycline.
- *E. coli* shows resistance in 76/78 (97.4%) antibiograms to Ampicillin, 73/78 (93.5%) antibiograms to Ciprofloxacin, 72/78 (92.3%) antibiograms to Levofloxacin, 75/77 (97.4%) antibiograms to Mezlocillin, 77/77 (100%) antibiograms to Moxifloxacin, 67/78 (85.9%) antibiograms to Trimeth/Sulfa, 58/78 (74.35%) antibiograms to Piperacillin and 52/77 (67.5%) antibiograms to Tetracycline.
- Studied *E. coli* had similarities in terms of sensitivity and resistance to tested antibiotics, probably as a result of the transmission of these resistant *E. coli* from pigs to humans.

Acknowledgement

The research was carried in the Animal Production Laboratory, in the frame of the project: *A bio-economical approach of the antimicrobial agents – use and resistance*, contract PCCDI 7/2018, code: PN-III P1-1.2-FPRD- 2017.

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