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COMPARATIVE LEVELS OF ANTIBIOTIC RESISTANCE IN PIGS RAISED UNDER DIFFERENT TECHNOLOGIES

Anca GOCAN (SECARĂ)[1], Carmen Dana ȘANDRU [1, 2], Diana OLAH[1], Gheorghiță DUCA[2], Constantin CERBU[1], Aurel VASIU[1], Emoke PALL[1, 2], Cristian VĂLEANU[1], Marina SPÎNU[1, 2]

[1]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca,
Faculty of Veterinary Medicine, 3-5 Mănăștur Street, 400372, Cluj-Napoca, Romania;
[2] Institute of Research and Development for Montanology Cristian,
147 Street XIII, Cristian, Sibiu, Romania

Corresponding author: marina.spinu@gmail.com

Abstract

Swine are considered one of the most important species of food animals worldwide, the majority of meat for human consumption in numerous countries being represented by pork. Sometimes heavily treated with antibiotics to maintain herd health, swine could be considered a sentinel species for humans in respect to antibiotic resistance.The research compared the dynamics of antibiotic resistance by distance between an intensive farm, where antibiotic use is considerable, and small households, with no antibiotic use, at 5 (A) and 10 (B) kilometers from the farm. Twenty-eight clinically healthy pigs (n = 16, farm, n = 6 A, n = 6 B) were sampled. Standard microbiological techniques, identification of the strains by Vitek 2 system and Kirby Bauer test to assess the antimicrobial resistance were applied. Of the total bacterial species identified, E. coli (72%) dominated while 10% were Gram+ bacilli and 10% Gram - unidentified coco-bacilli. The highest MAR (multiple antibiotic resistance) index was calculated in E. coli (MAR = 0.88, A and B) and also two other strains from the farm (MAR = 0.77). The high MAR indices stand for the presence of antibiotic resistance in untreated animals, urging for a more accurate surveillance of the phenomenon.

***Key words**: swine, farming technology, antibiotic resistance, spatial dynamics.*

INTRODUCTION

Swine are considered to be one of the most important farmed species of animals worldwide, owing it to their large-scale exploitation by which a partial coverage of the growing global meat demand - up to 13% increase by 2030 - is managed (OECD/FAO, 2021). Moreover, due to their anatomophisiology, sharing numerous similarities to that of humans, swine elicit an increasing interest in human medicine a donor model of various organs.

Antimicrobial resistance of pathogenic agents, a continuously expanding phaenomenon, originated in the exposure to antibiotics, either following therapy or horizontally, by simple exposure to bacteria hosting such genes (Munita and Arias, 2016). The spread of non-discriminating, extended and lengthy antibiotic use in farmed animals increased the prejudice to human health both through direct contact and food of animal origin (Peng et al., 2022). This also increased very much the farming costs (Founou et al., 2017).

Given the extended farming of swine communities, disease transmission poses a severe risk to animal health and welfare, therefore antibiotics represent a fast resourse to alleviate the symptoms and control microbial agents, being critically important in veterinary medicine (Holmer et al., 2019; Pyörälä et al., 2014). Thus, swine could be considered as a sentinel species for humans, hence the antibiotic resistance found in swine must also produce strong alarm signals in human medicine (Neil, 2015).

E. coli, a member of *Enterobacteriaceae,* inhabits the intestine and is considered an indicator of fecal pollution of the environment (Pholwat et al., 2020). It ubiquitous presence makes *E. coli* a perfect candidate for investigations on its antimicrobial resistance, as a measure of antimicrobial resistance in both hosts and habitat and aslo provides important information on the spread of MAR

Nevertheless, there are few studies on the multiple antibiotic resistance (MAR) or multi-drug resistance (MDR) and their spread on large farms, but also in the neighbouring premises and their broader environment.

Therefore, the aim of the work was to compare the level of antibiotic resistance of bacterial strains isolated from pigs reared on an intensive farm, representing an environment with extended use of antibiotics, with those isolated from pigs reared in households, at 5 and 10 kilometers distance from the farm, with much lesser if any, use of antibiotics.

MATERIALS AND METHODS

The isolation and identification of pathogenic and conditionally pathogenic bacteria and the evaluation of their antibiotic resistance were pursued, both in pigs belonging to an intensive.

For that, fecal samples were collected by use of sterile swabs from the rectum of clinically healthy pigs of which 16 from the farm, and 6 from each A and B villages, located 5 and 10 km away from the farm, respectively.

All samples were transferred to simple broth, cultivated for 24 h at 37°C and then spread to nutritious agar to obtain isolated colonies. After the expiration of the incubation time (24 h at 37°C), the morphology of the colonies allowed the first stage identification. Further, the samples were subjected to identification on chromogenic McConkey and Chapman agar media. The final identification was performed by the use of Vitek2 Compact system (BioMerieux, France) and 64 wells for biochemical testing.

The sensitivity to antibiotics in the isolated strains was tested against: oxytetracycline, tulathromycin, methicillin, cefquinome, enrofloxacin, neomycin, amoxicillin and clavulanic acid, colistin and chloramphenicol by the Kirby Bauer diffusion method and diametres of the inhibitions zones were measured. Due to its importance in human and animal pathology, *E. coli* was chosen in this experiment to exemplify the presence and spread of antimicrobial resistance.

The data were statistically analysed for significance of the treatment effect by using Microsoft Excel software.

RESULTS AND DISCUSSIONS

Of the total bacterial species identified, 72% were represented by *E. coli*, 10% by Gram-positive bacilli, 10% totaled Gram-negative coccobacilli unidentified following Vitek testing. Only 4% represented *Staphylococcus sciuri*, respectively *Citrobacter amalonaticus*.

The interpretation of the diameters of the inhibition zones of the strains taken in the study allowed the assessment of the effectiveness of each antibiotic used. Thus, the most effective antibiotics proved to be cefquinome, from the class of cephalosporins and neomycin, from the class of aminoglycosides, and the most ineffective was methicillin, an antibiotic from the class of penicillins. Out of 29 antibiograms performed, 24 of the tests showed total resistance to methicillin. The presence of resistant colonies was most frequently identified against chloramphenicol.

All studied strains, both from the farm and from households, showed total methicillin resistance, except for one *E. coli* strain isolated from farm pigs, one *E. coli* strain and one *Staphylococcus sciuri* strain isolated from in two distinct individuals from household B. The same two microorganisms showed sensitivity to all antibiotics.

In *E. coli* strains (n = 14) isolated from farmed pigs, the highest resistance was recorded to oxytetracycline, followed by amoxicillin/clavulanic acid, respectively methicillin.

No resistance was observed to tulathromycin and cefquinome (Figure 1).

In the *E. coli* strains (n = 5) isolated from pigs in household A, resistance to amoxicillin, methicillin and oxytetracycline was observed in the same proportion. No resistance to tulathromycin was noted (Figure 2).

In *E. coli* strains (n = 2) isolated from pigs in household B, the highest resistance was noted to oxytetracycline, followed by amoxicillin. No resistance to colistin was noted (Figure 3).

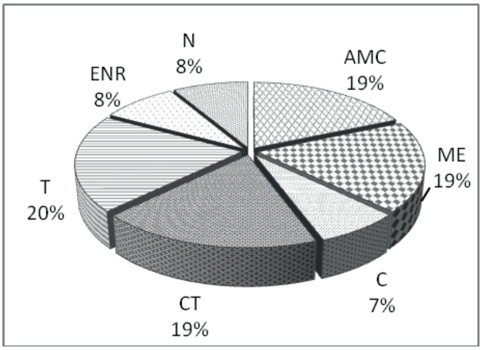

Figure 1. Resistance of *E. coli* strains isolated from the farmed swine

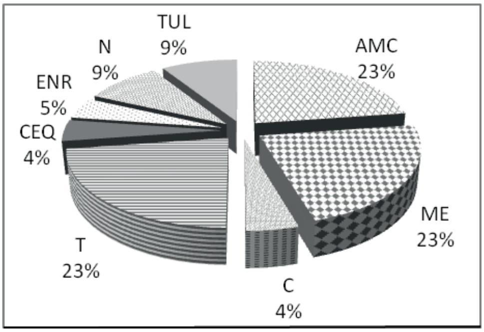

Figure 2. Resistance of *E. coli* strains isolated from household A

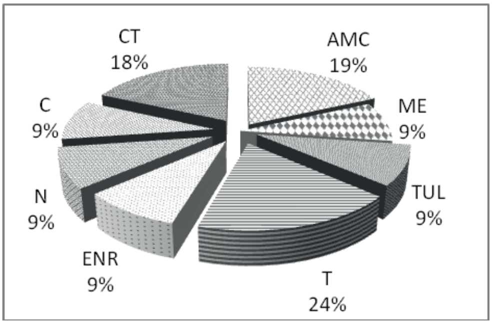

Figure 3. Resistance of *E. coli* strains isolated from household B

The MAR index is defined as the ratio of the number of antibiotics to which the strain was found to be resistant to the number of antibiotics to which the bacterial strain was exposed (Sandhu et al., 2016).

A MAR index > 0.2 indicates the existence of a multidrug-resistant bacterial strain from a source where antibiotics have been intensively used, while an index < = 0.2 suggests that the strain originates from a source where antibiotic treatment it was less often applied (Adenaike et al., 2016).

The highest MAR index was recorded in strains of *E. coli* from households A and B (MAR = 0.88) (Figure 5), followed by another strain of *E. coli* isolated from the farm (MAR = 0.77) (Figure 4).

Bacteria that showed a MAR index of 0 were represented by an *E. coli* strain from household B, another *E. coli* strain from the farm and *Staphyloccocus sciuri*, isolated from household B (Figure 6).

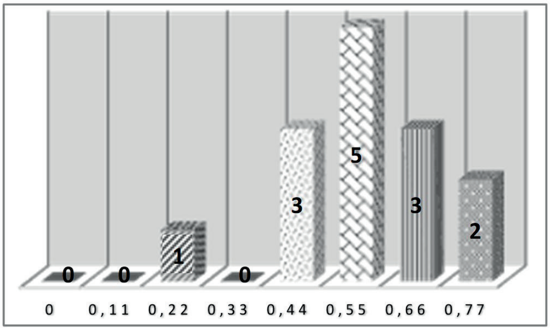

Figure 4. Correspondence between the number of resistant strains and their MAR index on the farm

Escherichia coli is commonly found in the digestive tract of both humans and animals. It is also isolated from soil, water or food, as a result of contamination through faeces or during the slaughter of animals for consumption. Shiga-toxin-producing *E. coli* (STEC) O157 emerged as a public health threat following its initial identification as a pathogen in a 1982 episode of illness associated with consumption of raw beef (Etcheverría and Padola, 2013). *E. coli* strains O157:H7 and O157:NM (non-motile) are recognized as major etiological agents in hemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) in humans (Bruyand et al., 2018).

The US Centers for Disease Control and Prevention estimates that *E. coli* O157:H7 causes approximately 73,400 illnesses and 60 deaths each year in the United States. Recent reports indicate that antimicrobial resistance of *E. coli* O157 is increasing (Abebe et al., 2023). However, the extent to which different antimicrobial use practices have contributed to the rise of antimicrobial resistance is not yet fully known (Zheng et al., 2012).

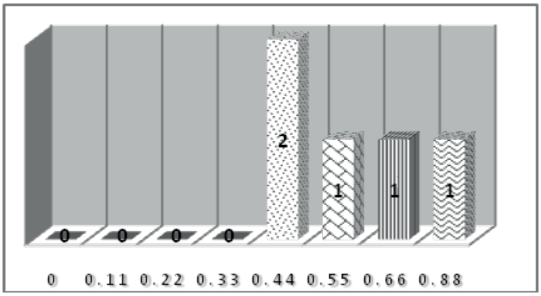

Figure 5. Correspondence between the number of resistant strains and their MAR index in household A

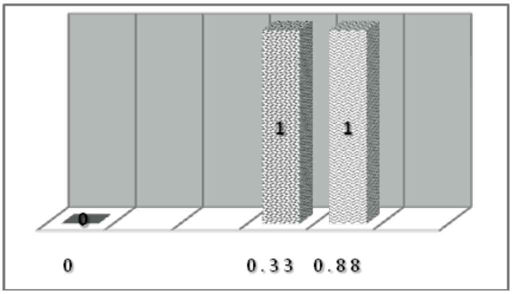

Figure 6. Correspondence between the number of resistant strains and their MAR index in household B

E. coli is one of the most common causes of infection in humans and animals worldwide. *E. coli* is associated with a wide variety of diseases, but there is a high degree of host and disease specificity for different serotypes. Thus, certain pathogenic *E. coli* serotypes dominate as causes of intestinal infections in humans and various animal species. In contrast, extraintestinal infections are caused by types of *E. coli* that are found in the normal intestinal flora (Chan et al., 2014).

Few studies describe antimicrobial susceptibility patterns among *E. coli* O157 isolates from humans and animals. Until near the end of the 20th century, most strains demonstrated susceptibility to antibiotic action, with resistance to ampicillin, streptomycin, sulfonamides and tetracycline being observed (Wegener et al., 1999). In poultry litter, it was found that probably the *groEL* gene fundamented *E. coli* resistance to ampicillin, colistin, tetracycline, sulphonamides, or cephalothin (Khong et al., 2023) A study conducted between 1984 and 1987 notes susceptibility of all 56 strains of *E. coli* isolated from human cases to all antibiotics tested (amoxicillin with clavulanic acid, ampicillin, ceftazidime, ceftriaxone, cefuroxime, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, streptomycin, sulfisoxazole, tetracycline, ticarcillin, tobramycin and trimethoprim-sulfamethoxazole). Of the 176 strains isolated between 1989 and 1991, 13 (7.4%) were resistant to streptomycin, sulfisoxazole, and tetracycline (Kim and Cha, 2021).

CONCLUSIONS

The results obtained in this study supported the initial working hypothesis, according to the book it was expected to quantify an increased MAR index in the farm (0.77), which decreases with increasing distance from it (0.55 at 5 km and 0.44 at 10 km from the farm) .

The very high values of the MAR indices, which correspond to the *E. coli* strains identified on the farm, confirm the hypothesis that the use of antibiotics at this level was intense.

Although the maximum number of strains with an intermediate MAR index were identified on the farm, strains with a MAR index of 0 were also isolated here, similar to households located at a distance of 10 km from it, which suggests the more frequent use of certain categories of antibiotics and avoidance of others in intensive rearing.

The existence of antibiotic resistance even at relatively large distances from the farm may indicate other mechanisms of installation of the phenomenon than strictly treating sick animals.

MAR indices with high values support the need for increased vigilance of the veterinarian and even corrective measures from the perspective of antibiotic use.

AUTHORS' CONTRIBUTION

All authors had equal contribution in the study design, sampling, sample processing, data processing and writing the paper.

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