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RESISTANCE PROFILE SCREENING IN PIG FARMS IN WESTERN ROMANIA

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Summary

Antibiotic resistance is no longer a medical novelty, being heavily studied worldwide by numerous agencies in charge of monitoring, evaluating and implementing procedures aimed to diminish the effects of this phenomenon in order to preserve the public health. The intense use of antimicrobial agents, as means of preventing or treating colibacili determine more and more frequently the emergence of the antibiotic-resistant strains in swine species. In this respect, the present study intends to follow the evolution of resistance in two counties from the Western part of Romania, in ten great swine units on piglets, at the weaning age category. After the diagnosis confirmation, by morphopathologic means, the identification and isolation of the etiological agents were followed by the classical methods known in microbiology. Of a total of samples (no. 167), we found pathogenically positive 75.44%, being also identified hemolytic strains (10.77%), and 13.79% negative samples. The (non-hemolytic) positive samples were tested by the Kirby-Bauer disk-diffusion test, where 11 antibiotics were used, and the obtained data were compared with the CLSI / 2009 standard. Results revealed comparatively a diverse evolution of the resistance to the tested antibiotics in both counties visited, probably due to the extensive antibiotherapy applied in these units. The resistance value of the antimicrobial structures evaluated was relatively similar: in Arad County increased values of resistance to lincospectin and doxycycline were reported and in Timis County to neomycin and respectively amoxicillin / clavulanic acid, confirming the insidious evolution of this phenomenon in the Romanian swine farms.

Keywords: antibiotic resistance, evolution, swine units

Digestive disorders are the following of a series of commensally or pathogenic bacteria that can cause symptoms which have a major impact on growth, meat's quality and decreasing the economic profit.

The genus Escherichia is the one that plays an important role in the development of the enteric syndromes. Worldwide, these bacteria are producing major losses on farms, the death percentage being influenced by many factors, including here the current legislation, which prohibit the usage of growth promoters in food.

In the current context, particular attention must be paid also to the youth category after weaning, due to the factors which can appear on the individuals by overloading the body with new feed conditions, changing intestinal flora and decrease of the immune status.

Thus, functional imbalances could appear by the emergence of pathological processes, an example could be E. coli strains in the small intestine at the piglets

(weaning diarrhea). The magnitude of casuistry with diarrheal manifestations in animal farms has concerned the attention of researchers, who claimed that these diseases are caused by pathogenic bacteria with multiple antibiotic resistances.
Among many, E. coli is considered the main factor in the appearance of the enteric syndrome, producing significant damage by decreased productivity, associated with the increasing cost of production and a low profitability index.
The purpose of the present research was to highlight some specific aspects about the etiopathogenesis of enteritis around the weaning age of the pig in the breeding units located in the Western part of Romania.

Objectives

In order to achieve the proposed goal, the following objectives were addressed as it follows:

- isolation of E. coli strains and gender-based biochemical properties;
- assessing the susceptibility and resistance profile of the various antimicrobial agents of isolated E. coli strains;
- statistical interpretation of the results with the Anova program (*t*-test).

Materials and methods

The experiment took part in the most important ten swine breeding and fattening units in the Western part of Romania. The units are constituted as intensive growth system and structured by age and weight categories.
After the pathological examination, hemorrhagic injuries were found in focal areas located in the small intestine, predominantly in the anterior third, along the entire duodenum tract and a small part of the jejunum. In addition to hemorrhagic chromatic changes, the intestinal distension by gas accumulation can be observed

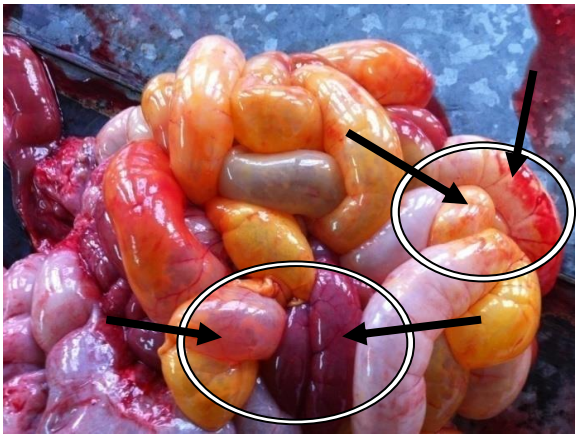

Fig. 1. Haemorrhagic duodenal-jejunitis inn outbreaks with intestinal distension with gas accumulation (Original by Doma)

The etiologic agent identification

From the biological matter, using a Pasteur pipette, samples have been collected, which then were introduced in bullion, and from there with the help of the Drigalsky loop the samples were moved to nutritive agar cast in Petri dishes.

For the best growth of microorganisms, the samples needed controlled temperature for 24 hours at 37 Celsius degrees. (Fig. 2 and 3)

Fig. 2. Incubation of the samples (Original by Doma)

Fig. 3. E. coli cultures identification on selective Levin medium (Original by Doma)

For our survey, we have chosen 10 animal raising units from the Timis and Arad counties and in the time period September 2011 - November 2014 we have examined females with cocobacillary enteritis. The certain diagnosis was based on specific symptoms gathered from the morphopatological examinations and laboratory exams in which we identified the pathogenic agent.

From the 167 biological samples (sections made from the small intestine, liver, kidneys, and lungs) examined, 75.44% turned out as positive, from them, a really small number of hemolytic ones, (10.77%.)

The remainder of 13.79% turned out negative which makes us to confirm that those cases have died from a different nature than bacteria.

In the study we have taken into consideration only the positive non-hemolytic samples tested with the disk-difusimetric Kirby-Bauer method, which has as the main function principle the diffusion of a substance in a solid medium.

Around the tablet, with the active solution it is created a well which is measured with the help of a caliper or a ruler, and the result is compared with the interpretative chart CLSI /2009 (Clinical Laboratory Standard Institute USA/2009).

The results are placed in three separated categories (Fig. 1.):

- sensitive (susceptible-S),
- mild sensitive (intermediate -I),
- resistant(R),
- non-susceptible.

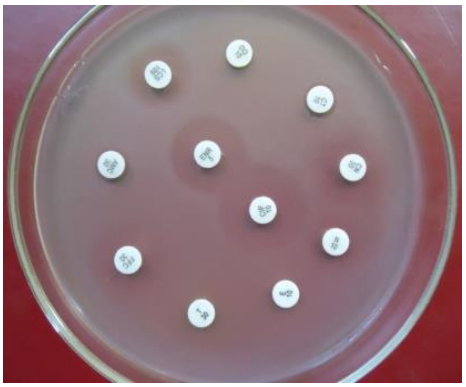

Fig. 4. Disk-Difusimetric Kirby-Bauer method (Original by Doma)

In Chart 2 is structured the technique used in the isolation and identification of the E. coli. colonies.

Chart 2:

Work technique for E. coli strains identifications.

Nr. crt.	Stage	Procedure
1.	Bacterial isolation	• confirming the existence of E. coli. strains; • isolating the colonies (picture 99 & picture 100).
2.	Preparing the inoculums.	• the colonies are dissolving in 5 ml of bullion; • incubation at 37 Celsius degrees for 24 hours; • mixing; • adjusting turbidity with standard solution 0.5 McFarland (1,5 x 108 CFU/ml).
3.	Insemination of research stems	• on Muller-Hinton agar, using Petri plates • releasing with the help of a sterile cotton-wool pad, successive in 3 directions for a better and a more uniform diffusion • for a better absorption, the plates will be exposed under a source of heat for 10-15 minutes.
4.	Application of micro-tablets	• using a pliers • the distance between tablets is approximately 30 mm • the distance between tablets and the edge of the plate is approximately 15 mm
5.	Incubation	• aerobic environment • the temperature of exactly 37C for 24 hours
6.	Reading and formulating the results	• using a ruler or a slide ruler • the measuring of the inhibition diameter is made from different directions for 2 up to 3 times • reporting the measurements to CLSI/2009

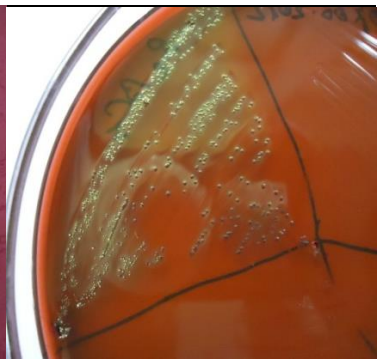

Fig. 5. Growing bacteria in test tubes with nutrient agar
Fig. 6. The microbiological aspect of E. coli culture on the selective environment

To determine the efficiency/resistance of a bacteriostatic and bactericidal substance we chose 11 antibiotics from chart 1:

Chart 1.

Commercial disks for antibiogram are from the Oxoid (UK) firm

DO	Doxycycline - 30µg
LCS	Lincospectin –109 µg
CT	Colistin – 25 µg
CN	Gentamicin – 10 µg
N	Neomycin –10 µg
CIP	Ciprofloxacin – 30 µg
ENR	Enrofloxacin –10 µg
AMC	Amoxicillin/ Clavulanic Acid – 30 µg
FFC	Florphenicols –30 µg
T	Tetracycline – 30 µg
E	Erythromycin – 15 µg

RESULTS AND DISCUSSIONS

Today, the phenomenon of antibiotic resistance does not represent anything new, compared with the last 50 years of the past century, when it was signaled for the first time. There are national and international agencies who's the main scope is to monitor the use of antimicrobial, evaluate the rates of resistance for mating animals and implement measures to mitigate this phenomenon, to maintain public health (1, 2).

The gusty use of substances with antimicrobial basis as a way of preventing the colibacili enteritis has determined the appearance of medicine-resistant stems. This phenomenon continues to expand both locally and globally, so the researcher's attention is turned to implementing some alternative measures to control the disease (4, 7).

The evolution of the resistance phenomenon from the two counties (Timis - 84 positive samples taken from 7 farms and Arad county 42 positive samples taken from three farms) of E. coli stems isolated from piglets, with ages around a lactation period, compared to the 11 antibiotics used , can be observed in the following figures. (fig. 7 and 8).

For many researchers, the resistance phenomenon of E. coli stems is still an interesting subject, as it is enigmatic.

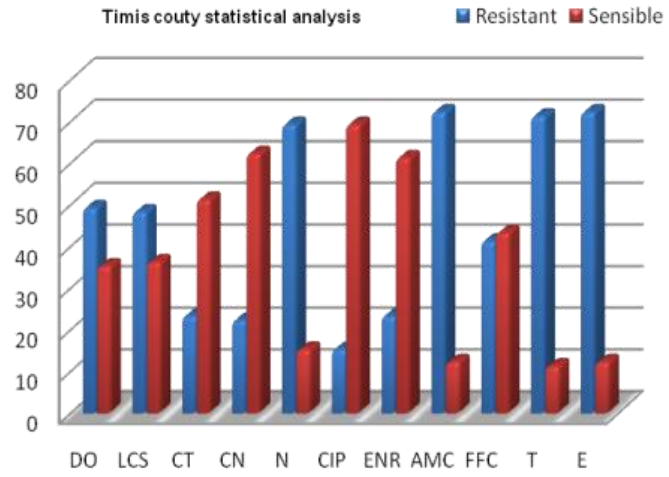

Fig. 7. Statistical interpretation of the results from Timis county through ANOVA program (t-test - P <0.001)

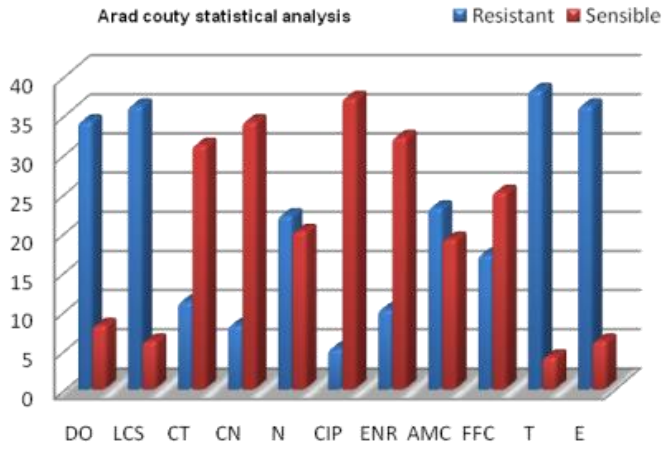

Fig. 8. Statistical interpretation of the results from Arad county through ANOVA program (t-test - P <0.001)

Also, in Croatia, following an experiment which took part in 8 farms (6), the rising evolution of oxytetracycline, streptomycin and ampicillin resistance was demonstrated. In Romania, following a similar experiment (9) the researchers, through lab exams, claim that they highlighted the resistance of the E. coli stem for enrofloxacin, streptomycin, tetracycline, and amoxicillin/clavulanic acid.
Other researchers have highlighted that the feeding of the livestock and elaborating rations based on age category, by lowering the pH with organic acids and adding zinc oxide or peptides, like colicine E1, can decrease the incidence of ablactating diarrhea (3, 5, 10).
Some authors claim that the evolution of antibiotic resistance depends on how often the antibiotics are used (4, 8).

CONCLUSIONS

Comparative results of antibiogram have highlighted a different evolution of the resistance phenomenon in Arad and Timis counties, therefore:
In Arad County, the high values of tetracycline and lincospectin resistance has been highlighted. In Timis County, the values were raised compared to tetracycline, and amoxicillin/clavulanic acid treatment. The evolution of antibiotic resistance depends on the location of the farms and therapeutic management.

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