

ANTIBIOTIC SUSCEPTIBILITY OF *ESCHERICHIA COLI* WILD STRAINSMaryna Kryvda*¹, Zhanna Rybachuk¹

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ABSTRACT

Escherichia coli is the normal microbe of the intestinal microflora but can cause different illnesses exception the intestinal tract. And the most problem of the illness with *E. coli* etiology is the rapid antibiotic resistance forming of the current pathogen. We had the aim to detect the antibiotic susceptibility of *E. coli* strains separated from the samples of the ill cattle in endometritis and mastitis. 12 types of antimicrobial preparations were tested. We have founded, wild *E. coli* strains were totally resistant to vancomycin, streptomycin, tetracycline, doxycycline, kanamycin, amoxicillin, and tylosin. Drugs of the cephalosporine range also were un-efficient. And just furazolidone, enrofloxacin, and gentamycin have shown the effective action to tested samples of *E. coli*. So, the testing of antibiotic susceptibility is the obligatory action in therapy-list formation. Just this action can demonstrate the efficient antimicrobial drugs that can provide the recovery of the illness animals.

Keywords: antibiotic resistance, bacteria, cattle, mastitis, endometritis, pharmacotherapy

INTRODUCTION

The age of antimicrobial pharmacology has been started in 1928 by Alexander Fleming. It was a revolution in medicine and has gave a hope for alive to millions (Puttaswamy *et al.*, 2018; Latek, 2020). It is well known, we are never alone from the birth to death: microbiota is not only always together with the macroorganisms but made their lives harmonic. And just a little part of microbiota is pathogenic for animals and humans. For these microbes part is necessary to have some drugs for inhibit or inactivate them (Da Silva & Domingues, 2017; Latek, 2020). And in this case, became especially important to understand pathogens' susceptibility to antibiotic drugs. First of all, using non-effective preparations forms the antimicrobial resistance. On the other hand, we have made an influence to autochthonous microbes without efficiency action to pathogens. So, the situation of unhelpful antibiotic drugs using has been created (Da Silva & Domingues, 2017; Popov *et al.*, 2018; Dadgostar, 2019). Puttaswamy *et al.* (2018) gave data about 700,000 deaths each year due to formed bacterias' antibiotic resistance. More other, this rate could be grown to 10 million deaths per year by 2050.

Escherichia Coli strains are well-known pathogens that can damage different systems: urinary and sexual tract, prostate, skin, udder, bloodstream, and other systems which are nonintestinal. Must be noted, *E.coli* is a typical resident of the intestinal. But from this reservoir, these bacteria can migrate to other body systems caused their damages (Manges *et al.*, 2019). There are data about the *E. coli* feature as one of the most transformations changed bacteria in a case of antibiotic resistance formation. It can be a flagman in antibiotic resistance biofilms formation (Mathlouthi *et al.*, 2021). This remains actual taking into account the speed of antibiotic resistance formation in veterinary and human medical practice (Popov *et al.*, 2018; Dadgostar, 2019; Kryvda & Rybachuk, 2021). So, we had an aim to detect the antibiotic susceptibility of the *E. coli* strains isolated from the ill cattle in mastitis and endometritis.

MATERIAL AND METHODS

Study design

The research was conducted at the research laboratory at the Veterinary faculty of the Polissya National University. The principles of the ethical conceptions with the animals were complimented in getting the samples for the study. Animals with mastitis (n=16) and post-calving complications (endometritis) (n=3) were tested in the Zhytomyr region. The samples (mastitis milk (n=16) and the flush from the genital tract (n=3)) were taken into the sterile individual transport tubes with applicators to the research laboratory. Each sample was detected in total microbial contamination and the microbial cultures with high spread and big growing speed were studied.

First, the samples from these animals were tested at the nutritious mediums into account of the discovered system. Total microbial contamination was checked by the deep inoculation method (the inoculated volume – 0.1 mL per plate). Microbial multiform was studied after surface inoculated samples on the nutrient plate. We

have used Nutrient Agar for total insemination testing, Agar Endo as a medium for Enterobacteriaceae family, Salt Selective Agar for Staphylococcus.

Total microbial insemination was studied by deep sowing. Inoculation on the Endo and Salt Agar was conducted by the strokes at the nutrient medium surface. The microbes with the highest growing speed and the greatest spread among other spaces were separated. All separated strains were studied and microscopied according to the well-known methodic (HiMedia Manual, 1998; Skibitskiy *et al.*, 2006; Puttaswamy *et al.*, 2018; Carroll & Pfaller, 2019). And the susceptibility to antimicrobial preparations was studied for these strains.

Study of the bacteria's susceptibility in antimicrobial preparations

From the isolated bacterial strains, *E. coli* isolates were in prefer because of their prevailed in the results of first sowing. We have studied its susceptibility to 12 types of antibiotic drugs. The methodic of discs antibiotic-impregnated was used according to the approved writes (Ministry of Health of Ukraine, 2007; Flanagan & Steck, 2018). After checking the areas of the microbe's growing stop (or inhibit) the conclusion about antibiotic susceptibility of the tested bacterial's strains were made. Mueller-Hinton agar was used in this kind of research (Ministry of Health of Ukraine, 2007).

RESULTS AND DISCUSSION

Bacterial identification from the enrichment culture

We had taken into account the cultural features of *E. coli*. So, *E. coli* colonies have metal shine on the dark pink-colored colonies (Garrity *et al.*, 2005). And the character view grow was identify in enrichment cultures (Fig. 1).



Figure 1 The stages of the study pathogens caused the illness in studied animals: enrichment and pure culture and microscopic study (magnification 1000, immersed) of the eliminate microbe strain (*E. coli*).

The quantity of *E. coli* number in the tested samples was dangerously. Must be remembered, *E. coli* present in the clinical samples means about the fecal waste (Odonkor & Addo, 2018). So, the problems with animals' hygiene are relevant for each tested sample, since *E. coli* is an active resident bacterial part of the gut. (Dadgostar, 2019). And the treatment of such illnesses has great dependence about this pathogen susceptibility in antimicrobial cure.

Bacteria's antibiotic susceptibility testing

Many strains of bacteria have got resistance to a lot of antibiotics due to mutation and forming of the unsusceptibility to a great part of the antimicrobial drugs. But scientists and practice specialists have made an efficient action – testing of the bacteria's susceptibility for the antimicrobial drugs. The rapid antibiotic susceptibility tests give us a possibility to make a positive effect on the treatment of bacterial infections. Due to excluding the antibiotics with low antibacterial activity, we can prevent of forming of multidrug-resistant bacteria (Popov *et al.*, 2018; Buz'n-Durbn *et al.*, 2018; Yang *et al.*, 2020). In this regard, we have looked for the efficient antimicrobial drug for the best use in ill animals treatment in current farm manufacture. Figure 2 demonstrates the research method (disc diffuse method) used in the efficiency antibiotics determination.

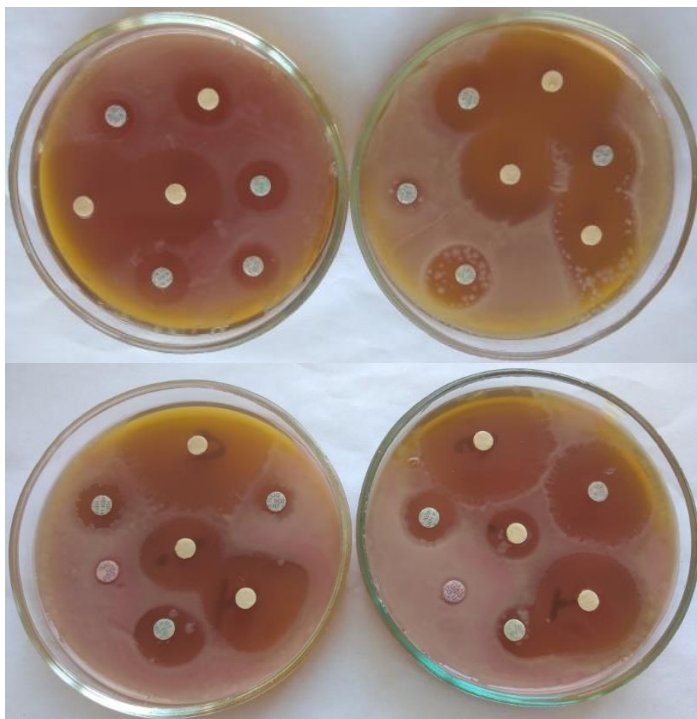


Figure 2 Method of paper disks in testing the antibiotic susceptibility of *E. coli* strains

This method of the bacteria's antibiotic susceptibility has some advantages like analysis spread (in 24 h), cheap, and simple application. This method allows getting useful and clear information in a short time that gives us an opportunity to get out economical damages and make cattle breeding more efficient (Ministry of Health of Ukraine, 2007; Popov *et al.*, 2018; Flanagan & Steck, 2018). Global researches have shown there is no total susceptibility of *E. coli* to some drug. Different strains have shown different receptivity to each antibiotic drug (Odonkor & Addo, 2018; Raeispour & Ranjbar, 2018).

According to gotten data, isolated strains *E. coli* were resistant to vancomycin, streptomycin, tetracycline, doxycycline, kanamycin, amoxicillin, and tylosin (Table 1).

Our received data showed almost totally similarity in obtained results. It can be explained by the similarity of the bacteria lines persistent in the farm manufacture. But the susceptibility of lines isolated from animals ill in mastitis and endometritis were different. This declares in difference *E. coli* lines caused these two kinds of diseases.

And our data have corresponded to world research. Odonkor & Addo (2018) have given data about the resistance of *E. coli* to tetracycline in 21.45% of cases of studied samples. But their research has shown a high range of susceptibility was fixed to penicillin. In our case, the penicillin derivative (amoxicillin) has shown total resistance.

The data of Raeispour & Ranjbar (2018) have demonstrated *E. coli* 100% susceptible to vancomycin and doxycycline. But our data is the opposite. In all tested samples were not been fixed susceptibility to each of these preparations (the zones of inhibited growing were absent).

In case of cephazolin, the susceptibility of *E. coli* wasn't the same in all tested samples. The efficiency of this cure was from semi-active (33.33 %) in case of isolated lines from endometritis ill cows to total inefficiency in case of bacterial lines isolated from the animals with mastitis illness. The other on cefasporine group drug - ceftazidime - has no shown any activity. But must be noted semi-active antibiotics can't be used in treatment schemes because of a high percentage of indifference for this drug bacteria. The data of Raeispour & Ranjbar (2018) have demonstrated *E. coli* resistance in cephalothin in 74% of cases. Odonkor & Addo (2018) and Podnecky *et al.* (2018) also demonstrate the resistance of the *E. coli* spaces to cephalosporin drugs, just only a little more than 50% of strains were susceptible to cefuroxime (52.58%) which is a semi-synthetic cephalosporin second-generation range antibiotic (Directory of medicines, 2021).

Table 1 The results of the antibiotic susceptibility testing of the *E. coli* strains

Tested antimicrobial drug	Susceptible, the large of the inhibited zone, mm*			The experimental data, diameter inhibited zone, mm
	No	The diapason	Yes	
Amoxicillin	<=13	14-17	>=18	0.0
Cephazolin	<=14	15-17	>=18	14.0±2.0
Ceftazidime	<=14	15-17	>=18	0.0
Doxycycline	<=12	13-15	>=16	0.0
Enrofloxacin	<=15	16-21	>=22	33.5±1.5
Furazolidone	<=14	15-16	>=17	17.7±0.3
Gentamycin	<=12	13-14	>=15	23.5±0.5
Kanamycin	<=13	14-17	>=18	15.0±1.0
Streptomycin	<=6	7-9	>=10	0.0
Tetracycline	<=12	13-15	>=16	10±0.3
Tylosin	<=14	15-16	>=17	16.0±0.5
Vancomycin	<=14	15-16	>=17	0.0

* - The normative data according to the Guideline (Ministry of Health of Ukraine, 2007).

Our research has shown the isolated strains of *E. coli* were susceptibility to furazolidone (75%), enrofloxacin (100%), and gentamycin (100%). All these drugs have shown bactericidal action to the tested *E. coli* strains. And according to pharmacological instruction for these preparations, it is natural. All of these drugs must have tidal action to Gram-negative spaces (Directory of medicines, 2021). And the biggest growing's inhibit zone has been shown by enrofloxacin. Enrofloxacin as a fluoroquinolones type is recommended for using in *Enterobacteriaceae* illnesses treatment by the World Health Organisation (Roth *et al.*, 2019).

Raeispour & Ranjbar (2018) have shown data in accordance with our, just only 10% of tested *E. coli* strains were resistant to furazolidone and 19% - to gentamycin, so this kind of antibiotics together with enrofloxacin can be tested for susceptibility first in the case of *E. coli*.

Must be emphasized, only a quarter of all tested antibiotics have effective action in wild strains of *E. coli*. And this is a global tendency. *E. coli* has different adaptations for antibiotic resistance formation (Syal *et al.*, 2017; Khrokalo *et al.*, 2020).

CONCLUSION

E. coli is a damaging pathogen that can cause different illnesses except for the intestinal tract where *E. coli* is the common space.

Antibiotic susceptibility testing of wild strains of *E. coli* separated from ill animals in endometritis and mastitis has shown its resistance to vancomycin, streptomycin, tetracycline, doxycycline, kanamycin, amoxicillin, and tylosin. Antibiotics of the cephalosporin range have not been efficient for tested *E. coli* isolates.

From 12 chosen antibiotics just only 3 had their efficient action in separated strains. They were furazolidone, enrofloxacin, and gentamycin.

In all illness cases with *E. coli* etiology testing in antibiotic susceptibility is the obligatory stage of therapy that provides efficient therapy and recovering of damaged animals.

Declaration of conflict of interest: We like the authors of the presented research declare there are no conflicts of interests in the current study. The research has not been externally sponsored.

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