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Features of Biofilm Formation of some Pathogenic and Commensal *Escherichia coli* Isolated from the Body of Dogs and Cats

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Abstract. The relevance of this study is conditioned upon epidemic growth of nosocomial infections, which include *Escherichia Coli* (*E. coli*). One of the factors of pathogenicity of such microorganisms is the ability to form a biofilm – a complex community, within which bacteria acquire increased resistance to environmental factors, primarily to antibacterial drugs, which considerably complicates the course of the infectious process. In this regard, the purpose of this study was to determine the features of the formation and dependence of the density of the formed biofilm on the antibiotic resistance of pathogenic and commensal *E. coli* strains isolated from dogs and cats. The resistance of *E. coli* isolates to antibacterial drugs was established according to the disk diffusion method, according to EUCAST recommendations. The ability of microorganisms to form biofilms and determine their density was investigated in sterile plastic 96-well plates. The ability to form biofilms was assessed visually and microscopically, the density of biofilms was determined in units, spectrophotometrically, by the optical density of the washing solution. The paper presents the results of a study of 63 samples of pathological (wound infections) and biological material. From them, 10 *E. coli* isolates were obtained (6 from dogs and 4 from cats), which were selected for further research. It was established that all *E. coli* isolates had the ability to form phenotypic biofilm. The study investigated the interdependence of antibiotic resistance of *E. coli* isolates and their ability to form biofilms. Thus, isolates that were parted from pathological material and had a positive reaction on the CHROMagar™ ESBL medium for the determination of extended-spectrum beta-lactamases had greater resistance to various groups of antibacterial drugs and formed high- and medium-density biofilms, while *E. coli* isolates parted from pathological and biological materials with a negative reaction on CHROMagar™ ESBL medium formed a low-density biofilm and had less resistance to different groups of antibacterial drugs. The results obtained allow searching for innovative, sometimes alternative, methods of treatment and prevention of pathologies caused by them

Keywords: microorganism, biofilm, antibiotic resistance, antibacterial drugs, wound infection

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Introduction

The constant evolution of pathogens of infectious diseases requires a more thorough study of their biological properties, and sometimes their mutual conditionality. Research in this area helps find innovative, and sometimes alternative, ways to treat and prevent the diseases that they cause.

Some microorganisms, apart from showcasing adhesive activity, have the property of producing an extracellular polymer substance that envelops the bacteria in a thin layer. This structural formation is known as a biofilm. The ability to form a biofilm can be evaluated as a manifestation of the powerful pathogenetic effect of microorganisms on the macroorganism. Therewith, the biofilm performs a protective function – it restricts direct contact of the microorganism with body defence factors and antibacterial drugs, which factually transforms the pathogen into an invulnerable target.

The category of bacteria with high variability includes *Escherichia coli* (*E. coli*), the modification of which is caused by the action of several factors, including the influence of antibiotics. Polyresistance to antibiotics of distinct groups acquired by individual strains of *E. coli* has become the cause of a global problem – the formation of nosocomial infections (ESKAPE). The results of earlier studies [1] of similar properties of other types of bacteria indicate the manifestation of a special level of resistance to factors of negative influence due to the formation of a specific form of protection in them – a biofilm. Regarding *E. coli*, the study of biofilm formation is particularly relevant, which is due to the rapid acquisition of a pathogenic state by commensal forms.

E. coli belongs to the group of bacteria that can show adhesive properties. The need for its detailed study became clear when this bacterium was included in the list of pathogens of nosocomial purulent-inflammatory infections. *Escherichia coli* is one of those microorganisms that can form biofilms, both in the animal body and outside it – on various surfaces. Cultivation of *E. coli* does not require special conditions and specific nutrient media, which makes it an indicative model for investigating the morphological and physiological features of biofilm formation [2].

Recently, in Ukraine, as in the entire world, a clear trend has been established regarding the increase in the level of resistance of *E. coli* strains to antibacterial drugs [3; 4]. At the same time, there are considerable fluctuations in the antibiotic sensitivity of these bacteria to certain groups and classes of antibacterial drugs, which causes increasing concern among the world community [5]. This situation has developed because up to 95% of cases of severe infections are treated without proper bacteriological examination [6].

Therefore, understanding the specific features of biofilm formation and the development of resistance to antibacterial drugs in *E. coli* strains will help open new areas in the diagnostics, treatment, and prevention of infectious diseases associated with biofilm-forming *E. coli* strains.

Biofilms are specific microbial communities formed on biotic and abiotic surfaces by secreting extracellular polymer substances that increase the level of adhesion to surfaces [7].

Bacteria inside the biofilm become more tolerant to the effects of various exogenous factors, such as antibacterial

drug [2]. Increased stability of biofilms is explained by several factors: 1) different rate of diffusion of substances; 2) the accumulation in the matrix of extracellular enzymes that destroy antibacterial drugs; 3) unavailability of bacteria due to clumping; 4) stable properties of the cells themselves, which are involved in the formation of a biofilm [8-10]. Thanks to these properties of the biofilm, pathogenic bacteria, which are the causative agents of many chronic infections, necessitate increased requirements for the disinfection of medical equipment and medical instruments [11]. Biofilm formation is an intra- and interspecific phenomenon that requires dynamic interactions between bacteria in mixed biofilm communities [12]. Bacterial species from biofilms interact through cell-to-cell communication, metabolic interaction, or spatial organisation [1].

Since its discovery in 1885, the status of *E. coli* has changed many times. Among the commensal *E. coli* strains, it was mostly shown that they do not have specialised virulence determinants and are useful for their host [13]. While among the pathogenic strains of *E. coli*, enterotoxigenic *Escherichia coli* (ETEC) and enteropathogenic *Escherichia coli* (EPEC) are recognised as the most common cause of bacterial putrefactive infections, especially in low-income countries with unsatisfactory sanitary conditions [14]. *E. coli* is a common inhabitant of the intestines of animals and humans, but can also occur in environmental objects, namely in water, soil, and vegetation. It is the leading causative agent of urinary tract infections [15] and is one of the most common pathogens that cause blood flow infections, wound infections, otitis media, and other complications in both animals and humans [5]. Unlike commensals and enteropathogenic strains, extraintestinal pathogenic *E. coli* cause infections of the urinary tract, bloodstream, cerebrospinal fluid, respiratory tract, and peritoneum. With such infections, the pathology can occur in the form of cholecystitis, bacteraemia, cholangitis, urinary tract infection, or neonatal meningitis. Infections caused by such pathogenic strains are widely reported in public places, as well as in veterinary clinics and long-term animal care facilities, which causes a considerable burden on medical and economic resources around the world [16; 17].

Although the current knowledge of bacterial biology is largely based on work carried out on planktonic cultures of *E. coli* laboratory strains, many isolates have the ability to form biofilms *in vivo* and *in vitro* [18].

Bacteria of the *Enterobacteriaceae* family are the most common pathogens of nosocomial infections that occur in veterinary clinics and medical institutions of Ukraine [19]. *E. coli* strains are polyresistant to antibacterial drugs of various groups and therefore belong to one of the most problematic pathogens of nosocomial infections – ESKAPE. The researchers found that 52.8% of enterobacteria strains isolated from surgical wounds were beta-lactamase producers. *E. coli* ranks second among beta-lactamase producers after *Klebsiella pneumoniae* and makes up 41.2% of the total number of strains [19].

Thus, M.D. Kukhtin et al. (2014) indicate that *E. coli* strains isolated from milking equipment in farms with good and satisfactory sanitary conditions, wherein antimicrobial drugs were widely used, formed biofilms of

high and medium density in 80% or more, which is 1.6-2.4 times more compared to farms with unsatisfactory sanitary conditions [20]. Furthermore, the results of the study by M.M. Mishyna et al. (2020) testify that the formation of biofilms in strains of microorganisms isolated from chronically ill patients who were treated with antibacterial drugs for a long time was more pronounced compared to patients with acute forms of infections [21]. According to Yu.Yu. Vishovan et al. (2021), all microorganisms isolated from sick and healthy animals can form biofilms [10]. Therewith, the highest density biofilm is observed in microorganisms described by a higher number of phenotypic and genotypic pathogenicity factors.

Therefore, *the purpose of this study* was to investigate the phenotypic features of biofilm formation, as well as to establish the interdependence between the density of the formed biofilm and resistance to antibacterial drugs of different groups of *E. coli* strains that were parted from pathological and biological materials isolated from cats and dogs.

Materials and Methods

The study was performed based on the Ukrainian Laboratory of Quality and Safety of Agricultural Products of the National University of Life and Environmental Sciences of Ukraine (Kyiv, Ukraine) and at the Department of Epizootology, Microbiology, and Virology of the National University of Life and Environmental Sciences of Ukraine (Kyiv, Ukraine) during 2020-2021.

For the study, 10 cultures of *E. coli* were selected among parted isolates from various species of sick and clinically healthy animals that were patients at the "Multivet" veterinary clinic (Kyiv Oblast, Ukraine). Cultures of *E. coli* were isolated as a result of bacteriological examination of pathological and biological materials from 36 dogs, of which 27 were clinically healthy, and 9 had purulent wound complications, and 27 cats, of which 18 were clinically healthy, and 6 had purulent wound complications.

Biological material was seeded on selective and differential diagnostic nutrient media: 5% blood agar produced by Graco (Poland), Endo medium produced by HiMedia (India), yolk-salt Agar produced by HiMedia (India), meat-peptone broth produced by HiMedia (India) with the addition of 5% glucose. Sowing was carried out by the quantitative method of sector seeding according to the Gold's method. Microorganisms were identified using a Vitek 2 compact bacteriological analyser (bioMérieux, France).

Sensitivity to antibacterial drugs of *E. coli* cultures was determined according to the disco-diffusion method using discs manufactured by HiMedia (India) and Muller-Hinton Agar manufactured by HiMedia (India) according to the EUCAST recommendations (European Committee for Antimicrobial Sensitivity Testing) [22]. Reference strains *E. coli* ATCC 25922 and *E. coli* ATCC 35218 were used for quality control of antimicrobial susceptibility tests.

Phenotypic determination of resistance factors was performed using CHROMagar™ ESBL manufactured by Graco (Poland).

The ability to form a biofilm in isolates and the interpretation of the results obtained were performed according to the modified method of M. D. Kukhtin [20]. This study was

performed using sterile polystyrene tablets (Greiner Bio-One GmbH, Germany) $n=96$, in each well of which 100 μL of cardiovascular broth produced by HiMedia (India) was added, and 10 μL of inoculant containing 0.5 McFarland daily culture of the *E. coli* isolates under study. Each individual isolate was cultured in 1 row with 12 wells in two different plates. Planchettes were cultured in a thermostat at 37°C for 24 hours. The remaining nutrient medium was carefully removed with a pipette dispenser. Planchette wells were washed three times from planktonic forms of *E. coli* isolates with a sterile phosphate buffer solution ($\text{KH}_2\text{PO}_4 \cdot \text{Na}_2\text{PO}_4 \cdot \text{H}_2\text{O}$), pH 7.2-7.4. The planchettes were air-dried and 100 μL of 96% ethanol was added to fix the resulting biofilms. The fixation exposure was 10 minutes. Then the fixing liquid was drained. Subsequently, the planchettes were divided into two groups. The first plate was stained with a 0.1% alcohol solution of crystal violet for 10 minutes; the second was dyed with a saturated aqueous Congo Red solution for 15 minutes. Subsequently, the planchettes were washed three times with a sterile phosphate buffer solution (pH 7.2) and dried. Then 100 μL of 96% ethanol was added and placed on a shaker to shake and then repel the biofilm from the well walls for 30 minutes. Finally, the contents of the planchette wells were pipetted and the amount of dye absorption by the biofilm was measured on an Evolution 300 spectrophotometer (Thermo Fisher Scientific, USA) at a wavelength of 570 nm for a tablet with a solution of crystal violet and 495 nm for a tablet with a saturated aqueous Congo Red solution. The density of the resulting biofilm was determined by measuring the level of dye adsorption with ethanol, which was expressed in units of optical density (OD) using a spectrophotometer.

When the optical density value is less than 0.10, it was assumed that the strains do not form biofilms, from 0.10 to 0.49 – the ability to form a film was considered low. If the optical density is from 0.50 to 1.0, it is the average density of the biofilm and its ability to form it. At values above 1.0 – high ability to form a biofilm and its high density.

Statistical analysis was performed in a Microsoft Excel 2010 spreadsheet. The Online EpiTools epidemiological calculator was used to estimate the 95% confidence interval (CI) [23].

Results and Discussion

This paper presents the results of the study of 10 *E. coli* cultures, which were selected among isolates parted from various species of sick and clinically healthy animals. This study investigated 6 strains of *E. coli* isolated from sick animals and 4 strains from clinically healthy individuals. Samples of the material from which the cultures under study were isolated were selected from the "Multivet" Veterinary Clinic (Kyiv Oblast, Ukraine) for the 2nd quarter of 2020.

E. coli cultures were isolated as a result of bacteriological examination of pathological and biological materials from dogs and cats: 4 isolates (40%) from dogs with purulent wound complications; 2 isolates (20%) from clinically healthy dogs, collected from the intestine and oral cavity; 2 isolates (20%) – from cats with purulent wound complications; 2 isolates (20%) from the intestines of clinically healthy cats (Table 1).

Table 1. The results of determining the presence of extended-spectrum beta-lactamases in *E. coli* cultures, which were selected among isolates parted from different animal species

No. Seq. No.	Strain	Isolation object	Locus	Presence of extended-spectrum beta-lactamases
1	EC1/21	Dogs	Wound	-
2	EC2/21	Dogs	Wound	+
3	EC3/21	Cats	Wound	+
4	EC4/21	Cats	Wound	-
5	EC5/21	Dogs	Wound	+
6	EC6/21	Dogs	Wound	+
7	EC7/21	Cats	Intestines	-
8	EC8/21	Cats	Intestines	-
9	EC9/21	Dogs	Intestines	-
10	EC10/21	Dogs	Oral cavity	-

The presence of extended-spectrum beta-lactamases for *E. coli* cultures was determined on the CHROMagar™ ESBL medium (Table 1, Fig. 1). Therewith, after cultivation for 24 hours, colonies of EC1/21, EC4/21,

EC7/21, EC8/21, EC9/21, EC10/21 strains on chromium agar had weak growth of small colourless colonies. Strains EC2/21, EC3/21, EC5/21, and EC6/21 formed lilac-coloured colonies.

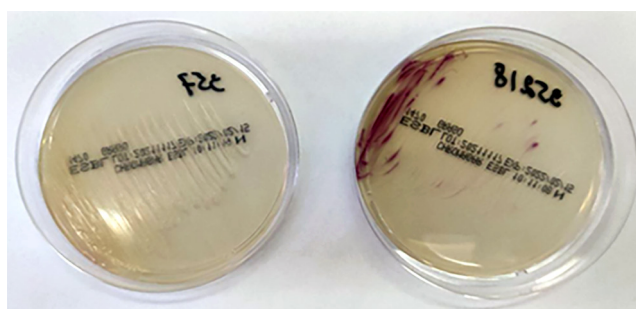


Figure 1. Phenotypic determination of resistance factors on CHROMagar™ ESBL

Note: EC4/21 culture dish on the left (negative), EC5/21 culture dish on the right (positive)

During the study of isolates for sensitivity to antibacterial drugs, the following data were obtained (Table 2): 60% of isolates were resistant to the penicillin group; 80% of isolates – to the cephalosporin group; 60% of isolates – to the fluoroquinolone group; 10% of isolates – to the carbapenem group; 30% of isolates – to the

aminoglycoside group. Therewith, all isolates were sensitive to tobramycin and ceftazidime/avibactam. Isolates selected from biomaterial from clinically healthy animals showed resistance to fluoroquinolones, while isolates from pathological material showed a higher percentage of sensitivity.

Table 2. Sensitivity of *E. coli* isolates to antibiotics of different groups according to EUCAST recommendations (M±m, n=6, P<0.01)

Anti-bacterial preparation	EUCAST (mm)		EC1/21	EC2/21	EC3/21	EC4/21	EC5/21	EC6/21	EC7/21	EC8/21	EC9/21	EC10/21
	S≥	R<										
Penicillins												
Piperacillin	20	20	6.1R	10.2R	9.1R	20.2S	9.6R	10.4R	6.1R	21.6S	24.7S	21.1S
Piperacillin / tazobactam	20	20	6.2R	22.4S	9.5R	24.9S	12.6R	21.4S	13.3R	25.3S	26.2S	22.1S
Ticarcillin / clavulanate	23	20	6.7R	25.3S	21.2I	9.3R	16.2R	15.9R	22.1I	15.5R	26.8S	10.4R
Ampicillin / sulbactam	14	14	16.2S	19.5S	9.5R	6.2R	14.5S	6.9R	19.2S	22.5S	24.7S	20.8S

Table 2, Continued

Amoxicillin / clavulanate	19	19	12.1R	21.1S	15.5R	14.2R	16.8R	20.6S	16.1R	14.2R	16.7R	12.2R
Fluoroquinolones												
Levofloxacin	23	19	6.2R	12.3R	16.1R	24.5S	26.1S	16.1R	28.1S	12.1R	10.2R	12.8R
Ciprofloxacin	25	22	6.5R	6.1R	9.2R	27.1S	20.1I	10.2R	27.2S	15.3R	16.2R	20.0R
Moxifloxacin	22	22	12.2R	22.1S	19.1R	26.2S	20.1R	13.8R	24.2S	19.1R	22.1S	17.4R
Ofloxacin	24	22	8.2R	26.8S	16.8R	28.9S	26.7S	14.2R	16.8R	18.9R	16.9R	15.2R
Cephalosporins												
Cefepime	27	24	26.4I	19.2R	11.8R	28.1S	14.4R	13.4R	18.8R	12.2R	19.8R	13.2R
Cefotaxime	20	17	12.9R	10.8R	9.1R	22.7S	13.1R	7.1R	11.7R	20.8S	22.8S	16.8R
Cefuroxime	50	19	16.8R	15.9R	10.7R	29.6I	16.9R	12.6R	14.5R	13.8R	16.3R	14.1R
Ceftazidime	22	19	10.1R	13.1R	13.2R	25.2S	17.0R	9.2R	12.1R	14.4R	21.2I	20.6I
Ceftazidime / avibactam	13	13	16.2S	18.3S	20.0S	16.9S	23.1S	16.4S	19.1S	22.1S	20.9S	25.3S
Carbapenems												
Meropenem	22	16	25.2S	23.3S	28.1S	26.2S	10.2R	29.1S	24.3S	23.2S	20.2I	29.1S
Imipenem	22	19	30.3S	14.6R	13.2R	29.1S	16.1R	20.1I	28.7S	13.1R	21.6I	10.9R
Aminoglycosides												
Amikacin	18	18	20.2S	21.9S	23.6S	22.9S	20.4S	25.3S	10.3R	9.2R	12.3R	14.4R
Tobramycin	16	16	20.1S	26.2S	19.2S	23.3S	25.2S	22.1S	17.2S	18.3S	17.6S	22.0S
Gentamicin	17	17	19.2S	15.1R	20.1S	23.8S	10.2R	13.9R	11.9R	16.1R	19.9S	8.2R
Other												
Aztreonam	26	21	10.8R	24.6I	11.3R	13.6R	19.0R	24.3I	27.1S	25.2I	29.2S	26.3S
Fosfomycin	21	21	13.0R	10.6R	18.2R	20.0R	11.3R	26.8S	13.8R	24.5S	26.7S	22.2S
Tigecycline	18	18	20.2S	23.9S	26.8S	25.1S	26.1S	20.1S	19.7S	6.8R	20.2S	9.8R

Based on the results of the study of the phenotypic formation of biofilms (Table 3, Fig. 2) upon using the crystal violet staining method, EC2/21, EC3/21 strains formed a

high-density biofilm; EC5/21, EC6/21 strains formed a medium-density biofilm; and EC1/21, EC4/21, EC7/21, EC8/21, EC9/21, EC10/21 strains formed a low-density biofilm.

Table 3. Results of phenotypic determination of *E. coli* biofilm formation (crystal violet and Congo Red staining)

No. Seq. No.	Strain	Density of the formed biofilm (n=12)	
		Upon crystal violet staining	Upon Congo Red staining
		λ 570	λ 495
1	EC1/21	0.421	0.462
2	EC2/21	1.006	1.063
3	EC3/21	1.144	1.178
4	EC4/21	0.524	0.518
5	EC5/21	0.635	0.696
6	EC6/21	0.596	0.554
7	EC7/21	0.298	0.356
8	EC8/21	0.293	0.334
9	EC9/21	0.427	0.498
10	EC10/21	0.436	0.496

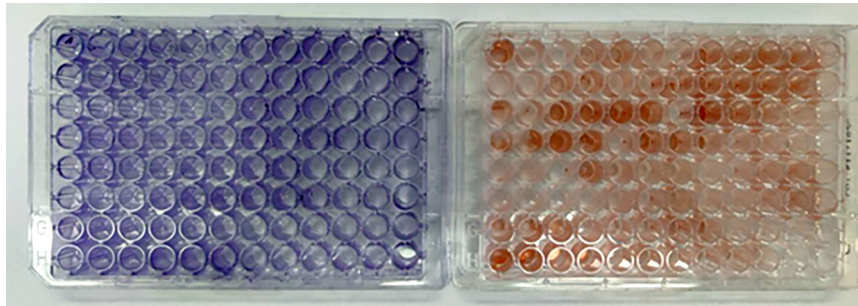


Figure 2. *E. coli* biofilm formation stained with crystal violet (left panel) and Congo Red (right panel)

In turn, for the study of the phenotypic formation of biofilms (Table 3, Fig. 2) upon using the Congo Red staining method, EC2/21, EC3/21 strains formed a high-density biofilm; EC/21, EC6/21 strains formed a medium-density biofilm; and EC1/21, EC4/21, EC7/21, EC8/21, EC9/21, EC10/21 strains formed a low-density biofilm.

In 4 *E. coli* strains isolated from dog wounds, the optical density of biofilm *in vitro* ranged from λ 0.421-1.006 for crystal violet and λ 0.462-1.063 for Congo Red. In 2 *E. coli* strains isolated from clinically healthy dogs, the optical density of biofilms was λ 0.427 (EC9/21) and 0.436 (EC10/21) for crystal violet and λ 0.354 (EC9/21) and 1.063 (EC10/21) for Congo Red. In 2 *E. coli* strains isolated from wounds in cats, the optical densities were λ 1.144 (EC3/21) and 0.524 (EC4/21) for crystal violet and λ 1.178 (EC3/21) and 0.518 (EC4/21) for Congo Red. In 2 *E. coli* strains isolated from the intestines of cats, the optical density was λ 0.298 (EC7/21) and 0.293 (EC8/21) for crystal violet and λ 0.356 (EC7/21) and 0.334 (EC8/21) for Congo Red.

According to the above results, there is an interdependence of antibiotic resistance of *E. coli* isolates and their ability to form a biofilm. The ability of isolates to form a higher-density biofilm was accompanied by better resistance to antibacterial drugs. Thus, EC2/21 and EC3/21 isolates parted from pathological material that had a positive reaction to CHROMagar™ ESBL medium for the determination of extended-spectrum beta-lactamases and greater resistance to antibacterial drugs (Table 2) formed a high-density biofilm with λ 1.006 (EC2/21) and λ 1.144 (EC3/21). Isolates from the pathological material isolated from dogs, EC5/21, EC6/21, which had a positive reaction to CHROMagar™ ESBL medium and high resistance to antibacterial drugs (Table 2) formed a medium-density biofilm with λ 0.635 (EC5/21) and λ 0.596 (EC6/21). Therewith, isolates from pathological and biological material that had a negative reaction to the CHROMagar™ ESBL medium formed a low-density biofilm. This indicates a positive correlation between the level of antibiotic resistance of isolates and the intensity of biofilm formation.

The results obtained regarding the biofilm formation of *E. coli* coincide with the results of the study by M.D. Kukhtin et al. [20], who indicated an increase in the density of the biofilms formed with the intensive use of antibacterial drugs. In the case under study, *E. coli*, which had less resistance to antibacterial drugs, formed a lower-density biofilm than bacteria that had higher resistance to

these drugs, indicating an increase in the biofilm-forming capacity of bacteria in response to the use of antibacterial drugs. Furthermore, the regularities established in this paper confirm the results of studies by M.M. Mishyna [21] and Yu.Yu. Vishovan [10], who note that an increase in both phenotypic and genotypic antibiotic resistance of microorganisms increases their ability to form biofilms, which is an essential factor in the mechanism of formation of resistance to antibacterial drugs.

Thus, upon developing an effective regimen for the treatment of purulent lesions with antibacterial drugs, it is necessary to conduct a bacteriological study for each individual isolate. Equally important is the monitoring of the antibiotic sensitivity of opportunistic and pathogenic bacteria to adjust the already available antibiotic therapy regimens. Furthermore, upon identifying isolates with a high level of antibiotic resistance, their increased ability to form biofilms should be considered. This may require added measures to disinfect medical equipment and instruments, as well as the need to introduce drugs that prevent the formation of biofilms into the treatment regimen.

Notably, an in-depth study of the correlation of antibiotic resistance among biofilm-forming strains of opportunistic and pathogenic bacteria will be the subject of the author's further research in this area.

Conclusions

As a result of the study of 63 samples of pathological and biological material, 10 *E. coli* strains were isolated – 6 from dogs and 4 from cats. Isolates parted from pathological material had higher resistance to antibacterial drugs than strains isolated from clinically healthy animals.

When investigating the results of phenotypic biofilm formation in a comparative aspect using the Congo Red and crystal violet staining method, no substantial differences were found. *E. coli* isolates with high resistance to antibacterial drugs were found to have the ability to form higher-density biofilms compared to more sensitive isolates. *E. coli* isolates that had the ability to form beta-lactamase formed a higher-density biofilm than isolates in which this enzyme was not synthesised.

To screen the ability of microorganisms to form extended-spectrum beta-lactamases, it is advisable to use chromium-agar, which will substantially speed up obtaining informative results.

References

- [1] Ushkalov, V., Salmanov, A., Kalachniuk, L., Vishovan, Y., Boianovskiy, S., Ushkalov, A., Granate, A., Huwiage, G.M., & Kepple, O. (2020). The influence of cultivation temperature on some phenotypic traits of *Yersinia pseudotuberculosis*. *One Health & Risk Management*, 1(2), 34–40. doi: 10.38045/ohrm.2020.1.14.
- [2] Tallawi, M., Opitz, M., & Lieleg, O. (2017). Modulation of the mechanical properties of bacterial biofilms in response to environmental challenges. *Biomaterials Science*, 5, 887–900. doi: 10.1039/C6BM00832A.
- [3] Akter, S., Chowdhury, A., & Mina, S.A. (2021). Antibiotic resistance and plasmid profiling of *Escherichia coli* isolated from human sewage samples. *Microbiology Insights*, 14. doi: 10.1177/11786361211016808.
- [4] Albert, M.J., Bulach, D., Alfouzan, W., Izumiya, H., Carter, G., Alobaid, K., Alatar, F., Sheikh, A.R., & Poirel, L. (2019). Non-typhoidal *Salmonella* blood stream infection in Kuwait: Clinical and microbiological characteristics. *Plos Neglected Tropical Diseases*, 13(4). doi: 10.1371/journal.pntd.0007293.
- [5] Mahmud, Z.H., Shirazi, F.F., Hossainey, M., Islam, M.I., Ahmed, M.A., Nafiz, T.N., Imran, K.M., Sultana, J., Islam, M.S., Islam, M.A., & Islam, M.S. (2019). Presence of virulence factors and antibiotic resistance among *Escherichia coli* strains isolated from human pit sludge. *Journal of Infection in Developing Countries*, 13(3), 195–203. doi: 10.3855/jidc.10768.
- [6] Gonzales-Rodriguez, A.O., Infante Varillas, S.F., Barrón Pastor, H.J., Llimpe Mitma, Y., Huerta Canales, D., Wong Chero, P.A., Gutierrez, C., & Suarez Cunza, S. (2020). Immunological and biochemical response of older adults with urinary tract infection to uropathogenic *Escherichia coli* virulence factors, *Rev Peru Med Exp Salud Publica*, 37(3), 527–531. doi: 10.17843/rpmesp.2020.373.4918.
- [7] Hall, C.W., & Mah, T.F. (2017). Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEMS Microbiology Reviews*, 41(3), 276–301. doi: 10.1093/femsre/fux010.
- [8] Del Pozo, J.L. (2018). Biofilm-related disease. *Expert Review of Anti-Infective Therapy*, 16(1), 51–65. doi: 10.1080/14787210.2018.1417036.
- [9] Huang, J., Liu, S., Zhang, C., Wang, X., Pu, J., Ba, F., Xue, S., Ye, H., Zhao, T., Li, K., Wang, Y., Zhang, J., Wang, L., Fan, C., Lu, T.K., & Zhong, C. (2019). Programmable and printable *Bacillus subtilis* biofilms as engineered living materials. *Nature Chemical Biology*, 15(1), 34–41. doi: 10.1038/s41589-018-0169-2.
- [10] Vishovan, Y., Ushkalov, V., Vygovska, L., Ishchenko, L., Salmanov, A., Bilan, A., Kalakailo, L., Hranat, A., & Boianovskiy, S. (2021). Biofilm formation and antibiotic resistance in *Staphylococcus* isolated from different objects. *EUREKA: Life Sciences*, (4), 58–65. doi: 10.21303/2504-5695.2021.001925.
- [11] Miquel, S., Lagrafeuille, R., Souweine, B., & Forestier, C. (2016). Anti-biofilm activity as a health issue. *Frontiers in Microbiology*, 7, 592. doi: 10.3389/fmicb.2016.00592.
- [12] Puligundla, P., & Mok, C. (2017). Potential applications of nonthermal plasmas against biofilm-associated micro-organisms in vitro. *Journal of Applied Microbiology*, 122(5), 1134–1148. doi: 10.1111/jam.13404.
- [13] Baker, K.S. (2015). Demystifying *Escherichia coli* pathovars. *Nature Reviews Microbiology*, 13(1), 5. doi: 10.1038/nrmicro3411.
- [14] Rohatgi, A., & Gupta, P. (2021). Natural and synthetic plant compounds as anti-biofilm agents against *Escherichia coli* O157:H7 biofilm. *Infection, Genetics and Evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases*, 95, article number 105055. doi: 10.1016/j.meegid.2021.105055.
- [15] Kot, B. (2019). Antibiotic resistance among uropathogenic *Escherichia coli*. *Polish Journal of Microbiology*, 68(4), 403–415. doi: 10.33073/pjm-2019-048.
- [16] Poolman, J.T., & Wacker, M. (2016). Extraintestinal pathogenic *Escherichia coli*, a common human pathogen: Challenges for vaccine development and progress in the field. *The Journal of Infectious Diseases*, 213(1), 6–13. doi: 10.1093/infdis/jiv429.
- [17] Raeispour, M., & Ranjbar, R. (2018). Antibiotic resistance, virulence factors and genotyping of uropathogenic *Escherichia coli* strains. *Antimicrobial Resistance and Infection Control*, 7, 118. doi: 10.1186/s13756-018-0411-4.
- [18] Roy, R., Tiwari, M., Donelli, G., & Tiwari, V. (2018). Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action. *Virulence*, 9(1), 522–554. doi: 10.1080/21505594.2017.1313372.
- [19] Potochylova, V., Rudnyeva, K., Pokas, O., & Vyshnyakova, H. (2020). Sensitivity to antibacterial drugs and phenotypic determination of resistance factors in microorganisms of the family *Enterobacteriaceae* – pathogens of wound infections. *Bulletin of Problems of Biology and Medicine*, 4(158), 259–263. doi: 10.29254/2077-4214-2020-4-158-259-263.
- [20] Kukhtin, M.D., & Krushelnyska, N.V. (2014). Formation of biofilms by microorganisms isolated from milking equipment. *Animal Biology*, 16(1), 95–103. doi: 10.15587/1729-4061.2017.110488.
- [21] Mishyna, M.M., Makieieva, N.I., Marchenko, I.A., Golovachova, V.A., & Osolodchenko, T.P. (2020). Biofilms formation by pyelonephritis causative agents in infants as a mechanism of resistance to antimicrobial agents. *Ukrainian Journal of Medicine, Biology and Sport*, 2(24), 104–111. doi: 10.26693/jmbs05.02.104.
- [22] Eucast. The European committee on antimicrobial susceptibility testing. Retrieved from <https://www.eucast.org/>
- [23] Sergeant, ESG. Epitools Epidemiological Calculators. Ausvet. (2018). Retrieved from <https://epitools.ausvet.com.au>.

Особливості біоплівкоутворення деяких патогенних та комменсальних *Escherichia coli*, ізольованих з організму собак і котів

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Анотація. Актуальність дослідження зумовлена процесом епідемічного росту внутрішньолікарняних інфекцій, до яких відноситься *Escherichia Coli* (*E. coli*). Одним із факторів патогенності таких мікроорганізмів є здатність до утворення біоплівки – складноорганізованого співтовариства, всередині якої бактерії набувають підвищеної стійкості до факторів довкілля, насамперед, до антибактеріальних препаратів, чим значно ускладнюють перебіг інфекційного процесу. У зв'язку з цим, метою роботи було визначити особливості формування та залежності щільності сформованої біоплівки від антибіотикорезистентності патогенних та комменсальних штамів *E. coli*, виділених від собак і котів. Диско-дифузійним методом, згідно з рекомендаціями EUCAST, встановлено стійкість ізолятів *E. coli* до антибактеріальних препаратів. Вивчення здатності мікроорганізмів формувати біоплівки та визначення їх щільності проводили у стерильних пластикових 96-лункових планшетах. Здатність формувати біоплівки оцінювали візуально та мікроскопічно, щільність біоплівок визначали в одиницях, спектрофотометрично, за оптичною густиною промивного розчину. Наведено результати дослідження 63 проб патологічного (ранові інфекції) та біологічного матеріалу. З них отримано 10 ізолятів *E. coli* (6 від собак і 4 від котів), які було обрано для подальшого дослідження. Встановлено, що всі ізоляти *E. coli* мали здатність до фенотипового утворення біоплівки. Досліджена взаємозалежність антибіотикорезистентності ізолятів *E. coli* та їх здатність утворювати біоплівку. Так ізоляти, які були виділені з патологічного матеріалу та мали позитивну реакцію на середовищі CHROMagar™ ESBL для визначення бета-лактамаз розширеного спектру, мали більшу резистентність до різних груп антибактеріальних препаратів і утворювали біоплівку високої та середньої щільності, тоді, як ізоляти *E. coli*, виділені з патологічного та біологічного матеріалів з негативною реакцією на середовищі CHROMagar™ ESBL утворювали біоплівку низької щільності та мали меншу резистентність до різних груп антибактеріальних препаратів. Отримані результати дають можливість пошуку нових, інколи альтернативних, методів лікування та профілактики викликаних ними патологій

Ключові слова: мікроорганізм, біоплівка, антибіотикорезистентність, антибактеріальні препарати, ранова інфекція