
BIOLOGICAL PROPERTIES OF *STAPHYLOCOCCI* DERIVED FROM CATS AND DOGS

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Abstract. Dogs and cats that live near humans are a source of zoonotic agents. The purpose of the study was to investigate domestic dogs and cats for staphylococcus carriers and to study their biological properties, biofilm ability, and antibiotic resistance in isolated cultures. A total of 44 samples were collected from 19 dogs and 25 cats of different age and sex groups. Staphylococci were detected in 54% of all samples selected. 25 cultures of *Staphylococcus* spp. were isolated. Plasma coagulation reaction was negative in 100% of strains isolated from cats and positive in 2 strains isolated from dogs (18%). Lecithinase activity was detected in 85.8% of strains isolated from cats and 72.8% from dogs. 71.5% of strains isolated from cats and 63.7% from dogs had the ability to induce hemolysis. Mannitol was fermented in 50.0% of strains isolated from cats and 54.6% from dogs. 78.6% of strains isolated from cats and 91.0% isolated from dogs grew on crystal violet lactose agar in the

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form of blue colonies. Simultaneously 28.5% of strains isolated from cats and 27.2% isolated from dogs showed the lecithinase activity, hemolysis, fermented mannitol, and grew on crystal violet lactose agar. The derived strains from cats and dogs had multiple resistance to Oxacillin, two or more groups of antibiotics. As a result of the study of biofilm formation, all 100% of isolates obtained from dogs had a high optical density ($OD > 1.0$) and the ability to form a biofilm. Only 21.4% of strains isolated from cats formed biofilms with a medium optical density ($OD > 0.5 < 1.0$), and the remaining 78.5% – high optical density ($OD > 1.0$) and the ability to form a biofilm.

Keywords: coagulase-positive and coagulase-negative *Staphylococci*, biofilms, antibiotic resistance

Introduction

Zoonoses are infectious diseases that spread between animals and humans and can be caused by microorganisms (bacteria, viruses, microscopic fungi) and parasites. They are among the most common diseases in the food chain of transmission (so-called food poisoning) and can also be transmitted to humans either in direct contact with a diseased animal or with a vector / carrier. According to a 2019 report of the European Food Safety Authority and European Center for Disease Prevention and Control (EFSA and ECDC), there are typically about 5 000 outbreaks of food zoonoses per year in the European Union, affecting more than 40 000 people.

In many cases, patients need hospitalization, and deaths are recorded. Together, they pose a major challenge to the health care system and have important implications for international trade.

Analysis of recent researches and publications

Dogs and cats as the main pets occupy a special place in human life. Often, dogs and cats have various diseases caused by *Staphylococci* microorganisms. The main pathogens are *S. aureus* (Weese & Duijkeren, 2010), *S. schleif-*

eri (Lee et al., 2019) *S. pseudointermedius*, *S. haemolyticus* (Kizerwetter-Świda et al., 2019), and many other coagulase-negative *Staphylococci*.

The widespread use of antimicrobials in the treatment of humans and their pets has led to the activation of resistance in certain types of microorganisms, in particular, in *Staphylococci*. One of the representatives of *Staphylococci* – the methicillin-resistant (MRSA) *S. aureus*. As a result of an in-depth study of the resistance of *Staphylococci* to antibiotics, a separate group of methicillin resistant (MRS) and methicillin susceptible (MSS) – *S. aureus* LA (livestock animals) was derived. *Staphylococcus* strains containing antibiotic resistance genes colonize the mucous membranes and skin of a wide variety of animal species, especially domestic dogs and cats. Numerous studies (Weese & Duijkeren, 2010; Hatch et al., 2012; Quitocoet al., 2013; Savini et al., 2013; Worthing at al., 2018; Gómez-Sanz et al., 2019; Loncaric et al., 2019) are devoted to the study of the transmission of *Staphylococci* from dogs and cats to humans and their biological properties. *Staphylococci* derived from dogs and cats are known to have a different set of pathogenicity features, such as the presence of coagulase lecithinase, hemolytic activity, toxin forma-

tion, biofilm formation, etc. (Rahman et al., 2018; Loncaric et al., 2019). At present, the phenomenon of biofilm formation needs to be thoroughly studied. It is known that they are formed due to the action of aggressive factors: detergents, disinfectants, antibiotics, and probiotics (Paharik & Horswill, 2016; Ahmadrabi et al., 2017; Stewart et al., 2017; Otto, 2018; Turko & Ushkalov, 2018; Vitale et al., 2019). In 2017, 80 MRS strains were isolated in Ukraine, including 77.5% from domestic animals, 11.3% from poultry, 6.3% from cattle, and 5% from pigs (Kozytska et al., 2019).

Purpose. In view of the above, the aim of our work was to investigate domestic dogs and cats as the *Staphylococcus* carriers and to study the biological properties of the selected cultures, in particular, the ability to biofilm and antibiotic resistance.

Materials and methods of research

Work was carried out in the Research Department of Microbiological Research of the Ukrainian Laboratory for Quality and Product Safety of the Agro-industrial Complex of the National University of Life and Environmental Sciences of Ukraine during August–March 2019–2020. The clinical research material was selected at the “Multivet” veterinary clinic in the Kyiv region. A total of 44 samples were collected from 19 dogs and 25 cats of different age and sex groups (Table 1).

Selection and identification of *Staphylococcus spp.* was carried out in accordance with DSTU EN 6888: 2003 “Microbiology of food products and animal feed”. Using the Baird-Parker agar medium (Merck, Germany). In the derived cultures, colony morphology, lecithinase activity, Gram staining, and catalase test were performed. Hemolytic properties were studied on Colombian blood agar (Biomérieux, France) at a temperature of 37 °C for 24 hours. The coagulase activity *in vitro* by lyophilized citrate plasma rabbit (Bioliik, Ukraine) was determined. The formed clots were evaluated visually after 2, 4, 18, and 24 hours of incubation at a temperature of 37 °C. The main characteristics of the colonies growth on Mannitol Salt agar (Merck, Germany) were determined. The sowing was performed by looping on the agar surface, cultured at temperature of 37 °C for 48 hours, and the colonies were evaluated visually. The presence of colonies on Crystal Violet Lactose agar (HiMedia, India) and their color were assessed visually.

Antibiotic resistance of cultures was determined by the disc diffusion method (Kirby-Bauer) using antibiotic discs and a Müller-Hinton agar medium (HiMedia, India), McFarland inoculum 0.5 optical density was determined using a DEN-1 densitometer (Biosan, Latvia) and McFarland standard (HiMedia, India). The incubation was carried out at a temperature of 37 °C for 18 ± 2 hours. The resistance of the obtained strains to Benzylpenicillin,

1. Quantitative characterization of selected samples by disease groups

Type	Number of samples	Pathologies				
		Skin	Eyes	Ears	Traumas	Clinically healthy
Dogs	19	2	4	2	5	6
Cats	25	3	5	3	2	12

Ampicillin, Oxacillin, Norfloxacin, Ciprofloxacin, Levofloxacin, Gentamicin, Tobramycin, Erythromycin, Tetracycline, Doxycycline, and Chloramphenicol was investigated. The results were evaluated in accordance with the recommendations of the European Committee for Antimicrobial Sensitivity EUCAST (version 10.0) and the national criteria for the assessment of antibiotic resistance – the methodological guidance “Determination of susceptibility of microorganisms to antibacterial drugs”.

The ability to form biofilms in the derived isolates was determined and the results obtained were interpreted (Kukhtyn & Krushelnyska, 2014). This study was performed using sterile polystyrene Petri dishes (Greiner Bio-One GmbH, Germany) of $d = 35$ mm, which were added to 5 mL of Tryptone-soy broth (HiMedia, India) and added 1 ml of inoculum with a cell content of 0.5 to MacFarland daily culture of studied *Staphylococci*. The plates were cultured in a thermostat at a temperature of 37 °C for 24 hours, the residues of the nutrient medium were carefully removed, the planktonic forms were washed three times with a sterile phosphate buffer solution ($\text{KH}_2\text{PO}_4 \times \text{Na}_2\text{PO}_4 \times \text{H}_2\text{O}$) (pH 7.2–7.4). The Petri dishes were air-dried and 5 mL of Ethanol 96 % was added to fix the formed biofilms. The fixation exposure was 10 minutes. Then the fixing liquid was drained and after it, the Petri dishes were stained with 0.1% alcohol solution of crystal violet for 10 min. Then washed the plates three times with sterile phosphate buffer solution (pH 7.2) and dried. Contributed to 5 mL of Ethanol 96% and placed on a shaker for shaking for 30 min. The contents of the Petri dishes were then pipetted and the amount of absorption of the biofilm of the dye was measured on an Evolution 300 spectrophotometer (Thermo Fisher Scientific, USA) at a wavelength of 570 nm. The density of the

formed biofilm was determined by measuring the adsorption level of the dye with ethanol measured in units of optical density (OD) using a spectrophotometer.

When the value of an optical density is less than 0.1, it was considered that the strains do not form a biofilm, from 0.1 to 0.49 – the ability to form a film was considered as a low. When the value of optical density is from 0.5 to 1.0 – the medium density of the biofilm and the ability to form it. At values above 1.0 – the high ability to form a biofilm and its high density (Kukhtyn & Krushelnyska, 2014).

Results of the research and their discussion

According to the results of the experiments, *Staphylococci* were detected in 54% of all samples. Total of 25 *Staphylococcus spp.* cultures were derived and investigated.

On the Baird-Parker agar, isolated cultures grew in the form of typical and not typical colonies. Typical colonies are black and gray, shiny, and convex of 1.0–2.5 mm in diameter, surrounded by a clear area. The atypical colonies were brilliant black colonies with a narrow white margin and gray colonies, with no clear area. Gram staining revealed gram-positive spherical bacteria, which occurred in the form of clusters that resemble grapes, fixed, did not form a spore. They gave a positive catalase reaction.

Plasma coagulation was negative in 100% of cat isolates and positive in 2 dogs isolates (18 %). Lecithinase activity was shown by 85.8% of the strains isolated from cats and 72.8% – from dogs (Fig. 1).

71.5% of strains isolated from cats and 63.7 % from dog possessed the hemolysis ability. Mannitol was fermented with 50.0% of the strains isolated from cats and 54.6% from dogs (Fig. 2, 3).

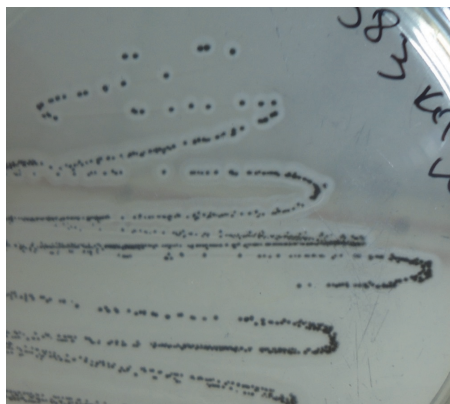


Fig. 1. Lecithinase activity on Baird-Parker agar (HiMedia, India)

On crystal violet lactose agar, 78.6% of the strains isolated from cats and 91.0% isolated from dogs were formed in blue colonies (Fig. 4). At the same time, the lecithinase activity, hemolysis, fermented mannitol and grew on crystal violet lactose agar exhibited 28.5% of strains isolated from cats and 27.2% isolated from dogs (Fig. 5).

28.5% of strains derived from cats and 54.5% of strains derived from dogs were resistant to Benzylpenicillin (Fig. 6). 28.5% of strains derived from cats and 27.2% of strains derived from dogs were resistant to Ampicillin. 28.5% of strains derived from cats and 45.4% of

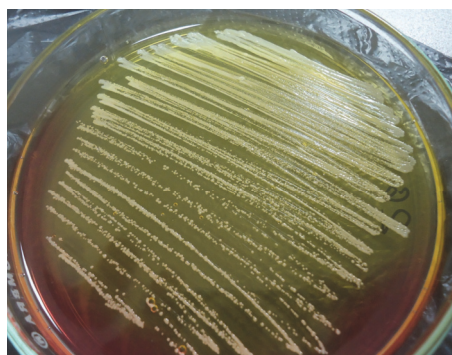


Fig. 2. Mannitol fermentation on Mannitol Salt agar (Merck, Germany)

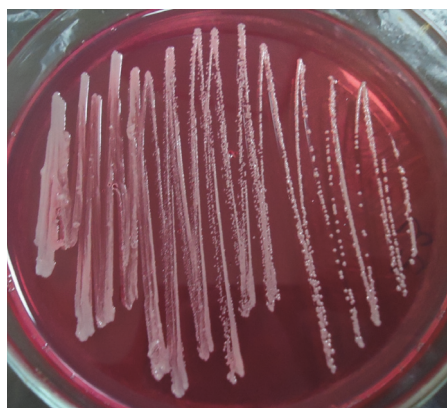


Fig. 3. No fermentation of mannitol on Mannitol Salt agar (Merck, Germany)

strains derived from dogs were resistant to Oxacillin (Fig. 7).

14.2% of strains derived from cats were resistant to Norfloxacin and Ciprofloxacin.



Fig. 4. Blue colonies on crystal violet lactose agar (HiMedia, India)

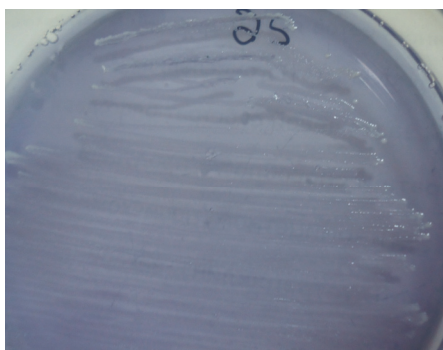


Fig. 5. White colonies on crystal violet lactose agar (HiMedia, India)

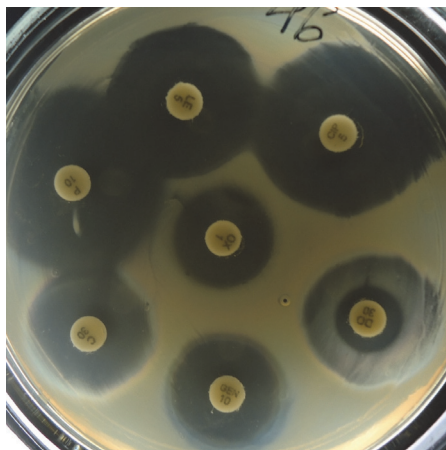


Fig. 6. Susceptible to Oxacillin and Benzylpenicillin strains of *Staphylococcus* spp.

27.2% of strains derived from dogs were resistant to Norfloxacin and Ciprofloxacin. 7.1% of strains derived from cats and 27.2% of strains derived from dogs were resistant to Levofloxacin. 21.4% of strains derived from cats and 27.2% of strains derived from dogs were resistant to Gentamicin and Tobramycin. 42.8% of strains derived from cats and 27.2% of strains derived from dogs were resistant to Erythromycin. 28.5% of strains derived from cats were resistant to

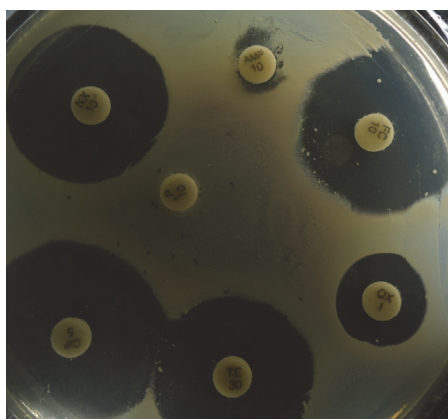


Fig. 7. Oxacillin, Ampicillin, and Benzylpenicillin resistant strains of *Staphylococcus* spp.

Tetracycline. 27.2% of strains derived from dogs were resistant and 9.0% were moderately resistant to Tetracycline. 14.2% of strains derived from cats and were intermediately resistant to Doxycycline. 18.1% of strains derived from dogs were resistant to Doxycycline. 14.2% of strains derived from cats and 36.3% of strains derived from dogs were Chloramphenicol-resistant. 21.4% of strains derived from cats and 36.3% of strains derived from dogs were resistant to Fusidic acid. The isolated strains from cats and dogs had multiple resistances to two or more antibiotic groups. Thus, a coagulase-negative strain resistant to all antibiotics except Levofloxacin, Fusidic, acid and moderately resistant to Doxycycline was derived from cats. Two resistant strains to the Penicillin group, Erythromycin, and Chloramphenicol, were also derived. A strain resistant to all antibiotic groups, except for Chloramphenicol and Fluoroquinolones, was also derived from cats. 35.7% of strains derived from cats were sensitive to all studied antibiotic groups.

Two coagulase-positive strains isolated from dogs exhibited different resistance. Thus, one strain was sensitive to Benzylpenicillin, Ampicillin, Erythromycin, Oxacillin, and, at the same time, was resistant to all other antibiotics. Another coagulase-positive strain was resistant to Benzylpenicillin, Gentamicin, Tobramycin, and Fusidic acid, and, at the same time, was sensitive to other antibiotics. Among the coagulase-negative strains isolated from dogs, one strain was resistant to all antibiotics, except for Doxycycline; one strain was resistant to all antibiotics, except for Ampicillin, Gentamicin, and Tobramycin. One strain resistant to the Penicillin and Erythromycin group and two strains resistant to the Penicillin group were also isolated. 27.2% of strains were sensitive to all groups of antibiotics tested.

2. The value of the optical density of biofilms at 570 nm and the ability to form biofilms in strains of *Staphylococci* isolated from cats and dogs ($M \pm m$, $n = 3$)

Strain No.	The value of the optical density of the strains derived from cats	The value of the optical density of the strains derived from dogs
1	3.184 ± 0.095	4.744 ± 0.188
2	0.909 ± 0.030	3.440 ± 0.134
3	1.651 ± 0.060	1.896 ± 0.065
4	1.243 ± 0.037	2.138 ± 0.088
5	0.908 ± 0.033	2.483 ± 0.052

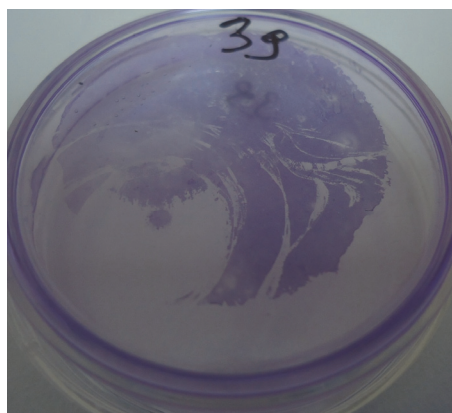


Fig. 10. The high density of the formed biofilm

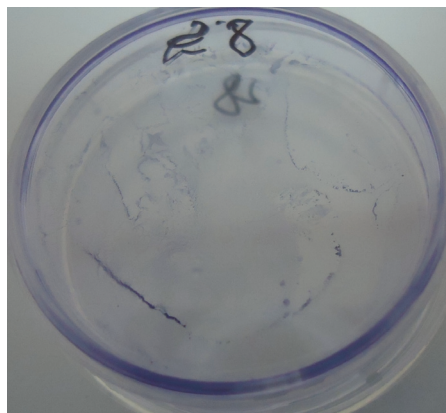


Fig. 11. The medium density of the formed biofilm

As a result of the study of biofilm formation, all 100% of the isolates isolated from dogs had a high optical density ($OD > 1.0$) and the ability to form a biofilm (Table 2).

Only 21.4% of strains isolated from cats formed biofilms with medium optical density ($OD > 0.5 < 1.0$), and the remaining 78.5% – with high optical density ($OD > 1.0$) and the ability to form a biofilm (Fig. 10).

Conclusion and future perspectives

Staphylococcus spp. was derived from 54.0% of studied animals. Selected cultures have different pathogenicity factors

(lecithinase, coagulase). Simultaneously showed lecithinase activity, hemolysis, fermented mannitol, and grew on crystal violet lactose agar 28.5% of strains isolated from cats and 27.2% isolated from dogs. The studied cultures of *Staphylococcus spp.*, had multiple resistance to Oxacillin, two or more groups of antibiotics. As a result of the study of biofilm formation, all 100% of isolates obtained from dogs had a high optical density ($OD > 1.0$) and the ability to form a biofilm. Only 21.4% of strains isolated from cats formed biofilms with a medium optical density ($OD > 0.5 < 1.0$), and the remaining 78.5% – high optical density ($OD > 1.0$) and the ability to form a biofilm. The data obtained by us indicate the need for full monitoring of antibiotic re-

sistance and biofilm capacity of *Staphylococci* isolated from domestic animals using polymerase chain reaction analysis.

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Анотація. Собаки й коти, які мешкають поруч із людьми, є джерелом збудників зоонозів. Метою роботи було дослідити домашніх собак і котів на носійство стафілококів і вивчити їхні біологічні властивості, здатність до біоплівкоутворення та стійкість до антибіотиків у виділених культур. Усього було відібрано 44 зразки від 19 собак та 25 котів різних вікових та статевих груп. У 54% з усіх відібраних зразків були виявлені стафілококи. Ізольовано 25 культур *Staphylococcus spp.* Реакція плазмокоагуляції була негативною у 100% штамів отриманих від котів і позитивною – у 2 штамів отриманих від собак (18%). Лецитиназну активність проявляли 85,8% штамів ізольованих від котів і 72,8% – від собак. Здатністю до гемолізу володіли 71,5% штамів виділених від котів і 63,7% – від собак. Ферментували маніт 50,0% ізолятів від котів і 54,6% – від собак. Росли на лактозному агарі з кристалічним фіолетовим у вигляді синіх колоній 78,6% штамів отриманих від котів і 91,0% – від собак. Одночасно проявляли лецитиназну активність, гемоліз, ферментували маніт і росли на лактозному агарі з кристалічним фіолетовим 28,5% штамів ізольованих від котів і 27,2% – від собак. Отримані штами від котів і собак володіли множинною резистентністю до оксациліну, двох і більше груп антибіотиків. Результати дослідження утворення біоплівок показали, що усі 100% ізолятів виділених від собак володіли високою оптичною щільністю ($OD > 1.0$) та здатністю до формування біоплівки. Лише 21,4% штамів ізольованих від котів утворювали біоплівки середньої оптичної щільності ($OD > 0.5 < 1.0$), а решта 78,5% – високої оптичної щільності ($OD > 1.0$) та здатності до утворення біоплівки.

Ключові слова: коагулазопозитивні і коагулазонегативні *Staphylococci*, біоплівки, антибіотикорезистентність