

ISOLATION OF POTENTIALLY ZONOTIC BACTERIA FROM THE SKIN OF SHELTER DOGS USING CHROMATIC DETECTION CULTURE MEDIA

IZOLAREA BACTERIILOR POTENȚIAL ZONOTICE DE PE PIELEA CÂINILOR DINTR-UN ADĂPOST FOLOSIND MEDII DE CULTURĂ CHROMATIC DETECTION

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ABSTRACT | REZUMAT

Skin samples from 20 mix-breed dogs, found in a shelter from Timisoara, were collected and processed for the detection of potentially zoonotic bacteria found on their skin, using MLT chromatic detection culture media. The election spots for the sample collection were the muzzle area, the interdigital area and the inguinal area. The samples were transported to the Faculty of Veterinary Medicine from Timisoara where they were further processed. The samples were placed on MLT Chromatic detection culture media and incubated for 24 hours at 36 degrees. Results revealed the presence of the following types of bacteria: *E. coli*-85% (17/20), *K. pneumoniae*-45% (9/20), *P. mirabilis*-15% (3/20), *P. aeruginosa*-0% (0/20), *Staphylococcus* spp. and *E. faecalis* - 100% (20/20).

Keywords: skin, bacteria, MLT chromatic detection, shelter, dogs

S-au recoltat probe de piele de la 20 de câini din rasa metis, proveniți dintr-un adăpost din Timișoara, cu scopul de a detecta bacterii cu potențial zoonotic, localizate la nivel cutanat, cu ajutorul mediilor de cultură MLT Chromatic Detection. Punctele de elecție pentru recoltarea probelor au fost zona botului, zona interdigitală și zona inghinală. Probele au fost transportate la Facultatea de Medicină Veterinară din Timișoara unde au fost prelucrate. Probele au fost utilizate pentru înșămânțarea mediilor de cultura MLT Chromatic Detection și incubate timp de 24 de ore la o temperatură de 36°C. Rezultatele au demonstrat prezența următoarelor tipuri de bacterii: *E. coli* - 85% (17/20), *K. pneumoniae*-45% (9/20), *P. mirabilis* -15% (3/20), *P. aeruginosa* - 0% (0/20), *Staphylococcus* spp. și *E. faecalis* - 100% (20/20).

Cuvinte cheie: piele, bacterii, MLT Chromatic detection, adăpost, câini

Normal skin microflora plays an important role in the skin defence mechanisms (immune modulation and prevention of pathogenic colonization) and it is essential in ensuring an optimal function of the skin. Several organisms are considered permanent inhabitants or *residents* of the skin such as *Micrococcus* spp., coagulase-negative staphylococci or coagulase-positive streptococci, all of which can be isolated from the skin and hair coat of healthy dogs (5). These organisms are found in low numbers and they live and multiply on the skin surface, forming micro-colonies, whereas *transient* microorganisms are mainly represented by contaminants from the environment which may take advantage of changes in the microenvironment of the skin surface and start colonies on the skin surface as well. Studies revealed that the anal, nasal and oral muco-cutaneous regions are important sites

for skin bacteria, as well as hair follicles (7, 12, 13, 19). Humidity and temperature play an essential role in the distribution of bacteria on the skin surface, thus, dry, exposed surfaces have much lower numbers of bacterial colonies compared to areas such as the axillae, groin and interdigital space which are occluded - fact that leads to increased local humidity and temperature which favours bacterial proliferation, especially for Gram-negative bacteria (16). *Staphylococcus* species are residents of the mucosae, especially the nasal mucosa, and it is spread on the skin through grooming activities. Thus, the most frequently and most heavily colonized hairy areas are those which come most in contact with the nose and with the anus.

Similar to humans, studies (7, 6) have shown high populations of *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroides* living on the skin of healthy dogs and humans. Animal-related factors such as breed, age or sex do not seem to alter the composition of the bacterial communities found on dog skin (14, 11, 21). Bacteria of the normal flora of the gut such as *E. coli* and *Enterococcus* spp. can be found on the skin

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of animals if they are kept in poor hygiene conditions (improper or rare cleansing of faeces in the shelter cages) (10). These bacteria, along with *S. aureus* and other representatives of the *Staphylococcus* family can all be used as indicators when studying antimicrobial resistance of the normal flora of dogs which is an emerging issue in the field of veterinary medicine since programs that monitor the issue of antimicrobial resistance have only been implemented for farm animals (2, 15). Previous studies proved the fact that dogs and owners share bacterial populations and dogs also share bacterial populations among themselves through co-habitation (20, 17).

The present study aimed to identify potentially zoonotic bacteria found on the skin of shelter dogs to gain knowledge on the risks that might occur regarding the transfer of bacteria from shelter dogs to their future adoptive families and to serve as a starting point for shelter owners, to acknowledge the deficits in hygiene and to take steps into solving this issue in order to avoid cross-contamination between animals and their future owners.

MATERIALS AND METHODS

The study was conducted during the period November - December 2019 on 20 mix-breed shelter dogs, of different ages (between 8 months and 10 years) and sexes (11 males and 9 females) from a dog shelter in Timisoara, Romania.

Samples were collected from three skin sites: interdigital skin (between all digits from the front paws), groin (inguinal area) and muzzle (lip commissure, nasal mucosa). For each skin site, we used one sterile culture swab (Fig. 1). Each swab was rubbed on the skin 20 times, then stored in the labelled tube and refrigerated before further processing.



Fig. 1. Swabs following collection of samples

The collected material was transferred onto the MLT Chromatic detection media using a sterile loop

and incubated at 36°C for 24 hours.

The MLT Chromatic Detection medium is used for the identification of microorganisms from clinical and food samples. Its typical formula is peptone, yeast extract, tryptone, sodium chloride, chromogenic mixture, and agar. The final pH of the medium is 7.2 ± 0.2 .

The special formula of the medium allows direct in-dole testing for the detection of *E. coli*.

RESULTS AND DISCUSSIONS

The results (Fig. 3, Fig. 4) were interpreted according to the key of the MLT chromatic detection medium (Table 1), after 24 hours of incubation.

Table 1

**Interpretation key
for MLT Chromatic Detection**

Microorganism	Typical aspect of colonies
<i>Escherichia coli</i>	Pink
<i>Klebsiella pneumoniae</i>	Blue-green
<i>Proteus mirabilis</i>	Brown
<i>Pseudomonas aeruginosa</i>	Yellow
<i>Staphylococcus aureus</i>	Cream
<i>Enterococcus faecalis</i>	Turquoise



Fig. 2. MLT Chromatic detection media

The presence of bacteria on various skin sites was as follows: *E. coli* was present in the interdigital space in 14/20 dogs (70%), the inguinal area in 11/20 (55%) and the muzzle area also in 11/20 (55%); *Klebsiella pneumoniae* was present in the interdigital space of 9/20 (45%), it was absent from the inguinal

area in all dogs but it was present in the muzzle area of 3/20 (15%); *P. mirabilis* was present only in the inguinal area of three dogs (15%) and *P. aeruginosa* was absent from all regions of all dogs. On the other hand, *Staphylococcus spp.* and *E. faecalis* had a very high incidence: 14/20 (75%) had *Staphylococcus spp.* in the interdigital space, 16/20 (80%) had it in the inguinal area and all 20 dogs were positive for the presence of *Staphylococcus spp.* in the muzzle area while the prevalence of *E. faecalis* was 100% in the interdigital space, 80% (16/20) in the inguinal space and muzzle area. The results are shown for each dog in Table 2.

In what regards the sex distribution, 9 females and 8 males were positive for *E.coli*, 5 females and 4 males were positive for *K. pneumoniae*, 2 males and 1 female were positive for *P.mirabilis* and all 9 females and 11 males were positive for both *Staphylococcus spp.* and *E.faecalis*. Having in view the almost equal distribution rate, we can say that sex is not an influencing factor in bacterial colonization of the skin.

The total prevalence of each type of bacteria (Fig.

5), regardless of the skin site, is as follows: *E. coli* - 85% (17/20), *K. pneumoniae* - 45% (9/20), *P. mirabilis* - 15% (3/20), *P. aeruginosa* - 0% (0/20), *Staphylococcus spp.* and *E. faecalis* - 100% (20/20).



Fig. 3. Close-up, MLT Chromatic detection medium

Table 2

Results after the incubation of MLT Chromatic detection media

Bacteria	E. coli			K. pneumoniae			P.mirabilis			P. aeruginosa			Staphylococcus spp.			E. faecalis			Age	Sex
	ID	I	M	ID	I	M	ID	I	M	ID	I	M	ID	I	M	ID	I	M		
Dog 1	+	+	-	+	-	+	-	-	-	-	-	-	+	+	+	+	+	+	5 y	M
Dog 2	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	2 y	M
Dog 3	-	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	10 y	F
Dog 4	+	-	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	7 y	F
Dog 5	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	5 y	M
Dog 6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	8 y	M
Dog 7	+	+	-	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	7 y	F
Dog 8	+	+	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	-	6 y	F
Dog 9	+	-	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	3 y	F
Dog 10	+	+	+	+	-	+	-	-	-	-	-	-	+	-	+	+	+	-	10 m	F
Dog 11	-	+	+	+	-	-	-	-	+	-	-	-	-	+	+	+	-	+	2 y	M
Dog 12	+	-	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	1 y	M
Dog 13	+	+	-	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	4 y	F
Dog 14	+	+	-	+	-	-	-	+	-	-	-	-	-	+	+	+	-	+	3 y	F
Dog 15	+	+	+	+	-	+	-	-	-	-	-	-	+	-	+	+	+	-	8 m	M
Dog 16	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	6 y	M
Dog 17	+	-	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	2 y	M
Dog 18	+	-	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	1 y	M
Dog 19	-	+	+	+	-	-	-	+	-	-	-	-	-	+	+	+	-	+	4 y	M
Dog 20	+	-	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	2 y	F

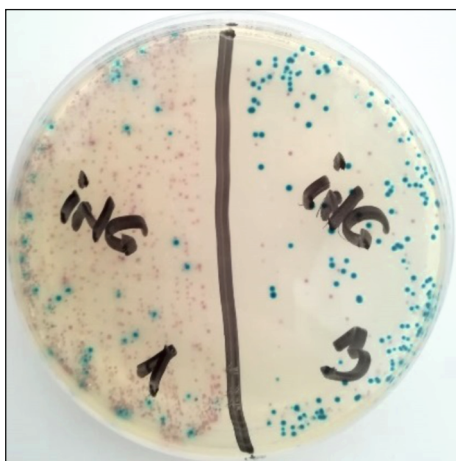


Fig. 4. Close-up MLT Chromatic detection medium

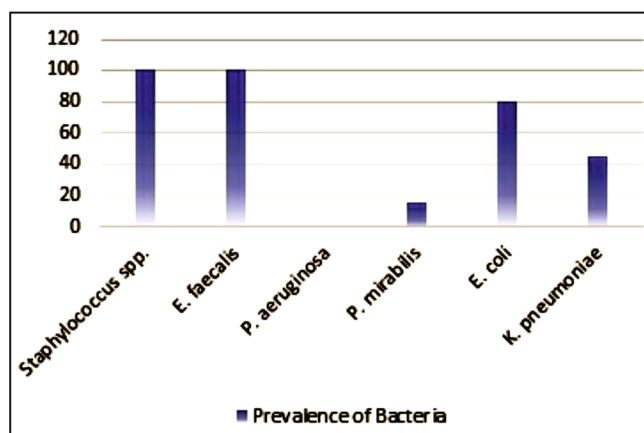


Fig. 5. Total prevalence of bacteria species

Reports regarding canine carriage of *S. aureus* vary widely probably due to the fact that culture-based differentiation between *S. aureus* and *S. intermedius* is quite difficult to make (5, 21). Having in view that our study limited the culturing techniques to the MLT chromatic detection media and further cultural methods are required to differentiate between *S. intermedius* and *S. aureus*, we cannot state for certain the percentage of *S. aureus* and *S. intermedius* so to avoid confusion, we will further refer to them as *Staphylococcus* spp.

Although *Staphylococcus* spp. was present in all sampled dogs and all three sampled skin sites, the number of colonies was reduced, showing that the colonization of the skin was not very extended. Results from similar studies (7, 8) have also shown a low presence of *Proteus* spp. and *Pseudomonas* spp. on dog skin.

Several studies (1, 13, 22, 23, 24) that researched the possibility of cross-transmission of *S. aureus*, especially MRSA-strains (4), between humans and their pets had results that indicated an 8.8% prevalence for *S. aureus* in pet dogs.

The difference between this study and ours is that

pet dogs live in far cleaner and better conditions than stray dogs in a shelter and this is probably the reason behind such a high incidence of *Staphylococcus* spp. on the skin of shelter dogs. The fact that *E. faecalis* and *E. coli* are as well in high numbers suggests the lack of proper hygiene in the shelter cages and the persistence of faecal matters and urine in the cages, which leads to skin contamination. There are however earlier studies that suggest a similar prevalence of *Staphylococcus* spp. on dog skin. The same study also suggests the presence of Gram-negative organisms such as *Proteus* spp., *E. coli* or *Pseudomonas* spp (11).

Guardabassi *et al.* (6) stated that animals are important reservoirs of bacteria which also affect humans, especially bacteria with acquired antibiotic-resistance describing cases of human infections with MRSA and vancomycin-resistant *E. faecalis*.

Similar to the cases of *S. aureus* or *E. faecalis* is the case of cephalosporin-resistant *Enterobacteriaceae* because it is known that humans and dogs can share identical strains of such bacteria, with the afferent resistance genes, making dogs a possible reservoir (3, 18). The same stands for transmission of *K. pneumoniae*, according to Hilty *et al.* (9) which found a sharing rate between humans and dogs living in the same household of 25%.

It is very important to consider the transmission possibility because dogs from the shelter will eventually be adopted and brought into households and it is of great importance to be aware of the risks the future owners might be exposed at. It is also important for shelters to acknowledge the level of bacterial colonization of dog skin and take action into lowering the rate as much as possible.

CONCLUSIONS

The study revealed the presence, in relatively high numbers, of potentially zoonotic bacteria such as *Staphylococcus* spp., *Enterococcus faecalis*, *Escherichia coli* and *Klebsiella pneumoniae* on the skin of shelter dogs from Timisoara.

This study represents the starting point for further studies based on determination of antimicrobial resistance in bacteria found on the skin of shelter dogs, that eventually come into contact with future owners and/or children, and to establish better hygiene routines in shelters in order to decrease the population of zoonotic bacteria on the skin of shelter animals.

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