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MONITORING THE SPECIES *STAPHYLOCOCCUS AUREUS* IN DOG FAECES, IN TIMISOARA PARKS: IS THERE A ZOONOTIC RISK?

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Abstract

Stray dogs have long been regarded as a potential source of zoonotic diseases (bacterial zoonotic risk) for human. In particular, host zoonotic bacteria and parasites in the intestine of dogs were found to pose a significant risk to human health. An ensemble social change, economic and environmental, across the globe, reflects on epidemiological characteristics and pathogenesis of diseases and pathogens. And the development and supervision of bacterial zoonosis, with particular reference to multiple antibiotic resistant staphylococci isolated from dog faces, were important changes, which we refer in this study.In fecal samples from dogs were isolated Staphylococcus aureus strains pathogenic to man (MRSA), so proving dog faeces role in urban areas as a reservoir of bacteria with multiple resistance. Because the genes coding for antibiotic resistance can be transmitted between bacteria and contact between pets and their owners is tighter than in the past, our study suggests that contamination parks for children with dog feces containing such microorganisms is a problem for public health and the environment.

Key words*: staphylococci, methicillin resistance, faeces, stray dog, Timisoara, parks.*

INTRODUCTION

Staphylococci are one of the most important groups of commensal bacteria that are isolated from the skin and the mucous membranes of dogs. Moreover, they are responsible for opportunistic infections acquired in hospitals and communities, affecting mostly skin and ears, and other anatomical areas (Euzéby, 2013; Guardabassi et al., 2004; Loeffler, 2008).
An ensemble social change, economic and environmental, across the globe, reflects on epidemiological characteristics and pathogenesis of diseases and pathogens. And the development and supervision of bacterial zoonosis, with particular reference to multiple antibiotic resistant staphylococci isolated from dog faces, were important changes, which we refer in this study.
Stray dogs have long been regarded as a potential source of zoonotic diseases (bacterial zoonotic risk) for human. In particular, host zoonotic bacteria and parasites in the intestine of dogs were found to pose a significant risk to human health. People are exposed to these pathogens through direct or indirect contact with infected dogs or their feces through accidental ingestion of a zoonotic agent.
It is also important to consider that exposure to zoonotic bacteria from feces of stray dogs could present a significant health problem in the urban areas (Simoons-Smit et al., 1997; Guardabassi et al., 2004).
Parks and playgrounds frequented by children as well as stray dogs are the main areas for such illnesses declared suspicious.
The reasons for which the owners have to collect feces after their four-legged friends are based on arguments related health risks.
Dog faeces contain bacteria and parasites. If you are abandoned in public space, we get to come into contact with the faces contaminated being exposed to serious diseases. For example, if it rains, the water dissolves them, clean shoes is inevitably contaminated, sprinkle us with goo formed on clothes; if it does not

rain, dry them, grind that you inhale especially when the wind blows.
Children have the highest risk of exposure because my hands on the floor, playing with objects that touch the ground and tend to take their hands dirty in the eyes, nose and mouth.
For children's and adolescents, who play with the ball or other toys in this area, exist the risk of contracting bacteria and parasites. Flies and other insects that lay excrement and then come to us are carriers of the kitchen to the said pathogen (Tarsitano et al., 2006).

MATERIALS AND METHODS

Fecal samples

To achieve its purpose, it was collected 60 fecal samples (29 fresh and 31 old) children's play parks, located in the City.
Fecal samples were collected at random.
Also feces collected were subjected to external factors (heat, drought, rainfall, wind, etc.).
There were counted four parks primarily for children, located in different areas of the city of Timisoara.
Actual crop were used plastic containers, sterile, individually wrapped (the need for urine culture), respectively spoon and disposable gloves.
A sample was individualized and is listed on the sample box number, area of origin and date (Fig. 1).

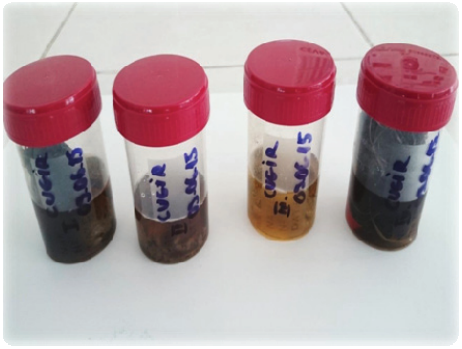

Fig. 1. Faecal samples (original).

After harvesting fecal samples were transported to the laboratory. Sample preparation was conducted in the Laboratory of Bacteriology of the Department of Infectious Diseases and Preventive Medicine in the period May-June 2015.
In the laboratory, the first step in the processsing of the stool samples was the achievement of a fecal suspension by the addition of a quantity of 5 ml of sterile physiological saline over the feces from the container used for the collection and maintenance of contact at room temperature (25-28° C) for 20-30 minutes. Subsequent the mixture was homogenized by gentle manual stirring.
For these suspensions were made insemination on a special chromogenic medium, Chromatic Detection (Mikrobiologie Labor Technik)

Chromatic Detection agar– description:

Chromogenic medium used for the enumeration and identification of microorganisms from clinical specimens and food.
Special formula allows also confirming directly the indole tests *Echerichia coli* (Table 1).

Table 1. Composition Chromatic Detection agar

Standardized formula	(g/l)
peptone	14.0
yeast extract	3.0
tryptone	6.0
sodium chloride	5.0
chromogenic mixture	13.125
agar	15.0
finale pH	7.2 +/- 0.2

Peptone, tryptone and yeast extract are a source of amino acids and vitamins. Sodium chloride maintains the osmotic balance of the environment.
Chromogenic mixture allows identification of microorganisms based on colony color and morphology.
Technique: medium surface inoculate 10 µl specimen using a sterile loop (loop bacteriological) or pharyngeal exudate rod for clinical trials.
Incubate at 37 ° C, under aerobic conditions in an incubator for 18-24 hours. Observe growth and colony color and interpretation is done according to the manufacturer, listed in the product data sheet (Table 2).

Table 2. Interpretation of results

Microorganism	Growth	Aspect of the typical colony
Escherichia coli	good	Pink
Staphylococcus aureus	good	Cream-colored
Klebsiella pneumoniae	good	Aquamarine
Proteus mirabilis	good	Brown
Pseudomonas aeruginosa	good	Yellowish
Enterococcus faecalis	good	Green Turquoise

Samples were collected using sterile cotton wool pads, attached to a plastic rod, pharyngeal exudate for harvesting.
Sowings were made by depletion of pathological material on a cotton ball on the agar surface. Next, the plates were incubated at 37 ° C in normal atmosphere for 18 -24 hours.
After interpreting the results of the Chromatic Detection agar, typical of *S. aureus* colonies were picked on nutrient broth medium with 5% sheep blood, to obtain fresh cultures necessary to carry out sensitivity testing. After 24 hour incubation, the obtained cultures were performed plating Muller Hinton medium, the specific Kirby Bauer technique (Codiţă and Buiuc 2008).

Susceptibility testing to antibiotics

Behavior towards antibiotics was tested all bacterial strains isolated Using diffusion method. A common method for determining the antimicrobial susceptibility, primarily in small laboratories and veterinary practices, is the agar diffusion test (diffusion method). This method uses paper disks impregnated with the antimicrobial substance, which are then applied to the surface of the agar medium previously impregnated with a pure culture of bacteria being tested. The diameter of the growth inhibition zone around the paper disk is inversely correlated with the minimal inhibitory concentration (MIC).
This diffusion technique is not difficult, however, it must be strictly observed and tracked area size standard for each drug separately. Any variation in the execution technique changes the relationship between the zone of inhibition and MIC, resulting in misinterpretation of results. The antibiotics tested were: methicillin - ME - 30 µg, gentamicin - CN – 10 µg, tetracycline - TE - 30 µg, ciprofloxacin - CIP - 30 µg, kanamycin - K - 30 µg, novobiocin - NV - 30 µg, doxycycline - DO - 30 µg, erythromycin - E - 15 µg, vancomycin - VA - 30 µg, ceftriaxone - CRO - 30 µg, cefoxitin - FOX - 10 µg, polymyxin B - PB - 50 IU, rifampicin - RA - 30 µg, lincomycin - L - 30 µg, cefaclor - CEC - 30 µg, pristinamycin - PT - 15 µg and ampicillin / sulbactan - SAM - 30 µg. All bio discs were manufactured by Liofilschen-Italy and interpretation of the results was performed in accordance with manufacturer's recommendations.

RESULTS AND DISCUSSIONS

After reading and interpretation of specific colonies on plates with Chromatic detection agar, seeded faecal samples from dogs were isolated microorganism with pathogenic potential for humans (Fig. 2). From 60 faecal samples, 18 samples were positive for *Staphylococcus aureus* (18/60; 30%). The results of the special chromogenic agar plating are shown in Table 3 and Fig. 2.

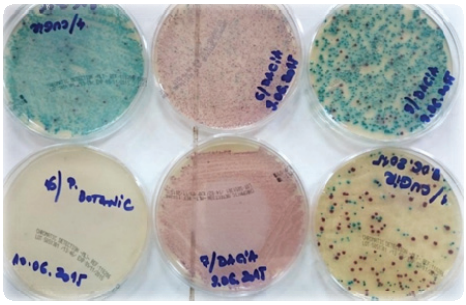

Fig. 2. Aspects of colonies in the Chromatic detection medium (original)

Enterobacteriaceae (*Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli*). Quantification of other bacteria not covered by this study.

Figure 3 presents *S. aureus* aspects of colonies in the Chromatic detection agar, according to the technical specifications given by the manufacturer. These colonies are cream colored.

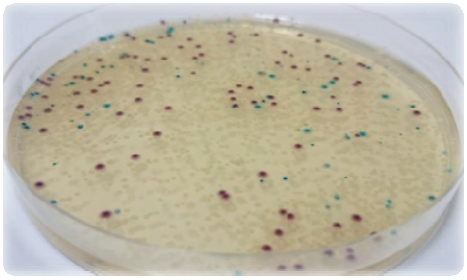

Fig. 3. Specific *S. aureus* colonies (cream) on Chromatic detection agar (original)

Table 3. Results of the inoculation in the Chromatic detection agar

Microorganism	Number of samples taken	Number of positive samples	
		No.	%
Escherichia coli	60	34	56.67
Staphylococcus aureus	**60**	**18**	**30.00**
Klebsiella pneumoniae	60	28	46.67
Proteus mirabilis	60	11	18.34
Pseudomonas aeruginosa	60	14	23.34
Enterococcus faecalis	60	43	71.67

Results of susceptibility testing to antibiotics

Most strains of staphylococci unexposed to the pressure of antibiotics are sensitive to these antimicrobials, but there have been cases where resistance was related phenomena.

Interpretation of results was done according to standards set by the Clinical and Laboratory Standards Institute (2006) Is better use for interpretation CLSI Vet 01-A4 and Vet 01-S2/2013. The results were classified into three categories: susceptible, intermediate sensitive and resistant.

The results obtained from testing the antibiotic susceptibility of strains of staphylococci isolated from the feces of dogs are given in Table 4.

Table 4. Sensitivity rate of *S. aureus* strains isolated (n = 18), compared to 17 antibiotics (Table 4 is glued to Table 3)

Antimicrobial substance name (Initials/MIC *)	interpretation sensitivity testing					
	susceptible		intermediate sensitive		Resistant	
	Nr.	%	Nr.	%	Nr.	%
Methicillin - ME - 30µg	17	94.45	-	-	1	5.56
Gentamycin – CN – 10µg	12	66.67	2	11.12	4	22.23
Tetracycline – TE – 30µg	9	50	1	5,56	8	44.45
Ciprofloxacin – CIP – 30 µg	18	100	-	-	-	-
Kanamycin – K – 30 µg	11	61.12	4	22.23	3	16.67
Novobiocin – NV – 30 µg	18	100	-	-	-	-
Doxycycline – DO – 30 µg	10	55.56	3	16.67	5	
Erythromycin – E – 15 µg	12	66.67	2	11.12	4	22.23
Vancomycin – VA – 30 µg	18	100	-	-	-	-
Ceftriaxone – CRO – 30 µg	18	100	-	-	-	-
Cefoxitin – FOX - 10µg	18	100	-	-	-	-
Polymyxin B – PB – 50UI	-	-	5	27.78	13	72.23
Rifampicin – RA – 30 µg	18	100	-	-	-	-
Lincomycin – L – 30 µg	18	100	-	-	-	-
Cefaclor – CEC – 30 µg	18	100	-	-	-	-
Pristinamycin – PT – 15 µg	18	100	-	-	-	-
Ampicillin / sulbactam – SAM – 30 µg	18	100	-	-	-	-

* MIC – The minimum inhibitory concentration

Analyzing the results of the table we can see that antibiotics sensitivity was variable depending on the group of antibiotics.

If antibiotics: novobiocin, rifampicin, pristinamycin, ciprofloxacin, vancomycin, ceftriaxone, cefoxitin, cefaclor and ampicillin / sulbactan,

considered the drug of choice for staphylococci, the number of sensitive strains were 100%, all isolates were sensitive (Table 4).
This suggests that the tested strains isolated from animals to which these antibiotics were not used. Also, it can be said that all of these antibiotics for staphylococci or kit is typically used in humans, in the treatment of staphylococcal infections in animals, respectively.
B-lactam used against (methicillin, ceftriaxone, cefoxitin, cefaclor, ampicillin with sulbactam), antibiotic sensitivity was highest, except *Staphylococcus aureus*, where they isolated one resistant strain for methicillin.
The phenomenon of antibiotic resistance, β-lactam in the case is based on genetic determinants of type plasmid and chromosomal β-lactamases governing synthesis, broad-spectrum, ensuring the resistance staph. Resistance to methicillin is transmitted by plasmids (R factor) and having a pattern common to other β-lactams (Weese 2008). For this reason, methicillin-resistant staphylococci are considered particularly with zoonotic risk, having a complex circuit or human-animal-human (Tarsitano 2006; Velescu and Tănase 2010; Bywater 2004; Weese and Van Duijkeren 2010).
Compared to aminoglycosides (gentamicin, kanamycin) and macrolides (erythromycin and vancomycin), antibiotics sensitivity was differrent, the maximum to vancomycin (Table 4). In the case of gentamicin-resistant strains were isolated four, three strains to kanamycin and 4 strains resistant to erythromycin (Table 4).
Most of the strains were resistant to polymyxin B (13 strains), through the use of topically applied preparations containing this antibiotic (Table 4).
Sensitivity to tetracycline's (tetracycline, doxycycline) was reduced 13 strains being resistant to this group of antibiotics to which resistance phenomenon is type plasmid and chromosomal (8 strains tetracycline and 5 strains to doxycycline) (Tables 4).
All strains tested were sensitive to ciprofloxacin, since the quinolone is not used in drug therapy in dogs the usual manner.
The development of resistance staphylococci to different antibiotics, it is a consequence of wasteful use in the treatment of diseases in pigs. Antibiotics used irrationally creates a selective pressure being selected and transmitted genetic determinants of type plasmid and chromosomally. Consequently, the phenomenon of multiple resistance intra- and interspecific that is transmitted. Methicillin resistance of special importance as it can be associated with resistance to β-lactams and other groups of antibiotics (Weese, 2008, Guardabasi et al., 2004, Bywater, 2004).
After testing strains of staphylococci isolated from the feces of dogs, against 17 antibiotics were identified methicillin-resistant strain and more type of resistance, β-lactams to, tetracyclines, macrolides and polymyxin B.
In the literature there is very little information available about the microbial flora present in fecal pets, especially dogs, despite the presence of Gram-positive cocci in feces dog has already been observed decades ago (Devriese and Pot, 1995; Murray, 1990, Tannock, 1995, Loeffler et. al., 2010).
In a study by Cinquepalmi et al. (2013) in Bari region - southern Italy, on a sample of 418 dog feces samples collected from the streets, have identified strains of MRSA (methicillin-resistant *S. aureus*) at a rate of 0.7%. In similar studies, Abbott et al. (2010) and Abdel-Moein et al. (2012) identified these bacteria in a proportion of 0.4% and 3%.
MRSA strains isolated from companion animals (dogs and cats) are also similar to disseminated hospital strains (Abbott et al., 2010). Dogs, for this reason, can pose major public health modules for dissemination outside hospitals MRSA strains (Abdel-Moein et al., 2012; Ferriera et.al., 2011; Morris et. al., 2012, Rich and Robert 2004).
Dog feces in urban areas can be an important source of pathogenic microorganisms with potential for both dog owners and for the community in that area, especially for children.

CONCLUSIONS

In fecal samples from dogs were isolated *S. aureus* strains pathogenic to man (MRSA), so Proving dog faeces role in urban areas as a reservoir of bacteria with multiple resistance.
Because the genes coding for antibiotic resistance can be transmitted between bacteria

and contact between pets and their owners is tighter than in the past, our study suggests that contamination parks for children with dog feces containing such microorganisms is a problem for public health and the environment.

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