

STAPHYLOCOCCI RESISTANT PHENOTYPES OF THE INTERMEDIUS GROUP ISOLATED FROM DOGS

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Abstract

Dogs have frequently infections with coagulase-positive and coagulase-negative staphylococci with different locations. A total of 49 samples consisting of pathological skin, auricular and genital secretions were bacteriologically examined using standard methodology.

The isolates were tested by disk diffusion Kirby-Bauer method, on which were used biodiscs with 19 antibiotics from different groups.

The staphylococci strains isolated from dogs included in Intermedius Group were susceptible to the antibiotics rarely or not used in the therapy of diseases in this species.

The isolates were methicillin-resistant strains, thus emphasizing the movement of these strains in the canine population, confirming the zoonotic risk of these strains.

After this study several resistant phenotypes of staphylococci strains included in Intermedius Group were identified, whose frequency was variable.

Key words: antibiotic resistance, Grup Intermedius, methicillin-resistant.

INTRODUCTION

Dogs have frequently infections with coagulase-positive and coagulase-negative staphylococci with different locations. Most common are the Intermedius Group staphylococci, including *S. intermedius* and *S. pseudintermedius*. They are coagulase-negative staphylococci and are part of the resident flora of the dogs. (Schissler, 2009)

Staphylococci in this group have also been found to pathogens for other animal species, including humans, and methicillin-resistant strains are considered as zoonotic risk strains, regardless of the species to which they belong. (Frank and Müller, 2012, Degi et al., 2012)

The research followed the frequency of resistant phenotypes of staphylococcal strains to antibiotics, included in this group, isolated from dogs with various diseases.

MATERIALS AND METHODS

A total of 49 samples consisting of pathological skin, auricular and genital secretions were taken from dogs with various diseases. The primary inseminations were made on agar with 5% defibrinated sheep

blood and the isolated strains were initially sorted based on cultural, morphological and tinctorial characters.

Isolated and purified staphylococcal strains were biochemically tested on Chapmann medium, on Difco agar with maltose, pH indicator and, finally, through the API Staph system.

The examined pathogenic factors were represented by hemolysins and clumping factor. (Degi et al., 2012)

The isolates were tested by disk diffusion Kirby-Bauer method, on which were used biodiscs with 18 antibiotics from different groups (table 1) and the results were interpreted according to the CLSI Standard. (Bemis et al., 2009)

RESULTS AND DISCUSSIONS

The bacteriological examination performed by the described methodology allowed the isolation of 45 strains included in Intermedius Group based on the main phenotypic tested characters.

The strains of staphylococci unexposed to the pressure of antibiotics are susceptible to these substances, while the strains isolated from dogs with various conditions, under the

pressure of antibiotics, due to therapy, may exhibit the occurrence of multiple resistance. The results obtained by testing the susceptibility to antibiotics of staphylococcal strains isolated from dogs are shown in Table 1.

Resistant phenotypes and the frequency of methicillin-resistant and ceftioxin-resistant strains were pursued at a total of 44 staphylococcal strains, using 19 antibiotics (Table 1).

Table 1. Methicillin-resistant phenotypes isolated from dogs

No.	Antibiotic	Antibiogram results						Total strains
		Susceptible		Intermediary		Resistant		
		No.	%	No.	%	No.	%	
1	Methicillin	35	79,5	2	4,5	7	15,9	44
2	Ampicillin / Sulbactan	44	100	-	-	-	-	44
3	Amoxicillin / Clavulanic acid	8	18,18	30	68,18	6	13,63	44
4	Ceftioxone	43	97,72	-	-	1	2,72	44
5	Cefoxitin	36	81,81	8	18,18	-	-	44
6	Cefaclor	36	81,81	8	18,18	-	-	44
7	Cefuroxime	-	-	36	81,81	8	18,18	44
8	Erythromycin	5	11,3	6	13,6	33	75	44
9	Lincomycin	6	13,6	6	13,6	32	72,72	44
10	Rifampicin	8	18,18	36	81,81	-	-	44
11	Gentamicin	7	15,9	37	84	-	-	44
12	Kanamycin	-	-	36	81,81	8	18,18	44
13	Tetracycline	2	4,5	9	20,4	33	75	44
14	Doxycycline	-	-	36	81,81	8	18,18	44
15	Ciprofloxacin	43	97,7	-	-	1	2,27	44
16	Vancomycin	8	18,18	36	81,81	-	-	44
17	Polymyxin B	-	-	8	18,18	36	81,81	44
18	Novobiocin	5	11,3	39	88,6	-	-	44
19	Pristinamycin	36	81,81	8	18,18	-	-	44

Analyzing the results from the table it can be seen that the rate of resistant phenotypes was variable depending on the groups of antibiotics.

Against the β -lactam group used (methicillin, ceftioxone, cefoxitin, cefaclor, ampicillin with sulbactan and amoxicillin with clavulanic acid) antibiotic susceptibility was highest to ampicillin, followed by cefuroxime, cefaclor, cefoxitin, methicillin and minimum to ceftioxone.

Intermediary antibiotic susceptibility was to ceftioxone and amoxicillin with clavulanic acid, and antibiotic resistance was shown to cefuroxime, amoxicillin with clavulanic acid and methicillin and absent to cefoxitin, cefaclor and ampicillin with sulbactan.

The various behaviour of the strains, tested to this group of antibiotics, is influenced, in dogs, by use of antibiotics mentioned in therapy, some of which are being used only for humans.

CHROBAK and col. notify similar results, observing that the staphylococci from Intermedius Group were resistant, in high proportions, to amoxicillin with clavulanic acid, ciprofloxacin, clindamycin and gentamicin.

The occurrence of antibiotic resistance in β -lactam group is based on genetic determinants located in plasmids and chromosomes, which governs the synthesis of β -lactamases with

large spectrum, that provides, thus, the staphylococci resistance.

Analyzing the results it can be observed that at the tested strains, methicillin resistance was of 15.9% and to cefoxitin resistant strains were not found.

The resistance to methicillin is governed by *mec* gene and transmitted by R plasmid, also having a common pattern to other β -lactams, therefore, oxacillin and cefoxitin can also be used for testing the presence of methicillin resistance in staphylococci, regardless of species or coagulase activity. (Frank and Müller, 2012)

For this reason, methicillin-resistant strains of staphylococci are considered strains with high zoonotic risk, that have a complex circuit, which is human-animal-human.

Against aminoglycosides used (gentamicin and kanamycin) antibiotic resistance was between 15.9% and 18.18%. Only these two antibiotics were used for testing, because in dogs aminoglycosides are rarely used, sometimes being used even products for humans, which may explain the high proportion of strains with intermediary behaviour.

The antibiotic resistance to this group of antibiotics is determined by a gene located either plasmidic or chromosomal, responsible for the synthesis of aminoglycoside acetyl transferase, enzyme that induce the resistance,

which explains the behaviour of isolated strains.

The resistance to macrolides (erythromycin) was of 75%, also indicating an inducible resistance to macrolides with 14 atoms that can be used in dogs, other animals and humans.

The resistance to tetracyclines was of 75% to tetracycline and 18.18% to doxycycline, procedure that can be explained by the fact that the doxycycline is less used in dogs.

Resistance to tetracycline group is governed by the *tet* gene present in the plasmid and the chromosomal genetic material and is common to all antibiotics of this group.

The resistance to fluoroquinolones, at the staphylococci isolated from dogs, it is rarely indicated, as this group of antibiotics gives various side effects to this species. Resistance was tested only to ciprofloxacin, which was in a proportion of 2.27%.

The resistance to lincomycin was of 72.72%, to polymyxin was of 81.81% and to vancomycin was absent. This behaviour is difficult to explain because these antibiotics are rarely used in dogs.

In case of novobiocin, rifampicin and pristinamycin, considered to be the elective antibiotics for staphylococci, there was no antibiotic resistance to the isolated and tested strains. This suggests that, in veterinary therapy, these three antibiotics are not used, but represents a kit for staphylococci in human pathology.

In case of staphylococci, the increase of resistance to various antibiotics is a consequence of the abuse in treatment of diseases in dogs, or of dog contamination with strains of human origin (from hospital).

As a result of testing the staphylococcal strains isolated from dogs to 19 antibiotics, there were identified methicillin-resistant strains and more resistant phenotypes to the groups of antibiotics used in the form of biosdiscs.

CONCLUSIONS

The staphylococci strains isolated from dogs included in Intermedius Group were susceptible to the antibiotics rarely or not used in the therapy of diseases in this species.

The isolates were methicillin-resistant strains, thus emphasizing the movement of these strains in the canine population, confirming the zoonotic risk of these strains.

After this study several resistant phenotypes of staphylococci strains included in Intermedius Group were identified, whose frequency was variable.

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