



Original Contribution

MICROBIOLOGICAL INVESTIGATION IN DOGS WITH PERIANAL SACCULITES

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ABSTRACT

Bacteriological analysis of samples obtained from dogs with inflammations of perianal glands was performed to find out the cause of these inflammations. Microorganisms belonging to different taxonomic groups were isolated. The *in vitro* behaviour of bacterial isolates to a number of antimicrobial drugs was studied. An analysis of antimicrobial resistance was made. Emphasis was placed on the methicillin/oxacillin resistance of staphylococcal isolates because of their potential to transfer this resistance in human infection.

Key words: dogs, perianal sacculites, bacterial flora, antibiotic therapy, resistance

INTRODUCTION

Perianal glands in dogs (known also as circumanal or hepatoid) are located in the subcutaneous connective tissue within a radius of 1-2 cm around the anus

According to some authors, perianal diseases prevalence is between 2 and 12.5% of canine pathological states. Most commonly, impaction states are diagnosed as well as inflammations with various pathogenesis. The latter are known as perianal sacculites or circumanal adenites. Frequently, they are purulent and have the features of abscesses (1, 2, 3).

The therapy of inflammations is predominantly conservative, but in very severe cases as well as in chronic situations, surgical intervention could be considered. The medication therapy is both local and systemic. It relies mainly on antiseptic washings and/or the application of various antimicrobial drugs. Their empirical use however, often results in appearance of resistance of aetiological microbial agents (4, 5).

That is why, in every single case, the performance of microbiological studies is necessary in order to elucidate the aetiology and to make an antibiogram with regard to the

aetiologic management.

The aim of the present study was to study the species diversity of microorganisms involved in the aetiology of perianal sacculites and to determine *in vitro* the behaviour of the different isolates to a number of antimicrobial drugs.

MATERIAL AND METHODS

Samples: In the period 2004-2005, a total of 55 swab samples obtained from dogs with clinically manifested circumanal adenites were analysed. There were patients of various clinics in Stara Zagora and Sofia originating from different parts of the country. The animals were from different breeds: 10 Pekingese, 10 Dachshunds, 9 Boxers, 6 Cocker Spaniels, 4 German Shepherds, 3 Bolognese, 3 Poodles, 2 Rottweilers, 2 Scottish collie, 1 Chow Chow, 1 Japanese Chin and 1 Dogue. The most investigated age group (50%) was five years.

Bacteriological studies: The transportation of swab samples was made on agar medium of Stuard (*NCIPD, Sofia*). Aseptically obtained samples were inoculated in liquid and solid nutrient media as follows: glucose broth, trypticase soy broth, blood agar with 5% sheep RBC, chocolate agar, McConkey's agar, citramide agar and mannitol salt agar.

The inocula were cultivated aerobically in a thermostat at 37°C for 24-48 h. After subcultivation for obtaining pure cultures, the

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isolates were identified through short and extensive identification protocols. The initial tests included microscopy of Gram stained preparations, catalase and oxidase tests, motility test as well as oxidation-fermentation test (O/F) of Hugh-Leifson with 1% dextrose.

Gram-negative isolates were also inoculated on Kligler polytropic medium. Depending on growth characteristics, the identification was pursued with additional biochemical tests (6).

Gram-positive isolates were tested for catalase and oxidase activity. Besides the haemolytic activity, the suspected staphylococcal cultures were also tested for plasmocagulase production, DNase, urease, alkaline phosphatase and sensitivity to novobiocin and polymyxin B (6).

The behaviour of microbial isolates to antimicrobial drugs was tested by the disk diffusion method in Muller-Hinton agar according to Performance Standards for Antimicrobial Disk Susceptibility Tests – Sixth Edition; Approved Standard. M2-A6, National Committee for Clinical Laboratory Standards, Vilianova, Pa. 1997 (7); Development of in vitro susceptibility testing criteria and quality control parameters for veterinary antimicrobial agents. Approved guideline, Second Edition, NCCLS, Documents M-37-A2, 2002 (8). Used disks were impregnated with amoxicillin (Ax) – 25 µg; amoxicillin + clavulanic acid (Ax+Cl) – 30 – 20/10 µg; carbenicillin (Cb) – 100 µg; gentamicin (G) – 10 µg; tetracycline (T) – 30 µg; chloramphenicol (C) – 30 µg; erythromycin (E) – 15 µg; enrofloxacin (Enr)- 5 µg. Their inclusion in the test was

differentiated according to the type of isolates. The results were interpreted on the three-degree scale of Bauer-Kirby as sensitive (S), intermediate (I) and resistant (R).

The methicillin/oxacillin resistance in staphylococcal strains was determined by the disk diffusion method (disk with 1 µg oxacillin) and the agar screening test in agar supplemented with 4% NaCl and 0.006 g/l oxacillin (9).

The phenotypic determination of acquired beta-lactamases in *E. coli* and *Proteus mirabilis* strains was done on Muller-Hinton agar-II and antibiotic disks containing as follows: amoxycillin (25 µg), ticarcillin (75 µg), amoxycillin and clavulanic acid (20/10 µg), cefalotin (30 µg), cefoxitin (30 µg), cefuroxime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), aztreonam (30 µg), imipenem (30 µg) of Becton- Dickinson and Oxoid (9).

The statistical analysis of data was done by calculation of the confidence limits using the angular transformation where $\varphi=2\arcsin\sqrt{p}$ and the confidence level was 0.95.

RESULTS

The species diversity of bacterial flora, isolated from perianal glands of dogs with circumanal adenites is presented on **Table 1**. It shows that 6 microbial species were isolated from dogs with perianal inflammations. *E. coli* was the most frequently encountered – in 53.2% of samples.

Table 1. Species diversity of bacterial isolates from dogs with sacculites (n=50)

Isolate	Number	%	Confidence limits
<i>E. coli</i>	23	47.0	33.4÷60.9
<i>Proteus mirabilis</i>	8	16.3	7.4÷27.8
<i>Proteus vulgaris</i>	3	6.1	1.2÷14.4
<i>Pseudomonas aeruginosa</i>	5	10.2	3.4÷20.1
<i>E. coli</i> + <i>P. mirabilis</i>	2+2	8.2	2.3÷17.4
<i>E. coli</i> + <i>Ps. aeruginosa</i>	1+1	4.0	0.4÷11.2
<i>Staphylococcus intermedius</i>	3	6.1	1.2÷14.4
<i>Staphylococcus epidermidis</i>	1	2.0	0.04÷7.7

Out of these, *E. coli* monocultures were present in 47% of cases and in 6.2% it was associated with other bacteria. The polymicrobial cultures with the participation of colibacteria included 4.2% associations in *Proteus* strains and mixed infection with *Pseudomonas aeruginosa* in one case.

The *Proteus* spp. strains were 26.6% of isolates. Out of them, 16.3% were monocultures of *Proteus mirabilis* and 6.1% – of *Proteus vulgaris*. In 8.2% of samples, *Proteus mirabilis* was associated with *E. coli*.

Pseudomonas aeruginosa strains isolated from dogs with sacculites amounted

to 12.2%: 10.2% of cases being monoinfections and in only one sample, the organism was associated with *E. coli*.

Only 4 (8.0%) of all isolates belonged to the *Staphylococcus* spp. Out of them, 3

(6.1%) were coagulase-positive and were identified as *Staphylococcus intermedius* and 1 (2.0%) were identified as *Staphylococcus epidermidis*.

Table 2. Percentages of sensitive (*S*), intermediate (*I*) and resistant (*R*) *E. coli* strains, isolated from dogs with perianal sacculites

Chemotherapeutics	<i>S</i>		<i>I</i>		<i>R</i>	
	%	CL	%	CL	%	CL
Amoxicillin	30.7	15.8÷49.4	38.4	21.0÷57.5	30.7	0÷14.4
Gentamicin	96.1	85.5÷100	3.8	0÷14.4	-	-
Tetracycline	50.0	31.3÷68.8	-	-	50.0	31.3÷68.8
Chloramphenicol	96.1	85.5÷100	3.8	0÷14.4	-	-
Enrofloxacin	88.5	73.6÷97.6	3.8	0÷14.4	7.7	0.78÷22.0

CL – confidence limits

Table 2 shows the results from the tests of *E. coli* strains isolated from dogs with perianal sacculites to a number of antimicrobial drugs. The highest sensitivity was observed against gentamicin and chloramphenicol (96.1%), as well as against enrofloxacin (88.5%), and the highest level of resistance – against

amoxicillin (30.7%). For the latter antibiotic, there was a high percentage of intermediate isolates (38.4), a sign for a trend of increasing resistance. The percentage of coltrains resistant to tetracyclines, was also high – 50.0%.

Table 3. Percentages of sensitive (*S*), intermediate (*I*) and resistant (*R*) *Proteus* spp. strains, isolated from dogs with perianal sacculites

Chemotherapeutics	<i>S</i>		<i>I</i>		<i>R</i>	
	%	CL	%	CL	%	CL
Amoxicillin	50.0	24.1÷77.0	37.5	14.2÷64.3	12.5	0.8÷35.2
Gentamicin	100	92.8÷100	-	-	-	-
Tetracycline	50.0	24.1÷77.0	12.5	0.8÷35.2	37.5	14.2÷64.3
Chloramphenicol	100	92.8÷100	-	-	-	-
Enrofloxacin	100	92.8÷100	-	-	-	-

CL – confidence limits

Table 4. Percentages of sensitive (*S*), intermediate (*I*) and resistant (*R*) *Pseudomonas aeruginosa* strains, isolated from dogs with perianal sacculites

Chemotherapeutics	<i>S</i>		<i>I</i>		<i>R</i>	
	%	CL	%	CL	%	CL
Amoxicillin clavulanic acid	100	84.5÷100	-	-	-	-
Gentamicin	100	84.5÷100	-	-	-	-
Tetracycline	40.0	7.8÷78.0	40.0	7.8÷78.0	20.0	0.4÷58.0
Carbenicillin	80.0	42.1÷99.6	20.0	0.4÷58.0	-	-
Enrofloxacin	80.0	42.1÷99.6	20.0	0.4÷58.0	-	-

CL – confidence limits

Table 3 presents the behaviour of *Proteus* strains, isolated from dogs with sacculites, to antimicrobial drugs. A sensitivity to gentamicin, chloramphenicol and enrofloxacin was exhibited. Only half of isolates was sensitive to amoxicillin and tetracycline – 50%. Intermediate behaviour was presented to amoxicillin and tetracycline 37.5% and 12.5%, respectively. The respective data for

isolated *Pseudomonas aeruginosa* strains are presented on **Table 4**. It could be seen that the highest percentage of sensitivity was shown against amoxicillin with clavulanic acid and to gentamicin – 100%. Sensitivity to carbenicillin was observed in 80.0% of isolates and to tetracycline – only 20.0%. Intermediate behaviour was exhibited to both carbenicillin and enrofloxacin with 20.00% as

well as to tetracycline (40.0%).

In the 4 staphylococcal isolates, resistance to amoxicillin, oxacillin, erythromycin and tetracycline was shown only by the coagulase-negative *S. epidermidis*. The methicillin/oxacillin resistance, observed for this strain by the disk diffusion method, was confirmed by the agar-screening test as well.

From coagulase-positive staphylococci *S. intermedius*, only one isolate was resistant to tetracycline. Methicillin/oxacillin resistance was not detected.

Phenotypes of resistance, related to production of beta-lactamases, were present in *E. coli* and *Proteus* spp. isolates.

Sensitivity to beta-lactams was present in 69.3% of *E. coli* isolates that formed a phenotypic profile corresponding to inherited beta-lactamases. The same phenotypic profile was observed in 87.5% of *Proteus* isolates. Only one of *E. coli* strains had a phenotypic profile corresponding to acquired beta-lactamases – plasmid penicillinase from the TEM-1, TEM-2, SHV-1 types. Such were not determined in *Proteus* strains.

DISCUSSION

Our data about the species diversity of microflora, isolated from canine perianal glands are closely similar to those of other investigators. Aside the microbial species we have identified, inflammations of these glands were shown to involve also *Enterococcus* spp., *Clostridium perfringens*, as well as *Bacillus* spp. and *Micrococcus luteus* (10).

In our study, the participation of enterobacteriae - *E. coli* and *Proteus* spp., prevailed whereas most authors communicated the involvement of staphylococci and yeasts, emphasizing on *Staphylococcus intermedius* (4, 11, 12).

The latter species was also observed by us, but in a significantly lesser percentage of samples. According to Harvey et al., 1994 (4), *S. intermedius* formed between 36 to 60% of the bacterial flora of anal mucosa in dogs. The authors also consider that anal sacs could serve as reservoirs for this species and from there, they could be transmitted onto the skin. This suggestion is supported by our results, showing similar sensitivity profiles against antimicrobial drugs in staphylococcal strains obtained from healthy dogs and dogs with pyoderma.

The absence of positive findings for yeast organisms in our study, especially

Malassezia spp., was somewhat surprising. Some investigators as Hajsig et al., 1985 (12) detected them in 46% of samples from the perianal sacs and thus are considered as reservoir of this species. The opinion of Pappalardo, 2002 (10) however is contrary, because in this study, they were rarely encountered.

In our study, the highest percentages of resistance were observed against tetracycline. It corresponded to other studies of ours, especially for *E. coli* isolates (13). The same was valid for *Proteus* strains – 50% of them was in the group of resistant or intermediate isolates with regard to tetracycline. Our data were somewhat similar to those of Maynard et al, 2004, who observed the highest resistance to tetracycline, ampicillin and sulphonamides in extraintestinal animal *E. coli* strains (14).

The relatively high resistance of isolates against amoxicillin – an antibiotic commonly used for treatment of circumanal adenites, were also interesting. This resistance should also be related to the other finding of ours – that, although at a low percentage, part of the resistance to beta-lactam antibiotics was due to acquired beta-lactamases, produced by isolates (15).

The data of increasing resistance of isolated strains to enrofloxacin should be interpreted from a merely practical point of view. This is especially important in our conditions having in mind the more frequent involvement of Gram-negative species in inflammations of perianal sacs.

Discussing the data about staphylococcal isolates, the fact about the presence of methicillin/oxacillin resistance in coagulase-negative *S. epidermidis*, could not be ignored. Moreover, this isolate exhibited a multiresistance against ampicillin, oxacillin, erythromycin and tetracycline. This fact is important from the point of view of its potential transmission from animals (including dogs) to humans (16, 17).

With regard to the data about *Pseudomonas* isolates, their high sensitivity to beta-lactam antibiotics and enrofloxacin corresponded to the results of Scol et al. (2002) (18) in dogs with various pseudomonad infections.

To summarize, the results of the present study emphasized the necessity of a bacteriological control of circumanal adenites in dogs and the application of an aetiologic treatment.

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