

OBSERVATIONS REGARDING BACTERIOLOGICAL AND MYCOLOGICAL MICROFLORA IN VARIOUS CAT AND DOG INFECTIONS

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ABSTRACT - *The aim of this study was the isolation and identification of bacteriological and mycological microflora from various dog and cat secretions and excretions in the Medical and Surgical Clinics of the Faculty of Veterinary Medicine, during October 2005 – April 2006. The analyses were conducted at the University Center of the Microbiology – Immunology Medical Research Laboratory from Iași. A number of 17 samples from the pathological material made of 13 dogs and four cats were carried out. After the microbiological examination, 27 bacteria stems and six mycological stems were isolated and identified. From the 27 bacteria stems, nine (33.33%) of them were Escherichia coli, five (18.51%), Staphylococcus spp., five (18.51%), Arcanobacter pyogenes, three (11.11%), Clostridium perfringens, three (11.11%), Streptococcus spp., one (3.70%), Pseudomonas aeruginosa, and one (3.70%), Proteus spp. From the six isolated mycetes stems, three (50%) of them were represented by Candida spp., two (33.33%) by Malassezia spp. and one (16.66%) by Cryosporium spp. For all the isolated species, pathogenity tests and antibiograma were carried out. The association of more bacteriological and/or mycotical species was frequent, being observed in five of the 17 analysed samples (four in dogs and one in cats).*

Key Words: microbiological exam, antibiograma, pathogenity

REZUMAT – *Observații privind asocierea florei bacteriene și micotice în diferite infecții la câine și pisică. Scopul acestui studiu îl reprezintă izolarea și identificarea microflorei bacteriene și micologice din diferite secreții și excreții ale câinilor și pisicilor, studiate la Clinica Medicală a Facultății de Medicină Veterinară, în perioada octombrie 2005-aprilie 2006. Analizele au fost realizate la Centrul Universitar al Laboratorului de Cercetare Microbiologică-Imunologică din Iași. Au fost examinate 17 probe de material patologic, provenite de la 13 câini și patru pisici. După examinarea microbiologică, au fost izolate și identificate 27 tulpini bacteriene și șase tulpini de miceti. Din cele 27 tulpini bacteriene, nouă (33,33%) din acestea au fost Escherichia coli, cinci (18,51%) Staphylococcus spp., cinci*

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(18,51%) *Arcanobacter pyogenes*, trei (11,11%) *Clostridium perfringens*, trei (11,11%) *Streptococcus spp.*, una (3,70%) *Pseudomonas aeruginosa*, una (3,70%) *Proteus spp.* Din cele șase tulpini de miceti izolate, trei (50%) au fost reprezentate de *Candida spp.*, două (33,33%) de *Malassezia spp.* și una (16,66%) de *Cryosporium spp.* Pentru toate speciile izolate, au fost realizate testele de patogenitate și antibiograma. Asocierea mai multor specii bacteriene și/sau micotice a fost frecvent întâlnită, fiind observată la cinci din cele 17 probe analizate (patru la câini și una la pisici).

Cuvinte cheie: examen microbiologic, antibiogramă, patogenitate

INTRODUCTION

The microbiological flora isolated from dogs and cats with different infections is various, bacteriological and mycotic species increasing most of the time their pathogenity. In majority of cases, the pathological material, which was represented by different secretions and excretions, contains an association of bacteriological and mycotic flora. The isolation and identification of involved species is very important in establishing the diagnosis, but for the veterinarians, the results of antibiograma on the entire flora, are important for an efficient treatment (Guguianu et al., 2002; Răucănescu, Bica Popii, 1986; Răpunțean et al., 2005; Zarnea, 1993-1990).

MATERIALS AND METHODS

A number of 17 samples of the pathological material from 13 dogs (aged between 7 months and 10 years) and four cats (aged between 4-9 years) of different breeds were analysed during October 2005 – April 2006: three samples of otic secretions, three samples of pharyngeal exudates, six urine samples, four dermal samples and one sample of vaginal secretion.

The pathological material was taken over under sterile conditions, and direct microscopic exam was carried out on Gram stained slides.

For the microbiological flora isolation and identification, the samples were cultured on usual and diagnosis mediums specific to aerobe and anaerobe bacteria, as well as to mycetes (agar, culture medium, Sabouraud, PDA, and medium with potato extract). The tubes were incubated in thermostat at 37°C, during 24-48 hours for bacteria, and at 22-25°C, during 3-5 days for mycetes. According to morphocultural characteristics, cultures were carried out on selective and identification mediums, (Chapmann, Levine, TSI, MIU, Simmons, and MacConkey).

The aim of the biochemical tests used was to point out catalysis activity, fermentation of manitol (*Staphylococcus spp.*), glucosis, lactose, saccharose and ketole (*Escherichia coli*) production.

In potentially pathogenic species, pathogenity was determined by using haemolytic test on sheep and rabbit erythrocytes, coagulation test of rabbit citrated plasma, and inoculation of laboratory animals.

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For determining the sensitiveness to antibiotics and chemotherapies of the isolated germs, we have done antibiograma, by diffusimetric method (OXOID Dispenser and antibiotics, compressed tablets OXOID and PHIZER: **AMP** – Ampycillin, **AMC**- Amoxycillin/Clavulanic acid, **NA** – Nalidixic acid 30µg, **CN** – Gentamycin 10µg, **E** – Erythromycin 15µg, **K** – Kanamycin - 30µg **NOR** – Norfloxacin 10µg, **OB** – Orbenin 25µg, **RD** – Rifampycin 30µg, **TE** – Tetracycline 30µg, **W** – Trimetoprim 5µg). Negative bacteriological samples were examined only mycologically, on native slides with lactophenol-eosine or glycerin.

RESULTS AND DISCUSSION

The studied cases and the results of the microbiological exam are presented in *Tables 1 and 2*.

Table 1

Synthesis of analysed samples and the results of microbiological exam and antibiograma in cats

Samples	Cats	Microbiological isolated species	Antibiograma results
Pharingian exudates	Tomcat, 5 year old - acute laryngotracheitis	<i>E. coli</i>	S - RD R - OB, AMP, TE, E, NA
Urine	Burmez tomcat, 9 year old - chronic nephritis	<i>Proteus spp.</i> <i>E. coli</i>	S - RD, CN R - NA, AMP, TE, E, K
	Persian cat , 4 year old -hemorrhagic cystitis	<i>E. coli</i> , <i>Cl.perfringens</i> <i>Candida spp.</i>	S - RD, K R - CN, TE, W, AMC, AMP, E
	Tomcat, 5 year old - chronic nephritis	<i>E. coli</i>	S - W, RD, NOR R -TE, E, CN

After the microbiological exam, 27 bacteriological stems and six mycetes stems were isolated and identified. From the 27 bacteria stems, nine (33.33%) of them were *Escherichia coli*, five (18.51%), *Staphylococcus spp.*, five (18.51%), *Arcanobacter pyogenes*, three (11.11%), *Clostridium perfringens*, three (11.11%), *Streptococcus spp.*, one (3.70%), *Pseudomonas aeruginosa*, and one (3.70%), *Proteus spp.* From the six isolated mycetes stems, three (50%) of them were represented by *Candida spp* 2 (33.33%), two (33.33%) by *Malassezia spp.* and one (16.66%) by *Cryosporium spp.* For all the isolated species, pathogenity tests and antibiograma were carried out.

The association of more bacteriological and/or mycotical species was frequent, being observed in five (29.41%) of the 17 analysed samples (four from dogs and one from cats). *Staphylococcus spp.* was isolated in association with *Malassezia spp.*, *Arcanobacter pyogenes* (one case) and *Escherichia coli* (two cases) with *Candida spp.*, *Malassezia spp.* with *Cryosporium spp.* (one case).

Table 2

**Synthesis of analysed samples and the results of microbiological exam
and antibiograma in dogs**

Samples	Dogs	Microbiological isolated species	Antibiograma results
Otic secretions	Rottweiler, 5 year old	<i>Staphylococcus spp.</i> <i>Malassezia spp.</i>	S - CN, AMP , RD R - K, E, TE
	Cocker, 7 year old - otitis	<i>Pseudomonas aeruginosa</i> <i>Arcanobacter pyogenes</i>	S - RD R - CN, E, TE, K, W, AMC, AMP
	Ciobănesc german , 7 year old	<i>Cryosporium spp.</i> , <i>Malassezia spp.</i>	-
Pharyngian exudates	Cocker , 5 year old - pharyngitis	<i>Arcanobacter pyogenes</i>	S - RD R - CN, E, TE, K, AMC, AMP, W
	Ciobănesc mioritic, 7month old	<i>Staphylococcus spp.</i> , <i>E. coli</i> <i>Arcanobacter pyogenes</i>	S - NOR R - TE, E, AMP, AMC, RD, W
Urine	Female dog , 9 year old – cystitis	<i>Arcanobacter pyogenes</i> <i>Candida spp</i>	MS - AMP , RD R - OB, K, CN, NA, E, TE
	Boxer, 10 year old - chronic nephritis	<i>E. coli</i>	S - NOR R - TE, AMP, AMC, E, CN
	Peke, 3 year old - nephritis	<i>Streptococcus spp.</i> <i>E. coli</i>	S - AMP, R - TE, K, RD, E, AMC, W
Dermal samples	Dog, 7 year old – dermatitis of bacteriological hypersensibilization	<i>Staphylococcus spp.</i> <i>Cl. perfringens</i>	S - OB, RD, R –NA, AMP, TE, E
Plantar exudates	Cocker, 1 year old	<i>Arcanobacter pyogenes</i> <i>Cl. perfringens</i>	S -RD, CN R - E, K, TE, W, AMC, AMP
	Ciobănesc german, 9 month old – pyodermatitis	<i>Streptococcus spp.</i> <i>Staphylococcus spp.</i> <i>E. coli</i>	S - NOR, R - W, RD, TE, AMC, AMP, CN, E
Plantar exudates	Bull terrier, 2 year old	<i>Streptococcus spp.</i> <i>Staphylococcus spp</i>	S -RD R -TE, NOR, W, CN, E
Vaginal secretion	Female dog, 5 year old	<i>E. coli</i> <i>Candida spp.</i>	S - NOR R - W, RD , TE, AMC, AMP, CN, E

Microorganism sensibility to the action of different antibiotics and chemotherapy varied according to the association of microbiological flora, but differences of sensibility in different stems from the same identified species were noticed.

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Statistical results presented in *Tables 1 and 2* have shown a variable sensibility in more frequently isolated species to the action of various antibiotics:

Staphylococcus spp. (Gram positive):

- in association with *Malassesia spp.*, it was sensitive to Ampicillin action, in the other cases being resistant (Togoe, Dobrea, 2006);
- in association with Gram positive (*Arcanobacter pyogenes* or *Streptococcus spp.*), and Gram negative germs (*Escherichia coli*), it was sensitive to Norfloxacin action and resistant to Rifampicin action;
- in association with another Gram positive germ (*Streptococcus spp.*), it was sensitive to Rifampicin action and resistant to Norfloxacin action.

Arcanobacter pyogenes (Gram positive):

- alone or in association with other Gram positive germs (*Clostridium perfringens*), it was sensitive to Rifampicin and Gentamicin action, to which, Gram negative flora was resistant.
- in association with Gram negative flora (*Pseudomonas aeruginosa*), it was sensitive only to Rifampicin action.
- in association with mycetes (*Candida spp.*), it was sensitive to Ampicyllin action, to which it was usually resistant.

Escherichia coli (Gram negative):

- isolated in pure culture, it was sensitive to Rifampicin and Norfloxacin.
- in association with Gram positive flora (*Staphylococcus spp.*, *Streptococcus spp.*) and mycetes (*Candida spp.*), it was resistant to Rifampicin.
- in association with *Streptococcus spp.*, it was sensitive to Ampycillin, to which it was usually resistant.

The analysis of antibiograma results pointed out that isolated species from all samples were sensitive to Rifampicin and resistant to Erythromicin, Tetracycline and Kanamycin.

CONCLUSIONS

A number of 17 samples of pathological material from 13 dogs and four cats (three samples of otic secretions, three samples of pharyngian exudates, six urine samples, four dermal samples and one sample of vaginal secretion) have been analysed.

From the 27 bacteria stems, nine (33.33%) of them were *Escherichia coli*, five (18.51%), *Staphylococcus spp.*, five (18.51%) *Arcanobacter pyogenes*, three (11.11%), *Clostridium perfringens*, three (11.11%), *Streptococcus spp.*, one (3.70%), *Pseudomonas aeruginosa*, and one (3.70%), *Proteus spp.*

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From the six isolated mycetes stems, three (50%) of them were represented by *Candida spp.*, two (33.33%) by *Malassezia spp.* and one (16.66%) by *Cryosporium spp.*

Association of more bacteriological and/or mycetical species was frequent, being observed in five (29.41%) of the 17 analysed samples (four from dogs and one from cats). *Staphylococcus spp.* was isolated in association with *Malassezia spp.*, *Arcanobacter pyogenes* (one case) and *Escherichia coli* (two cases) with *Candida spp.*, *Malassezia spp.* with *Cryosporium spp.* (one case).

Isolated bacteriological species from all the samples are sensitive to Rifampicin (except some bacteriological associations: *Staphylococcus spp.*- *E.coli* – *Arcanobacter pyogenes*, *Streptococcus spp.*— *E.coli*, *Streptococcus spp.*— *E.coli* - *Staphylococcus spp.* or bacterio-mycotic association: *E.coli* – *Candida spp.*) and resistant to Erythromycin, Tetracycline, and Kanamycin.

Association between *Staphylococcus spp.* and *Arcanobacter pyogenes* with *Malassezia spp.* or *Candida spp.* gives sensitivity to Ampicillin, to which Gram positive bacteria are resistant.

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