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Fungal microbiota of the hair coat of laboratory animals

Microbiota fúngica da pelagem de animais de laboratório

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Abstract

With the refining of handling techniques for laboratory animals kept in animal rooms in which different work rules are followed, it is fundamental to evaluate the microbiota of the raising environment, as well as of the animals in this environment. It is known that several saprobe fungi are isolated from hair and skin of experimental animals, being potentially pathogenic in specific situations. The aim of this research was to determine the fungal microbiota on the coats of laboratory animals from the Central Animal Room of the University of Ceará. Samples of hair and skin of 355 healthy animals (150 rats, 150 mice, 40 guinea pigs and 15 hamsters) were analyzed. Samples were collected by vigorously brushing the back of the animals and then cultured on Sabouraud's dextrose and Mycosel agar. Species identification was based on the analysis of the macroscopic appearance of colonies and microscopy features of the fungi. Development of fungi was observed in 63.95% of the animals. The most frequent fungi were: *Scopulariopsis brevicaulis* (34.7%), *Aspergillus* sp. (30%) and *Penicillium* sp. (16.7%). In conclusion, fungal microbiota on coat of these animals is diversified, and saprobe fungi occurs in a great number of experimental animals.

Keywords: fungal microbiota, coat, laboratory animals.

Resumo

Com o afinamento das técnicas de manejo de animais de laboratório mantidos em biotérios que seguem diferentes normas de trabalho, é fundamental que se avalie a microbiota dos ambientes de criação, bem como dos animais que deles fazem parte. É sabido que várias espécies fúngicas sapróbias são isoladas da pele e pelos de animais experimentais, podendo em situações específicas ser potencialmente patogênicas. A presente pesquisa objetivou conhecer a microbiota fúngica da pelagem de animais de laboratório do Biotério Central da UECE. Para tanto, foram analisadas amostras de pele e pelos de 355 animais clinicamente saudáveis (150 ratos, 150 camundongos, 40 cobaias e 15 *hamsters*). As amostras foram coletadas mediante escovação vigorosa do dorso desses animais e semeadas em ágar Sabouraud dextrose e ágar Mycosel, sendo a identificação das espécies baseada na análise das características macro e microscópicas das colônias fúngicas. Houve o desenvolvimento de fungos em 63,95% dos animais. Os fungos mais incidentes foram: *Scopulariopsis brevicaulis* (34,7%), *Aspergillus sp*. (30%) e *Penicillium sp*. (16,7%). Frente aos resultados obtidos, conclui-se que a microbiota fúngica da pelagem desses animais é bem diversificada, ficando evidente a ocorrência de fungos sapróbios em grande número de animais experimentais.

Palavras-chave: microbiota fúngica, pelagem, animais de laboratório.

There has been increasing interest in the development of procedures involving the breeding of laboratory animals. With the development of handling techniques for laboratory animals kept in animal rooms in which different work rules are followed, it is fundamental to evaluate the microbiota of the raising environment, as well as of animals in this environment (Ishikawa et al. 1996, Park et al. 2006). The skin of animals is contaminated by numerous fungi, some of which are opportunistic pathogens or allergens (Baker 1998, Connole et al., 2000, Pollock 2003). Thus, fungal microbiota is defined as the group of filamentous fungi and yeasts associated to animal tissues without causing disease or that are commonly found in natural or urbanized environments developing itself harmoniously in the habitat. Several investigations have reported the occurrence of saprophytic fungi in apparently healthy skin of domestic and wild animals (Abarca et al. 1989, Aho 1983, Bagy et al. 1998, Connole et al., 2000, Pollock 2003). Therefore, the present research aimed to identify fungal microbiota from the coat of laboratory animals of the Central Animal Holding Room of UECE. The experimental group was composed of a total of 355 animals: 150 rats (*Rattus norvegicus*), 150 mice (*Mus musculus*), 40 guinea pigs (*Cavia porcellus*) and 15 hamsters (*Syrian hamsters*), male and female, different ages and breeds, with no skin alteration and clinically normal from a dermatological point of view. Skin scales and hair collection were made with

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vigorously brushing the backs of the animals with sterile brushes. The brushes containing the clinical samples were rubbed on the surface of tubes with Sabouraud's dextrose agar increased with chloramphenicol (Sanofi®) and Mycosel agar® (BBL). Tubes were incubated at room temperature (@ 28ºC) for two weeks, and examined on a daily basis. After fungal colonies had appeared, a triage was made through macroscopic characteristics of all different types of colonies. The chosen colonies were then subcultured on Sabouraud's dextrose agar increased with chloramphenicol. After purification, the identification of colonies was based both on the macroscopic appearance of colonies and microscopy features, according to methodology cited by Sidrim et al. (2004) and De Hoog et al. (2000). Data were determined by analysis of variance. The present research was approved by the Committee of Ethics in Research of the State University of Ceará – CEP/UECE.

It was observed that there was an exclusive growth of saprophytic fungi in 63.95% of the animals (227 samples), with any isolation of primary pathogenic fungi in the studied families. A total of 12 fungal genera were isolated, with *Scopulariopsis* sp. (79 samples – 34.7%), *Aspergillus* sp. (68 samples – 30%) and *Penicillium* sp. (38 samples – 16.7%) being the most commonly found. The other nine genera were less frequently observed, as demonstrated in Tab.1. Regarding the specter of the identified fungi, it is composed predominantly by hyalines filamentous deuteromycetes. The only representatives of the leaven group were *Trichosporon* sp., *Rhodotorula rubra* and *Candida* sp. Concerning only the samples with fungal growth, 1 and 2 genera were isolated from 158 and 69 samples, respectively. In total, 128 samples were negative for fungal development (Tab.2). Saprophytic fungi, which grow in 63.95% of the animals, were isolated from all guinea pigs and hamsters, 62% of the rats, and 53% of the mice. Although the literature correlates a higher incidence of fungi in young animals, in this research, the age and weight of the animals in relation to the presence of saprophytic fungi were not statistic significant.

The isolation of these contaminant fungi, mainly the more frequent genera, can be explained by the fact that these fungi are cosmopolitans and are found in great quantities in nature and in environments occupied by humans. The high incidence of *Scopulariopsis brevicaulis* (34.7%) found was unexpected, especially as it is not routinely described as a member of cutaneous microbiota either in humans or animals. This fungus is traditionally described as a habitual saprobe of the ground and decomposed vegetables. Mantovani et al. (1982) describe the role of animal substratum as fungal growth factors, which the authors call "animalization". Animal debris, such as hair, skin scales, sweat and other organic materials of animals in the ground or wood shavings of cages from captivity animals may create environmental conditions favorable to the development of keratinophylic fungi, specially the geophylic ones. The predominance of the genera *Penicillium*, *Cladosporium*, *Aspergillus*, *Mucor*, *Aureobasidium*, *Alternaria*, *Scopulariopsis* and *Trichoderma* had been already described by Aho (1983) and Bagy et al. (1998), who verified that these fungi were the main components found in the coats of domestic and laboratory animals. The higher frequency of saprophytic fungi isolated from guinea pigs and hamsters correlates with the greater surface area of these animals in relation to mice, as well as the denser coat which promotes fungal development. Regarding influence that presence of these fungi can cause in tests with experimental animals, Junior et al. (1999) discuss that presence of genera of non-dermatophyte filamentous fungi may be associated with host susceptibility to immunosuppressive factors such as stress caused by captivity. Hyalines filamentous fungi, represented by the genera *Scopulariopsis* sp., *Aspergillus* sp. and *Penicillium* sp. were the only ones present in all strains of animals, possibly due to its ample ubiquitous distribution. Therefore, the majority of isolated genera on this study were expected for this habitat. From the obtained results it can be concluded that the isolated fungi are probably integrants of normal microbiota in the coats of laboratory animals. In addition, it was observed the occurrence of saprophytic fungi in a great number of experimental animals.

Table 1: Relative participation (%) and number of isolates from different fungal genera in 355 dermatological healthy animals

Fungal isolates	Total positive samples	Rats	Mice	Guinea pigs	Hamsters	% Total isolates	Total animals
Acremonium sp.	2	2	-	-	-	0.9	2
Aspergillus sp.	68	26	19	14	9	30	68
Candida sp.	4	2	2	-	-	1.8	4
Cunninguamella sp.	1	1	-	-	-	0.4	1
Curvularia sp.	4	3	-	1	-	1.8	4
Fusarium sp.	1	-	-	1	-	0.4	1
Penicillium sp.	38	16	11	7	4	16.7	38
Rhinocladiella atrovirens	1	1	-	-	-	0.4	1
Rhodotorula rubra	7	4	1	-	2	3	7
Scopulariopsis brevicaulis	79	36	15	18	10	34.7	79
Trichoderma sp.	15	5	3	5	2	6.9	15
Trichosporon sp.	7	2	2	3	-	3	7
TOTAL	227	98	53	49	27	100	227*

*** 69 samples were positive for two genera of contaminant fungi**

Table 2: Distribution of the number of fungal isolates per sample

Samples	Total of samples	Rats	Mice	Guinea pigs	Hamsters	% Total
Negatives	**128**	57	71	-	-	**37.5**
Positives						
	1 fungus for sample					
	158	64	55	28	11	**43.0**
	2 fungi for sample					
Aspergillus sp. and *Penicillium* sp.	39	17	12	7	3	9.1
Aspergillus sp. and *Scopulariopsis brevicaulis*	22	8	9	5	-	8.4
Penicillium sp. and *Scopulariopsis brevicaulis*	6	3	3	-	-	1.7
Rhodotorula rubra and *Scopulariopsis brevicaulis*	2	1	-	-	1	0.3
Total	**69**	29	24	12	4	**19.5**

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