

Fungal microbiota isolated from healthy pig skin*

Microbiota fúngica isolada da pele de suínos sadios

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ABSTRACT

Background: Researches have been developed to observe the normal microbiota of different animal species. This subject is of major importance for the control of potential infection risks. Fungi can be found in various substrates, foodstuffs (cereals, meat, milk, vegetables) and also in the skin, mucosae, respiratory and gastrointestinal tracts of animals. With the dissemination of immunosuppressive diseases in swine herds over the last years, the number of concomitant diseases caused by opportunist microorganisms is gradually increasing in literature. The objective of this study was to determine the microbiota of pig skin with no apparent lesions.

Materials, Methods and Results: A number of 261 pigs from 11 swine farms located in six municipalities of the State of Rio Grande do Sul, in Southern Brazil, were used for the study, in the period from April 2005 to April 2006. After being cleaned with water and 70% ethanol, skin samples were collected by friction of circular and sterile hair brushes against the posterior ventral region of the animals, on an area of no more than 10 cm. After sample collection, the brushes were wrapped with the same aluminium foil used in the sterilization process. Within the next 24 hours, the material was streaked onto agar and incubated at 25°C to 30°C for up to four weeks. Micromorphology was used for mold identification purposes, and the process employed lactophenol cotton blue staining. Whenever an initial identification was not possible due to the absence of characteristic structures, the isolate would be picked onto Potato agar to stimulate the development of reproductive structures. Yeasts and yeast-like fungi were characterized by physiological routine assays and differential tests, such as chlamydoconidia production and germ tube tests, and also by cultivation in HiCrome Agar. Isolates that produced arthroconidia were classified into the genera *Geotrichum* or *Trichosporon*. A number of 501 isolates were obtained, of which 297 were molds and 204 yeasts. Among the molds, the hyalohyphomycetes prevailed with 211 isolates, followed by 53 pheohyphomycetes and 33 zygomycetes. Two hundred and four yeast samples were identified as *Candida albicans* and *Trichosporon* spp., in addition to other far less frequent species, such as *C. glabrata*.

Discussion: The varied range of species isolated from the skin of pigs in this study can be explained by a number of factors, such as type of management, swine farm installations and environmental variations. The diversity of the microbiota found in relation to other studies demonstrates the necessity of this kind of research, because knowledge of the prevailing microbiota in a determined region facilitates the evaluation of potential impacts of sporadic or emerging new fungal diseases in herds, particularly in immunosuppressed animals. The observation of 17 *Scopulariopsis brevicaulis* isolates is worth pointing out, as its presence, associated with environmental and host factors, may favor the infection of pigs and the clinical development of the Dermatitis in the species. Knowledge of the diversity of mycological agents that are in direct contact with healthy animals may assist the diagnosis of exotic etiologies, particularly in animals with immunosuppressive diseases, considering that these are being diagnosed in swine with an increasing frequency.

Keywords: fungal microbiota, pig skin, yeasts, hyalohyphomycetes, pheohyphomycetes, zygomycetes, *Scopulariopsis brevicaulis*, Pustular Psoriasiform Dermatitis.

Descritores: microbiota fúngica, pele de suínos, leveduras, hialohifomicetos, feohifomicetos, zigomicetos, *Scopulariopsis brevicaulis*, Dermatite Pustular Psoriaforme.

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INTRODUCTION

Microbial agents are a potential risk, both in the dissemination of intra and interspecies diseases and in the production and preservation of foodstuffs destined to human and animal consumption [4,16].

Fungi can be found in various substrates, foodstuffs (cereals, meat, milk, vegetables) and also in the skin, mucosae, respiratory and gastrointestinal tract of animals [32]. Furthermore, breeding in installations where a complete cleaning is not possible while animals are housed, in addition to contact with feces, will favor the multiplication and dissemination of the microbiota. Studies developed to evaluate the contamination of the environment and of grains used as foodstuff demonstrate the presence of several bacterial and mycological agents, even when the installations are undergoing a period of rest before re-filling [19,35].

With the dissemination of immunosuppressive diseases in swine herds over the last years, the number of concomitant diseases caused by opportunist microorganisms is gradually increasing in literature, affecting different systems such as the integumentary, respiratory, and gastrointestinal [2,9,31]. In this new scenario, mycoses are being diagnosed with an increasing frequency, because an animal with impaired immunity often becomes unable to fight infection, even at low levels caused by direct or environmental contact.

Therefore, the objective of this study was to determine the microbiota of swine skin with no apparent lesions, in order to assess the degree of exposure to several potentially pathogenic agents.

MATERIALS AND METHODS

1. Animals

A number of 261 samples from healthy pigs were obtained for the study, originated from 11 swine farms located in six municipalities of the State of Rio Grande do Sul, in Southern Brazil, in the period from April 2005 to April 2006.

2. Collection of Samples

The material was collected by means of friction of circular and sterile hair brushes against the posterior ventral region of the animals, on an area of

no more than 10 cm. The brushes were sterilized by overnight exposure to ultraviolet light. A negative control of the incubation was made after each brush sterilization process.

The samples were collected after the skin was cleaned with water and 70% ethanol. After collection of the samples, the brushes were wrapped with the same aluminium foil used in the sterilization process. The material was cultured within less than 24 hours.

3. Fungal Isolation

The material was streaked onto Sabouraud's dextrose agar with 0.5 g/L chloramphenicol and incubated for a period of up to four weeks at a temperature of 25°C to 30°C [14,22].

3.1 Mold Identification

Micromorphology was made employing lactophenol cotton blue staining. Whenever an initial identification was not possible due to the absence of characteristic structures, the isolate would be picked onto Potato agar to stimulate the development of reproductive structures [14,21,33].

3.2 Identification of Yeasts and Yeast-like Fungi

The yeasts were characterized through physiological routine assays [3,38] and differential tests, such as chlamydoconidia production and germ tube tests [21] and through cultivation in HiCrome Agar. Isolates producing arthroconidia were classified in the genera *Geotrichum* or *Trichosporon*.

RESULTS

A number of 501 isolates were obtained among the 261 samples, of which 297 (59%) were molds and 204 (41%) yeasts. Among the molds, the hyalohyphomycetes prevailed with 211 (71%) isolates (genera *Penicillium*, *Aspergillus*, *Geotrichum*, *Scopulariopsis* and *Fusarium*), followed by 53 (18%) pheohyphomycetes (genera *Cladosporium*, *Curvularia*, *Alternaria* and *Aerobasidium*) and 33 (11%) zygomycetes (*Mucor* e *Absidia*) (Table 1). The 204 yeast specimens were identified as being *Candida* spp. (45%), *Candida albicans* (35.3%), *Trichosporon* spp. (10.7%), *Candida krusei* (2.4%), *C. tropicalis* (2%), *Candida parapsilosis* (1.5%) in addition to other far less frequent species, such as *C. glabrata*, *Rhodotorula* sp., *Candida famata* and *Pichia ohmeri* (Table 2).

DISCUSSION

Researches to observe the normal microbiota of different animal species have been developed [1,18,25]. This is of major importance for the control of potential infection risks, particularly the ones associated with species under confinement, where an unbalance of the environmental microbiota may influence the animal-environment interaction, with the consequent development of diseases.

Molds and yeasts are widely distributed in nature, being found in the terrestrial environment, aquatic environment or as a part of the anemophile microbiota. Several studies identifying the fungal

microbiota, both in installations and in swine food, have been already published [12,13,19,30,35]. *Absidia* spp., *Alternaria* spp., *Aspergillus flavus*, *Cladosporium* spp., *Fusarium* spp., *Penicillium* spp., *Rhizopus* spp., and *Scopulariopsis* spp. have been already identified in swine installations [13,19].

In the present study, 211 (71%) hyalohyphomycetes (genera *Aspergillus*, *Penicillium*, *Geotrichum*, and *Fusarium*) have been isolated. Researches involving foodstuffs used in swine food, particularly corn, indicate the isolation of *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., *Alternaria* spp., and *Cladosporium* spp. [12,30,35]. The most

Table 1. Diversity of molds isolated from the skin of healthy pigs from swine farms located in municipalities of the State of Rio Grande do Sul, Southern Brazil, during one year (April 2005 to April 2006).

Molds	Number (%)	Isolates	Number (%)
hyalohyphomycetes	211 (71.0)		
		<i>Penicillium</i> spp.	52 (17.7)
		<i>Aspergillus flavus</i>	29 (9.7)
		<i>A. fumigatus</i>	21 (7.0)
		<i>Geotrichum</i> spp.	21 (7.0)
		<i>Scopulariopsis brevicaulis</i>	17 (5.7)
		<i>Aspergillus</i> spp.	8 (2.7)
		<i>Fusarium</i> spp.	6 (2.0)
		<i>Aspergillus niger</i>	2 (0.7)
		<i>Mycelia sterilia</i>	55 (18.5)
pneohyphomycetes	53 (18.0)		
		<i>Cladosporium</i> spp.	46 (15.5)
		<i>Curvularia</i> spp.	3 (1.0)
		<i>Alternaria</i> spp.	3 (1.0)
		<i>Aerobasidium</i> sp.	1 (0.3)
zygomycetes	33 (11.0)		
		<i>Mucor</i> spp.	21 (7.0)
		<i>Absidia</i> spp.	4 (1.3)
		Not identified	8 (2.7)

frequently genus found among the hyalohyphomycetes was *Aspergillus* spp. (20.1%), with 60 isolates.

The observation of 17 *Scopulariopsis brevicaulis* isolates must be pointed out as the presence of this agent, associated to environmental and host-related factors, may favor the infection and clinical development of the Porcine Pustular Psoriasiform Dermatitis [6,8,26,27]. Additionally, *S. brevicaulis* could also be the agent of dermatomycoses in humans and animals [11,23,24].

Among 53 (18%) pheohyphomycetes, the genus *Cladosporium* was observed with 46 isolates. Pheohyphomycetes are associated with opportunist infections that are being found with an increasing frequency in human and animal pathology; the most frequent clinical form involves cutaneous and/or subcutaneous tissues, but dissemination to several organs may occur in animals with an impaired immune system [7,15,36]. The presence of melanin in their cell walls may be a virulence factor for these

fungi. Human cases of endocarditis are mostly reported on bioprosthetic valves, in particular those of porcine origin [28].

With regards to zygomycetes, the genus *Mucor* has widely prevailed. This is an omnipresent fungus, but that is little associated with diseases, both in animals and in humans. These fungi have an acknowledged potential to infect a wider variety of organisms in comparison to other filamentous fungi [29]. Zygomycoses are diseases of an opportunist nature, in which the degree of resistance of the host, the amount of inocula and the virulence factors of the microorganism are important characteristics for the development of the clinical picture. The occurrence of gastric zygomycosis in a pig affected by postweaning multisystemic wasting syndrome has been recently described [34]. This mycosis has been already described in swine, equines, bovines, ovine, and dogs [32].

With regard to yeast, *Candida albicans* is the species that has been most frequently isolated and

Table 2. Diversity of yeasts isolated from the skin of healthy pigs from swine farms located in municipalities of the State of Rio Grande do Sul, Southern Brazil, during one year (April 2005 to April 2006).

Genera	Number (%)	Isolates	Number (%)
<i>Candida</i>	179 (87.8)		
		<i>Candida albicans</i>	72 (35.3)
		<i>C. krusei</i>	5 (2.4)
		<i>C. tropicalis</i>	4 (2.0)
		<i>C. parapsilosis</i>	3 (1.5)
		<i>C. glabrata</i>	2 (1.0)
		<i>C. famata</i>	1 (0.5)
		<i>Candida</i> spp.	92 (45.1)
<i>Trichosporon</i>	22 (10.7)		
		<i>Trichosporon</i> spp.	22 (10.7)
<i>Rhodotorula</i>	2 (1.0)		
		<i>Rhodotorula</i> spp.	2 (1.0)
<i>Pichia</i>	1 (0.5)		
		<i>Pichia ohmeri</i>	1 (0.5)

described, both in human and veterinary medicine. The infections caused by the development of this species are usually not regarded as opportunist due to their endogenous nature, as the genus *Candida* is a skin, mucous and digestive tract commensal of mammals and birds [37]. In this study, *C. albicans* was found most frequently, representing 35.3% from a total of 204 yeast specimens. Its presence may be influenced by a reduced competition among agents, ease of contamination from matrices due to the successive contact with their feces, continuous use of medication, use of a number of stressing managing techniques (dental wear, vaccinations, docking, among others), which could allow its colonization and establishment of the disease [13,19,31,37,39].

Candidosis in swine can occur when the defenses of the host are reduced. The disease has been observed in piglets fed with food leftovers and which are kept under precarious sanitary conditions, and it can affect up to 40% of the herd [6]. The disease has been reported in pigs bred both in complex commercial systems and in extensive systems [9,39]. These animals, after being affected by viral diseases, presented a clinical picture of candidosis, demonstrating the importance of this fungal agent in the production chain.

The existence of eventual errors in some production segments, such as a high population index, sudden temperature variations and an increase in the environmental humidity rate, among other factors, generally allows the occurrence of diseases. Such situations, associated with the presence of immunosuppressive agents, contribute to decrease the resistance of the animals, leaving them susceptible to the action of several microorganisms [25].

Zygomycetes, *Aspergillus* and *Candida* spp., for instance, can be considered nowadays potential pathogens in swine pathology, after the onset of immunosuppressive diseases in herds from various continents [10,31,34,39].

CONCLUSIONS

The varied range of species isolated from skin of pigs, as shown in this study, can be explained by a combination of factors, such as management type, swine farm installations and environmental variations. The diversity of the microbiota found in relation to other studies demonstrates the necessity for this kind of study, as knowledge of the prevailing microbiota in a determined area facilitates the evaluation of potential impacts of sporadic or emerging new fungal diseases in herds, particularly in immunosuppressed animals.

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