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Fungal colonization on body surfaces of dead south American Sea lions on sandy beaches, and an evaluation of risk of contamination to humans at southern Brazil

Colonização fúngica em superfícies corporais de leões marinhos mortos da América do Sul em praias arenosas, e uma avaliação do risco de contaminação para os seres humanos no sul do Brasil

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ABSTRACT
At the interface between public health and marine ecology, there is a knowledge gap concerning the extent to which the carbon that enters sandy sediments when large biomass marine mammals decompose changes the sediment, bringing risks to human health. This study aimed to: 1) identify the fungal microbiota present in decomposing carcasses of South American sea lions (Otaria flavescens) found on beaches on the coast of southern Brazil, and 2) verify the extent to which the fungal microbiota are dispersed in the sandy sediment around the carcasses, presenting risks to human health. Samples of the corporal surface of 10 carcasses of O. flavescens deposited on beaches along the southern coast of Brazil, in a moderate decomposition state, as well as of the sand around the carcasses, were collected for analysis. Fungal microbiota was identified to genus level based on their macro- and micro- morphological characteristics.From carcasses were identified: Trichoderma, Aspergillus, Penicilium, Cladosporium and Fusarium. Aspergillus and Cladosporium were the most frequently taxa in the sand around the carcasses and in the control samples. Furthermore, their distribution around the carcasses was not compatible with the findings of fungi from the respective parts of the body, suggesting that these microorganisms were of a marine origin, and that their presence in the sand is not exclusively associated with the presence of the carcasses. The presence of Aspergillus in the carcass and sandy sediment reaffirms its regional distribution and the human risk due to dispersion of the spores from carcasses caused by wind.

keywords: Mycotic infections, marine mammals, zoonosis, carcasses

RESUMO
Na interface entre a saúde pública e a ecologia marinha, existe uma lacuna de conhecimento relativamente à medida em que o carbono que entra nos sedimentos arenosos quando grandes mamíferos marinhos de biomassa se decompõem altera o sedimento, trazendo riscos para a saúde humana. Este estudo teve como objectivo: 1) identificar a microbiota fúngica presente nas carcaças em decomposição dos leões marinhos sul-americanos (Otaria flavescens) encontrados nas praias da costa do sul do Brasil, e 2) verificar até que ponto a microbiota fúngica está dispersa no sedimento arenoso em torno das carcaças, apresentando riscos para a saúde humana. Foram recolhidas amostras da superfície corporal de 10 carcaças de O. flavescens depositadas nas praias da costa sul do Brasil, num estado de decomposição moderada, bem como da areia em redor das carcaças, para análise. A microbiota fúngica foi identificada ao nível do género com base nas suas características macro e micro morfológicas. A partir das carcaças foram identificadas: Trichoderma, Aspergillus, Penicilium, Cladosporium e Fusarium. Aspergillus e Cladosporium foram os taxa mais frequentes na areia em redor das carcaças e nas amostras de controlo. Além disso, a sua distribuição em torno das carcaças não era compatível com as descobertas de fungos das respectivas partes do corpo, sugerindo que estes microrganismos eram de origem marinha, e que a sua presença na areia não está exclusivamente associada com a presença das carcaças. A presença de Aspergillus na carcaça e no sedimento arenoso reafirma a sua distribuição regional e o risco humano devido à dispersão dos esporos das carcaças provocada pelo vento.

Palavras-chave: Infecções micóticas, mamíferos marinhos, zoonose, carcaças

1 INTRODUCTION

Knowledge concerning coastal and marine ecosystems is in constant evolution. Coastal regions represent transition zones between continents and oceans [1], and as such, in these regions physical-chemical variations and changes in the retention rates of organic matter, pollutants, sediments and nutrients from the continent occur. Among the peculiar characteristics of these ecosystems, it must be considered the abiotic and biotic events responsible by innumerable modifications and interactions such as the availability and capacity of carbon multidirectional recycling and relative stable equilibrium [2]. Domestic waste, including human and animal discharges represent pollutants that can contaminate the sea, and may disseminate a vast variety of pathogenic bacteria and fungi to this ecosystem [3]. Marine animals that are in contact with contaminated water can be infected, altering their physiological condition and causing illnesses, and they are often then found in a weakened condition in the coastal zone [4]. At the interface between public health and marine ecology, there is a knowledge gap concerning the extent to which the carbon that enters sandy sediments when large biomass marine mammals decompose changes the sediment, bringing risks to human health.

Many fungal species are present in the environment as saprobes or commensals, coexisting with animals without affecting them [5]. These fungi are part of the ecosystem, are found in high concentration and are widely dispersed throughout the ecosystem, as they have a great capacity to colonize different substrates and to grow in extreme environmental conditions. However, many fungi can eventually cause diseases in human beings, animals and plants [6]. Fungi that are aerially dispersed, or anemophilous fungi [7], can be inhaled by animals and humans, but are rarely pathogenic in healthy organisms [8]. This is because the development of a fungal infection depends on the immune state of the host, the opportunity of interaction between the host and the fungus, and the infectious dose. Other factors that can be relevant include the host’s age, epidemiological exposures and risk factors.

In Brazil, fungal infections in marine mammals are relatively rarely reported compared with reports of viral, bacterial and helminthic diseases [9]. Lobomicosis has been recorded in the skin of bottlenose dolphins in Southern Brazil [10], as well as in other marine mammals around the world [11]. Nevertheless, it is believed that fungal organisms may contribute, in a significant manner, to the mortality of marine mammals [12]. Brazil’s south coast is characterized by a rectilinear coastline with a great wealth of marine fauna that includes several species of birds and mammals [13]. The presence of pinnipeds in this region is common during the winter and spring months, when many of them are weakened or, even, arrive dead at the coast, having been carried there by marine currents [14, 15]. According to previous study [16], in polluted waters the incidence of soil fungi is high. Twelve genera of fungi have been found in waters where the present study was carried out and, *Aspergillus* and *Penicillium* were frequently reported [17]. These genera were also dominant in the study of fungal diversity conducted in the Brazilian northeast, where they were associated with high salinity and alkaline waters [18]. *Penicillium*, *Aspergillus* and *Trichoderma* have also been isolated from sandy sediments of impacted urban beaches [17], in the present study their constant presence in carcass and around it suggests colonies growing due high organic matter available.

The South American sea lion, *Otaria flavescens* (Shaw 1800) is one of the species that occurs frequently in this region, the higher concentration out of the reproductive colonies are in two points of coast line in Rio Grande do Sul, Brazil [19,15]. Most of the individual of this species that are found dead along the beaches in the south of Brazil are non-breeding males, and these individuals generally occupy the extremes of the reproductive colonies (in Uruguay), in areas dominated by older male individuals that can reach a weight of 300 kg [20]. Due to the topography and the urban isolation of certain coastal areas, there is a high incidence of carcasses of this species that remain on the sandy sediment until they have totally decomposed [14, 15, 21]. It is often assumed that such events will cause the spreading of pathogenic microorganisms, as well as altering the structure of fungal colonization in the sediment, and therefore, the carcasses could act as transmitters of diseases to the beach's visitors [22]. However, data concerning the microbial components on body surfaces of healthy or sick sea lions that come to the Brazilian coast, as well as the fungal microorganisms that colonize them after death and decomposition of carcasses on sandy sediments, are not available in the literature.

Research concerning marine animal zoonosis have important implications for public health and for the determination of suitable measures to protect public health, via an understanding of the possible transmission mechanisms of these diseases. This study aimed to: 1) identify the fungal microbiota present in decomposing carcasses of South American sea lions found on beaches in southern Brazil, and 2) verify the extent to which the fungal microbiota are dispersed in the sandy sediment around the carcasses, presenting risks to human health.

2 MATERIALS AND METHODS

Superficial samples were collect from 10 carcasses of *O. flavescens*, found during systematic and weekly field trips in beaches along the Brazilian south coast between Torres (29°20' S) and Arroio do Chuí (33°15'S). The state of decomposition of carcasses was mild to moderate (level 2 and 3 according to the modified protocol from [23]. Only those carcasses deposited next to the humid zone of the beach were used for this study, excluding those partially or totally submerged in the sea and those that were completely dehydrated.

Four microbial samples were collected from the surface of each carcass using a sterile swab, corresponding to four regions of the body: mouth, anus, back and anterior flipper (thoracic member). The swabs were stored in isothermal boxes to move them to the lab, where the material was seeded by the streaking methodology in Potato Dextrose Agar (PDA) and incubated in a bacteriological incubator at 25 °C for 7 days.

To evaluate sand contamination, four samples of superficial sandy sediment were collected from around each carcass. Each sample comprised of 225 cm^3, taken from a maximum depth of 5 cm. These sediment samples were strategically removed at 10 cm distances from the following points: mouth, anus and right and left anterior flippers. To analyze these samples, aliquots of 25 g of each sediment sample were diluted in 250 mL of peptone water 0.1 % and submitted to a serial dilution, in base 10, until a dilution of 10^3. Volumes of 100 μL of each dilution were seeded in the culture medium Potato Dextrose Agar (PDA), and incubated in a bacteriological incubator at 25 °C, for 7 days. As a control, another seven sand samples were collected from places without the presence of the carcasses and were processed similarly to the sand samples collected around the carcasses.

Both body surface samples and those from sandy sediment were submitted to fungal colonies identification. Identification to genus level was based on macro- and micro- morphological characteristics.

3 RESULTS

A total of 87 samples were analyzed including the body surface samples, the sediment samples from around each carcass, and the controls. Considering all samples, the taxonomic genus and the percentage of occurrence of fungi at each point of sampling (carcass, sand and sand control) are shown in **Fig 1**.

Five fungi genera were identified on the carcasses: *Trichoderma* spp., *Aspergillus* spp., *Penicilium* spp., *Cladosporium* spp. and *Fusarium* spp. It is possible verify a clear distribution of fungi colonization on different parts of the body. The filamentous fungi *Trichoderma* spp. and *Aspergillus* spp. were the most frequent genera in all samples. However, *Trichoderma* spp. was found only in the corporal surface of the sea lions, not occurring in the sand. Both fungi were observed in all sampled points of the body. *Penicillium* spp. was identified on the flippers and back, being absent in samples from the mouth and anus, where *Aspergillus* and *Trichoderma* were dominant. *Cladosporium* spp. was found only on skin from the flippers and mouth, in the sandy sediment close these parts of the carcasses, and also in the control samples (**Figs 2, 3**).

The samples of sandy sediment from around the carcasses were colonized by fungi from four genera: *Aspergillus* spp., *Fusarium* spp, *Penicilium* spp and *Cladosporium* spp. Of these fungi,

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Aspergillus spp. and *Cladosporium* spp. were the most frequently isolated (**Fig 3**).

Five fungi genera were identified from control sand samples (not influenced by carcasses): *Aspergillus* spp., *Rhizopus* spp., *Paecilomyces* spp., *Alternalia* spp. and *Cladosporium* spp. (**Fig 1**).

4 DISCUSSION

Few studies have been carried out concerning fungi colonization in carcasses of free-living pinnipeds, with the aim of evaluating risks to the human population. The present work is, to the best of our knowledge, the first with this focus, and shows the real possibility of an emergent disease to occur related with carcass manipulation. The fungal microbiota of the body surface of these animals (live or dead) is not known, and little information about mycotic infections in marine mammals is available in the literature [9,24]. On the other hand, many studies focused on biology or veterinary aspects of this group of mammals have been performed using carcasses found along the coastline, where individuals have died offshore or inshore, and arrive partially decomposed on the beaches, and where they are then often manipulated for the collection of biological samples and data [14,19,25,26]. It is important to emphasize that fungal organisms can contribute to the mortality of marine mammals in general [9], as well as acting as a source of infection to humans [27]. Among marine mammals, pathogenic fungi that have been recorded previously include the genera *Blastomyces*, *Candida*, *Coccidioides* and *Histoplasma* [28,29,]. However, only some endemic fungi are pathogenic and able to promote infection in healthy individuals, as occurs with *B. dermatitidis*, *C. immitis* and *H. capsulatum* [9].

In the present study, *Cladosporium* spp and *Aspergillus* spp. were present in the carcass samples, in the sediment samples and in the control, suggesting the marine origin of these microorganisms, which are not exclusively associated with the presence of the carcass where show equal or low occurrence compared to other samples. *Cladosporium* has more than 50 species, many of which are cosmopolites and found in high concentration in the atmosphere, colonizing several environments and substrates, including the marine environment [30,6]. Although not commonly associated with vertebrate infections, more recently ten new species of the genus have been isolated from human and animal clinical specimens from the USA [31]. Most of them were isolated from human respiratory samples, which might be explained by the fact that *Cladosporium* conidia are easily dispersed by air [32]. However, the clinical relevance of the species of this genus, at least to produce invasive disease, has been questioned by their inability to grow at 37 °C [33, 31]. The genus *Aspergillus* is responsible for aspergillosis, an opportunistic infection that can be severe in immunocompromised individuals [34,35]. Species of this genus are found in the soil and organic matter in decomposition [36], their spores are dispersed by the wind [35]. Inhalation of the spores can cause pulmonary diseases such as allergic bronchopulmonary aspergillosis and invasive aspergillosis [37]. The symptoms vary from local inflammation of the airways to severe infections and this pathology is considered as an opportunistic mycosis [38,39]. Manifestation of aspergillosis is relatively uncommon in mammals, however there are reports in canines [40], felines [41], equines [42], bovines [43], and primates [44]. Aspergilosis is one of the main causes of mortality in birds [40] and in the marine environment it is a problem, mainly in weakened birds. Aspergillosis in Magellanic penguin (*Spheniscus magellanicus*) have been reported in rehabilitation centers [45]. Penguins, among other birds, are particularly susceptible, despite the fact that this mycosis rarely attacks these marine birds in free life [46]. This pathology is considered to be the most frequent fungal disease in captivity, corresponding to nearly 30 % of the mortality causes of penguins in rehabilitation centers and zoos [47, 38]. *Aspergillus* sp. is also the organism most frequently isolated in mycotic pneumonia in marine mammals [9]. The identification of this fungus in the carcasses and sandy sediments reaffirms its regional distribution, and the frequent infection in marine weakened birds, shows its opportunistic feature and infective capacity [40]. The weather conditions along the coastline of southern Brazil, with frequent windy days, are favorable to the dispersal of fungal spores. Furthermore, caution is required to avoid inhalation of fungal spores during manipulation of carcasses *in situ,* and we recommend moistening the carcass prior to manipulation.

Contamination of the carcasses by *Trichoderma* spp. may originate from the tidal washes on the carcasses, since this genus is often found in the marine environment [48] and reports of pathologies in pinnipeds and cetaceans associated to this genus have not been reported [9,27]. The exclusive presence in *O. flavescens* carcasses, taking into account the differences observed in the composition of fungal communities in the sandy sediment it is suggestive of a possible suppressive action by other genera in the same substrate, as observed previously with other mycotic colonizations [49]. The genera *Rhizopus* spp, *Alternalia* spp and *Paecilomyces*, are considered to be cosmopolites, and in this study were identified only in the control samples, suggesting that there presence is not related to the presence of the carcasses in the sand. All identified fungal genera are anemophilous, microorganisms frequently found in the environment and not considered primary pathogens. However, they are known to cause allergic processes and severe opportunistic infections in humans. Filamentous fungi, such as *Fusarium* and *Aspergillus,* can cause diverse clinical manifestations determined by a deficiency in the immune response of the host. Thus, when the immune system is weakened, the probability of occurrence of infections caused by *Aspergillus* increases, due to its opportunistic nature [35]. Considering the biogeographical region were the study was performed, *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicilium*, and *Rhizopus* have already been identified in the sand and water and indicated as having the potential to cause mycotic and allergic pathology in humans [50].

The present study is novel in terms of its aim to evaluate the potential risks when manipulating dead *O. flavescens*, whose carcasses remain on the coast of the southern coast of Brazil, until their total degradation. It is believed that the fungal genera isolated from the carcasses come from the environment and colonize the body surface of the animals after they arrive on the coast. In addition, it was verified that the surface of the carcasses do not carry fungi of zoonotic potential, except for individuals who are immunologically depressed or debilitated. Based on the findings of the present study, physical protection (latex gloves and hospital masks) are recommended for professionals who will manipulate the carcasses on the beach.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

STATEMENT ON THE WELFARE OF ANIMALS

The present study used only naturally deceased animals. The samplings were performed in cooperation with the Laboratory. of Megafaune from Institute of Oceanography of University of Rio Grande.

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ANNEXES

FIGURE LEGENDS

Fig 1 General distribution (in percentage) of fungal genera colonization in: carcasses of South American sea lions (*O. flavescens*) (n=10, 40 samples); sandy sediment where the carcasses were deposited (40 samples); and control sand samples (7 samples), along the southern coastline of Brazil.

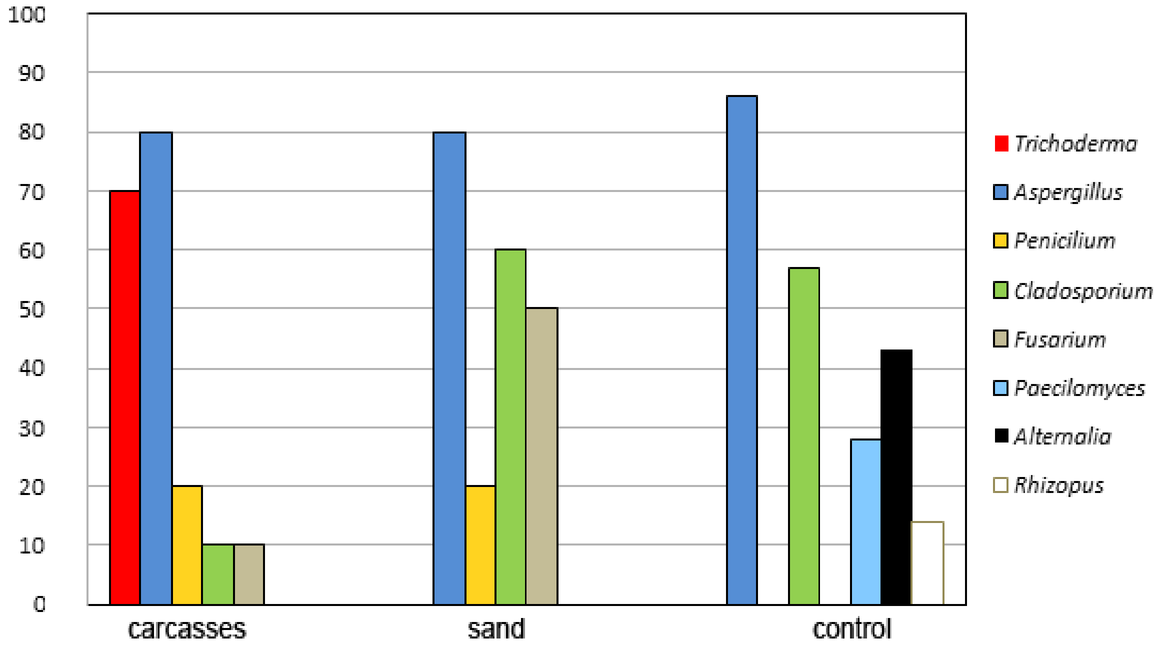

Fig 2 Isolated fungal genera, percentage according to body surface regions from carcasses of South American sea lions (*O. flavescens*) (n=10, 40 samples), along the southern coastline of Brazil.

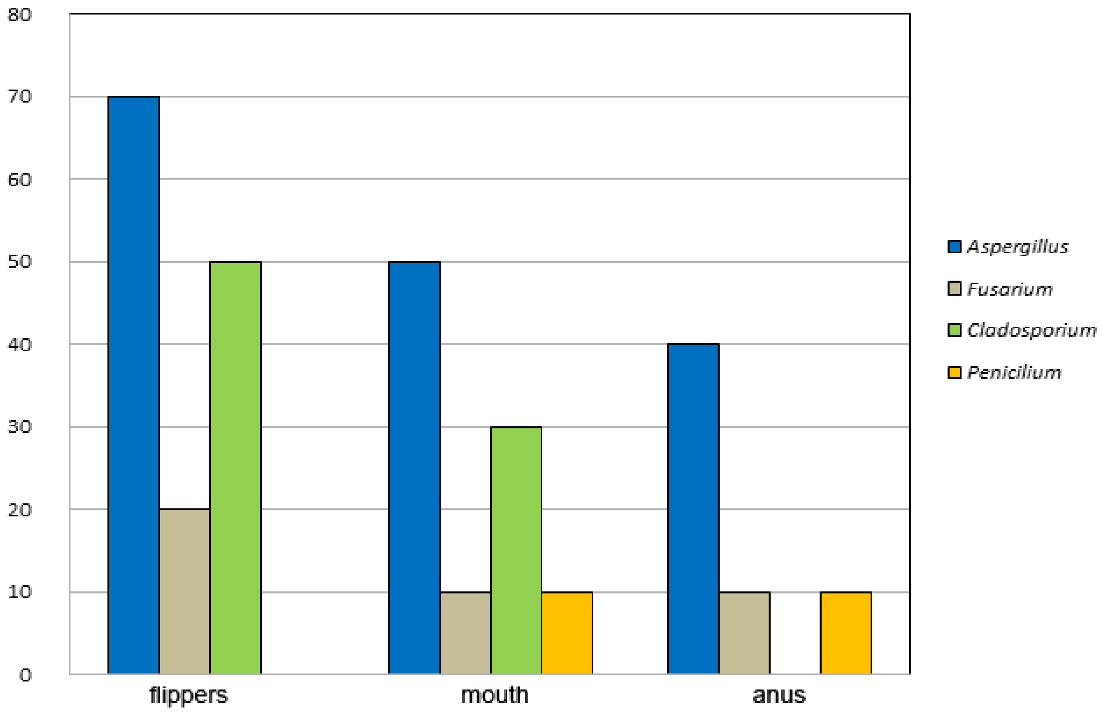

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Fig 3 Isolated fungi from sandy sediment (40 samples), percentage according to regions near to collection points of carcasses surface of South American sea lions (*O. flavescens*) (N=10) in decomposition, along the southern coastline of Brazil.