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Mycobiota recovered from the trachea and lungs of pigeons (*Columba livia*) captured in a grain mill

Micobiota recuperada da traqueia e pulmões de pombos (*Columba livia*) capturados em um moinho de grãos

Micobiota recuperada de tráquea y pulmones de palomas (*Columba livia*) capturadas en un molino de granos

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Abstract

Respiratory diseases are commonly related to grain mills in association with fungi. Pigeons (*Columba livia*) are a potential source of pathogenic fungi and a public health concern, causing economic damages in urban areas. We aimed to determine the tracheal and pulmonary mycobiota of pigeons captured in a grain mill, identifying potentially pathogenic fungi. Captured pigeons were euthanized for the collection of tracheal secretion and lungs in saline suspensions for culture (100µL) in Sabouraud dextrose agar with chloramphenicol (48h/28°C). Yeasts were evaluated after Gram staining in microscopy and cultured in Hicrome®*Candida* agar, while lactophenol cotton blue solution was used in filamentous fungi/molds. Mycological identification keys were used for the genus determination. 207 fungal (9 genus) were isolated from the trachea and lungs of 21 pigeons captured: 199 molds (96.14%) and 8 yeast (3.86%). *Aspergillus* spp., *Rhizopus* spp., *Penicillium* spp. and *Candida* spp. were the most prevalent, mainly in lungs. We confirmed yeasts and molds from pigeons, increasing the potential risks for chronic or acute diseases after contact with several fungi from pigeons. A greater control of the presence of pigeons in the work environment is necessary.

Keywords: Filamentous fungi; *Aspergillus*; *Candida*; Mycobiota; Pigeons.

Resumo

As doenças respiratórias são comumente relacionadas aos moinhos de grãos em associação com fungos. Visitantes frequentes devido ao hábito granívoro, pombos (*Columba livia*) são vetores em potencial de fungos patogênicos e um problema de saúde pública, causando prejuízos econômicos em áreas urbanas. Nós objetivamos determinar a micobiota traqueal e pulmonar de pombos capturados em moinho de grãos, identificando fungos potencialmente patogênicos. Os pombos capturados foram eutanasiados para coleta de secreção traqueal e pulmões em suspensões salinas para cultivo (100µL) em ágar Sabouraud dextrose com cloranfenicol (48h/28°C). As leveduras foram avaliadas após coloração de Gram em microscopia e cultivadas em ágar Hicrome®*Candida*, enquanto a solução azul algodão de lactofenol foi utilizada em fungos filamentosos. Chaves de identificação micológica foram utilizadas para a determinação do gênero. Um total de 207 fungos (9 gêneros) foram isolados da traqueia e pulmões de 21 pombos capturados: 199 filamentosos (96,14%) e 8 leveduras (3,86%). *Aspergillus* spp., *Rhizopus* spp., *Penicillium* spp. e *Candida* spp. foram os mais prevalentes, principalmente nos pulmões. Confirmamos leveduras e fungos filamentosos potencialmente patogênicos no sistema respiratório dos pombos, o que pode aumentar os riscos potenciais para doenças crônicas ou agudas após contato com os diversos gêneros identificados. É necessário um maior controle da presença de pombos no ambiente de trabalho.

Palavras-chave: Fungos filamentosos; *Aspergillus*; *Candida;* Micobiota; Pombos.

Resumen
Enfermedades respiratorias se relacionan comúnmente con los molinos de granos en asociación con hongos. Las palomas (*Columba livia*) son una fuente potencial de hongos patógenos y un problema de salud pública, causando daños económicos en zonas urbanas. Nosotros determinamos la micobiota traqueal y pulmonar de palomas capturadas en molino de granos, identificando hongos potencialmente patógenos. Las palomas capturadas fueron sacrificadas para la recolección de secreción traqueal y pulmones en suspensiones salina para cultivo (100 µl) en agar Sabouraud dextrosa con cloranfenicol (48h/28°C). Las levaduras se evaluaron después de la tinción de Gram en microscopía y se cultivaron en agar Hicrome®*Candida*, mientras que lactofenol azul se usó en hongos filamentosos. Se utilizaron claves de identificación micológicas para la determinación del género. 207 hongos (9 géneros) fueron aislados de la tráchea y pulmones de 21 palomas capturadas: 199 mohos (96.14%) y 8 levaduras (3.86%). *Aspergillus* spp., *Rhizopus* spp., *Penicillium* spp. y *Candida* spp. fueron los más prevalentes, principalmente en pulmones. Confirmamos levaduras y mohos de palomas, aumentando los riesgos potenciales de enfermedades crónicas o agudas tras el contacto con varios hongos de palomas. Es necesario un mayor control de la presencia de palomas en el entorno laboral.
Palabras clave: Hongos filamentosos; *Aspergilus*; *Candida*; Mycobiota; Palomas.

1. Introduction

Pigeons (*Columba livia*) are cosmopolitan and a potential source of pathogenic fungi, causing economic damages in urban areas as a public health concern, due to environmental contamination by the deposition of their feathers, excrements and even fungi scattered in the respiratory process (Ferreira et al., 2016; Lee et al., 2017). Their granivorous habits affect *in natura* or industrialized grain-food industries from the processing to the final consumer, besides the importance as a vector of pathogens (Ferreira et al., 2016). Studies are scarce about the dust from activity in grain mill, which is the main responsible for respiratory clinical conditions in workers in these industries (Straumfors et al., 2021), as well as in the surrounding community (Theisinger; Smidt, 2017; Permatasari & Kolibu, 2020), being fungi important etiological agents involved in these processes.

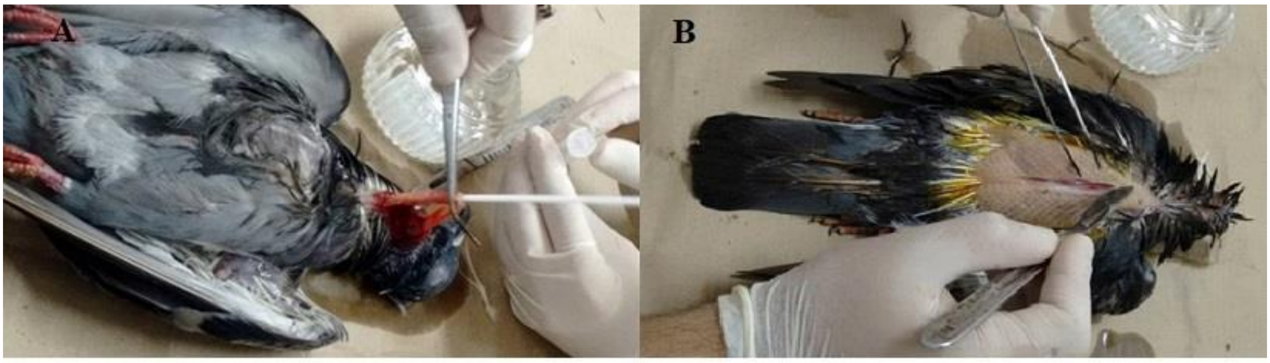
The grain processing is related to several respiratory diseases caused mainly by mycotic agents, and the presence of pigeons increases the problem (Ferreira et al., 2016). Thus, the present study aimed to characterize the mycobiota of the respiratory system of pigeons captured in a grain mill in Alagoas to identify the coexisting fungi in their respiratory tract, focusing two different sites.

2. Material and Methods

Our study is descriptive observational (Patel; Patel, 2019), when pigeons were captured at the Moinho Motrisa S/A, (Maceió, Alagoas, Brazil) by a specialized company and submitted to anesthetic overdose euthanasia with intravenous thiopental sodium (DCB 086338/40 mg/kg-^{1}) via brachial vein under aseptic conditions, performed in Histology and Embryology Laboratory (LHE/ICBS/UFAL) and approved by Animal Ethics Committee (n°58/2015).

Confirmed death, tracheal samples were obtained by introducing a sterile cervical brush into the trachea after an incision in the medial portion (Figure 1A), adapted method to collect by Garcia et al. (2007) and Zampieri et al. (2015), and samples were immersed in sterile saline (1 ml) for vortex (1 min). Lungs were extracted after incisions in the dorsal portion (Figure 1B), with the adoption of 2 methods in both lungs: (a) Lung fragments were inoculated directly on Sabouraud dextrose agar with chloramphenicol (ASDC) at 5 equidistant points; (b) Lung fragments were macerated with a sterile swab in a microtube with sterile saline (1 ml) for vortex (1 min). Right and left lobes were evaluated separately, and carcasses were incinerated following biosafety standards.

Figure 1 - (A) Introduction of the sterile cervical brush into the trachea to collect secretion sample and (B) dorsal incision for removal of the right and left lungs.



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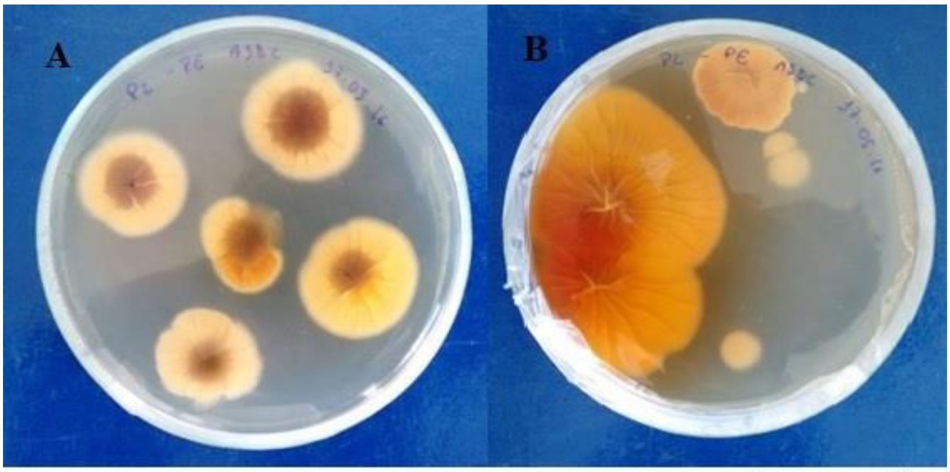
Aliquots of the tracheal and pulmonary suspensions (100 µL) were processed in the Clinical Microbiology Laboratory (LMC/ICBS/UFAL) for individual cultives on ASDC (48h; 28-32°C); fungal colonies were subcultivated in potato-dextrose-agar (Sigma) to yield pure cultures. Yeasts were evaluated by Gram stain for microscopic identification (40x-1600x) and cultured in Hicrome®*Candida* Differential Agar (Himedia) for presuntive identification of the medically important *Candida* species (Algrawal et al., 2014), while lactophenol cotton blue solution was used in filamentous fungi (40x-1600x) and mycological identification keys were used for the genus determination (Hoog et. al, 2000; Kidd et al., 2016).

3. Results and Discussion

Twenty-one pigeons were captured for collection in 2 anatomic sites, a total of 207 fungal isolates were obtained from the tracheal and pulmonary samples (Figure 2), with 199 filamentous fungi (96.14%) and only 8 yeasts (3.86%). The highest frequency of yeasts and filamentous fungi was represented by *Candida* spp. (3.38%) and *Aspergillus* spp. (58.45%), respectively (Table 1).

Figure 2 - (A) Fungal colonies in Sabouraud dextrose agar with chloramphenicol medium after cultive of lung fragments and (B) after scattering on medium of the macerated lung fragments suspension (100µl).



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Probably, there is a greater fungi proliferation in the grain mill, an environment with suspension of spores and a higher rate of fungi inhalation (Permatasari & Kolibu, 2020), although all the captured pigeons in the dependencies of grain mill were clinically healthy.

Table 1 - Filamentous fungi and yeasts identified in different anatomical sites of the respiratory system of pigeons (n=21).

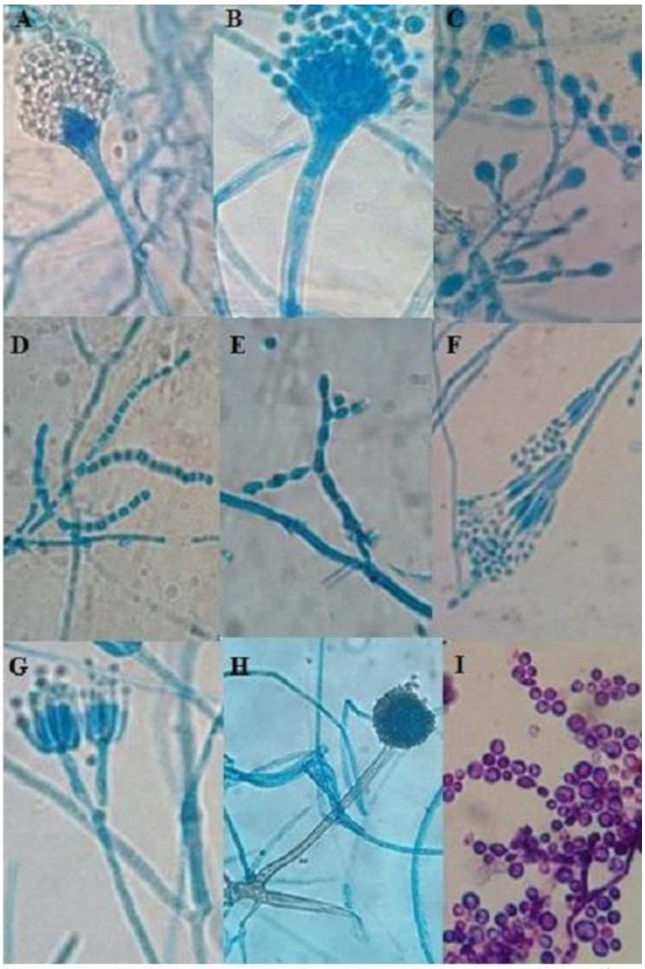
Isolated fungi	**Anatomic sites**							
	Trachea		**Right lung**		**Left lung**		**Total**	
	n	%	n	%	n	%	n	%
Filamentous/ Molds	20	9.66%	88	42.51%	91	43.97%	199	96.14%
Absidia sp.	4	1.93%	6	2.90%	6	2.90%	16	7.73%
Aspergillus sp.	12	5.80%	57	27.54%	52	25.12%	121	58.45%
Chrysosporium sp.	-	-	1	0.48%	-	-	1	0.48%
Mycelia sterilia	-	-	5	2.42%	9	4.35%	14	6.76%
Neurospora sp.	1	0.48%	-	-	-	-	1	0.48%
Paecilomyces sp.	-	-	2	0.97%	2	0.97%	4	1.93%
Penicillium sp.	3	1.45%	8	3.86%	9	4.35%	20	9.66%
Rhizopus sp.	-	-	9	4.35%	13	6.28%	22	10.63%
Yeasts	1	0.48%	4	1.93%	3	1.45%	8	3.86%
Candida albicans	-	-	-	-	1	0.48%	1	0.48%
Candida krusei	-	-	2	0.97%	-	-	2	0.97%
Candida tropicalis	1	0.48%	2	0.97%	1	0.48%	4	1.93%
Geotrichum sp.	-	-	-	-	1	0.48%	1	0.48%
Total	21	10.14%	92	44.44%	94	45.42%	207	100%

Source: Authors.

Nine genera were identified (Figure 3), in addition to the Mycelia sterillia group (n = 14); *Aspergillus* spp. was the most prevalent (121 isolates), followed by *Rhizopus* spp. (n = 22), *Penicillium* spp. (n = 20), *Absidia* spp. (n = 16). Less frequently, we identified *Candida* spp. (n = 7), *Paecilomyces* sp. (n = 4) and 1 of each *Chrysosporium* sp., *Geotrichum* sp. (yeast-like) and *Neurospora* sp.

Bird respiratory microbiota studies address normally bacterial communities in beef animals (Glendinning et al., 2017; Abundo et al., 2021), with the mycobiota being contemplated in few studies (Mulholland et al., 2021). The respiratory microbiota provides environmental and health information for understanding and management of respiratory diseases (Tomic et al., 2021), especially in birds (Furian et al., 2018; Arne et al., 2021).

Figure 3 - Fungi identified in trachea and lungs of pigeons (*Columba livia*): Filamentous fungi in lactophenol cotton blue solution and yeast after Gram stain (Optical microscope examination: 1.600x). (A) *Absidia* sp., (B) *Aspergillus* sp., (C) *Chrysosporium* sp., (D) *Geotrichum* sp., (E) *Neurospora* sp. (F) *Paecilomyces* sp., (G) *Penicillium* sp., (H) *Rhizopus* sp. and (I) *Candida* sp. (Gram stain).



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Twenty filamentous fungi (9.66%) and only 1 yeast *Candida* sp. (0.48%) were isolated in the trachea (10.14%), the less colonized site, while 186 isolates (89.85%) were obtained in lungs: 92 (44.44%) from the right lung (88 molds/4 yeast) and 94 (45.42%) from the left lung (91 molds/3 yeast-like) (Table 1). Among the *Candida* species, in the chromogenic medium it was possible to identify 1 *C. tropicalis* from trachea and 1 *C. tropicalis*, 2 *C. krusei* and 1 *C. albicans* from lungs.

The immunological status of the birds, the habitat and methodology used for collecting biological samples can have changed the colonization profile in birds. We performed the collection with the introduction of a cervical brush in the trachea, without any contact with the oropharynx, different from the methodology that Garcia et al. (2007) and Zampieri et al. (2015) applied in birds from rehabilitation centers (RC), with the introduction of a swab into the oropharynx, which may increase the risk of contamination with yeasts from the oropharynx (Melville et al., 2004; Silva et al., 2019). Garcia et al. (2007) reported 42.59% of wild birds in RC with fungi isolated from the trachea, yeasts (24.53 %) and molds (12.5%), mainly *C. albicans* and *C. parapsilosis*. Birds in rehabilitation are more susceptible to stress, and weakness and/or drug therapy by corticosteroids and antibiotics facilitate the yeast proliferation (Silva et al., 2014). An increase was also observed by Zampieri et al. (2015) in seabirds (40% of *Candida* sp.), which are more due to the aquatic environment (Monaphati et al., 2020).

A study in birds of prey (Silva et al., 2019) confirmed a higher occurrence of yeasts isolated in oropharynx (93.3%) compared to trachea (6.7%), the majority *Candida* spp. (80%) isolated from *Rupornis magnirostris* (Roadside hawk/Gavião-carijó) (73.7%). All birds of prey were clinically healthy and normally do not inhabit aquatic environments (Silva et al., 2019), which could explain the low number of yeasts isolated from the trachea, similar to our research.

High concentrations of airborne fungi were identified at the grain mill from Lithuania, where allergenic genus such as *Penicillium*, *Cladosporium* and *Geotrichum* prevailed (Straumfors et al., 2021). Thus, it is possible that fungal propagules have been inhaled by the birds, indicating, therefore, that they could also be inhaled by grain mill workers and cause acute respiratory symptoms through occupational exposure by the inhalation of large fungal inoculum (Theisinger & Smidt, 2017; Permatasari & Kolibu, 2020; Straumfors et al., 2021). All fungi isolated from the respiratory tract of pigeons are saprophytic and coexist on diverse organic matters, and the majority is considered as potentially pathogenic and involved in acute respiratory symptoms, increasing the possibility of interfering in the health of workers or the surrounding community (Senbeta & Gure, 2014; Pelliser et al., 2016; Theisinger & Smidt, 2017).

4. Conclusion

Our findings confirm that pigeons are frequently colonized by *Aspergillus* and *Candida* species. An exposure to their conidia may provoke adverse health effects such as allergic rhinitis or asthma by opportunistic pathogens involved also in systemic diseases in immunosuppressed individuals. Therefore, a better maintenance of the area is necessary, avoiding pigeon access and dispersion of fungi, as well as performing microbiological studies on air quality in grain mill dependencies to guarantee the health of the workers and surrounding community.

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