Short Paper

Molecular identification of non-*Cryptococcus* yeasts associated with pigeon droppings in Shiraz, Southern Iran

Pakshir, K.[1*]; Zareshahrabadi, Z.[2]; Zomorodian, K.[1]; Ansari, S.[3]; Nouraei, H.[2] and Gharavi, A.[4]

[1]Department of Parasitology and Mycology, Basic Sciences in Infectious Diseases Research Center, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran; [2]MSc in Medical Mycology, Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran; [3]Department of Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [4]BSc in Microbiology, Department of Parasitology and Mycology, Basic Sciences in Infectious Diseases Research Center, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

[*]***Correspondence:*** *K. Pakshir, Department of Parasitology and Mycology, Basic Sciences in Infectious Diseases Research Center, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran. E-mail: pakshirk@sums.ac.ir*

(Received 28 Aug 2018; revised version 15 Jan 2019; accepted 13 Feb 2019)

Abstract

Background: Birds are considered as a reservoir for pathogenic and non-pathogenic fungi. Pigeon droppings have the potential for spreading these fungi to the environment. *Cryptococcus* species are important fungi associated with pigeon droppings. In this regard, there are many types of yeast associated with guano that is important for human and animal health. **Aims:** The main objective of this study is the identification of non-*Cryptococcus* yeasts isolated from pigeon dropping in Shiraz, Southern Iran. **Methods:** A total of 100 unknown yeasts, which were previously screened and identified as non-*Cryptococcus* from pigeon guano through the conventional methods, were used in this study. Identification of the isolates was performed based on conventional methods and DNA sequence analysis of internal transcribed spacer (ITS) rDNA gene region. The sequence results were deposited in NCBI database using the Basic Local Alignment Search Tool (BLAST). **Results:** A total of 16 species belonging to 7 genera were identified as *Candida* spp. 51% (8 species), *Rhodotorula* sp. 24%, *Trichosporon* spp. 21% (3 species), *Rhodosporidium* 2%, *Saccharomyces* 1%, *Rhizoctonia* 1%, and *Meyerozyma* 1%. The predominant isolates were *Rhodotorula rubra* (24%), *Candida famata* (20%), and *Trichosporon asahii* (13%). The other species were *Rhodosporidium kratochvilovae* 2 (2%), *Saccharomyces cerevisiae* 1 (1%), *Rhizoctonia solani* 1 (1%), and *Meyerozyma caribbica* 1 (1%). **Conclusion:** Pigeon excreta examined in this study were associated with several kinds of opportunistic yeasts which could cause diseases in prone human and animals.

Key word: *Candida*, DNA sequence, Non-*Cryptococcus*, Pigeon

Introduction

Avian dropping is known as the reservoir of different pathogenic and opportunist organisms (Chee and Lee, 2005). Pigeons are reported as a major carrier of *Cryptococcus* species including *C. neoformans*, which is the most deadly opportunist yeast known (Costa *et al*., 2010; Soltani *et al*., 2013), and is considered as reservoir of zoonotic yeasts and other pathogenic fungi (Medina *et al*., 2017).

In an urban area, pigeons could have a threating role in public health, as they live close to people in public parks, on the roof of hospital buildings, rooms near the air handler units, and even in sacred places where they are fed for religious reasons (Haag-Wackernagel and Moch, 2004).

On average, a well-fed pigeon could deposit 25 pounds of guano a year (Haag-Wackernagel and Moch, 2004). Naturally, by the wind and during raining or after some disasters like flooding, their droppings disseminate and come into close contact with people, especially the susceptible ones.

Although *Cryptococcus* has received much attention in medicine, it is not the only pathogen that affects human health. Recently, other opportunistic fungi such as *Candida* spp., *Trichosporon* spp., *Rhodotorula* spp., *Geotrichum* spp., *Mucor* spp., and *Aspergillus* spp. have been isolated from pigeon droppings (Khosravi, 1997; Lanzafame *et al*., 2001; Costa *et al*., 2010; Abulreesh *et al*., 2015; Lee *et al*., 2017). In recent years, opportunistic fungal infections by uncommon yeast species are increasing (Lanzafame *et al*., 2001; Munoz *et al*., 2005). Candidiasis is known as the most common fungal infection in human and birds of prey (Deem, 2003). Several cases of candidemia with uncommon *Candida* species such as *C. fumata* and *C. lusitania* have been reported, which are less known concerning their epidemiological features and ways of transition (Chen *et al*., 2009). Identification of these species helps to understand the importance of these fungi and risk of encountering and provides useful information for epidemiological and ecological studies.

Although the yeast composition of pigeon faeces has been reported by many studies from different regions of

Iran and around the world, transmission of the pathogens depends on climatic condition, stability, in the environment, and environmental factors such as temperature and humidity (Tsiodras *et al*., 2008).

Although there are many studies conducted based on culture-dependent methods, they are not precise enough to identify the yeast species (Rastogi and Sani, 2011). In this regard, the molecular-based method is more specific and sensitive than conventional methods. Taxonomic identification of fungi based on DNA sequence analysis is one of the best techniques applied to identify non-coding rDNA spacer regions.

The internal transcribed spacer (ITS) includes the ITS1 and ITS2 noncoding regions that are located between the *18S rRNA* and *28S rRNA* genes and are quite variable among related species. By amplification and sequencing of these regions, we can achieve a higher resolution compared to other methods (Bellemain *et al*., 2010). Using this method, Cafarchia could identify the yeasts species isolated from hens faeces (Cafarchia *et al*., 2018) but there is less data regarding identification of yeasts associated with bird droppings by molecular techniques.

In our previous study, we identified many yeast isolates as *Cryptococcus* species by molecular and basic conventional methods (Pakshir *et al*., 2018). In the present work, we also isolate many unknown yeast species associated with pigeon guano. The main aim of this study is to identify the pathogenic and non-pathogenic yeasts isolates associated with pigeon dropping in Shiraz, Southern Iran by ITS sequence analysis.

Materials and Methods

Isolates preparation

In this study, 100 unknown frozen yeast stock isolates from pigeon guano - previously identified as non-*Cryptococcus* (our previous study) - were analyzed (2014). The samples were subcultured on Sabouraud's Dextrose Agar pH = 5.6 ± 0.2 (Merck, Germany), supplemented with chloramphenicol (50 mg/L), incubated at 25°C for 48 h, and inspected daily for growth.

Conventional identification methods

Primary identification of isolates was based on micromorphological analysis of yeast shape using the teased mount preparation results (round, ovoid or rectangular shape), growth on Corn Meal Agar Tween 80 (Merck, Germany) for detection of pseudohypha and chlamydoconidia, production of germ tube in serum and colony color on CHROMagar *Candida* media (Paris, France).

Molecular method

DNA preparation

Genomic DNA was extracted through the boiling method described by Makimura *et al*. (1994) with small modifications. Briefly, a small amount of the yeast colony was suspended in 100 µL of lysis buffer containing 100 mM Tris-HCl, 0.5% sodium dodecyle sulfate (SDS), and 30 mM ethylenediaminetetraacetic acid (EDTA) and boiled for 15 min at 100°C. A solution of potassium acetate (2.5 M) was then added to the lysate, held on ice for 1 h, centrifuged at 16125 × g for 5 min, and the supernatant was transferred to a new tube. The yeast DNA in the supernatant was washed twice with ethanol and air dried and re-suspended in 50 µL of distilled water (DW) prior to use for polymerase chain reaction (PCR). The purity and quantity of DNA were evaluated by absorbance ratio of A260/A280, and also nano-drop reading results.

Amplification and sequencing of the ITS regions

A set of universal primers (ITS1 5´-TCC GTA GGT GAA CCT GCG G-3´ and ITS4 5´-TCC TCC GCT TAT TGA TAT GC-3´) (Meta-Bion International, Martinsried, Germany) were employed for amplification of ITS region of an ITS1-5.8S-ITS2 segment of the ribosomal DNA gene. PCR amplification was carried out in a final volume of 50 µL consisting of 5 µL of 10 × PCR buffer, 1.5 mM $MgCl_2$, 0.8 mM deoxynucleoside triphosphates (0.2 mM each), 1.2 U of Taq DNA polymerase (Roche Molecular Biochemicals, Mannheim, Germany), 0.5 µM of each primer, 2 µL of DNA template, and eventually with DW to a final volume (50 µL). An initial denaturation step at 95°C for 5 min was followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 50 s, and extended at 72°C for 1 min, with a final extension step at 72°C for 7 min and kept at 4°C for 5 min. Negative controls were also used in each set of reactions. The PCR product was electrophoresed on 1.2% agarose gel and stained with ethidium bromide. Then, the PCR products were purified and sequenced (Bioneer Company, South Korea). The sequence results were processed using the web-based blasting program and Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST), and the data were compared with those in the NCBI/Genebank database.

Results

Germ tube formation was observed in eight isolates of *Candida albicans* (8%). *Candida albicans*, *C. krusei*, and *C. glabrata* present green, white-pink, and purple colony colors respectively and the other yeast species had no color and remain white.

ITS region of extracted DNA was amplified in all samples (Fig. 1) and sequence results of PCR products demonstrate 16 species of fungi, belonging to 7 genera (Table 1), and compared with the other data (Table 2).

Genus *Candida* has the highest prevalence and *Rodotorula rubra* 24%, *Candida famata* 20%, and *Trichosporon asahii* 13% were the most dominant species isolated from pigeon extra.

Table 1: Result of yeast species identified from pigeon dropping in Shiraz

Yeast species	Number	Percent	Yeast species	Number	Percentage
Candida famata	20	20%	*Rhodotorula rubra*	24	24%
Candida albicans	8	8%	*Trichosporon asahii*	13	13%
Candida glabrata	8	8%	*Trichosporon coremiiforme*	6	6%
Candida cutenulata	7	7%	*Rhodosporidium kratochvilovae*	2	2%
Candida krusei	2	2%	*Trichosporon moniliiforme*	1	1%
Candidia colliculosa	2	2%	*Saccharomyces cerevisiea*	1	1%
Candida lusitania	1	1%	*Rhizoctonia solani*	1	1%
Pichia anomala	3	3%	*Meyerozyma caribbica*	1	1%
Total	100				

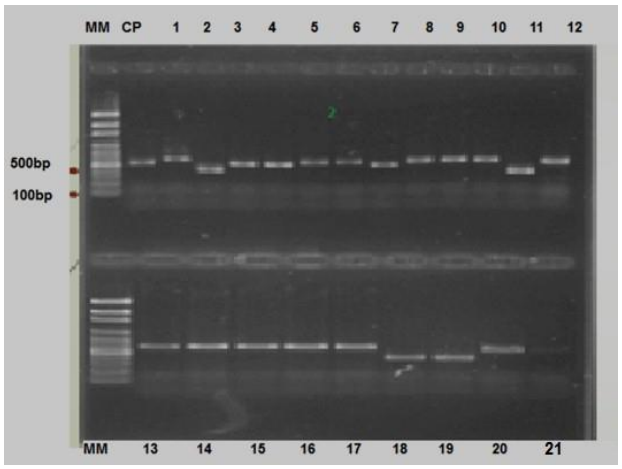

Fig. 1: Results of PCR products electrophoresis. Line MM: 100 bp molecular marker, Line CP: Standard positive control, Lines 1, 5, 6: *Rhodotorula rubra*, Lines 2, 11, 18, 19: *Candida cutenulate*, Line 3: *Saccharomyces cerevisiea*, Lines 4, 7: *Trichosporon asahii*, Lines 8-10 and 12-17: *Candida famata*, Line 20: *Cryptococcus albidus*, and Line 21: Negative control (distilled water)

Table 2: Comparison of the most common yeast species isolated from pigeon dropping in Iran and the other countries

Yeast species	Iran	Other countries
Cryptococcus	Yes	Yes
Candida	Yes	Yes
Trichosporon	Yes	Yes
Rhodotorula	Yes	Yes
Saccharomyces	Yes	Yes
Geotrichum	Yes	Yes
Pichia	Yes	Yes
Rhodosporidium	Yes	No
Rhizoctonia	Yes	No
Meyerozyma	Yes	No
Torulopsis	No	Yes
Malassezia	No	Yes
Wallemia	No	Yes
Debaromyces	No	Yes
Zygoseccharomyces	No	Yes

Discussion

Pigeon droppings, which are found in public areas such as streets, parks and gardens, old buildings and hospitals, and pigeon towers, are considered as a possible source of infections to human and animals. Nitrogen-rich environment contaminated with birds dropping provides a suitable condition for bacteria and fungi growth (Harrigan, 1998). The chemical properties and composition of pigeon faeces (pH, uric acid, and nitrogen) create an excellent substrate for fungal spores to propagate. In this regard, the abundance of pathogenic fungi has been linked to weather (humidity, temperature, and radiation), vegetation, and microbes associated with guano (Lee *et al*., 2017). Excreta of pigeons also were reported as an ideal environment for yeast replication, especially *Candida* and *Trichosporon* species (Medina *et al*., 2017).

If there was any evidence for transmission of the pathogen to humans from the avian species, it could classify as direct/indirect transmission. Although there is little known about the actual direct/indirect transmission of these pathogens, the risk exists through ingestion of water contaminated from faeces or exposure to inanimate surfaces contaminated by bird secretions or droppings (Tsiodras *et al*., 2008). Pigeons are an important source in the spread and maintenance of *C. neoformans*. In addition, various species of pathogenic/non-pathogenic yeasts are associated with pigeons that are important in human and animal health (Nweze *et al*., 2015; Simi *et al*., 2018). To date, approximately 48 species of potentially pathogenic fungi from 28 genera have been isolated from pigeons globally (Abulreesh *et al*., 2015; Lee *et al*., 2017).

Among various diagnostic methods, molecular techniques such as DNA sequence analysis of ITS region are the most accurate techniques that have been used in many countries like Korea and China (Cafarchia *et al*., 2006; Lee *et al*., 2017). Using this method, we could identify 17 species belonging to 8 genera. In Iran, many studies (by non-molecular techniques) report that the highest prevalence of non-*Cryptococcus* yeasts associated with pigeon dropping belongs to genus *Candida* (Khosravi, 1997; Soltani *et al*., 2013). Similar to those reports, in our study, *Candida* spp. was the most prevalent genus isolate but among the species, *C. famata* was the predominant one; however, this data was not in agreement with the other data reported from Iran, Egypt, and Brazil (Khosravi, 1997; Costa *et al*., 2010; Soltani *et al*., 2013; Abulreesh *et al*., 2015).

In this study, the other uncommon *Candida* species associated with pigeons were *C. catenulata*, *C. colliculosa*, and *C. lusitania*. These species are responsible for candidiasis but diseases occurred by these organisms are less frequently encountered. *Candida*

colliculosa is found in fruits and dairy products like grapes and milk and was responsible for fungal endocarditis in a Turkish gardener patient (Kaygusuz *et al*., 2003). *Candida catenulata* and *C. famata* also were isolated from droppings of captive birds in Italy (Mancianti *et al*., 2002). Elsewhere, *C. catenulata* was reported as the causative agent of fungemia in a patient with gastric cancer (Radosavljevic *et al*., 1999).

Pichia anumala (anamorphic stage of *Candida pelliculosa*) was another yeast identified in our study. Murphy *et al*. (1986) report nosocomial outbreaks of fungemia due to *P. anomala* in Liverpool. Another study has reported the same species responsible for an outbreak of nosocomial fungemia in the pediatric wards of a hospital in India (Chakrabarti *et al*., 2001). *Candida* species was also reported as main causative infections in different animals like dogs, buffalo, cattle, horse and goat (Jadhav and Pal, 2013).

Rhodotorula rubra was the second most frequent isolate from pigeon excreta in this study. This genus is mostly associated with bird droppings. Cafarchia *et al*. (2006) reported this fungus as the highest number of isolates from cloacae of migratory wild birds in Italy while Costa *et al*. (2010) reported it in Brazilian pigeons. Although this yeast is not as dangerous for humans, it could occasionally cause diseases like meningitis in hospitalized patients (Lanzafame *et al*., 2001). There are several reports of skin infections in chickens, lung infection in sheep, dermatitis in cat and respiratory tract in dog all of which are caused by *Rodotorola* species (Wirth and Goldani, 2012; Biegańska *et al*., 2018).

Trichosporon sp. was the third most frequent genus isolate including *T. asahii* (13%), *T. coremiiforme* (6%), and *T. moniliforme* (1%). This genus is also isolated from pigeon droppings in Egypt and Brazil (Costa *et al*., 2010; Abulreesh *et al*., 2015). *Trichosporon* species is considered as one of the most important etiological causes of nosocomial infections (Ghiasian *et al*., 2006; Miceli *et al*., 2011) and has been reported as the second- or third-most-common agent of yeast fungemia (Chagas-Neto *et al*., 2009).

Trichosporon asahi species that accounted for the highest percentage of systemic invasive infection in susceptible patients, as well as *T. coremiiforme* and *T. moniliforme*, cause superficial skin infections (Rodriguez-Tudela *et al*., 2005; Asada *et al*., 2006). This fungi could also cause diseases in dogs and cats (Karnik *et al*., 2009; Biegańska *et al*., 2018).

Another yeast identified in our study was *Saccharomyces cerevisiae*. This is a useful yeast that is used as a probiotic in the bakery, food, and brewing industries around the world. However, *S. cerevisiae* was reported as the causative agent of nosocomial infections that can cause a wide range of clinical syndromes such as peritonitis, cellulitis, and liver abscess. Munoz *et al*. (2005) reported three cases of fungemia in an intensive care unit of a hospital in Spain and present another 57 cases of *S. cerevisiae* fungemia in their literature review.

The yeast *Meyerozyma caribbica* was another isolate in our study that belongs to genus *Colletotrichum*. These fungi in this genus are phytopathogens and responsible for fruit rot such as mango. The yeast *M. caribbica* was used against this genus as a biological control but there is no evidence of pathogenicity in human being yet (Bautista-Rosales *et al*., 2013).

The yeast *Rhizoctonia solani* isolated from a skin lesion of a patient in India (Kaore *et al*., 2012) but human mycosis by this fungus is rare and less common. Finally, most of the yeasts associated with pigeon droppings in Iran were similar with those reported from the other countries as mentioned in result section.

In this study, we identified many species of yeasts associated with pigeon excreta in Shiraz city and more than 90% of the isolates had a history of diseases in the human and animals as opportunistic fungi. Pigeons are important reservoirs and carriers for zoonotic yeast in the environment.

Acknowledgements

This study was funded by Deputy of Research and Technology of Shiraz University of Medical Sciences, Shiraz, Iran (Grant No. 7220) and was extracted from an MSc thesis by Z. Zareshahrabadi.

Conflict of interest

The authors report no conflicts of interest.

References

Abulreesh, HH; Organji1, SR; Elbanna, K; Haridy Osman, GE; Almalki1, MHK and Abdel-Mallek, AY (2015). First report of environmental isolation of *Cryptococcus neoformans* and other fungi from pigeon droppings in Makkah, Saudi Arabia and *in vitro* susceptibility testing. Asian Pac. J. Trop. Dis., 5: 622-626.

Asada, N; Uryu, H; Koseki, M; Takeuchi, M; Komatsu, M and Matsue, K (2006). Successful treatment of breakthrough *Trichosporon asahii* fungemia with voriconazole in a patient with acute myeloid leukemia. Clin. Infec. Dis., 43: e39-e41.

Bautista-Rosales, PU; Calderon-Santoyo, M; Servín-Villegas, R; Ochoa-Álvarez, NA and Ragazzo-Sánchez, JA (2013). Action mechanisms of the yeast *Meyerozyma caribbica* for the control of the phytopathogen *Colletotrichum gloeosporioides* in mangoes. Biol. Control. 65: 293-301.

Bellemain, E; Carlsen, T; Brochmann, C; Coissac, E; Taberlet, P and Kauserud, H (2010). ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. BMC Microbiol., 10: 189.

Biegańska, MJ; Rzewuska, M; Dąbrowska, I; Malewska-Biel, B; Ostrzeszewicz, M and Dworecka-Kaszak, B (2018). Mixed infection of respiratory tract in a dog caused by *Rhodotorula mucilaginosa* and *Trichosporon jirovecii*: a case report. Mycopathologia. 183: 637-644.

Cafarchia, C; Camarda, A; Romito, D; Campolo, M; Quaglia, NC; Tullio, D and Otranto, D (2006). Occurrence of yeasts in cloacae of migratory birds. Mycopathologia. 161: 229-234.

Cafarchia, C; Iatta, R; Danesi, P; Camarda, A; Capelli, G and Otranto, D (2018). Yeasts isolated from cloacal swabs, feces, and eggs of laying hens. Med. Mycol., 57: 340-345.

Chagas-Neto, TC; Chaves, GM; Melo, ASA and Colombo, AL (2009). Bloodstream infections due to *Trichosporon* spp.: species distribution, *Trichosporon asahii* genotypes determined on the basis of ribosomal DNA intergenic spacer 1 sequencing, and antifungal susceptibility testing. J. Clin. Microbiol., 47: 1074-1081.

Chakrabarti, A; Singh, K; Narang, A; Singhi, S; Batra, R; Rao, KLN; Ray, P; Gopalan, S; Das, S; Gupta, V; Gupta, AK; Bose, SM and McNeil, MM (2001). Outbreak of *Pichia anomala* infection in the pediatric service of a tertiary-care center in Northern India. J. Clin. Microbiol., 39: 1702-1706.

Chee, HY and Lee, KB (2005). Isolation of *Cryptococcus neoformans* var. grubii (serotype A) from pigeon droppings in Seoul, Korea. J. Microbiol., 43: 469-472.

Chen, SCA; Marriott, D; Playford, EG; Nguyenb, Q; Ellis, D; Meyer, W; Sorrella, TC and Slavin, M (2009). Candidaemia with uncommon *Candida* species: predisposing factors, outcome, antifungal susceptibility, and implications for management. Clin. Microbiol. Infec., 15: 662-669.

Costa, AK; Sidrim, JJC; Cordeiro, RA; Brilhante, RSN; Monteiro, AJ and Rocha, MFG (2010). Urban pigeons (*Columba livia*) as a potential source of pathogenic yeasts: a focus on antifungal susceptibility of *Cryptococcus* strains in Northeast Brazil. Mycopathologia. 169: 207-213.

Deem, SL (2003). Fungal diseases of birds of prey. Vet. Clin. North Am. Exot. Anim. Pract., 6: 363-376.

Ghiasian, SA; Maghsood, AH and Mirhendi, SH (2006). Disseminated, fatal *Trichosporon asahii* infection in a bone marrow transplant recipient. J. Microbiol. Immunol. Infect., 39: 426.

Haag-Wackernagel, D and Moch, H (2004). Health hazards posed by feral pigeons J. Infect., 48: 307-313.

Harrigan, WF (1998). *Laboratory methods in food microbiology*. London, UK, Gulf Professional Publishing.

Jadhav, VJ and Pal, M (2013). Human and domestic animal infections caused by *Candida albicans*. J. Mycopathol. Res., 51: 243-249.

Kaore, NM; Atul, AR; Khan, MZ and Ramnani, VK (2012). A rare case of human mycosis by *Rhizoctonia solani*. Indian J. Med. Microbiol., 30: 361-363.

Karnik, K; Reichle, JK; Fischetti, AJ and Goggin, JM (2009). Computed tomographic findings of fungal rhinitis and sinusitis in cats. Vet. Radiol. Ultrasound. 50: 65-68.

Kaygusuz, I; Mulazimoglu, L; Cerikcioglu, N; Toprak, A; Oktay, A and Korten, V (2003). An unusual native tricuspid valve endocarditis caused by *Candida colliculosa*. Clin. Microbiol. Infec., 9: 319-322.

Khosravi, AR (1997). Isolation of *Cryptococcus neoformans* from pigeon (*Columba livia*) droppings in northern Iran. Mycopathologia. 139: 93-95.

Lanzafame, M; De Checchi, G; Parinello, A and Cattelan, MTAM (2001). *Rhodotorula glutinis*-related meningitis. J. Clin. Microbiol., 39: 410.

Lee, WD; Fong, JJ; Eimes, JA and Lim, YW (2017). Diversity and abundance of human-pathogenic fungi associated with pigeon faeces in urban environments. Mol. Ecol., 26: 4574-4585.

Makimura, K; Murayama, SY and Yamaguchi, H (1994). Detection of a wide range of medically important fungi by the polymerase chain reaction. J. Med. Microbiol., 40: 358-364.

Mancianti, F; Nardoni, S and Ceccherelli, R (2002). Occurrence of yeasts in psittacines droppings from captive birds in Italy. Mycopathologia. 153: 121.

Medina, IR; Fuentes, LR; Arteaga, MB; Valcárcel, FR; Arbelo, FA; Castillo, DP; Suárez, SD; Quintana, OF; Gutiérrez, BV; Sergent, FS and Acosta-Hernández, B (2017). Pigeons and their droppings as reservoirs of *Candida* and other zoonotic yeasts. Rev. Iberoam. Micol., 34: 211-214.

Miceli, MH; Díaz, JA and Lee, SA (2011). Emerging opportunistic yeast infections. Lancet Infect. Dis., 11: 142-151.

Munoz, P; Bouza, E; Cuenca-Estrella, M; Eiros, JM; Jesús Pérez, M; Sánchez-Somolinos, M; Rincón, C; Hortal, J and Peláez, T (2005). Saccharomyces cerevisiae fungemia: an emerging infectious disease. Clin. Infect. Dis., 40: 1625-1634.

Murphy, N; Damjanovic, V; Hart, CA; Buchanan, CR; Whitaker, R and Cooke, RWI (1986). Infection and colonisation of neonates by *Hansenula anomala*. Lancet. 327: 291-293.

Nweze, EI; Kechia, FA; Dibua, UE; Charles, EZE and Uwakwe, SONOJA (2015). Isolation of *Cryptococcus neoformans* from environmental samples collected in Southeastern Nigeria. Rev. Inst. Med. Trop. S. Paulo. 57: 295-298.

Pakshir, K; Fakhim, H; Vaezi, A; Meise, JF; Mahmoodi, M; Zomorodian, K; Javidnia, J; Ansari, S; Hagen, F and Badali, H (2018). Molecular epidemiology of environmental *Cryptococcus* species isolates based on amplified fragment length polymorphism. J. Mycol. Med., 28: 599-605.

Radosavljevic, M; Koenig, H; Letscher-Bru, V; Waller, J; Maloisel, F; Lioure, B and Herbrecht, R (1999). *Candida* catenulata fungemia in a cancer patient. J. Clin. Microbiol., 37: 475-477.

Rastogi, G and Sani, RK (2011). Molecular techniques to assess microbial community structure, function, and dynamics in the environment. In: Ahmad, I; Ahmad, F and Pichtel, J (Eds.), *Microbes and microbial technology*. (1st Edn.), New York, Springer-Verlag. PP: 29-57.

Rodriguez-Tudela, JL; Diaz-Guerra, TM; Mellado, E; Cano, V; Tapia, C; Perkins, A; Gomez-Lopez, A; Rodero, L and Cuenca-Estrella, M (2005). Susceptibility patterns and molecular identification of *Trichosporon* species. Antimicrob. Agents Chemother., 49: 4026-4034.

Simi, W; Leite-J, DP; Paula, CR; Hoffmann-Santos, HD; Takahara, DT and Hahna, RC (2018). Yeasts and filamentous fungi in psittacidae and birds of prey droppings in midwest region of Brazil: a potential hazard to human health. Braz. J. Biol., 79: 414-422.

Soltani, M; Bayat, M; Hashemi, SJ; Zia, MA and Pestechian, N (2013). Isolation of *Cryptococcus neoformans* and other opportunistic fungi from pigeon droppings. J. Res. Med. Sci., 18: 56.

Tsiodras, S; Kelesidis, T; Kelesidis, I; Bauchinger, U and E. Falagasde, M (2008). Human infections associated with wild birds. J. Infect., 56: 83-98.

Wirth, F and Goldani, LZ (2012). Epidemiology of *Rhodotorula*: an emerging pathogen. Interdiscip. Perspect. Infect. Dis., 2012: 465717.