Multidrug resistant yeasts in synanthropic wild birds

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Abstract

Background: The aim of this study was to investigate the presence of multidrug resistant yeasts in the faeces of synanthropic wild birds from the Bangsar suburb of Kuala Lumpur.

Methods: Species characterisations of yeast isolates and determinations of antimycotic susceptibility profiles were undertaken using the commercial characterization kit, Integral System Yeasts Plus (Liofilchem, Italy).

Results: Fourteen species of yeasts were detected in the bird faecal samples. Candida albicans was present in 28.89% of bird faecal samples, Candida krusei (13.33%), Candida tropicalis (4.44%), Candida parapsilosis (2.22%), Candida lumbica (2.22%), Candida stellatoidea (2.22%), Candida rugosa (2.22%) and Candida lusitaniae (2.22%). Amongst the non-candidal yeast isolates, Cryptococcus laurentii was present in 6.67% of bird faecal samples, Cryptococcus uniguttulatus (4.44%), Saccharomyces cerevisiae (4.44%), Trichosporon pullulans (2.22%), Trichosporon pullulans/Cryptococcus albidus (8.89%) and Rhodotorula rubra/Rhodotorula glutinis (4.44%). Of the isolated yeasts, 18.1% (or 26/144) were found to be resistant to all 11 antimycotic agents they were tested against i.e. Nystatin, Amphotericin B, Flucytosine, Econazole, Ketoconazole, Clotrimazole, Miconazole, Itraconazole, Voriconazole, Fluconazole 16 and Fluconazole 64. 45.8% (or 66/144) of the bird faecal yeast isolates were resistant to four or more of the 11 antimycotic agents they were tested against.

Conclusions: This finding is of public health significance as these synanthropic wild birds may be reservoirs for transmission of drug resistant yeast infections to humans.

Background

Wild birds that inhabit urban regions have long been known to harbour human pathogens with zoonotic potential. Amongst reported cases are Listeria monocytogenes in wild birds around Helsinki [1], Chlamydophila psittaci in pigeons and other free-living species in Zagreb [2], Campylobacter spp. in ducks in Washington [3], Salmonella spp. in gulls in the Czech Republic [4], pathogenic Escherichia coli, Enterobacter cloacae, Salmonella spp., Aeromonas hydrophilia and Providencia alcalifaciens in Canada geese found in London parks [5], Cryptosporidium spp. and Giardia spp. in Canada geese in Maryland [6], Eimeria magna (3%), Eimeria hermania (14%), Eimeria truncata (2%) and Tyzzeria parvula in Canada geese in Ontario [7], Histoplasma capsulatum in chickens, pigeons, starlings, blackbirds and bats [8] and West Nile Virus in urban birds in Georgia [9]. In Malaysia, a recent study done by Hwee Yong et al [10] showed the presence of the Enterobacteriaceae and protozoan parasites in the stool of the large billed Crow (Corvus spp.) in Kuala Lumpur. Perhaps the best known example occurring globally is the current situation with Highly Pathogenic Avian Influenza Virus (HPAIV) in poultry and migratory wild birds [11]. As such, although the presence of these birds, upon occasion, is aesthetically pleasing in parks and recreational bodies of water, and without doubt, they do function as pollinators within cities, large numbers when present, particularly in the vicinity of high risk areas such as hospitals, are a potential health hazard.

This study focuses on the Bangsar area, an affluent residential suburb of Kuala Lumpur, the capital city of Malaysia. It is heavily populated and endowed with numerous open air restaurants and road side food stalls. Many of the sampling sites chosen for this study were

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in close proximity to these eateries and had the potential to become contaminated with pathogens that were harboured in bird faeces that littered the surrounding areas. Bangsar also boasts one major hospital, clinics and other nearby hospitals.

**Methods**

**Sample collection**

A total of 45 wild bird faecal samples were collected from 12 designated sampling sites from the Bangsar suburb of Kuala Lumpur. Collection of bird faecal samples from these sites was randomised. Fresh faecal samples were collected at two week intervals at 7 pm when the wild birds came to roost for the night. Faecal samples were loaded into eppendorf tubes containing sterile Sabouraud’s Dextrose Broth supplemented with 0.5 g/L chloramphenicol (SDB-chmp) to inhibit bacterial growth. Samples were stored in a cooler box and transported to the research laboratory immediately. Upon arrival, samples were weighed, vortexed briefly, then stored at 4°C before processing.

**Viability Assay**

Cold stored samples were vortexed and plated onto Sabouraud’s Dextrose Agar plates supplemented with 0.5 g/L chloramphenicol (SDA-chmp). Immediately after plating, plates were incubated at 37°C in the dark for five days. On the sixth day of incubation, viable counts were taken. Counts were expressed as x CFU per milliliter fresh weight faeces.

**Species characterizations and determinations of antimycotic susceptibility profiles**

From each of the 45 faecal samples, seven isolates were randomly selected from the SDA-chmp viability assay plates and subcultured twice for purification. Species characterisations of these isolates and determinations of antimycotic susceptibility profiles were undertaken using the commercial characterization kit, Integral System Yeasts Plus (Liofilchem, Italy).

**Results**

Fourteen species of yeasts were detected (Additional File 1). Candida albicans was present in 28.89% of bird faecal samples, Candida krusei (13.33%), Candida tropicalis (4.44%), Candida glabrata (4.44%), Candida parapsilosis (2.22%), Candida lambica (2.22%), Candida stellatoidea (2.22%), Candida rugosa (2.22%) and Candida lusitaniae (2.22%). Amongst the non-candidal yeast isolates, Cryptococcus laurentii was present in 6.67% of bird faecal samples, Cryptococcus uniguttulatus (4.44%), Saccharomyces cerevisiae (4.44%), Trichosporon pullulans (2.22%), Trichosporon pullulans/Cryptococcus albidus (8.89%) and Rhodotorula rubra/Rhodotorula glutinis (4.44%).

A significant proportion of the yeasts isolated from the bird faecal samples were found to be drug resistant or multidrug resistant (Additional Files 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16). 18.1% (or 26/144) were found to be resistant to all 11 of the antimycotic agents they were tested against. 45.8% (or 66/144) of the bird faecal yeast isolates were resistant to four or more of the 11 antimycotic agents they were tested against (Table 1).

The following proportions of isolates demonstrated resistance to four or more antimycotic agents: 13.2% (or 7/53) of the C. albicans isolates, 33.3% (or 1/3) of the C. glabrata isolates, 85.7% (or 6/7) of the C. krusei isolates, 66.7% (or 2/3) of the C. lusitaniae isolates, all (or 2/2)
of the *C. tropicalis* isolates and all (or 1/1) of the *C. stel- 

datoidea* isolates. Of the noncandidal isolates, 100% (or 

8/8) of the Cryptococcus laurentii isolates, 100% (or 2/2) 

of the *T. pullulans* isolates, 87.5% (or 7/8) of the 

*T. pullulans/C. albicans* isolates, 40% (or 2/5) of the 

*R. rubra/R. glutinis* isolates and 69.2% (or 27/39) of 

the unidentified yeast isolates were resistant to four or 

more antimycotic agents.

**Discussion**

The findings of this study indicate the presence of mul-

tidrug resistant yeasts in the faeces of synanthropic wild 

birds in Bangsar. Furthermore, many of the species of 

yeasts detected are documented human pathogens with 

zoonotic potential [12].

28.89% of the bird faecal samples harboured *C. albici-

cans* and this was the most prevalent species of yeast in 

the faecal samples. European surveys indicate that this 

species is responsible for more than half the cases of 

invasive candidaemia; however, the occurrence of non-

albicans related disease appears to be increasing [13]. 

During the past decade there has been an increasing 

trend of systemic and fatal infections with non albicans 

species such as *Candida tropicalis, Candida glabrata, 

Candida parapsilosis, Candida lusitaniae* and *Candida 

lipolytica*. Identification of these species is essential for 

effective therapy in view of the emergence of resistance 

to antifungal drugs in these species [14]. *Candida parap-

silosis* is a major emerging human pathogen that has 

dramatically increased in significance and prevalence 

over the past two decades, and is now one of the leading 

causes of invasive candidal disease. Individuals at the 

highest risk for severe infection include neonates and 

patients in intensive care units [15].

Non-neoformans cryptococci have traditionally been 

thought of as saprophytes but the incidence of human 

infection with these species has increased with *Crypto-

coccus laurentii* and *Cryptococcus albidus* together 

responsible for 80% of non-neoformans reported cases 

[16]. These species are emerging fungal pathogens to be 

reckoned with. All eight *C. laurentii* isolates obtained 

from this study were resistant to all 11 of the antymyc-

otic agents they were tested against. *C. laurentii* has 

been reported as a rare cause of CAPD associated peritonitis, 

pulmonary and cutaneous infections [17], fungaemia in 

a premature neonate [18] and invasive disease in a nine-

year-old boy with X-linked hyper-immunoglobulin M 

syndrome [19]. *C. albidus* has been reported to cause 

cutaneous infections [20], eye and blood infections in 

HIV patients [21], pulmonary infection and fungaemia in 

a leukaemia patient [22] and mucormycosis empyema in 

a haemodialysis patient [23].

It has been reported that antifungal primary prophyl-

axis with either itraconazole or fluconazole is effective 

in reducing the incidence of cryptococcal disease in 

adults with advanced HIV disease [24]. The results of 

this study are of concern as the *C. laurentii* isolates 

obtained are resistant to both itraconazole, fluconazole 

and all the other antymycotic agents they were tested 

against.

*Rhodotorula rubra* has been implicated in patients suf-

fering from meningitis and keratitis, albeit in the debili-

tated and the immunocompromised [25,26]. *Rhodotorula glutinis* on the other hand has been 

reported to cause meningitis in an immunocompetent 

patient [27]. *Trichosporon pullulans* has been reported 

to have caused pulmonary infection in a leukaemia 

patient [28], infections in a renal transplant patient [29] 

and in two patients with chronic granulomatous disease 

[30], amongst others.

Perhaps what may also be significant are the isolates 

that have not been identified as they were beyond the 

resolving power of the commercial identification kit 

used. 69.2% of them were resistant to four or more anti-

mycotic agents. Some of these isolates may be human 

pathogens or may have the potential of being human 

pathogens.

Nosocomial fungal infections are seen to be increas-

ingly significant amongst the critically ill. Fungi are now 

amongst the most frequently isolated organisms in 

intensive care units. Reports describe potentially fatal 

fungal infections that are resistant to many commonly 

used antifungal agents [31], reminiscent of the drug 

resistant and multidrug resistant yeasts isolated in this 

study. It is therefore important to recognize and mini-

mize the major risk factors associated with infection, 

including the existence of synanthropic animal reser-

voirs. This study supports previous reports that describe 

the potential of synanthropic birds as carriers, reservoirs 

and disseminators of pathogens.

In the Bangsar area, the predominant species of bird 

are wild crows. Not being of the domesticated variety, 

these birds would not have encountered antifungal che-

motherapy. As such, the drug resistant yeasts obtained 

in this study are either originally human strains that 

evolved resistance during human therapy with antifun-

gals and subsequently were disseminated to these wild 

birds, or alternatively, different mechanisms of antifun-

gal resistance development are at work. Possibly, poly-

specific transporters, if they exist in these drug resistant 

yeasts, may have evolved in response to toxins/poisons 

that are commonly encountered by the birds that har-

bour these yeasts, either in the wild or in the cities 

where they frequently dwell. As such, it would be of 

epidemiological interest to determine whether (1) sylva-

tic crows living in proximity to Bangsar harbour simi-

larly drug resistant strains of yeasts, and (2) whether the 

drug resistant yeast strains obtained in this study are
the same as that occurring in hospitals and birds associated with hospitals in the vicinity of the study site.

While mass culling and disposal is the well prescribed method of dealing with contagion in domesticated and wild animals, it is expensive, labour-intensive and does not in any way address the root causes of the problem. As such, it is not a long-term solution and when applied, often does not prevent recurrence of the problem. If colonization with these yeasts is directly associated with the immune status of the birds, then the root of the problem lies in the factors that lower bird vitality. The latter include ecological changes that result from urbanization and growth of megacities, and the lack of or breakdown in sanitation that often ensues. Chemical pollution, noise pollution and climate change as a result of shifting temperatures and precipitation levels, are yet other potential contributory factors to colonization and the emergence of disease, by way of modifying both bird and microbial behaviour.

Conclusions
This study demonstrates that synanthropic wild birds in the Bangsar area harbour multidrug resistant Cryptococcus laurentii and other drug resistant and multidrug resistant yeasts. Both the candidal and noncandidal yeast species isolated have been associated with opportunistic and disseminated infections in man, particularly in those with some form of underlying immune suppression, including those on high-dose corticosteroid therapy, having neutropenia or human immunodeficiency virus (HIV) infection [32-35]. In view of the burgeoning immunosuppressed populations both in Malaysia and globally [36,37], the existence and evolution of drug resistant and multidrug resistant strains of yeasts in synanthropic birds that commonly inhabit urban regions, is a source of public health concern.

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Authors’ contributions
Authors’ contributions are in the order of listed names. ATKL participated in the study design, carried out the bench work and conducted an extensive review. KM and SS participated in the study design and contributed to writing of the manuscript. SA contributed ideas and his broad experience in the field of environmental health. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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References
1. Hellstrom S, Kiviniemi K, Auto T, Korkeala H: Listeria monocytogenes is common in wild birds in Helsinki region and genotypes are frequently similar with those found along the food chain. J Appl Microbiol 2008, 104(3):883-888.
2. Prukner-Radošević E, Horvatek D, Gottstein Z, Ciglar I, Grozdanić, Mazić J: Epidemiological investigation of Chaemydophila psittaci in pigeons and free-living birds in Croatia. Vet Res Commun 2003, 29(Supplement 1).
3. Pacha RE, Clark GW, Williams EA, Carter AH: Migratory birds of central Washington as reservoirs of Campylobacter jejuni. Can J Microbiol 1988, 34:80-82.
4. Cizek A, Leterak J, Hejilcik K, Treml F, Smola J: Salmonella contamination of the environment and its incidence in wild birds. J Vet Med 1994, 41(5):320-327.
5. Feare CJ, Sanders MF, Blasco R, Bishop JD: Canada goose (Branta canadensis) droppings as a potential source of pathogenic bacteria. J Royal Soc Health 1999, 119(3):146-155.
6. Graczyk TK, Fayer R, Trout JM, Lewis EJ, Farley CA, Sulaiman I, Lal AA: Giardia sp. cysts and infectious Cryptosporidium parvum oocysts in the feces of migratory Canada geese (Branta canadensis). Applied and Environmental Microbiology 1996, 647(7):2757-2758.
7. Skene RC, Remmler O, Fernando MA: Coccidia of Canada Geese (Branta canadensis) at Kortright Waterfowl Park, Guelph, Ontario, Canada, with description of Isospora anseris n. sp., J Vet Zool 1981, 59:493-497.
8. Canadian Centre for Occupational Health & Safety: Histoplasmosis. [http://www.oscahcanada/oschanswers/diseases/histopla.html].
9. Willis J: Wild bird mortality and West Nile Virus surveillance: biases associated with detection, reporting and carcass persistence. J Wildlife Dis 2006, 42(1):92-106.
10. Lee HY, Ambu S, Devi S, Maung M: Detection of protozoan and bacterial pathogens of public health importance in faeces of Corvus spp. [large-billed crow]. Trop Biomed 2008, 25(2):134-139.

11. World Health Organisation: H5N1 Avian Influenza: Timeline of major events. [http://www.who.int/csr/disease/avian_influenza/timeline090727.pdf].

12. Weber W: Pigeon Associated People Diseases. [http://www.avianweb.com/pigeonassociateddiseases.html].

13. Lass-Floir C: The changing face of epidemiology of invasive fungal disease in Europe. Mycoses 2009, 52(3):197-205.

14. Ng KP, Sav TL, Na SL, Hoo TS: Systemic Candida infection in University Hospital 1997-1999: the distribution of Candida biotypes and antifungal susceptibility patterns. Mycopathologia 2001, 149:141-146.

15. Trofa D, Gácker A, Nasonchuk JD: Candida parapsilosis, an emerging fungal pathogen. Clin Microbiol Rev 2008, 21(4):606-25.

16. Khawcharoenporn T, Apisarnthanarak A, Mundy LM: Non-neoformans cryptococcal infections: a systematic review. Infection 2007, 35(2):51-53.

17. Mycology Online: Cryptococcus laurentii.[http://www.mycology.adelaide.edu.au/Fungal_Descriptions/Yeasts/Cryptococcus/C_laurentii.html].

18. Cheng MF, Chiou CC, Liu YC, Wang HZ, Hsieh KS: Cryptococcus laurentii fungemia in a premature neonate. J Clin Microbiol 2001, 39(4):1608-1611 [http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=87860].

19. Simon G, Erdös M, Maródi L: Invasive Cryptococcus laurentii disease in a nine-year-old boy with X-linked hyper-immunoglobulin M syndrome. Pediatr Infect Dis J 2005, 24(10):935-7.

20. Narayan S, Batta K, Colloby P, Tan CY: Cutaneous Cryptococcus infection due to C. albicans associated with Sezary syndrome. Br J Dermatol 2000, 143:612-4.

21. Garellick JM, Rhodabakhsh AJ, Lopez Y, Barmi M, Lister M: Scleral ulceration caused by Cryptococcus albicans in a patient with acquired immune deficiency syndrome. Cornea 2004, 23:790-1.

22. Wells GM, Gajjar A, Pearson TA, Hale KL, Shene JLP: Brief report: Pulmonary cryptosporidiosis and Cryptococcus albicans fungemia in a child with acute lymphocytic leukemia. Med Pediatr Oncol 1998, 31:544-6.

23. Horowitz ID, Blumberg EA, Kvedar L: Cryptococcus albicans and mucormycosis empymema in a patient receiving hemodialysis. South Med J 1993, 86:1070-2.

24. Chang LW, Phipps WT, Kennedy GE, Rutherford GW: Antifungal interventions for the primary prevention of cryptococcal disease in adults with HIV. Cochrane Database Syst Rev 2005, 3:CD004773.

25. Elias ML, Soliman AK, Mahoney FJ, Karam El-Din AZ, El-Kebbi RA, Ismail TF, Wasfy MM, Mansour AM, Sultan YA, Pimentel G, Earhart KC: Isolation of Cryptococcus, Candida, Aspergillus, Rhodotorula and Nocardia from meningitis patients in Egypt. J Egypt Public Health Assoc 2009, 84(1-2):169-81.

26. Thakur K, Singh G, Agarwal S, Rani L: Meningitis caused by Rhodotorula rubra in an human immunodeficiency virus infected patient. Indian J Med Microbiol 2007, 25(2):166-8.

27. Lanzaferme M, Cechini G, Patinello A, Treverzoli M, Cartelan AM: Rhodotorula glutinis-Related Meningitis. J Clin Microbiol 2001, 39(1):410.

28. Shigehara K, Takahashi K, Tsunematsu K, Koba H, Katoh S, Asakawa M, Suzuki A: A Case of Trichosporon pullulans Infection of the Lung with Adult T-cell Leukemia. Japanese Journal of Medicine 1991, 30(2):135-137.

29. Hughes CE, Senstock D, Wilson BD, Payne W: Infection with Trichosporon pullulans. Annals Of Internal Medicine 1969, 108(3):722-723.

30. Holland S, Shea Y, Kwon-Chung JI: Trichosporon pullulans infection in 2 patients with chronic granulomatous disease. Journal of Allergy and Clinical Immunology 2004, 114(1):205-206.

31. Toscano CM, Jarvis WR: Clinical Updates: National Foundation For Infectious Diseases: Epidemiology and Clinical Aspects of Unusual Fungal Nosocomial Infections.[http://www.nfid.org/publications/fungal_archive/nosof.html].

32. Hidalgo JA: Candidiasis.[http://emedicine.medscape.com/article/213853-overview].

33. Maves RC: Trichosporon Infections.[http://emedicine.medscape.com/article/230705-overview].

34. Rhodotorula spp. [http://www.doctorfungus.org/thefungi/rhodotorula.htm].

35. Ventriculitis Due to Cryptococcus uniguttulatus: Discussion. [http://www.medcapeview.com/viewarticle/410704_3].

36. Chong PP, Ching-Soo DC, Lee YL, Hafeez A, Nor Shamsudin M, Heng FS, Ng KP: Recurrent candidaemia in a neonate with Hirschsprung’s disease: flucanazole resistance and genetic relatedness of eight Candida tropicalis isolates. J Med Microbiol 2006, 55:423-428.

37. Nguyen MH, Peacock JE, Morris AJ, Tanner DC, Nguyen ML, Snyderman DR, Wagener MM, Rinaldi MG, Yu VL: The changing face of candidemia: emergence of non-Candida albicans and antifungal resistance. Am J Med 1996, 100:617-623.