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The Risk Of Several Fungi Associated With Bird Waste

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ABSTRACT: In Babylon province and rounded cities , samples of dried waste were obtained and studied for the presence of fungi of potentially pathogenic nature. There was a high proportion of Candida spp , Cryptococcus neoformans and Rhodotorula mucilaginosa and other fungi obtained from the dry droppings. The aim of the present study was to survey filamentous fungi and yeasts associated with dropping of domestic birds in the Babylon province and rounded cities. During 2013–2014, 191samples of dropping west were collected cultured and examined by dilution plate method .Filamentous fungi and yeasts colonies growths appearing on the Sabouraud's Dextrose Agar (SDA)medium were microscopically examined and the cultures obtained were identified on the basis of their microscopic and colonial morphology. A total of 2506 fungal isolates (included 2104 yeast isolates and 402 filamentous isolates were collected, Candida spp. constituted the majority (38.22 %of the 2104 isolates, represented by mainly C.albicans and C.Krusei, Geotrichum spp. Blastomycetes spp., Macrorhabdus and Cryptococcus. These yeasts had proteolytic activities .conclusion indicated that Blastomycetes spp. Macrorhabdus and Cryptococcus were recorded for the first time associated with bird waste in Iraq. Cryptococcus had Phospholipase activities .waste of birds is a finding be sources of risk on the public health.

Keywords: Bird ,dropping ,filamentous fungi ,yeasts, Phospholipase , .Iraq.

1.Introduction

Large populations of roosting birds may present a disease sources, the most serious health risks from contact or inhaling of the fungal spores from disease organisms that grow in the nutrient-rich accumulations of bird droppings and dispersal , feathers and debris under a roost the association between dried droppings and the isolation of fungi was first described by Emmons (1955). Birds and their droppings can carry over 60 other diseases many of them are airborne and can be transferred to humans just by being around droppings(Littman and Borok, 1968; Zarrin et al., 2010). Most of early studies on the fungi associated with bird dropping were performed before 1970s .These studies repotted that yeast has been isolated from pigeon droppings in London by Randhawa, Clayton, and Riddell (1965) and by Partridge and Winner (1965). The relationship of *H. capsulatum* to avian habits has been reviewed by Ajello (1964).and the organism has been reported in droppings from chickens, pigeons, and bats (Lehan and Furcolow, 1957; Stoker, 1964).
Most Candidiasis and Cryptococcosis infections are mild and occur without symptoms(Lazera et al., 1996). Diffuse pulmonary infection is often asymptomatic and unrecognized. Persons with weakened immune systems, however, are more susceptible to symptomatic infection. (Ellis *et al*., 1990,).Fungal flora was representative the possible sources of infection to man (Mwaba *et al*, 2001) .

Although the possibility of their droppings having been collected is small,Feathers and hairs are a rich source of keratin and are regarded as one of the reservoirs of keratin-degrading microorganisms. Birds carry keratinophiilic fungi passively through the intact feather (Mandeel and

recovery of keratinophilic fungi with the addition of hair-bait. Hence the latter technique was employed for all the samples of feace. The plates were incubated at 30°C . filamentous fungi and yeasts were identified based on Wattab 2002 .CHROMagar candida was used as confirmatory tests for *Candida* spp.(Nadeem et al 2010) Yeast were cultured on egg yolk agar plates for detection Phospholipase activities .

2.2.Isolation and identification of dropping fungi

Loop full of dropping suspension transferred to plates of Sabouraud dextrose agar (Hi Media, Mumbai, India) supplemented with chloramphenicol (Falcon Chemox Pvt, Ltd, Ghaziabad, India) at a concentration of 0.05 mg/ml,. The cultures were microscopically examined to check for purity and sub-cultured to get pure cultures. The purified fungal isolates were identified up to species level as far as possible by a detailed study of their colonial characters and microscopic morphology in lacto-phenol blue mounts, and comparison of their characteristics with the descriptions of the species in the standard books and manuals (10–12). The study was approved by the local Ethics Committee.

Frequency %= No. of observation in which colony appear / total number of observation recorded x100(Adhikari et al.,2004)

Occurrence %= No. of fungus in which sample appear / total number of samples x100(Maria and Sridhar,2003)

2.3.Determination of phospholipase activity

The phospholipase activity of *C.albicans* and *Cryptococcus* was detected by the method of (Samaranayake *et al*, 1984) .Approximately 5 µL of standard inoculum of test strain containing 10^8 Candida cells/mL was aseptically inoculated onto egg yolk agar. The plates were dried at room temperature and then incubated at 37°C for 48 h.

Mancianti 2001). Some of these fungi are species of non-dermatophytes or dermatophytes known to cause cutaneous infections of keratinized tissues, *viz*skin, feather, hair and nail (dermatophytoses) of humans and animals (Efuntoye and Fashanu ,2002). several workers have investigated the occurrence of Candidiasis fungi , Coccidiodes and other fungi on a wide variety of birds in different countries as in Euripi, Africa ,Austere Filiphich and Parker, 1993 ;Multu *et al.*, 1997; Schulze and Heidrich 2001; Hanka *et al*., 2010 ;Martins *et al*., 2006 ; Phalen *et al*., 2007;; Jansson *et al.*, 2008;;. Unfortunately, no previous studies about fungi associated with birds dropping were performed in Iraq. The aim of the present study was to survey filamentous fungi and yeast associated with dropping of domestic birds in the Babylon province and rounded cities.

2. Material and Methods

2.1.Collection and processing of samples of bird dropping

Samples collected from around Egg chickens, Meat chickens fields and cages of domestic birds . The dropping west were collected 8 different species of birds: Egg chickens, Meat chickens, *Sirnus leucopygius, Sernius canaria , Melopsittacus undulates, Nymphicus hollandicus, Aggpornis* spp. and Pigeon. Throughout period extended from October 2013-April2014 dry droppings from Babylon and rounded cities ,191Samples of dropping originated from were collected in plastic pages . 0.5 g of the dropping samples were diluted in sterile water 0.5ml of suspention were powered into Sabouraud's Dextrose Agar medium with 0 05 g/l chloramphenicol. The samples were placed in clean plastic zip bags which were labelled and transported promptly to the laboratory. The plates containing the samples of dropping were incubated at room temperature (22–30 °C) for 5-7 depending on the rate of growth, and periodically moistened with small quantities of SDW. Initially some samples of feathers were processed on soil plates. A comparison of the results showed better

Rhizomucor	**20**	**28**	**0.25**	**1.11**
Cladosporium	**1**	**4**	**10.471**	**0.2**
Aspergillus flavus	**8**	**10**	**4.18**	**0.4**
A. terreus	**7**	**11**	**3.66**	**0.43**
A.fumigatus	**10**	**53**	**5.23**	**2.11**
A.niger	**29**	**39**	**15.18**	**1.6**
A.candidus	**5**	**9**	**2.61**	**0.35**
***Cryptococcus* sp.**	**5**	**5**	**2.617**	**0.2**
***Candida* spp.**	**73**	**710**	**38.2**	**28.32**
Macrorhabdus	**8**	**900**	**4.189**	**35.9**
Blastomycetes	**2**	**2**	**1.04**	**0.079**
Geotrichum	**17**	**497**	**8.90**	**19.82**
المجموع	**191**	**2506**		**~=100 %**

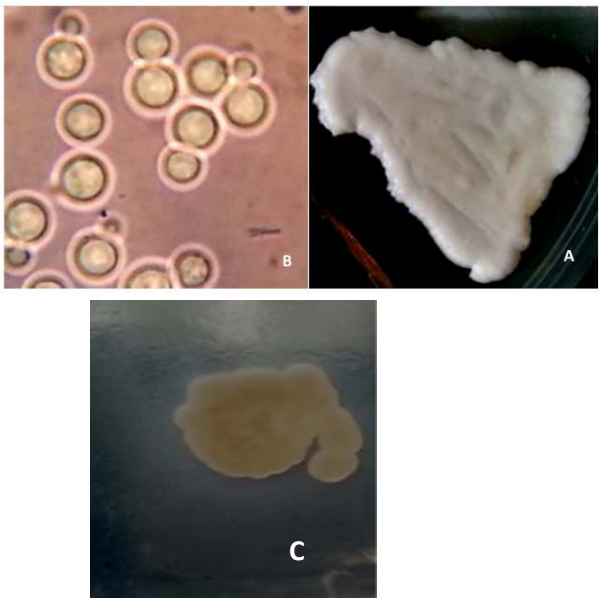

Figure 2:*Cryptococcus* sp isolated from bird dropping A-colony,B-Capsule of yeast,C-Phospolipase production.

Other species of fungi recovered from waste in the present study included 92 isolates

The plates were examined for the presence of precipitation zone around the colony. The presence of precipitation zone indicated expression of phospholipase enzyme.

3.Results :

Out of 191samples of dropping waste of 8 species of birds examined, 2506 isolates of filamentous fungi and yeasts were recovered. The distribution of the isolates according to the species of birds investigated is more with Egg chickens, Meat chickens, , *Sernius canaria* and *Sirnus leucopygius*. *Aspergillus* spp., a well-known genus of filamentous fungi, constituted the majority (4.5%) followed by *Penicillium* spp (2.9%)of the 2506 isolates. Regarding the relative distribution of different species of filamentous fungi, . *Macrorhabdu* was the commonest species (35.9%) followed by *Geotrichum* and *Candida* spp (19.82,28.3%) respectvelly (Table 1).*Candida* spp showed different colors on CHROMagar(Figure 1). and *Cryptococcus* were showed phospholipase activity (Figure 2).

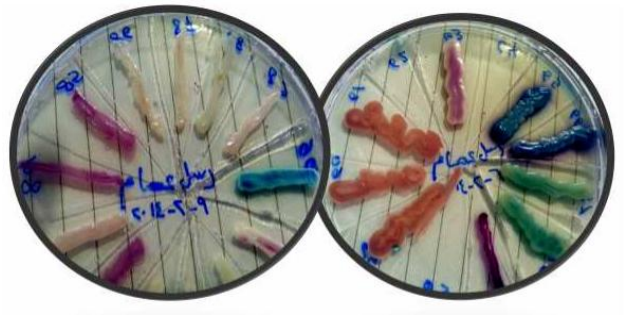

Figure 1:Colonies color of *Candida* spp and *Cryptococcus* sp isolated from bird dropping on CHROMagar.

Table1:Fungi associated with bird dropping

Fungi	Samples No.	Colonies No.	% Occurrence	% frequency
Rhizopus	**44**	**92**	**23.036**	**3.66**
Penicilliu m	**17**	**72**	**8.90**	**2.87**
Fusarium	**2**	**2**	**1.047**	**0.1**
Mucor	**31**	**82**	**16.23**	**3.27**

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of *Rhizopus*,(28) *Rhizomucor* (2),*Fusarium* and five isolates of *Cryptococcus* sp., Table1.

4.DISCUSSION

The present report on the occurrence of yeasts and filamentous fungi on dropping of birds in Babylon province and rounded cities constitutes the first study of its kind in Iraq. The predominance of *Geotrichum* spp ,*Candida* spp.is in agreement with that reported in surveys of bird dropping associated yeasts and filamentous fungi in several other countries (Filiphich and Parker, 1993 ;Multu *et al.*, 1997; Schulze and Heidrich 2001;; Phalen *et al*., 2007;Martins *et al*., 2006; Jansson *et al.*, 2008; Hanka *et al.*,2010). *Macrorhabdus. Geotrichum* and *C.albicans* were the most frequent species, followed by mucorales fungi, these results coincidence with Rad (2013). Phospholipases facilitate the invasion of the host mucosal epithelia by hydrolyzing one or more ester linkages in glycerophospholipids Singhai *et al.* ,2012 . In our study phospholipase was the major virulent factor expressed by *Cryptococcus* isolates . Screening of phospholipase production in *Cryptococcus* isolates can be used as an important parameter to differentiate invasive strains from noninvasive colonizers. our conclusion referred to presence of many of pathogenic and opportunistic filamentous and yeasts representative sources of risk fungal disease on the human health.

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