ZANCO Journal of Pure and Applied Sciences
The official scientific journal of Salahaddin University-Erbil
https://zancojournals.su.edu.krd/index.php/JPAS

**ISSN (print ):**2218-0230**, ISSN (online):** 2412-3986**, DOI: http://dx.doi.org/10.21271/zjpas**

RESEARCH PAPER

# Isolation and identification of some pathogenic yeast from pigeon droppings in Erbil city-Kurdistan region-lraq

Neveen N. Rajab[1], Nadeem A. Ramadan[2]
[1]Department of Biology, College of Science, Salahaddin University-Erbil, Kurdistan Region, Iraq
[2]Hadbaa University College, Mosul, Iraq

**A B S T R A C T:**

One hundred sixty-four samples of local pigeon droppings were collected in Erbil city and its suburbs from 28 regions, 1161 isolates of yeasts were obtained using Niger bird seed agar while 547 obtained using Sabouraud dextrose agar media respectively.

The yeasts were identified through VITEK 2 system which belongs to 4 genera (Candida, Cryptococcus, Rhodotorula and Trichosporon). Four species belonging to the genus Candida, one species for each Cryptococcus and Rhdotorula, two species for Trichosporon, have been diagnosed on Niger bird seed and on Sabouraud dextrose agar media includes four genera (Candida, Cryptococcus, Rhodotorula and Trichosporon) two species of Candida (C.rugosa and C.ciferrii) and two species of Cryptococcus (C. albidus and C. laurentii), while one species were identified for both Rhodotorula (R. glutinis) and Trichosporon (T. mucoides) respectively.The frequency of Trichosporon spp. was the highest (40.5 %) reaching 694 colonies, followed by Rhodotorula spp. (305) then Cryptococcus spp. (265), and finally Candida spp. (226).

KEY WORDS:
DOI: http://dx.doi.org/10.21271/ZJPAS.35.SpD.22
ZJPAS (2023) , 35(SpD);195-201 .

## 1.INTRODUCTION:

The genus *Cryptococcus* is cosmopolitan and may be an agent of opportunistic mycoses in immunocompromised and sometimes immunocompetent individuals. *Cryptococcus* species are frequently isolated from trees and bird excreta in the environment and infection occurs by inhalation of propagules dispersed in the air (Brito et al., 2019).

The genus *Cryptococcus* involves many species which are capable of resulting in infections in humans and animals such as superficial mycoses, central nervous system mycoses as well as pulmonary diseases. *Cryptococcus neoformans* and *C. gattii* are the major pathogens (Khawcharoenporn, et al., 2007).

*C. albidus* and *C. laurentii* are uncommon species of this genus that in early decades have increasingly caused opportunistic infections in humans, more frequently in immunocompromised patients; the best therapy for such infection is not known (Heitman et al., 2011).

Other *Cryptococcus* species have classically been considered to be non-pathogenic. However, *C. albidus, C. laurentii, C. luteolus, C. uniguttulatus,* have emerged as opportunistic pathogens over the last few years (Pamela et al., 2017). Several factors such as the rise of immune-suppressed patients who are more susceptible to opportunistic fungal infections are behind the increase of infections that are caused by species (Roudbary et al. 2012).

*C. laurentii* infections have also been documented in human beings including dispersed disease with an invasion of the central nervous system (Vlchkova et al., 2004). However, other types of species have also been isolated from environmental sources and rarely from patient

* **Corresponding Author:**
Neveen N. Rajab1
E-mail: neveen.rajab@su.edu.krd
**Article History:**
Received: 13/01/2023
Accepted: 01/09/2023
Published: 01/11 /2023

samples (Brito et al., 2019).

*Cryptococcus* species are pathogenic and non-pathogenic basidiomycete yeasts that are found widely in the environment. Based on phenotypic methods, this genus has many species (Kamari et al.,2017).

The purpose of this research is to verify the presence of Cryptococcus spp. and some pathogenic yeast also to identify their species in the droppings of pigeons from different locations in the city of Erbil and its environs.

**Materials and methods: -**

**A- Sample Collection:**

One hundred and sixty-four samples of pigeon droppings were collected from 28 different regions in Erbil city and its suburbs from November 2021 to October 2022. Samples have been collected and transferred in sterilized and sealed containers into the advanced mycology laboratory at Science College/ Salahaddin University.

**B-Isolation of** Pathogenic **yeast.**

A biosafety cabinet has been used for the purpose of homogenizing and processing all samples. 20 grams of Pigeon (*Columbia livia.)* dropping samples were suspended in sterilized phosphate-buffered saline (PBS) at a ratio of 1:5 by vortexed for 5 minutes and centrifuged at $500^{X}$ g for 5 min, 100 µL of the supernatant from each tube was inoculated onto each Niger Bird Seed Agar **(**NBSA**)** medium and onto Sabouraud dextrose agar (SDA) medium plates containing 1.0 g creatinine and 40 mL chloramphenicol (50 µg/mL) 3 replicates for each medium. The Petri dishes were incubated in the dark at 25 °C in humid conditions for 3~10 days (Ed-berg et al.,1980; Oh, and Hwang, 2005; Sirag et al., 2021).

C-Identification method: -

A sterile swab or applicator stick is used to transfer a sufficient number of colonies of pure culture and to suspend the microorganism in 3.0 mL of sterile saline (aqueous 0.45 % to 0.50 % NaCl, pH 4.5 to 7.0) in a 12 × 75 mm clear plastic (polystyrene) test tube. The turbidity is adjusted accordingly (Table,1) and measured using a turbidity meter (Densi Chek$^{TM}$). Then, the yeasts were identified in a Raparin children's hospital utilizing VITEK2system.

Table-1: Suspension turbidities used for card inoculation

| **Product*** | **McFarland Turbidity rang** |
|---|---|
| GN (Gram-negative fermenting and non-fermentingbacilli) | 0.50-0.63 |
| GP (Gram-positive cocci and non-spore-formingbacilli) | 0.50-0.63 |
| YST (yeasts and yeast-like organisms) | 1.80-2.20 |
| BCL (Gram-positive spore-forming bacilli) | 1.80-2.20 |

***** Pincus, (2006).

**Results** One hundred sixty-four samples of local pigeon excrete were collected in Erbil city and its suburbs from 28 regions, and 1161 isolates of yeasts were obtained using Niger bird seed medium. The yeasts were identified through VITEK 2 system which belongs to 4 genera (*Candida, Cryptococcus, Rhodotorula and Trichosporon).* Six species belonging to the genus *Candida*, one species each to the genus *Cryptococcus and Rhodotoreula*, two species to the *Trichosporon* have been diagnosed (Table 2). The frequency of the isolated species was the highest for *Trichosporon mucoides* in the percentage of 21.5%, then followed by *Candida lusitaniae* with a percentage of 12.9% while only one single isolate belonging to the genus (*Cryptocuccos laurentii*) in three regions (Berkot New, Tayrawa and Taajeel) with a frequency of 0.8, 6.0 and 2.0 %, respectively.For the first time, *Cryptocuccos laurentii* and *Cry. albidus* were isolated from pigeon droppings in Erbil and its suburbs in Iraq.On the other hand, a total of 547 isolates were obtained from Sabouraud dextrose agar media which belong to four genera (*Candida, Cryptococcus, Rhodotorula* and *Trichosporon*) which include two species of *Candida* (*C. rugosa* and *C. ciferrii*) and two species of *Cryptocucoos* (*Cryp. albidus* and *Cryp. laurentii*), while one species was identified for both *Rhodotorula* (*R. glutinis*) and *Trichosporon* (*T. mucoides*) respectively. As shown in (Table 3) the most frequent isolates were *Trichosporon mucoides* (27.4%) in 121Gulan followed by *Cryptocuccus laurentii* (10.9%) in Tayrawa region.

**Table-2:** The identity and quantity of yeasts isolated from pigeon dropping on Niger bird seed agar medium.

| No. | Sample source | Yeast | No. Colonies | % Frequency |
|---|---|---|---|---|
| 1. | Bahar new | *Candida ciferrii* | 5 | 0.4 |
| 2. | Bahar Old | *C.rugosa*<br>*C. albicansRhodotorula*<br>spp. | 20<br>4<br>20 | 1.7<br>0.3<br>1.7 |
| 3. | Berkot New | *C. lusitaniae*<br>*C. albicans*<br>*C. rugosa*<br>*Cryptococcus laurentii*<br>*Trichosporon asahii*<br>*T. mucoides*<br>*Rhodotorula* spp. | 150<br>5<br>28<br>9<br>155<br>100<br>35 | **12.9**<br>0.4<br>2.4<br>**0.8**<br>13.3<br>8.6<br>3.0 |
| 4. | Bnaslawa | *Rhodotorula* spp. | 30 | 2.5 |
| 5. | Badawa | *Rhodotorula* spp. | 10 | 0.8 |
| 6. | Barzan | *Rhodotorula* spp. | 15 | 0.3 |
| 7. | Tayrawa | *Rhodotorula* spp.<br>*Cry. laurentii* | 6<br>70 | 0.5<br>**6.0** |
| 8. | Toraq | *Rhodotorula* spp. | 0 | 0.0 |
| 9. | Chwar chra | - | 0 | 0.0 |
| 10. | Chnar | *Rhodotorula glutinis*<br>*C. rugosa* | 12<br>19 | 1.0<br>1.6 |
| 11. | Daratu | *C. parapsilosis* | 2 | 0.2 |
| 12. | Darwzae Shar | *Rhodotorula glutinis* | 20 | 1.7 |
| 13. | Taajeel | *Rhodotorula* spp.<br>*Cry. laurentii* | 14<br>17 | 1.2<br>**1.5** |
| 14. | Swere gchka | - | 0 | 0.0 |

| | | | | |
|---|---|---|---|---|
| 15. | Saydawa | *C. rugosa* | 0 | 0 |
| 16. | Shawes | *Rhodotorula* spp.<br>*C. albicans*<br>*C. rugosa* | 15<br>6<br>24 | 0.3<br>0.5<br>2.0 |
| 17. | Farmanbaran | *Rhodotorula* spp.<br>*C. albicans*<br>*C. famata* | 17<br>8<br>10 | 1.5<br>0.7<br>0.8 |
| 18. | Qarabu | *T. asahii* | 28 | 3.2 |
| 19. | Karezan | *T. asahii* | 11 | 0.9 |
| 20. | Mhala Arab | *C. ciferrii* | 12 | 1.0 |
| 21. | Science College | - | 0 | 0.0 |
| 22. | Mala Omer | *Rhodotorula* spp. | 6 | 0.5 |
| 23. | Masif Salahddin | *Rhodotorula* spp. | 13 | 1.1 |
| 24. | Zanko | *Rhodotorula* spp. | 5 | 0.4 |
| 25. | Xanaqa | *Rhodotorula* spp. | 6 | 0.5 |
| 26. | Roshenbere | *Rhodotorula* spp. | 4 | 0.3 |
| 27. | Zhean | - | 0 | 0 |
| 28. | 121 Gulan | *T. mucoides* | 250 | **21.5** |
| | **Total** | | 1161 | |

Results of table (2 and 3) show that there were 4 locations in which samples of bird droppings had negative results based on Niger bird seed agar medium, while 11 locations gave no results on Sabouraud dextrose agar medium, and for this reason the first medium is considered in general better in isolating yeasts, but the second medium is better in searching for species of *Cryptococcus*, where the two species *Cryptococcus albidus* and *Cryptococcus laurentii* were isolated using Sabouraud dextrose agar medium from 11 regions, while *Cryptococcus laurentii* was isolated from two regions using Niger bird seed agar medium

Table-3: The identity and quantity of yeasts isolated from pigeon dropping on Sabouraud dextrose agar medium.

| **No.** | **Sample source** | **Yeast** | **No. Colonies** | **% Frequency** |
|---|---|---|---|---|
| 1. | Bahar nwey | - | 0 | 0.0 |
| 2. | Bahar kon | *Rhodotorula glutinis*<br>*Cryptococcus albidus* | 36<br>5 | 6.5<br>0.9 |
| 3. | Berkot nwey | - | 0 | 0.0 |
| 4. | Bnaslawa | *Cry. laurentii*<br>*Rhodotorula* spp.<br>*Cryp. albidus* | 5<br>21<br>17 | 0.9<br>3.8<br>3.1 |
| 5. | Badawa | - | 0 | 0.0 |
| 6. | Barzan | *Candida rugosa* | 27 | 4.9 |
| 7. | Tayrawa | *C. rugosa*<br>*Cry. laurentii* | 15<br>60 | 2.7<br>10.9 |
| 8. | Toraq | *Cry. albidus* | 10 | 1.8 |
| 9. | Chwar chra | *C. ciferrii* | 40 | 7 |

| | | | | |
|---|---|---|---|---|
| 10. | Chnar | *C. rugosa*<br>*Cryptococcus laurentii*<br>*Cry. albidus* | 61<br>2<br>2 | 11.5<br>0.3<br>0.3 |
| 11. | Daratu | - | 0 | 0.0 |
| 12. | Darwzae shar | - | 0 | 0.0 |
| 13. | Taajeel | *Rhodotorula* spp.<br>*Cry. laurentii* | 2<br>11 | 0.3<br>2.0 |
| 14. | Swere gchka | *Cry. laurentii* | 4 | 0.7 |
| 15. | Saydawa | - | 0 | 0.0 |
| 16. | Shawes | *Cry. albidus* | 5 | 0.9 |
| 17. | Farmanbaran | *Rhodotorula* spp. | 20 | 3.6 |
| 18. | Qarabu | *Cry. laurentii* | 20 | 3.6 |
| 19. | Karezan | - | 0 | 0.0 |
| 20. | Mhala Arab | *Cry. laurentii* | 21 | 3.8 |
| 21. | Science College | *C. parapsilosis* | 3 | 0.5 |
| 22. | Mala Omer | *Cry. albidus* | 7 | 1.3 |
| 23. | Masif Salahddin | - | 0 | 0.0 |
| 24. | Zanko | - | 0 | 0.0 |
| 25. | Xanaqa | *Rhodotorula* spp. | 3 | 0.5 |
| 26. | Roshenbere | - | 0 | 0.0 |
| 27. | Zhean | - | 0 | 0.0 |
| 28. | 121 Gulan | *Trichosporon mucoides* | 150 | 27.4 |
| | **Total** | | 547 | |

Table 4 shows that four genera of yeasts were isolated, namely *Candida* (4 species), *Cryptococcus* (2 species) *Rhodotorula* (2 species), and *Trichosporon* (2 species). It was shown that the yeast belongs to species *Trichosporon* was the highest, reaching 604 colonies, followed by *Rhodotorula* (305) then *Cryptococcus* (265), and finally *Candida* (226) *Cryptococcus laurentii* was isolated with a frequency rate of 12.9, and it was previously considered a saprophytic yeast, but there are studies that have proven that it is pathogenic to humans in its areas of spread.

**Table-4**: Total yeast colonies isolated from the dropping pigeons in Erbil and its suburbs.

| **Yeast** | **Media** | | **Total** | **Frequency** | **Total of genera** | **Frequency** |
|---|---|---|---|---|---|---|
| | **NBSA** | **SDA** | | | | |
| | **Total No. of Colonies** | | | | | |
| *Candida albicans* | 23 | 0 | 23 | 1.5 | 439 | 28.1 |
| *Candida ciferrii* | 17 | 40 | 57 | 3.6 | | |
| *Candida famata* | 10 | 0 | 10 | 0.6 | | |
| *Candida lusitaniae* | 150 | 0 | 150 | 9.6 | | |
| *Candida parapsilosis* | 2 | 3 | 5 | 0.3 | | |
| *Candida rugosa* | 91 | 103 | 194 | 12.4 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Cryptococcus albidus* | 0 | 46 | 46 | 2.9 | 265 | 17.0 |
| *Cryptococcus laurentii* | 96 | 123 | 219 | 14 | | |
| *Rhodotorula* spp. | 196 | 46 | 242 | 15.5 | 310 | 19.9 |
| *Rhodotorula glutinis* | 32 | 36 | 68 | 4.3 | | |
| *Trichosporon asahii* | 194 | 0 | 194 | 12.4 | 694 | 31.9 |
| *Trichosporon mucoides* | 350 | 150 | 500 | 32 | | |
| Total | **1161** | **547** | **1558** | - | - | - |

## Discussion

Pigeon droppings, including the feces of domestic birds, are a good environment for many microorganisms, including fungi. In this study, 164 samples of domestic bird droppings were collected to isolate the fungi present in these droppings.The genus *Cryptococcus* comprises 19 species, loosely characterized as a variety of en-capsulated yeasts. There continue to be occasional reports of human infections with several of these non-neoformans and non-gattii species, such as *Cryptococcus albidus* and *Cryptococcus laurentii* (Smith *et al.*,2017).*C laurentii* is a psychrophilic organism and it grows poorly at temperatures above 30 $^{o}$C. while an optimal growth temperature of 15$^{o}$C has been reported for this species, this yeast is cryotolerant and can be successfully cultured in near freezing conditions (Molina-Leyva *et al.*,2013).The overall prevalence of *Cryptococcus spp.* was 14.9% (265) retrieved from all 28 regions studied from 1708 isolates, 2.7% (46) and 12.98% (219) were identified to represent *C. albidus* and *C. laurentii* respectively. The results are comparable with other findings reported by Jang *et al.*, who stated the occurrence of 14.30% *Cryptococcus albidus* and 7.9% *Cryptococcus laurentii* in pigeon droppings from China (Jang *et al.*, 2011).*Cryptococcus albidus* was another species in the *Cryptococcus* genus isolated in this investigation. It is an uncommon cause of infection in humans and should be considered as a potential pathogenic agent of corneal ulcer (Morales-López and Garcia-Effron, 2021). Ragupathi and Reyna, (2015) reported the first case of. *C.albidus* peritonitis in a patient infected with hepatitis C who was undergoing peritoneal dialysis due to renal failure. The patient was treated with amphotericin B; however, infections of non-neoformans cryptococcal species presented a clinical challenge because they are complicated to diagnose and treat. *Cryptococcus albidus* has five varieties, namely *C. albidus var. aerius, C. albidus var. albidus, C.albidus var. diffluens, C.albidus var.kuetzingii, and C.albidus var.ovalis* (Kamari et al.,2017).The prevalence of different species of yeasts from the feather is reported by Mohammed et al., 2017 which reported that *Candida albicans* was the most frequency isolated yeast (22.4%) followed by *C. famata* (18.6%), *C. pelliculosa* (12%), *C. tropicalis* (7.7%), *C. krusei* (7.2%), *C. rugosa* (6.3%), *Trichosporon asahii* (13.8%), *Geotricum klebahnii* (5.8%), *G. candidum* (2.4%) and *Cryptococcus laurentii* (3.8%), these results are partially agree with our results. *Cryptococcus laurentii* is an extremely rare human pathogen. This yeast was previously considered saprophytic and nonpathogenic to humans, but it has been isolated as the etiologic agent of infections such as skin infection, keratitis, endophthalmitis, lung abscess, peritonitis, meningitis, and fungemia.*Cryptococcus albidus* and *Cryptococcus laurentii* are uncommon species of this genus that in recent decades have increasingly caused opportunistic infections in humans, mainly in immunocompromised patients (Smith et al., 2017). *Cryptococcus laurentii* is the most common yeast found in tundra, Antarctica, and prairie soils, as well as in the phyllosphere of numerous ecosystems. The fecal matter of healthy birds has been identified as an important repository for cryptococcal fungi (Molina-Leyva et al.,2013; Mohammed et al., 2016).

## Acknowledgement

The authors would like to thank and acknowledge the department of Biology, College of Science, University of Salahaddin Erbil-Iraq for enabling this work. Also, authors would like to thank

Raparin Educational Hospital for Children for their help.

## Reference:

Brito, M.; Bessa, M.; Menezes, R.; Röder, D.; Penatti, M.; Pimenta ,J,; Aguiar, P. and Pedroso, R.D.S.(2019). Isolation of *Cryptococcus* species from the external environments of hospital and academic areas. *J. Infect. Dev. Ctries. 13(6):545-553. doi: 10.3855/jidc.10849. PMID: 32058990.*

Ed-berg, S. C.;S. J. Chaskes,; E. Alture-Werber, and J. M. Singer. (1980). Esculin-based medium for isolation and identification of *Cryptococcus neoformans*. *J. Clin. Microbiol.* 12:332–335.

Heitman,J. ; Kozel,T.R. ; Kwon-Chung,K.J. ; Perfect,J.R. ; Casadevall, (2011).*Cryptococcus*: From Human Pathogen to Model Yeast. *ASM Press. 647pp. ISBN : 9781555815011*

Jang,Y.H; Lee, S.J, Lee; J.H, Chae; H.S, Kim and S.H, Choe, N.H. (2011). Prevalence of yeast-like fungi and evaluation of several virulence factors from feral pigeons in Seoul, Korea. *Lett Appl Microbiol.* ;52(4):367- 371.

Kamari,A.;Sepahvand A. and Mohammadi, R. (2017). Isolation and molecular characterization of *Cryptococcus* species isolated from pigeon nests and Eucalyptus trees. *Curr. Med .Mycol*.;3(2):20-25. doi: 10.29252/cmm.3.2.20. PMID: 29354777; PMCID: PMC5763894.

Khawcharoenporn,T.;Apisarnthanarak, A. and Mundy, L.M.(2007). Non neoformans cryptococcal infections: a systematic review. *Infection35(2):51-8*. doi: 10.1007/s15010-007-6142-8..

Mohammed,A.R.; Ismael,M.H. and Abdulrahman, Z.F. (2016). Conventional and Molecular Identification of Pathogenic and Keratinophilic Fungi Isolated from Feathers of Bird in Erbil City. https://www.researchgate.net/publication/323515801

Molina-Leyva,A,Ruiz-Carrascosa,J.C,Leyva-Garcia,A.Husein-Elahmed,H..(2013) . Cutaneous *Cryptococcus laurentii* infection in an immunocompetent child . *Int.J.Infect.Dis. (12):e1232-3*. doi: 10.1016/j.ijid.2013.04.017.

Morales-López,S. E. and Garcia-Effron, G. (2021) . Infections due to Rare *Cryptococcus* species. A Literature Reviewv. *J. Fungi , 7,279.* https:// doi.org/ 10.3390 /jof7040279.

Oh, K. S., and S. M. Hwang. (2005).Isolation and characterization of *Cryptococcus neoformans* from environmental sources in Busan. *Mycobiol. 33:188–193.*

Pamela ,T.;E. Mayayo,L. López-Fernández;J. Guarro and J. Capilla , .(2017). Combined antifungal therapy against systemic murine infections by rare *Cryptococcus* species. *Mycoses ;60(2):112-117. doi: 10.1111/myc.12569*.

Pincus, D. H.(2006). Microbial Identification Using The Biomérieux Vitek®2. System. *Encyclopedia of Rapid Microbiological Methods* .pg4.https:// store .pda.org/Table of Contents / ERMM_V2_Ch01.pdf

Ragupathi, L, and Reyna, M.(2015). Case report of *Cryptococcus albidus* peritonitis in a peritoneal dialysis patient and a review of the literature. *Per Dial Inter.; 35(4):421-7.*

Roudbary, M; Kumar, S; Kumar A; Černáková, L; Nikoomanesh, F and Rodrigues C.F.(2012).Overview on the Prevalence of Fungal Infections, Immune Response, and Microbiome Role in COVID-19 Patients . *J. Fungi (Basel) ;7(9):720. doi: 10.3390/jof7090720.*

Sirag, Khidir, E.S ; Dumyati M.; Sindi B.; Alsinnari, M.; Faidah H and Ahmed A (2021) *Cryptococcus neoformans* and other opportunistic *Cryptococcus* species in pigeon dropping in Saudi Arabia: Identification and characterization by DNA Sequencing . *Front in Microbiol . 12:726203. Doi: 10.3389/fmicb.2021.726203*

Smith, N,:Sehring,M,:Chambers,J,:Patel,P.(2017) . Perspectives on non-*neoformans* cryptococcal opportunistic infections. J.Community Hosp. *Intern. Med. Perspect. 19;7(4):214-217. doi: 10.1080/20009666.*

Vlchkova,L.; S.Kamberova,; A.Starova,; N.Tsatsa-Biljanovska,; V.Janevska and M.Petrovska, .(2004).Cutaneous *Cryptococcus laurentii* infection in a human immunodeficiency virus-negative subject . *JEADV .18(1) :99-100.https: // doi.org/10.1111/j.1468-3083.2004.00434*.