Isolation, Serodiagnosis and Antimycotic Susceptibility of *Cryptococcus neoformans* isolated from pigeon droppings

ISSN 1817 – 2695

Basil Abdulzahra Abbas , Mohammed Hasan Khudor , Hassan Mohammed Jasim Al-Tamemy

Dep. of Microbiology - College of Veterinary Medicine - Basrah University

((Received 2/3/2010 , Accepted 26/10/2010))

Summary

A total of 250 samples of dried pigeon excreta, were collected from several sites in Basrah province. The study showed that out of 250 dried pigeon excreta 40 (16%) samples were positive for the presence of *Cryptococcus neoformans* isolates in 9 of the 15 investigated locations. In addition; the highest percentage for positive samples were recorded in Alhussein Quarter at 15 (37.5%) and local Basrah market for birds at 14 (35%) out of 40 total positive samples, while the highest percentages of isolation were in the local Basrah market for birds at 14 (36.8%) out of 38 samples, Alhussein Quarter 15 (28.8%) out of 52 samples, Shat-Alarab Center 2 (28.6%) out of 7 samples and Alzubair 2 (28.6%) out of 7 samples respectively. Seroinvestigation for the serums of 25 pigeon breeders and 25 leukemia patients revealed a presence of antibody titer against the *Cryptococcus* in six (24%) pigeon breeders and four (16%) leukemia patients serum samples. In addition it had been noticed that the presence of cryptococcal antigenemia in four (16%) out of 25 leukemia patients serum samples. By using disc diffusion method for three antifungal drugs, ketoconazole , miconazole and amphotericin B, toward 41 *C. neoformans* isolates, Ketoconazole showed a significant growth inhibition capability compared with other two tested antifungal drugs ($P < 0.01$) while there were no significant differences between the inhibition efficacy of miconazole and the amphotericin B.

Keywords: Cryptococcus neoformans, antifungal susceptibility , cryptococcosis serodiagnosis.

Introduction

Cryptococcus neoformans, the causative agent of cryptococcosis, is a common encapsulated fungus that can cause a range of illnesses including a lethal infection of the central nervous system (CNS) leading to meningoencephalitis, predominantly in immunocompromised individuals treated with corticosteroids or those with lymphoreticular malignancies (leukemia and lymphoma), sarcoidosis , AIDS (1, 2). With the eruption of the HIV epidemic, *C. neoformans* has only emerged as a serious human pathogen during the last 30 years and has become the leading mycological cause of morbidity and mortality among AIDS patients. It is estimated that 6% to 10% of the patients with AIDS in the United States, Western Europe, and Australia and up to 50% of AIDS patients in sub-Saharan Africa countries are infected with life-threatening cryptococcal meningitis (3). *C. neoformans* is the second commonest opportunistic CNS infection in patients with cancer and mainly associated with lymphoma (4). Patients with leukemia develop several problems affecting their immune system and immune function. Although chemotherapy is effective at eliminating the leukemia, it also depletes normal immune cells, particularly T cells (5).

Two varieties (Currently Two species) were primarily implicated in human and animal disease, *C. neoformans* var. *neoformans* and *C. neoformans* var. *gattii*. Several studies suggested that *Cryptococcus* spp. may have a different host preference based on the immunological status of the patient and that the *C. neoformans* infections are notorious for eliciting very different types of inflammatory responses in both immune-compromised and immunocompetent hosts (6). Epidemiological data showed that the infection by *C. neoformans* preferentially affects immunosuppressed populations (7). The cause of this host preference remains unclear. In human the infection is initiated by the inhalation

of the *C. neoformans* into the lung where the spreading of the infection to various organs subsequently initiated (8).

The major environmental sources of *C. neoformans* have been shown to be either soil contaminated with pigeon excreta in case of *C. neoformans* or eucalyptus trees and decaying wood forming hollows in living trees in case of *C. gattii* (9). Epidemiological studies have been shown to be effective for finding geographic differences in antifungal susceptibility patterns and the extent of antifungal resistance among *C. neoformans* (10).

Studies in Iraq have considered some aspects related to *C. neoformans* in north and central parts of Iraq. To our knowledge this is the first study that tackled the relatedness of *C. neoformans* to pigeons in Basrah governorate. The purpose of this study was to determine the presence of the *C. neoformans* and its varieties in Basrah province environment; investigate serologically the presence of the *C. neoformans* among the immune-compromised (Leukemia patients) and in the immune-competent (pigeon breeders); and to detect the antifungal susceptibility *in vitro* of the locally isolated strains .

Materials and Methods

Isolation and Identification:

Small quantities of desiccated pigeons (*Columba livia* var. *domestica*) excreta were collected from several sites in Basrah province (a total of 250 dried pigeon excreta sample) and placed in separate plastic test tubes and were tightly closed with flexible screw caps closures. All samples were processed within 1 to 3 days after collection. About Two grams from each sample was suspended in 10 ml of sterile normal saline. Each of the suspensions was incubated for 2 hrs. at room temperature with constant agitation at 150 rpm. The dilution was prepared from each suspension with 1:10 sterile normal saline. (11). Each sample suspensions (0.2 ml of the 1:10) and the undiluted sample were evenly spreaded separately with a sterile glass rod on the surface of sunflower agar plates and incubated at 30 °C for 7 days with daily observation (12,13). Suspected colonies especially mucoid brown colonies were carefully examined grossly to demonstrate pigment production in addition to the morphological tests and the ability to grow at 37 ^{o}C. Rice extract agar Tween-80 was used to demonstrate the morphological status of the yeast (14).

Biochemical tests included the following tests: growth inhibition by cycloheximide (15), phenoloxidase activity (12) , urease test (16), potassium nitrate assimilation test (17) , biotyping by using canavanine-glycine - bromothymol blue (CGB) (18) and API 20 C AUX Diagnostic Strips (BioMérieux France). Local reference isolate of *C. neoformans* (From University of Baghdad – Mycology section) has been passed throughout all the morphological and or biochemical tests for to match results.

Sero-Diagnosis :

Fifty human blood samples were collected aseptically from two groups. Twenty five blood samples were collected from leukemia patients in Basrah maternity hospital (Leukemia and Cancer Units) and Basrah training hospital from under 18 years children while another 25 blood samples were collected from the pigeon breeders during the period of July – August , 2008. Additional Serum samples information was collected from each patient using a prepared form that includes age, sex, date of the visit and additional pathological sequels to leukemia. Two systems for serodiagnosis were used: the Latex-Cryptococcus Antigen test and the YA-Crypto antibody tube agglutination System (Immuno-Mycologics-IMMY /USA).

Antifungal Susceptibility:

Tubes containing Sabouraud dextrose broth were inoculated with two loopfulls of purified yeast colonies to be tested from 1-2 days Sabouraud dextrose agar old cultures. Tubes were incubated overnight at 30 ^{o}C , then diluted 1:100 with sterilized normal saline then was shaken vigorously. The suspension was adjusted to 10^{6} cell / ml by hemocytometer and Mcfalrand standard methods (19). Yeast inocula suspension (0.2 ml) was piptted on the surface

of the Emmon's modified Sabouraud's dextrose agar (ESDA) plates and spread evenly. Disc diffusion method at a concentration of (10 µg / disc) for Ketoconazole , Amphotericin B and Miconazole was conducted according to (15, 20).

Statistical analysis :

In order to determine the statistical significances among different variables SPSS program (Statistical program for social sciences) version 11, was used. Chi-square and analysis of variance tests were applied to test the obtained results ($P<0.01$ and $P > 0.05$) respectively.

Results

Isolation:

Forty dried pigeon samples 16% out of 250 samples were positive for the presence of *C. neoformans* isolates in 9 of the 15 investigated locations (Table 1).

Serodiagnosis:

Pigeon Breeders: All the 25 samples were negative for *Cryptococcus* antigens in the tested serum samples while Six (24%) out of 25 pigeon breeders serum samples showed antibody positive agglutination. Of the six Positive specimens the number of positive samples were 2,3 and 1 at 1:2, 1:4 and 1:8 dilutions respectively (Table -2).

Table (1) Percentage of positive sample of *C. neoformans* isolated from pigeon excreta from different areas in Basra.

No.	Area	No. of Samples	No. Positive Samples	*	**
1	Jobaila	49	0	0	0.0
2	Alia	10	0	0	0.0
3	Kut Alhujaja	4	0	0	0.0
4	Abo Alkhaseeb	20	0	0	0.0
5	Hay Alhussein	52	15	37.5	28.8
6	Shat Alarab Center	7	2	5	28.6
7	Zubair	7	2	5	28.6
8	Alhady	11	1	2.5	9.1
9	Qibla	13	2	5	15.4
10	Jomhorya	13	2	5	15.4
11	Hartha	6	1	2.5	16.7
12	Qurna	6	1	2.5	16.7
13	Aal amlak	6	0	0	0.0
14	Alburadyaa	8	0	0	0.0
15	Local Basrah Market	38	14	35	36.8
	Total	**250**	**40**	**100**	**16.0**

*** % of positive samples in each region (No. of all +ve) / (40)**
**** % of +ve samples from the total number of isolates (250) in all regions**

Leukemia Patients:

Four (16%) out of 25 leukemia patients serum samples were with antigen positive agglutination. Of the Four positive specimens number of positive samples were (1and3) at 1:4 and 1:8 dilutions, respectively. In addition four (16%) out of 25 leukemia patients serum samples showed a positive antibody

agglutination. In (Table -2), of the four positive specimens the numbers of positive samples were 2 at both 1:2 and 1:4 dilutions respectively. Out of the 8 positive antigen and antibody leukemia serum samples one leukemia serum sample (Leukemia # 15) showed agglutination at both antigen and antibody tests.

Antifungal Susceptibility:

The total mean values of the inhibition zones for the tested isolates were ketoconazole 31.5 mm, miconazole 12.1 mm and amphotericin B 11.5 mm. Ketoconazole showed a significant ($P<0.01$) inhibition capability compared with other antifungal drugs while there were no significant differences between the efficacy of Miconazole and the Amphotericin B (Table -3)**.**

Table (2) Antigens – Antibodies presence in the serum samples of pigeon breeders and leukemia patients

Test	Pigeon Breeders		No. of Positive Titrations			Leukemia Patients		No. of Positive Titrations		
	(+ve)	(%)	1:2	1:4	1:8	(+ve)	(%)	1:2	1:4	1:8
Ag in the Serum	0/25	0	0	0	0	4/25	16		1	3
Ab in the Serum	6/25	24	2	3	1	4/25	16	2	2	0

Discussion

There is an association between *C. neoformans* and pigeon. However, pigeons do not acquire cryptococcosis, most likely because *C. neoformans* cannot grow at the pigeon's normal body temperature of 42°C. According to the recent studies, the environmental habitat of *C. neoformans* appears to be related to trees and plant materials. It is likely that the environmental niche of the fungus is vegetation but that it is easily isolated from avian excreta because it provides good media for growth and can favor the domestic contamination (21). In a study in Brazil (22), and out of 824 environmental samples *Cryptococcus neoformans* was isolated in 37 areas including indoor and outdoor locations. The isolation from pigeon dropping was more significant 12.7 % and it was indicated that pigeon droppings have a relation in the degree of domestic contamination with *Cryptococcus neoformans* and this could influence the possibility of infection. In the current study, some of the studied areas showed high isolation rates of *Cryptococcus* and this may increase the risks of the infection among the vulnerable groups due to the domestic contamination in *neoformans*.

Table (3) *In Vitro* antifungal susceptibility test for *C. neoformans* isolates

Inhibition zones in millimeters				Inhibition zones in millimeters			
Isolate No.	**Keto.**	**Mico.**	**Ampho. B**	**Isolate No.**	**Keto.**	**Mico.**	**Ampho. B**
101	30	12	12.5	**122**	36	13.5	12.5
102	32.5	12	13	**123**	32	15	12
103	34	13	13.5	**125**	35.5	13	11.5
104	31.5	13	12.5	**126**	31	13	10
105	34.5	12	13.5	**127**	32	11.5	12
106	32.5	13	9.5	**128**	35	12	11
107	31.5	11	12.5	**129**	26	11	11
108	32	9	10	**130**	28	13	11
109	30	12.5	11	**131**	28	11	11
110	26	10.5	10	**132**	30.5	12	12.5
111	30	10.5	10	**133**	30	11	12
113	30	11.5	10.5	**134**	31	13	13
114	32.5	11.5	10	**135**	32	17	9.5
115	30.5	11.5	10.5	**136**	32	10	9
116	33	11	11.5	**301**	33	12	12
117	34	11	11	**302**	33	11	11
118	32.5	12	11	**303**	31	13	15
119	27	13.5	9.5	**305**	33	14.5	14
120	31.5	9	11.5	**307**	32	11.5	12.5
121	28.5	14.5	12	**308**	33	11.5	9.5
Total Mean	**31.5 a**	**12.1 b**	**11.5 b**	**Total Mean**	**31.5 a**	**12.1 b**	**11.5 b**
($P < 0.01$). LSD=1.294							

The differences in the isolation rates among the tested locations can be attributed to a number of factors that can affect the prevalence of the fungus in the excreta of pigeons and could explain the degree of isolation of the fungus from the environment. Of these factors are: sunlight exposure to the pigeon habitats due to the susceptibility of this fungus to the ultraviolet radiation, avian alimentary habits and breeders habits such as the way and the frequency of cleaning the coops and the humidity of the samples. The yeast cells of *C. neoformans* were found to survive for 2 years in dry pigeon excreta that was protected from the sun (23, 24)**.**

The present study showed that the percentages of environmental isolation of the fungus differs among the geographical areas in Basrah. This can be attributed to the large number of dried samples and the poor management of bird's sellers in these two locations. The pigeons were kept in crowded unclean cages in which birds from different areas in Basrah are exchanged on a daily basis and such conditions may favor the yeast (25). The results are in consistent with (25) who reported that after taking 450 wet (200) and dry (250) pigeon excreta samples in Baghdad city 35 samples were positive and the highest *C. neoformans* isolation percentage in an area was 12.8 and 18 % . In addition the percentage of positive samples was higher in the dry samples 29 (11.6%) positive samples for the isolation of the *C. neoformans* in comparison with wet samples 6 (3%). In contrast, our isolation percentage was higher compared with (26) who mentioned that out of 70 dried pigeon samples, 3 (4.28%) were positive for *C. neoformans* and this could be attributed to the low number of the dry pigeon dropping samples collected in that study and that our samples were all from indoor pigeon nests and this could influence the isolation rates (25).

This study also showed that none of the 25 pigeon breeders have cryptococcal antigenemia in their serum. This is consistent with what had been mentioned in that the immune competent individuals are more resistant for infection. In contrast antibody test showed that 6 (24%) out of 25 of the tested serum samples for the pigeon breeders have a positive serum antibodies titers for *Cryptococcus* at 1:2, 1:4 and 1:8 dilutions. These positive dilutions indicate the low level of antibodies in the six positive samples. It is apparent that immunocompetent individuals have some degree of immunity to *C. neoformans* polysaccharide antigens (3).

The results of *C. neoformans* serum diagnosis in the current study were low compared with (26) who reported that 42 % of the 50 tested serums samples for the pigeon breeders and poultry workers were positive for cryptococcal antibodies at 1:2-1:32 titers. This could be attributed to the usage of less sensitive passive haemagglutination test method used in that study that used polyclonal antibodies with less specificity compared with commercial latex test. Commercial cryptococcal antigens latex test typically demonstrate 90-100 % sensitivity and 97-100 % specificity compared with culture and clinical diagnosis. The sensitivity for antigens in CSF samples ranges from 93-100 % and for serum samples from 83-97 % while cryptococcal antibody test have 50% of sensitivity (27).

The results indicated the presence of antigenemia in 4 of those positive patients and the possibility of cryptococcosis. It is reported that a positive antigenemia with 1:2 dilution and over is indicative for the infection. In a study on serum of AIDS patients using latex test the cryptococcal antigenemia was recorded in 59 (18%) out of 327 tested samples at a titer of at least 1:2 and that the sensitivity for antigenemia indicative for detection of cryptococcosis were at a titer of $\geq$1:8 or > 1:64 in 97.6 % and 90.2 % of patients (28) while in a similar study the antigenemia was recorded in 22 (5.8 %) out of 377 of these 96 % were at a titer > 1:4 (29). In the current study and out of the 8 positive cryptococcal antigens and antibodies leukemia serum samples .

The result showed that ketoconazole, miconazole and amphotericin were effective on the tested 41 *C. neoformans* isolates. There were significant differences between the tested antifungal ($P< 0.01$) of which ketoconazole showed the highest inhibition rate compared with the other two antifungal drugs while there was no statistical difference in the inhibition efficacy between miconazole and the amphotericin B. In a study the ketoconazole, miconazole and the amphotericin B. were effective against *C. neoformans* isolates by using the minimum inhibitory concentration for 90% of the growth (MIC_{90}) method at a concentration ketoconazole (0.5), miconazole (0.5) and amphotericin B (0.25) µg (30), while in another study it was for Ketoconazole (0.064), Amphotericin B (0.094) µg (31). The differences in the susceptibility may be attributed to the methodological differences and /or the strains variations. The antifungal susceptibility testing results depend on a variety of test conditions like inoculums size , medium components which may antagonize drug activity especially when complex media are used for testing , medium pH , incubation temperature and time of reading results can also influence antifungal effects (32). In addition, unless the antifungal agents are stable in the test medium, the susceptibility testing procedure may give false results because of the breakdown of the drug (33).

It is reported that there were a susceptibility differences between serotypes A and D and the reasons for these differences are not clear. One of the possible mechanisms could be related to the relative differences in their ability to synthesize melanin. This is suggested by reports that pigmented *C. neoformans* cells were much more resistant to killing by amphotericin B possibly by a mechanism in which melanin present in the cell wall prevents the drug from reaching its active site (34). This may partially explain the differences between the susceptibility of the tested isolates in the current study toward the antifungal drugs as there were differences in the pigmentation potency of those isolates when tested on the caffeic and the sun flower seed agars. Noteworthy all our tested isolates except the reference isolate were environmental isolates that were not likely subjected to the effects of the antifungal agents before. In our study we conclude that there was no primary resistance against amphotericin B, ketoconazole and miconazole among the local environmental isolates of *C. neoformans* of Basrah.

References

1- **P. Kumari ; P.R. Shahapur and P.S. Rao.** Corticosteroid induced *Cryptococcus* meningitis. Ind. J. Med. Microbiol. 32: 145-50. **(2001)**

2- **E. Blasi ; R. Barluzzi ; R. Mazzolla; P. Mosci and F. Bistoni.** Experimental model of Intracerebral infection with *Cryptococcus neoformans:* Roles of phagocytes and opsonization. J. Infect. Immun. 60(9): 3682-3688. **(1992).**

3- **J. Schop.** Protective immunity against *Cryptococcus neoformans* infection. M.J.M.10(1): 35-43. **(2007).**

4- **J. Klastersky and M. Aoun.** Opportunistic infections in patients with cancer. Annals of Oncology. 4: 329–335.**(2004)**

5- **V. Kumar ; R. S. Cotran; S. L Robbins.** Basic pathology. 7th . ed. Saunders, Philadelphia, U.S.A. Pp: 158-164. **(2003).**

6- **T.R. Kozel.** Virulence factors of *Cryptococcus neoformans*. Trend. Microbiol. 3(8): 295-298. **(1995).**

7- **M. Bovers; F. Hagen; E.E. Kuramae and T. Boekhout.** Six monophyletic lineages identified within *Cryptococcus neoformans* and *Cryptococcus gattii* by multi-locus sequence typing. J. Fungal Genetics and Biology. 45 : 400–421. **(2008)**

8- **J. Capilla; C.M.L. Maffel; K.V Clemons; R.A. Sobel and D.A. Stevens.** Experimental systemic infection with *Cryptococcus neoformans* Var. *grubii* and *Cryptococcus gatti* in normal and immunodefficient mice. Med. Mycol. 44: 601 -601. **(2006)**

9- **T. Baro´; J.M. Torres-rodri´guez ; M. H. De mendoza; Y. Morera and C. Ali´a.** First identification of autochthonous *Cryptococcus neoformans* var. *gattii* isolated from goats with predominantly severe pulmonary disease in Spain. J. Clin. Microbiol. 36(2): 458–461. **(1998).**

10- **M. A. Pfaller; S. A. Messer; L. Boyken ; C. Rice; S. Tendolkar ; R. J. Hollis; G.V. Doern and D.J. Diekema.** Global trends in the antifungal susceptibility of *Cryptococcus neoformans* (1990 to 2004). J. Clin. Microbiol. 43(5): 2163–2167. **(2005)**

11- **A. P. Litvintseva; L. Kestenbaum ; R. Vilgalys and T. G. Mitchell.** Comparative analysis of environmental and clinical populations of *Cryptococcus neoformans*. J. Clin. Microbiol. 43(2): 556–564. **(2005).**

12- **M. Pal.** First report of isolation of *Cryptococcus neoformans* var. *neoformans* from avian excreta in Katmandu, Nepal . Rev Iberoam. Micol. 14:181-188. **(1997).**

13- **F. Staib ; M. Seibold ; E. Antweller ; B. Frohlich ; S. Weber and A. Blisse.** The brown color effect (BCE) of *Cryptococcus neoformans* in diagnosis , control and epidemiology of *Cryptococcus neoformans* in AIDS patients. Zbl. Bakt. Hyg., A 266: 167-177. (Cited by 26). **(1987).**

14- **Himedia. (2003). Manual for microbiliogy and cell culture laboratory practice.** P. 373

15- **M. R. McGinnis (1980).** Laboratory handbook of medical mycology. Academic press, New York. Pp: 661

16- **G.M. Cox; J. Mukherjee; G.T. Cole; A. Casadevall and J.R. Perfect.** Urease as a virulence factor in experiment cryptococcosis. J. Infect. Immun. 68(2):443-448. **(2000)**

17- **D.H. Pincus ; I.F. Salkin; N.J. Hurd ; I. L. Levy and M.A. Kemna.** Modification of potassium Nitrate assimilation test for identification of clinically important yeasts. J. Clin. Microbiol. 26(2): 366- 368. **(1988).**

18- **M.M. Nishikawa; O.D. Sant'Anna ; M.S. Lazera and B. Wanke.** Use of d-proline assimilation and CGB

medium for screening Brazilian *Cryptococcus neoformans* isolates. J. Med.Vet. Mycol. 34:5, 365 – 366. **(1996).**

19- **Z. Odabasi; V. Paetznick; B. P. Goldstein; J. H. Rex and L. Ostrosky-Zeichner.** Disk diffusion-based methods for determining *Candida parapsilosis* susceptibility to anidulafungin. J. Antimicrob. Agen. Chemoth. 47(9): 3018–3020. **(2003).**

20- **M. H. Khudor.** A study on vaginal candidiasis in Basrah women and effect of five antifungal drugs on some clinical isolates. M. Sc. Thesis., College of science, University of Basrah. **(1998).**

21- **E. J. Anaissie; M. R. McGinnis; M.A. Pfaller.** Clinical Mycology. 1st .ed. Churchill Livingstone, New York. Pp: 1-608.**(2002).**

22- **L.F.C. Passoni; B. Wanke; M. M. Nishikawa and M.S. Lazera.** *Cryptococcus neoformans* isolated from human dwelling in Rio de Janeiro, Brazil: an analysis of the deomestic environment of AIDS patients with and without cryptococcosis. J. Med. Mycol. 36:305-311. **(1998).**

23- **K. J. Kwon-Chung and J. E. Bennett.** Medical mycology. Lea and Febiger , Philadelphia, U.S.A. Pp: 397-439. **(1992).**

24- **T. C. Sorrell & D. H. Ellis.** Ecology of *Cryptococcus neoformans.* Rev. Iberoam. Micol. 14: 42-43. **(1997).**

25- **H. A. A. Aldelemy.** Screening study for *Cryptococcus neoformans* yeast isolated from pigeon excreta in Baghdad governorate. M. Sc. Thesis., College of Science, University of Almustansiryah (In Arabic). **(1998).**

26- **S. S. M. Aldelemy.** Immunologic and diagnostic study of cryptococcosis. M. Sc. Thesis., College of veterinary medicine, University of Baghdad. (In Arabic). **(2003)**

27- **D.L. Kiska; D.R. Orkiszewski; D. Howell and P.H. Gilligan.** Evaluation of new monoclonal antibody-based latex agglutination test for detection of cryptococcal polysaccharide antigen in serum and cerebrospinal fluid. J. Clin. Microbiol. 32(9): 2309-2311. **(1994).**

28- **R. Micol; O. Lortholary ; D. Laureillard ; C. Ngeth ; J. Dousset ; H. Chanroeun ; L. Ferradini ; P.J. Guerin ; F. Dromer; and A. Fontanet.** Prevalence, determinants of positivity, and clinical utility of cryptococcal antigenemia in Cambodian HIV-infected patients. J. Acquir. Immune Defic. Syndr. 45 (5): 555-559. **(2007).**

29- **C.A. Liechty; P. Solberg; W. Were; J. P. Ekwaru ; R.L. Ransom; P.J. Weidle ; R. Downing; A. Coutinho and J. Mermin.** Asymptomatic serum cryptococcal antigenemia and early mortality during antiretroviral therapy in rural Uganda. Tropical Med. Intern. Health. 12(8): 929–935. **(2007).**

30- **K. G. Davey; A. D. Holmes; E. M. Johnson ; A. Szekely and D. Warnock.** Comparative evaluation of FUNGITEST and broth microdilution methods for antifungal drug susceptibility testing of *Candida* species and *Cryptococcus neoformans*. J. Clin. Microbiol. 36(4): 926–930. **(1998).**

31- **Z.U. Khan; H.S. Randhawa; T. Kowshik; A. Chowdhary and R. Chandy.** Antifungal susceptibility of *Cryptococcus neoformans* and *Cryptococcus gattii* isolates from decayed wood of trunk hollows of *Ficus religiosa* and *Syzygium cumini* trees in north-western India. J. Antimicrob. Agen. Chemoth. 60: 312–316. **(2007).**

32- **J.N. Galgiani .** Mini review: Antifungal Susceptibility tests. J. Antimicrob. Agen. Chemoth. 31(12): 1867-1870. **(1987).**

33- M. Ghannoum ; A. S. Ibrahim ; Y. Fu ; M.C. Shafiq ; J. E. Edwards and R.S. Criddle. Susceptibility testing of *Cryptococcus neoformans*: a microdilution technique. J. Clin. Microbiol. 30 (11): 2881-2886. **(1992).**

34- Z.U. Khan; H.S. Randhawa; T. Kowshik; A. Chowdhary and R. Chandy. Antifungal susceptibility of *Cryptococcus neoformans* and *Cryptococcus gattii* isolates from decayed wood of trunk hollows of *Ficus religiosa* and *Syzygium cumini* trees in north-western India. J. Antimicrob. Agen. Chemoth. 60: 312–316. **(2007).**

العزل والتشخيص المصلي وتحديد الحساسية للمضادات الفطرية ضد المكورات الخبيئة المعزولة من فضلات الحمام

باسل عبد الزهرة عباس ، محمد حسن خضر ، حسان محمد جاسم التميمي

قسم الاحياء المجهرية / كلية الطب البيطري / جامعة البصرة

الخلاصة

تم جمع 250 عينة من فضلات الحمام الجافة من أماكن متفرقة في محافظة البصرة حيث أظهرت الدراسة أن أربعين عينة (16%) كانت موجبة لتواجد عزلات خميرة المكورات الخبيئة من النوع *Cryptococcus neoformans* في تسعة من بين خمسة عشر موقعا تم التحري فيها. وقد ظهر أن أعلى نسبة للعينات الموجبة تم تسجيلها في حي الحسين في 15 (37.5%) وفي سوق البصرة المحلي للطيور في 14 (35%) من مجموع 40 عينة موجبة في حين ان أعلى نسبة عزل في العينات المأخوذة كانت في سوق البصرة المحلي للطيور في 14 (36.8%) من مجموع 38 عينة ، وفي حي الحسين في 15 (28.8%) من مجموع 52 عينة ، وفي مركز منطقة شط العرب في 2 (28.6%) من مجموع 7 عينات ، وفي الزبير في 2(28.6%) من مجموع 7 عينات على التوالي.

أظهر التحري المصلي لمصول 25 من مربي الحمام و 25 من مرضى اللوكيميا وجود عيارية من الأضداد ضد خميرة المكورات الخبيئة في كلا المجموعتين في 6 (24%) من مجموع 25 من مربي الحمام وفي 4 (16%) من مجموع 25 من مرضى اللوكيميا ، كذلك تم ملاحظة وجود مستضدات خميرة المكورات الخبيئة في المصل في 4 (16%) من مجموع 25 عينة مصل من مرضى اللوكيميا.

باستخدام طريقة أقراص الانتشار لثلاثة مضادات فطرية (amphotericin , miconazole , ketoconazole) B ضد 41 عزلة *C. neoformans* اظهر ال ketoconazole مقدرة تثبيط عالية المعنوية ($P< 0.01$) مقارنة مع بقية المضادات الفطرية التي تمت دراستها ، في حين لم يكن هناك اختلاف معنوي في بين كفاءة التثبيط لكل من ال miconazole و ال amphotericin B.