International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 3 Issue 5, August 2019 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

Pigeon Excreta: A Potential Source of *Cryptococcus Neoformans* and their Antifungal Susceptibility Profile

Richa Gumasta[1,2], Shankar Mohan Singh[1,2], Ravi Prakash Mishra[1], Shesh Rao Nawange[1,2], Abhijeet Garg[3], Anuranjan Singh Rathore[4]

[1]Department of Biological Sciences, Rani Durgavati University, Jabalpur, Madhya Pradesh, India
[2]Centre for Medical Mycology, Fungal Disease Diagnostic and Research Center, SRDTHFD, Jabalpur, Madhya Pradesh, India
[3]School of Biotechnology, Devi Ahilya Vishwavidyalaya, Indore, Madhya Pradesh, India
[4]National Institute of Research in Tribal Health, Indian Council of Medical Research, Jabalpur, Madhya Pradesh, India

How to cite this paper: Richa Gumasta | Shankar Mohan Singh | Ravi Prakash Mishra | Shesh Rao Nawange | Abhijeet Garg | Anuranjan Singh Rathore "Pigeon Excreta: A Potential Source of Cryptococcus Neoformans and their Antifungal Susceptibility Profile" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-3 | Issue-5, August 2019, pp.233-238, **https://doi.org/10.31142/ijtsrd25250**

IJTSRD25250

ABSTRACT

Globally the pigeon droppings are a known ecologic niche for cosmopolitan pathogenic yeast Cryptococcus neoformans, an etiological agent of deadly disease cryptococcosis. In this prospective study between 2015- 2017, we analyzed the isolation of C. neoformans strains from a total of 305 pigeon excreta samples of caged pigeons with a pH of 6-8, from different sites of Central India. NCCLS broth microdilution methodology was employed on the isolated strains against amphotericin B, fluconazole, itraconazole, ketoconazole, and voriconazole. C. neoformans were found positive from fifty-five dry guano feces. Maximum positive samples found for the pathogens were from caged pigeon excreta collected from the 12 different sites in city Jabalpur 23 (46 %), 9 (18 %) from four sites katni, followed by 3 sites from each city Betul 8 (16 %), Satna 6 (12%) and Rewa 4 (.08 %). The highest frequency of C. neoformans was recorded from site 2 (60%), followed by site 24 (37.5%), site 17 (27.27%), whereas site 3, 6, 10, 15 and 19 found negative for pathogenic yeast. the present study of antifungal susceptibility profile for C. neoformans revealed resistance against ketoconazole (25.5%) and fluconazole (8.5 %). The highest susceptibility was observed for amphotericin B (100 %) followed by voriconazole (97.9 %) and itraconazole (78.7%) No resistance was found for polyene drug amphotericin B. Fluconazole (46.8 %) and ketoconazole (36.2%). This data of prevalence and colonization of this pathogen suggests that the dry excreta provides a more favorable environment for growth inside the cages and is more concerned with health hazards of the humans in proximity and further comprehensive study is required to reinforce the antifungal spectrum for the prudent therapy of cryptococcosis.

KEYWORDS*: Pigeon droppings, Cryptococcosis, Cryptococcus neoformans, Central India, NCCLS, antifungal susceptibility*

INTRODUCTION

Cryptococcosis is a major life-threatening acute, sub-acute or chronic systemic mycosis caused by encapsulated opportunistic yeasts belonging to genus Cryptococcus. *Cryptococcus* is a cosmopolitan basidiomycetes opportunistic fungal pathogen prevalent ubiquitously in the environment, however only very few are usually pathogenic to men and animal hosts. Majority of infections are caused by *C. neoformans* and *C. gattii* species complex while other *Cryptococcus* species are rarely been reported to cause disease till date. *C. neoformans* is isolated predominantly from avian or areas contaminated with avian feces, therefore, pigeon droppings are a known ecologic niche for this pathogen [1].

Although the occurrence of *C. neoformans* was also studied from domestic and wild animals include macaw, swan, parakeet, Guenon monkey, fox, potoroo, and sheep [1]. the droppings of exotic and migratory birds like Munia birds [2], Bats [3], Chickens [4-5], Canaries [6], Parrots [7], Beccari's crowned pigeon [8], Barlett's bleeding heart pigeon [9], a macaw [10], a thick-billed parrot [11] and Moluccan Cockatoo [12].

In Central India for the first time the natural occurrence of *Cryptococcus neoformans* var. *neoformans* was reported in the soil contaminated with pigeon excreta in Jabalpur [13] and in Betul [14]. Out of 29 samples examined, 9 (31%) proved to be positive for *C. neoformans* from which eight were collected from pigeon houses, which were located inside the residential places.

15 clinical cases of avian cryptococcosis (Congo African Grey parrot, African Grey parrot, 2 case of Eclectus parrot, King parrot, Sulphur Crested Cockatoo, 3 cases of Long-billed

Corella, 2 cases of Gang Gang Cockatoo, Racing pigeon and 3 cases of North Island brown kiwi.) were reported from Australia and New Zealand [15]. In Australia *Cryptococcus* developed an invasive disease of the upper respiratory tract of parrots whereas, severe invasive disease, with the involvement of the lower respiratory tract or dissemination to internal organs of bird residing outside Australia. *Cryptococcus* biotypes were determined, three of which was reported to be *C. n var. gattii* and the other as *C. n var. grubii.*

C. neoformans var. neoformans was isolated from feces of four captive bird's species in Nigeria at the Jos Wildlife Park [16]. Total five isolates belonged to serotype A, which were isolated from a White face duck (*Dendrocygna viduata*), eagle owl (*Bubo africanus cinerascene*) and peacock (*Pavo cristatus*) while two were serotype D isolated from Spotted Eagle Owl.

Cryptococcosis has been rarely reported in birds [17]. The source of *C. neoformans* in avian dropping remains a mystery. Since infection is associated with their high body temperature (41.5- 43.3 ^{o}C), but experimental infections in pigeons revealed that they have been produced by the intracerebral inoculation of the yeast [18]. Part of this study was presented in mp young scientist 2018.

MATERIAL AND METHODS

A. Sampling

In the present investigation, a total of 305 desiccated pigeon excreta samples were collected from different localities of central India (12 sites of Jabalpur city, Rewa (3), Satna (3, Katni (4) and 5 of Betul (Figure 1). Pigeon excreta samples were aseptically collected using long spatulas, transferred to clean sterilized plastic bags, and properly labeled according to site and date. The average sample weight was around 500 g. Samples were taken to the laboratory and were used immediately or in case of delay, samples were stored at room temperature and processed within 48 hours.

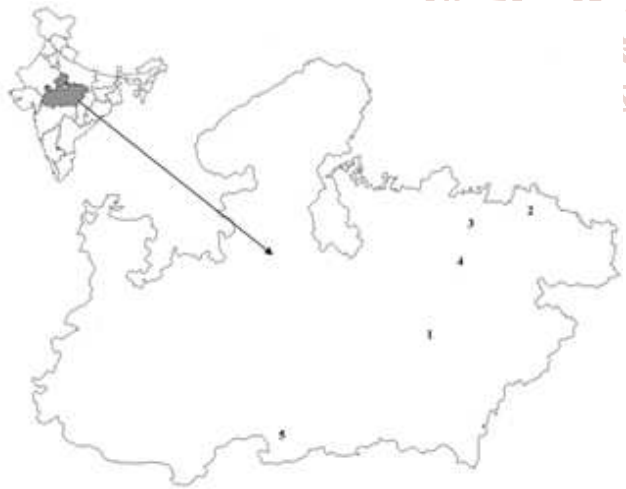

Figure1: India map: location of five cities of Central India (1. Jabalpur, 2. Rewa, 3. Satna, 4. Katni and 5. Betul), where *C. neoformans* strains were isolated from pigeon excreta samples.

B. Isolation and sample processing

Selective isolation of *C. neoformans* sp. complex was done by swabbing and Direct Plating Method using Staib's *Guizotia abyssinica* Creatinine agar (GACA- Staib's) medium (50 g of pulverized Niger seeds, 1 gm dextrose, 20 gm agar, 1 gm KH_2PO_4, 1 gm creatinine, and pH of medium was set at 5.5. The medium was cooled to approximately 50ºC to which streptomycin sulfate (40 µg / ml), penicillin (20 µg / ml) and biphenyl (1gm biphenyl in 10 ml absolute alcohol per 1000 ml of the culture medium) were added and plates were prepared [17, 19].

In the direct plating technique, approximately 5 g of each sample was suspended in 45 ml of sterilized distilled water containing 20 mg/ml penicillin and 40 mg/ml streptomycin. The suspension was shaken vigorously for a few minutes and then was allowed to settle at 37 °C for 1 hour. Serial dilution i.e. 1:10, 1:100, 1:1000 of the suspension was prepared and 0.1 ml of each dilution was plated on GACA- Staib's medium [6] After 3–4 days of incubation at 28° C, the number of chocolate brown yeast-like colonies compatible with the *C. neoformans* species complex appearing on GACA- Staib's medium plates were counted visually and were subjected to biochemical, physiological and morphological identification tests in order to identify Cryptococcus species [17, 19]. Canavine glycine bromothymol medium was used to screen the varieties.

C. Antifungal susceptibility testing

In-vitro sensitivity of different antifungal drugs was studied against 47 isolates of *C. neoformans* environmental isolates. Clinical Laboratory Standard Institute- National Committee for Clinical Laboratory Standards, a methodology of the reference microdilution method M27-A3 (1:2 fold broth dilution method) was followed [20]. For the determination of MIC, following five antifungal drugs were used amphotericin B (AMFOCAN, Dabur India Ltd.), ketoconazole (NIZRAL; Johnson and Johnson India Ltd) , itraconazole capsules (CANDISTAT; E Merk India Ltd), fluconazole (Flustan TM ; Dr. Reddy's Lab. Ltd. Novartis India Ltd), voriconazole (VFEND Pfizer India Ltd). A serial dilution was prepared from the stock solution of the 5 anti-fungal agents to have final concentration ranges of 0.156 -640 µg/ml.

D. Quality Control

Quality control testing as per CLSI (NCCLS) guidelines was followed. Three reference strains for MIC determination used were obtained from Microbial Type Collection Centre (MTCC) PGI Chandigarh India. These reference strains were *C. albicans* ATCC 90028 and *C. tropicalis* ATCC 750 and *C. glabrata* ATCC 90030.

E. Interpretation of results

For *In-vitro* sensitivity testing, MIC is defined as the lowest concentration in mg/ml of an antifungal drug that substantially inhibits 80% (azoles) and 100% (amphotericin B) growth of an organism in comparison with drug free control growth. The tested strains were categorized into three groups: Susceptible S), Susceptible Dose Dependent (SDD) and Resistant (R). Amphotericin B susceptibility breakpoint-< 0.25µg / ml (S), Breakpoints for fluconazole were < 8µg / ml (S), 16-32µg / ml (SDD), > 64 µg / ml (R), for itraconazole < 0.125 µg / ml (S), 0.25-0.5µg / ml (SDD), > 1µg / ml (R), for ketoconazole < 0.0625µg / ml (S), > 0.125 µg / ml (R) and for voriconazole < 0.125 µg / ml (S), 0.25-0.5µg / ml (SDD), > 1µg / ml (R).

F. Result Analysis-

WHONET-5.6 software was used to analyze and interprete MIC results [21].

G. Statistical Analysis-

Independent "t" test was used to find significance between both the species of *C. neoformans* isolated from environment, against all the five antifungals using SPSS software.

RESULT AND DISCUSSION

In the present investigation, population density and isolation frequency of *Cryptococcus neoformans* var. grubii and *Cryptococcus neoformans* var. *neoformans* were investigated in pigeon excreta collected from various sites of Jabalpur, katni, Betul, Satna and Rewa cities of Central India. The highest population density (2.6 x 10^5 cfu/g) of C. *neoformans* was obtained from site 5 of Jabalpur city site 10 (1.7 x 10^5 CFU/g), site 14, Rewa and site 16, Satna (1.6 x 10^5 cfu/g), followed by site 17, Satna (1.4 x 10^5 cfu/g) etc.

Table1 Isolation of *Cryptococcus neoformans* strains from Pigeon excreta samples of Central India.

City	sites	Total no. of samples	Positive samples	Isolation frequency	cfu/g	Melanin production	Isolated strains
Jabalpur	1	26	4	15.38 %	0.6 x 10^4	+++	*C.neoformans*
	2	10	6	60 %	1.1 x 10^4	++	*C.neoformans*
	3	08	0	0 %	-	++++	*C.neoformans*
	5	12	3	25 %	2.6 x 10^5	++	*C.neoformans*
	6	10	0	0 %	-	++	*C.neoformans*
	7	17	1	5.88 %	0.6 x 10^5	++++	*C.neoformans*
	8	16	2	12.5 %	0.8 x 10^4	+++	*C.neoformans*
	9	15	2	13.33 %	1.3 x 10^4	+++	*C.neoformans*
	10	16	0	0 %	-	++	*C.neoformans*
	11	10	3	0.3 %	2 x 10^4	++	*C.neoformans*
	12	23	2	8.69 %	0.6 x 10^4	+++	*C.neoformans*
Rewa	13	15	3	20 %	0.9 x 10^4	+++	*C.neoformans*
	14	09	1	11.11 %	1.6 x 10^5	+++	*C.neoformans*
	15	6	0	0 %	-	++	*C.neoformans*
Satna	16	18	1	5.55 %	1.6 x 10^5	+++	*C.neoformans*
	17	11	3	27.27 %	1.4 x 10^5	++++	*C.neoformans*
	18	19	2	10.52 %	0.7 x 10^4	+++	*C.neoformans*
Katni	19	10	0	0 %	-	++	*C.neoformans*
	20	6	1	16.66 %	0.9 x 10^5	+++	*C.neoformans*
	21	4	2	50 %	0.8 x 10^4	++++	*C.neoformans*
	22	10	6	60 %	1.1 x 10^5	++	*C.neoformans*
Betul	23	10	1	10 %	0.8 x 10^5	++	*C.neoformans*
	24	16	6	37.5 %	2.1 x 10^4	+++	*C.neoformans*
	25	08	1	12.5 %	1.4 x 10^4	++	*C.neoformans*

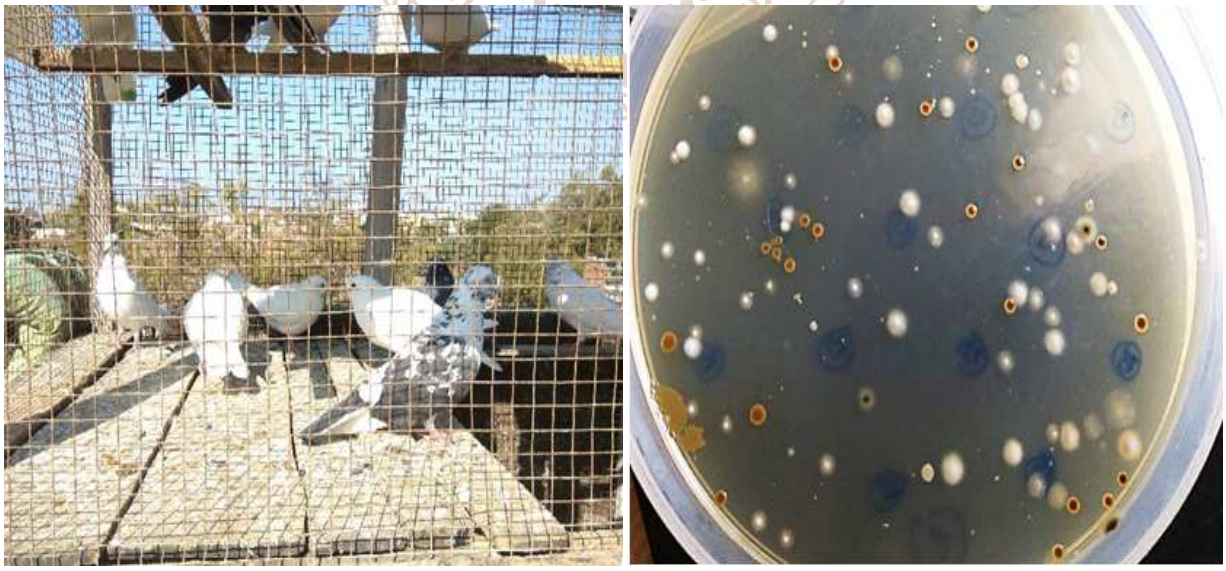

Figure2: Showing isolation of *Cryptococcus* yeast (brown colonies) from pigeon excreta samples on Staib (GACA) medium

These *C. neoformans* isolates obtained from pigeon excreta samples were examined using CLSI M27-A3 susceptibility testing to check their antifungal susceptibilities towards the five commonly used antifungal drugs (amphotericin B, fluconazole, itraconazole, ketoconazole, and voriconazole). The result shows the percentage resistance (% R), percentage intermediate (% I), percentage susceptibility (% S), MIC50, MIC90, geometric mean of *C. neoformans* strains and their MIC's range.

Table2 Antifungal susceptibility results of *C. neoformans* isolates using WHONET 5.6 software

Code	Antibiotic Name	Breakpoints	%R	%I	%S	%R 95%C.I	MIC50	MIC90	Geom. Mean	MIC range
AMB_NM	Amphotericin B	S<=.25 R>=1	0	0	100	0.0-9.4	0.125	0.25	0.131	0.0625 - 0.25
FLU_NM	Fluconazole	S<=8 R>=64	8.5	44.7	46.8	2.8-21.3	16	32	11.567	1- 64
ITR_NM	Itraconazole	S<=.125 R>=1	0	21.3	78.7	0.0-9.4	0.064	0.25	0.082	0.03 – 0.5
KET_NM	Ketoconazole	S<=.062 R>=.124	25.5	38.3	36.2	14.4 -40.6	0.064	0.125	0.065	0.03 - 2
VOR_NM	Voriconazole	S<=.125 R>=1	0	2.1	97.9	0.0-9.4	0.064	0.125	0.057	0.03 – 0.25

Figure 3 shows the percentages of resistant strains of *C. neoformans* against five antifungal drugs (amphotericin B, fluconazole, itraconazole, ketoconazole, and voriconazole). Maximum resistance shown was 25.5% by the drug ketoconazole, followed by fluconazole i.e, 8.5%. Whereas no resistance (i.e. 0%) was exhibited by drugs amphotericin B, itraconazole and voriconazole.

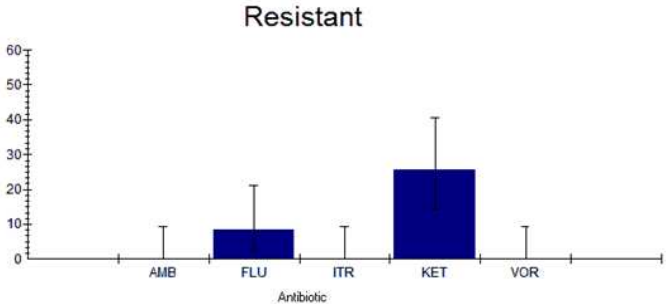

Figure3. Percentage of resistance showed by *C. neoformans* isolates against five antifungal drugs (amphotericin B, fluconazole, itraconazole, ketoconazole, and voriconazole)

The percentages of *C. neoformans* isolates were shown for the drug amphotericin B, fluconazole, itraconazole, ketoconazole, and voriconazole, in different categories of MIC. Results are shown in Figure 7.

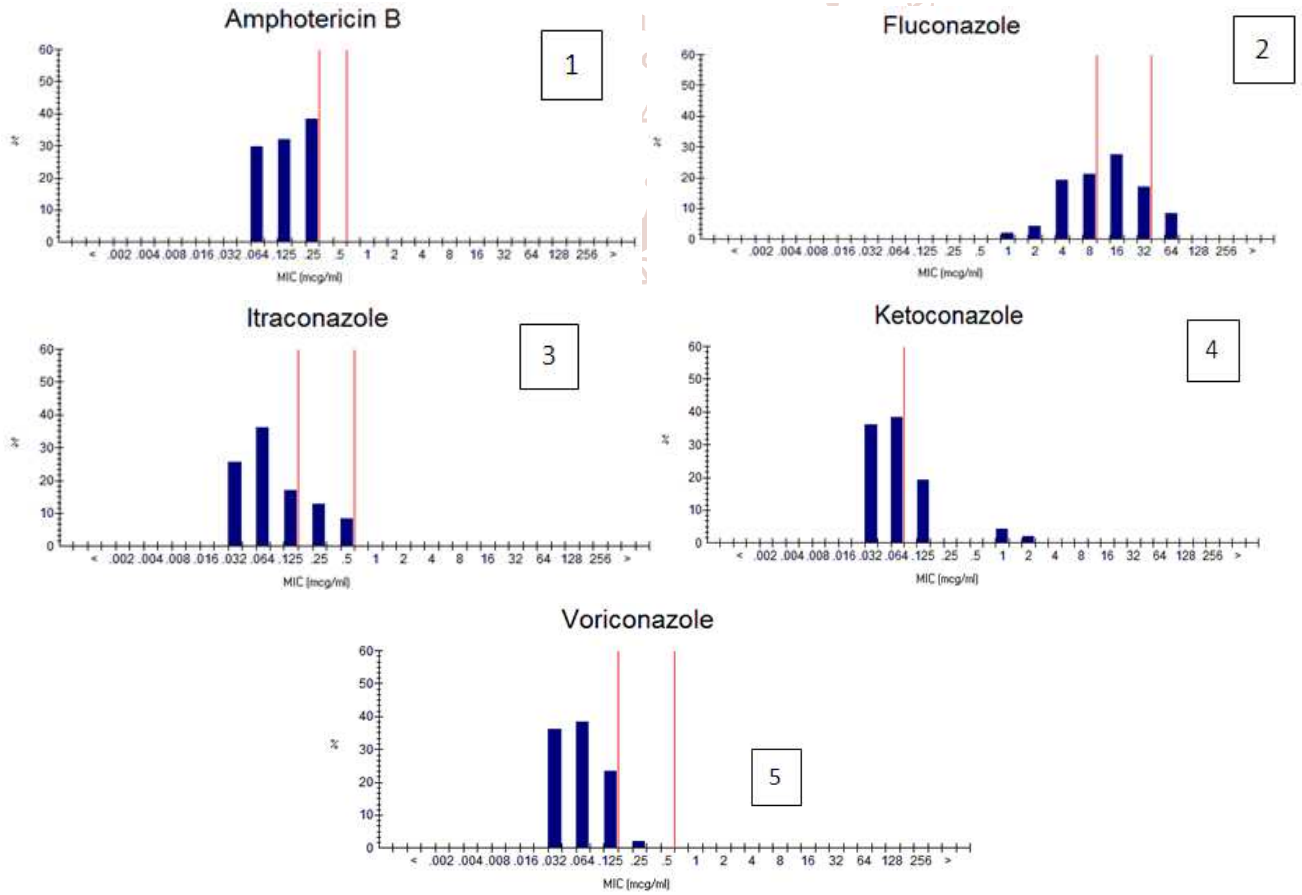

Figure4. MIC results show the percentages of *C. neoformans* isolates, for the antifungal drugs 1. amphotericin B, 2. fluconazole, 3. itraconazole, 4. ketoconazole and 5. voriconazole at different categories of MIC.

Maximum positive samples found for the pathogens were from caged pigeon excreta collected from the 12 different sites in city Jabalpur 23 (46 %), 9 (18 %) from four sites katni, followed by 3 sites from each city Betul 8 (16 %), Satna 6 (12%) and Rewa 4 (.08 %). However, no isolates of the pathogens were obtained from site 3, 6, 10 of Jabalpur city, site 15, Rewa and site 19, Katni. (Table 1).Though no statistically significant difference ($P > 0.05$) was found in the isolation of both species from any particular site.

As previously reported [22], the association of *C. neoformans* (serotype AD) was also found with pigeon excreta [23]. In contrast, 34 samples of pigeon droppings were found positive for *C. neoformans* var. *grubii* (Molecular type VNI) with mating type αA and were confirmed to be the prevalent genotype in the samples of pigeon excreta [24]. According to our results, positive strains belonged to *C. neoformans* var. *grubii* and *C. neoformans* var. *neoformans* isolated from pigeon excreta and highest population density (2.6×10^5) of pathogen was recorded from the Hanumantal locality (site 2) of Jabalpur city.

Our study reveals 8.6% R for fluconazole against *C. neoformans* strains. These results were similar to [25] who demonstrated higher MIC of fluconazole for *C. neoformans*. The MIC 50 and MIC 90 values for fluconazole (4 & 16) and itraconazole (0.032-0.125) which is slightly higher than our results for fluconazole (16 & 32) and itraconazole (0.064-0.25).

Our study represents 8.6% resistance for the drug fluconazole by *C. neoformans* strains which was contrary [26]. In our results we were getting moderately high (8.6%) fluconazole MIC's which is in correspond to the study [29]. Our MIC 50 and MIC 90 values for the drugs were as fluconazole (16 & 32 µg/ml), itraconazole (0.64 & 0.25 µg/ml), ketoconazole (0.064 & 0.125 µg/ml), amphotericin B (0.125 & 0.25 µg/ml) and voriconazole (0.064 & 0.125µg/ml).

MIC 90 susceptibility ranges for fluconazole (4 µg/ml), ketoconazole (0.064 µg/ml) and itraconazole (0.094 µg/ml) [27]. Our results were slightly higher for MIC 90 of fluconazole (32 µg/ml), ketoconazole (0.125 µg/ml), itraconazole (0.25 µg/ml) and showed susceptibility for *C. neoformans* amphotericin B. The lowest activity of fluconazole (48.4%) *in-vitro* susceptibility [28]. Our results also indicate the lowest activity of fluconazole for *C. neoformans* strains (46.8%), in addition, the lowest susceptibility of 36.2% was for the drug ketoconazole.

Our study reveals the colonization and dispersion of *C. neoformans* in the pigeon excreta samples of different localities in Central India.it can be concluded that the pathogen can colonize various sites via contamination with avian excreta and the wind serves as the best source for its dispersion associated with the infection risk in a given population. These investigations substantiate pigeon's droppings as the important factor of yeast infection and habitat of *C. neoformans* in the urban areas.

ACKNOWLEDGMENT

The authors wish to thank the Vice-Chancellor of R. D University, Head of the Department of Biological Science of RD University for providing laboratory facilities and Prof S.M Singh for his valuable guidance. I owe a deep sense of gratitude to Dr. S.R Nawange, (SRDTHFD) for providing facilities required for my research work.

REFERENCE

[1] Casadevall and Perfect J.R. (1998). *C. neoformans.* ASM Press, Washing DC: ASM Press

[2] Pal, M. (1989). *Cryptococcus neoformans* var. *neoformans* and munia birds. Mycoses, 32; 250-252

[3] Lazera MS., Wanke B., Nishikawa MM. (1993). Isolation of both varieties of *Cryptococcus neoformans* from saprophytic sources in the city of Rio de Janeiro, Brazil. J Med Vet Mycol, 31: 449–454

[4] Ajello L. (1958). The occurrence of *Cryptococcus neoformans* in soils. Am J Hyg, 67(1):72-7

[5] Emmons, C. W. (1955). Saprophytic sources of *Cryptococcus neoformans* associated with the pigeon (*Columba livia*). Am. J. Hyg, 62, 227–32

[6] Staib F. (1962). *Cryptococcus neoformans* and *Guizotia abyssinica* (syn. G. oleifera D. C.) (Farbreaktion fur*C. neoformans*). Z Hyg, 148:466-475

[7] Bauwens L, Swinne D, Vroey CDE, Meurichy WDE. (1986). Isolation of *Cryptococcus neoformans* var. neoformans in the aviaries of the Antwerp Zoological Gardens. Mykosen, 29:291–4

[8] Ensley, PK, Davis CE, Anderson MP, Fletcher KC. (1979). Cryptococcosis in a male Beccari's crowned pigeon. J. Am. Vet. Med. Assoc, 175:992–994

[9] Griner LA, Walch HA. (1978). Cryptococcosis in columbiformes at the San Diego Zoo. J Wildl Dis, 14(3):389-94

[10] Clipsham RC, Britt JO Jr., (1983). Disseminated cryptococcosis in a macaw. J Am Vet Med Assoc, Dec 1;183(11):1303-5

[11] Rosskopf WJ, Jr., Woerpel RW. (1984). Cryptococcosis in a thick-billed parrot. Avian/Exotic Pract, 1: 14–18,

[12] Fenwick B, Takeshita K, Wong A. (1985). A moluccan cockatoo with disseminated cryptococcosis. J Am Vet Med Assoc, 187: 1218–1219

[13] Dhindsa, M. K., Grewal, P. K. and Singh, S. M., (1994). Natural occurrence of *Cryptococcus neoformans* in soil contaminated with pigeon excreta at Jabalpur (Madhya Pradesh). Vislesana Research Journal, 1; 139-143

[14] Nawange, S. R., Pandey, A., Singh, S. M. and Sharma, B. (2000). Occurrence of *Cryptococcus neoformans var. neoformans* in Betul soil of Central India. Vishlesana Res. J. (Sci). Rani Durgavati University Jabalpur, 7 (2); 91 – 102.

[15] Malik R, Krockenberger MB, Cross G, Doneley R, Madill DN, Black D, McWhirter P, Rozenwax A, Rose K, Alley M, Forshaw D, Russell-Brown I, Johnstone AC, Martin P, O'Brien CR, Love DN. (2003). Avian cryptococcosis. Med Mycol, 41(2):115-24

[16] Irokanulo EO, Makinde AA, Akuesgi CO, Ekwonu M. (1997). *Cryptococcus neoformans* var. *neoformans* isolated from droppings of captive birds in Nigeria. J Wildl Dis, 33: 343–345

[17] Kwon-Chung, K. J. and Bennett, J. E. (1992). Medical Mycology, Lea and Febiger Publishers, Philadelphia, London.

[18] Littman, M. L., and R. Borok. (1968). Relation of the pigeon to cryptococcosis: natural carrier state, heat resistance and survival of *Cryptococcus neoformans*. Mycopathol. Mycol, Appl. 35:329–345

[19] De Hoog, G. S., Guarro, J., Gene, J. and Figueras, M. J. (2000). Atlas of Clinical Fungi, 2nd Edition, Vol I. ASM Press. Utrecht, The Netherlands.

[20] CLINICAL AND LABORATORY STANDARDS INSTiTUTE (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts: third edition (M27-A3). Wayne, PA: CLSI. (2008)

[21] WHONET Software Version 5.6. Available at:http://www.who.int/drugresistance/whonetsoftware/en. 19;10, (2010)

[22] Dromer, F., Mathoulin-Pelissier, S., Launay, O., Lortholary, O. (2007). Determinants of disease presentation and outcome during cryptococcosis: the Crypto A/D study. *PLOS Medicine*. 4(2); 297-308

[23] Nawange, S. R., Singh, S. M., Naidu, J., Sethi, R., Jain, R. Y., Tiwari, A. and Singh, K. (2011). Serotype distribution of *Cryptococcus neoformans* and *C. gattii* in patients and in the environment of Jabalpur, a city of Madhya Pradesh in India. Asian Journal of Microbiology and Environmental Sciences. 13; 735-742

[24] Ellabib MS, Aboshkiwa MA, Husien WM, D Amicis R, Cogliati M. (2016). Isolation, Identification and Molecular Typing of *Cryptococcus neoformans* from Pigeon Droppings and Other Environmental Sources in Tripoli, Libya. Mycopathologia, 181(7-8):603-8

[25] Fernandes, O de F. L., Passos, X. S., Souza, L. K. H., Miranda, A. T. B., Cerqueira, C. H. P. V., and Silva, M. R. R. (2003). *In-vitro* susceptibility characteristics of *Cryptococcus neoformans* varieties from AIDS patients in Goiania, Brazil. Memorias do Instituto Oswaldo Cruz, 98; 839-841

[26] Pedroso, S. R., Lavrador, M. A. S., Ferreira, J. C., Candido, R. C. and maffei C. M. L. (2010). *Cryptococcus neoformans* var. *grubii*- Pathogenicity of environmental isolates co-related to virulence factors, susceptibility to fluconazole and molecular profile. Mem Inst Oswaldo Cruz, Rio de Janeiro, 105 (8); 993-1000

[27] Costa AKF, Sidrim JJC, Cordeiro RA, Brilhante RSN, Monteiro AJ, Rocha MFG. (2010). Urban pigeons *(Columbia livia)* as a potential source of Pathogenic yeast: A focus on antifungal susceptibility of *Cryptococcus* strains in Northeast Brazil. 169; 207-213

[28] Khan, Z. U., Randhawa, H. S., Kowshik, T., Chowdhary, A. and Chandy, R. (2007). 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 Chemother. 60: 312-316

[29] Trpkovic A, Pekmezovic M, Barac A, Crncevic Radovic L, Arsic Arsenijevic V. (2012). *In-vitro* antifungal activities of amphotericin B, 5-fluorocytosine, fluconazole and itraconazole against *Cryptococcus neoformans* isolated from cerebrospinal fluid and blood from patients in Serbia. J Mycol, Med.22(3):243-8