International Journal of Microbiology and Application
2015; 2(1): 6-11
Published online January 20, 2015 (http://www.openscienceonline.com/journal/ijma)

Spectrum of opportunistic mould infections in suspected pulmonary tuberculosis (TB) patients

Yahaya H.[1, *], Taura D. W.[2], Aliyu I. A.[1], Bala J. A.[1], Yunusa I.[3], Ahmad I. M.[3], Ali B.[4]

[1]Department of Medical Laboratory Science, Faculty of Allied Health Sciences Bayero University, Kano, Nigeria
[2]Department of Microbiology, Faculty of Science, Bayero University, Kano, Nigeria
[3]Department of Biochemistry, Faculty of Science, Kano University of Science and Technology, Wudil, Kano, Nigeria
[4]Dept of Biology, Faculty of Applied and Natural Sciences, Jigawa State University, Kafin Hausa, Jigawa, Nigeria

Email address

hyahaya.mls@buk.edu.ng (Yahaya H.)

To cite this article

Yahaya H., Taura D. W., Aliyu I. A., Bala J. A., Yunusa I., Ahmad I. M., Ali B.. Spectrum of Opportunistic Mould Infections in Suspected Pulmonary Tuberculosis (TB) Patients. *International Journal of Microbiology and Application*. Vol. 2, No. 1, 2015, pp. 6-11.

Abstract

The opportunistic fungi are potential pathogens in the immunocompromised patients, those with the pre – existing disease and long history of antibiotics. The study was designed to document the prevalence of TB associated with respiratory mould infections in Dambatta Kano, Nigeria. The study included induced sputum samples from 300 patients with complaints of symptoms suggestive of Tuberculosis (TB) infections. The TB was diagnosed by sputum Ziehl – Neelsen staining technique. Identification of Mould isolates was done by direct microscopy and culture on two sets of SDA and Corn Meal Agar. Of the 300 sputum samples examined, 28(9.3%) patients were positive to AFB microscopy while fourteen different species were isolated from 26(8.7%) patients mainly caused by the genus Aspergillus. *A. niger* was isolated in 3(1%) of the patients, while *A. fumigatus, A. nudilans* and *A. terreus* were isolated from 3(1%), 1(0.3%) and 2(0.6%) patients respectively. Other fungal agents isolated include, *Penicillium viridicatum* 3(1%), *Rhizopus oryzae* 3(1%), *Rhizomucor pusillus* 1(0.3%). The genus Fusarium had the prevalence of 5(1.5%) comprising of *F. oxysporum* 2(0.6%), *F. nivale* 2(0.6%) and *F. tricinctum* 1(0.3%). The genus Trichophyton had a prevalence of 3(1%) consisting of *T. concentricum* 1(0.3%) and *T. rubrum* 2(0.6%). The least prevalence of 1(0.3%) was observed in *Malbranchea saccardo* and *Phoma saccardo* respectively. Mould and TB co – infection was 5(1.6%) with male patients having 4(1.3%) while females had 1(0.3%) ($P = 0.06145$). Co – infection of mould and TB exists and the prevalence of array of these mould species is apparently important considering the immunocompromised status and inadequate response to anti – tubercular drugs of these patients.

Keywords

Mould, Rhizomucor, Tuberculosis, Mycoses, Infection, Dambatta

1. Introduction

The opportunistic fungi are potential pathogens in the immunocompromised patients, those with some pre – existing disease and those with long history of antibiotics (Chugh, 2000).Tuberculosis patients are immunocompromised and they take prolong treatment of antibiotics and vitamins tablets ie immunosuppressive agents, hence fungal infection occurs in early stage of TB infection (Sunita and Rai, 2008). Aspergillus and candida are the classical examples of such fungi were earlier reported from various plants as pathogens, but now they are known to cause disease in human beings (Gupta *et al.,* 2003). The synergistic growth promoting association of fungi and Mycobacterium has raised increased concern for studying the various opportunistic fungal agents and their significance in pulmonary tuberculosis patients. The major opportunistic fungal pathogens include: *Candida* species causing candidiasis *Aspergillus* species causing aspergillosis, *Cryptococcus neoformans* causing cryptococcosis, *Mucor* species causing mucormycosis (Mandanas, 2011).

Pulmonary tuberculosis is contagious bacterial infection caused by *Mycobacterium tuberculosis* (Ganguly, 2000). It is aerobic non motile bacillus (Jasmer *et al.,* 2002).The lungs are primarily involved, but the infection also occurs in other organs. Tuberculosis is caused by a group of organisms, tuberculosis patients are immunocompromising patients hence mycotic infection also occurs in lungs (Ganguly, 2000).

Nigeria was ranked 11th among the 22 highly burden countries in the world. The 22 high burden countries accounted 80% of global TB burden with a total of 180,000 cases occurring annually in Nigeria (WHO, 2013). In present study, we focus the attention on opportunistic fungal pathogens, their infections and identification of such fungi that are present in pulmonary TB patients and relate demographic characteristics to such opportunistic mycoses.

2. Materials and Methods

2.1. Study Site

The site for the current study is Pathology unit, Dambatta General Hospital located at Dambatta Local Government Area, Headquarter of Kano State. It is located between latitude 12º 25′ 59″ N and Longitude 8º 30′ 55″ E (Steve *et al.,* 2006).

2.2. Study Population

The study population includes patients of all ages and sexes enrolled in Directly Observed Treatment Short course Chemotherapy (DOTS) and patients with suspected pulmonary tuberculosis disease under National Tuberculosis and Leprosy Control Programme (NTBLCP).

2.3. Sample Size

A total of 300 samples were collected from the subjects who completed consent form and questionnaire. The minimum sample size was calculated to be 145 according to Henderson and Sundareshy (1982).

2.4. Inclusion and Exclusion Criteria

2.4.1. Inclusion Criteria

All patients suspected of Tuberculosis who presented the symptoms of the pulmonary disease that attended the DOTS Clinic and referred to the laboratory for AFB microscopy were enrolled in the study.

2.4.2. Ethical Clearance/Consent Form/Questionnaire

Before commencement of the research, the ethical approval was first sought from Kano State Hospital Management Board's Ethical Committee. The ethical approval was then taken to Danbatta General Hospital for permission to use the Laboratory for the research. Consent form was given to each participant to sign for his/her acceptance and Questionnaire seeking for demographic characteristics and possible risk factors was also administered.

2.4.3. Sample Collection

A total of three hundred (300) TB suspects who consented and completed questionnaire were sampled. Early morning sputum samples from them were collected as they come to the laboratory for TB diagnosis with the assistance of experienced Medical laboratory scientists. The sputum was expectorated from lower respiratory tract and collected in sterile screw capped containers to avoid contamination from external sources in the following order as described by (Brooks *et al.,* 1991; USAID, 2010).

2.4.4. Collection Procedure

Patients were asked to produce the samples in an open air space away from other people to avoid aerosol spread. The patients were instructed to inhale deeply 3 to 4 times before coughing out from the chest. The sputum produced was carefully spit into the container without contaminating the outside of the container. The lid of the container was screwed tightly before being processed, with utmost care not wrapping the container with the laboratory request form (USAID, 2010).

2.4.5. AFB Staining

The specimens for AFB staining were treated on the same day of collection according to the method of Collins *et al.,* 1997.

2.5. Mycological Analysis

2.5.1. Direct Microscopy

With the use of Pasteur pipette, few drops of 10% KOH were placed on the center of a clean glass slide and using a sterile wire loop, this was mixed with a portion of sputum. The preparation was flattened under cover slip and examined with the magnification × 40 Objective for the presence of fungal hyphal fragments.

2.5.2. Cultural Method of Isolation

All samples irrespective of the outcome of direct microscopy were cultured given that the full identification of mycotic agents is achieved through culture. Sputum specimens were cultured according to the procedure of John (2002). This was achieved by streaking 0.01ml, using sterile inoculation loop on to the surfaces of pre – dried SDA streptomycin (50mg/ml) and Corn Meal agar with tween 80 plates. Duplicate cultures of SDA and Corn Meal agar media were incubated at 27ºC for 48 hours, incubation continued for 21 days at room temperature to maximize detection of slow growing agents such as *Histoplasma capsulatum*.

2.5.3. Cultural Identification

After appropriate incubation, the growth form**,** rate of growth, surface and reversed coloration on SDA plates were noted. Pure isolates were obtained by sub culturing on new plates and colonies growing out of the inoculation areas were regarded as contaminants. Moulds isolated were identified with their distinctive morphological features associated with the characteristic sporing heads, septa formation etc according to Collier *et al.* (1998).

In the case of culture for mould and yeasts, positive results of mycological examinations were accepted only if the direct examination was positive and if the two parallel media (SDA and Corn Meal Agar) culture growths of the same fungus specimen were observed and subsequently confirmed by microscopy. Cases failing these criteria were regarded as negative and were not included in the study.

2.5.4. Statistical Analysis

The data generated in this study was analyzed for statistical significant difference in the association between demographical characteristics (i.e. age, sex), using Pearson Chi – Square with the aid of Open Epi version 2.2.1.

3. Results

3.1. Positive and Negative AFB Results

Acid – fast bacilli: Reddish – purple Colour, Non – acid-fast bacilli: Blue Colour

A total of 300 fresh sputum samples were collected and examined for the presence of opportunistic mycotic agents and *M. tuberculosis*, out of which 28(9.3%) patients were positive to AFB microscopy (Table 1), while fourteen different species were isolated from 26(8.7%) patients (Table 1), mainly caused by the genus Aspergillus. *A. niger* (figure 1) was isolated in 3(1%) of the patients, while *A. fumigatus* (figure 2) *A. nudilans* and *A. terreus* (figure 3) were isolated from 3(1%), 1(0.3%) and 2(0.6%) patients respectively. Other fungal agents isolated include, *Penicillium viridicatum* 3(1%), *Rhizopus oryzae* 3(1%), *Rhizomucor pusillus* (figure 4) 1(0.3%). The genus Fusarium had the prevalence of 5(1.5%) comprising of *F. oxysporum* 2(0.6%), *F. nivale* (figure 5) 2(0.6%) and *F. tricinctum* 1(0.3%). The genus Trichophyton had a prevalence of 3(1%) consisting of *T. concentricum* 1(0.3%) and *T. rubrum* (figure 6) 2(0.6%). The least prevalence of 1(0.3%) was observed in *M. saccardo* (figure 7) and *Phoma saccardo* (figure 8) respectively (Table 2). Mould and TB co – infection was 5(1.6%) with male patients having 4(1.3%) while females had 1(0.3%) though not statistically significant (P – Value = 0.06145) (Table 1).

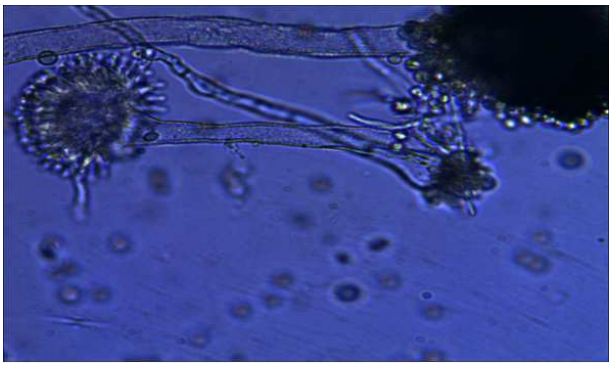

Figure 1. *Aspergillus niger showing dark brown biseriate conidial head with brown conidia on Saboraud's Dextrose Agar at 37°C*

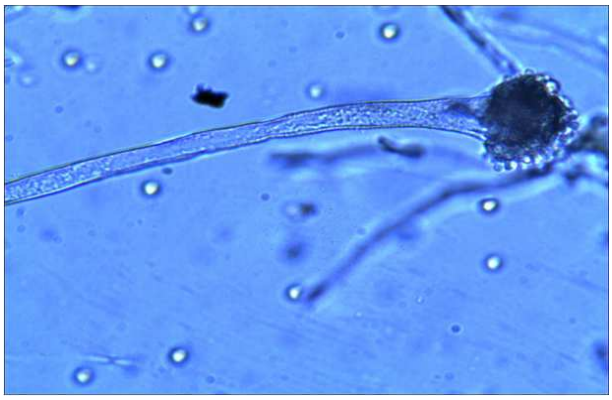

Figure 2. *Wet mount of Aspergillus fumigatus showing uniseriate conidium grown on Saboraud's Dextrose Agar at 37°C*

Table 1. *Occurrence of Acid Fast Bacilli, Moulds and Yeasts based on Sex*

Sex (n=300)	AFB	Moulds	AFB and Mould
Males	22(7.3%)	14.4(4.7%)	4(1.3%)
Females	6(2.0%)	12(4.0%)	1(0.3%)
Total	28(9.3%)	26(8.7%)	5(1.6%)
P – value			0.06145
Chi – Square			3.498

Key: AFB: Acid Fast Bacilli

Table 2. *Distribution of Moulds Isolates based on Age*

Age Group (n=300)	*A. niger*	*A. fumigatus*	*A. nudilans*	*A. terrreus*	*M. saccardo*	*P. saccardo*	*P. viridicatum*
0 - 5	0	0	0	0	0	0	0
6 – 10	0	0	0	0	0	0	0
11 – 15	0	0	0	0	0	0	0
16 – 20	0	0	0	0	0	0	1
21 – 25	1	1	0	0	0	0	1
26 – 30	0	0	0	1	1	0	0
31 - 35	0	1	0	0	0	0	0
36 – 40	0	1	0	1	0	0	0
41 – 45	1	0	1	0	0	1	0
46 – 50	0	0	0	0	0	0	1
51 – 55	0	0	0	0	0	0	0
56 – 60	0	0	0	0	0	0	0
61 – 65	0	0	0	0	0	0	0
66 - 70	1	0	0	0	0	0	0
71 – 75	0	0	0	0	0	0	0
76 – 80	0	0	0	0	0	0	0
Total	3(1.0%)	3(1.0%)	1(0.3%)	2(0.6%)	1(0.3%)	1(0.3%)	3(1.0%)

Table 2. *Continued*

Age Group (n=300)	*R. oryzae*	*R. pusillus*	*F. oxysporum*	*F. nivale*	*F. tricinctum*	*T. Concentricum*	*T. rubrum*
0 - 5	0	0	0	0	0	0	0
6 – 10	0	0	0	0	0	0	0
11 – 15	0	0	0	0	0	0	0
16 – 20	0	0	0	1	0	1	0
21 – 25	0	0	0	0	1	0	0
26 – 30	2	0	0	0	0	0	1
31 - 35	0	0	1	0	0	0	0
36 – 40	0	1	0	0	0	0	1
41 – 45	0	0	0	0	0	0	0
46 – 50	0	0	0	0	0	0	0
51 – 55	0	0	0	0	0	0	0
56 – 60	0	0	0	1	0	0	0
61 – 65	0	0	0	0	0	0	0
66 - 70	1	0	1	0	0	0	0
71 – 75	0	0	0	0	0	0	0
76 – 80	0	0	0	0	0	0	0
Total	3(1.0%)	1(0.3%)	2(0.6%)	2(0.6%)	1(0.3%)	1(0.3%)	2(0.6%)

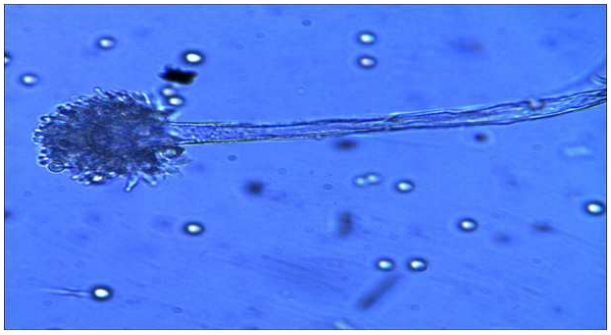

Figure 3. *Aspergillus terrreus showing biseriate conidial head covering the entire conidia on Saboraud's Dextrose Agar at 37°C*

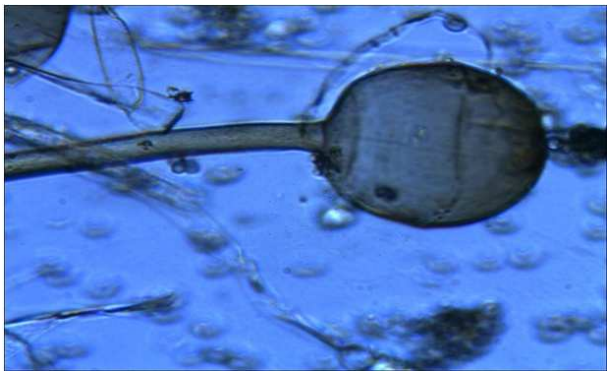

Figure 4. *Rhizomucor pusillus showing brown sporangium with base septation, aseptate sporangiophore on Saboraud's Dextrose*

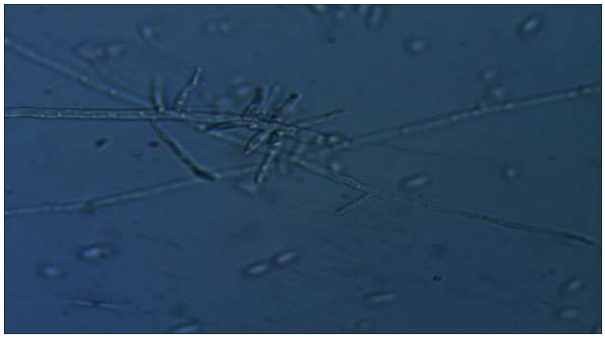

Figure 5. *Wet preparation of Fusarium nivale showing bunch of macroconidia attached to hyphae ×40 objective*

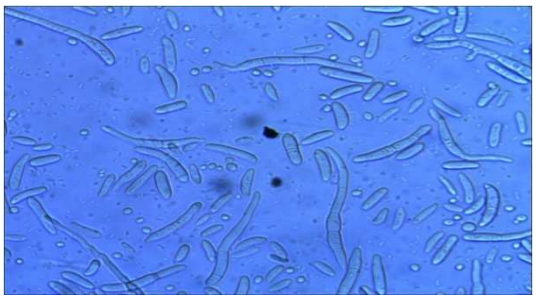

Figure 6. *Wet preparation of Trichophyton rubrum: showing septate macro and micro conidia using × 40 objective grown on Saboraud's Dextrose Agar at 37°C*

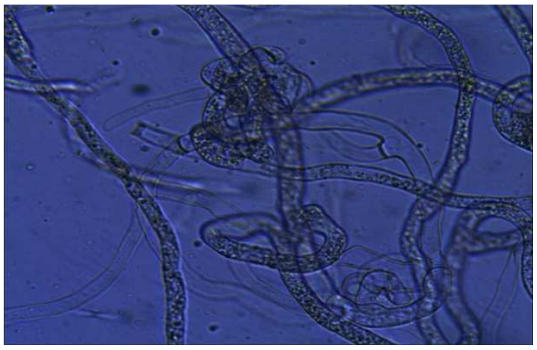

Figure 7. *Malbranchea saccardo with alternate arthroconidia produced in terminal fertile portions of the hyphae*

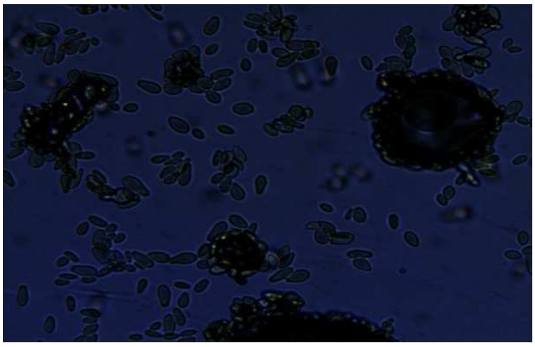

Figure 8. *Wet preparation of Phoma saccardo grown on Saboraud's Dextrose Agar showing dark spindle shaped conidia and pycnidia with no mycelia using × 40 objective*

4. Discussion

Fungal infections of lungs are important infective processes which are being encountered more and more in today's practice (Panda, 2004). Although active mycosis may be an independent marker of advanced immunosuppresion, it may also act as co – factor in accelerating and amplifying the clinical course of tuberculosis disease (Whalcn *et al.,* 2000).

In the present study, the sex – wise distribution of TB infection is sex dependant with males having higher prevalence of 22(7.3%) more than females with the prevalence of 6(2.0%). This agrees with the findings of WHO Report (2012), which puts the males as more prone to TB infection than females. This might be attributed to more exposure of males to external environment than females. Also agrees with the findings of Elizabeth (2011) (Table 1).

In this study, as far as sex distribution is concerned, Table 1 revealed that mould infections are dependent on the sex, although, according to this study, the difference on the occurrences of fungal infections between the two sexes was very low, with the prevalence rates of 14 and 12 for mould infections, This result was in agreement with that of Bansod and Rai (2008), who observed that mould infection was higher in males compared to females as men are more vulnerable to fungal infections than females due to their great exposure to the surrounding (Table 1). This finding disagrees with the finding of Hidalgo and Vazquez (2004) in which it depicted that sex is independent of the distribution of the fungal infections.

AFB and Mould co – infections prevalence rate was observed in the study, 5(1.6%). This established relationship was also observed by Panda *et al.* (1998) and possible justification for this is the fact that TB remains the most important cause of sub acute and chronic respiratory morbidity which most often leaves behind a scarred pulmonary parenchyma, vulnerable to fungal colonization, therefore, TB of the lung can be seen as a predisposing factor for colonizing aspergillosis in case with aspergilloma.

This finding also suggests that the age is dependant of these mycotic infections as lower ages below 10 years did not show the presence of the systemic infection and old ages greater than 76 years also show very low prevalence with middle age brackets showing high prevalence possibly due to high environmental exposure of these age brackets, particularly in secondary infections (Table 2).

Table 2 Shows the clinical moulds isolated based on age. In this study, a total of fourteen different species of moulds with different prevalence rates were isolated. The total prevalence was 26(8.7%). Genus aspergillus had the highest prevalence of 9(2.9%) consisting of four species (Table 2). This agrees with the findings of Kuan – yu Chen *et al.* (2001) and Shahid *et al.* (2007) where aspergillus species were the most common pathogens isolated in patients with pulmonary TB infections.

Fusarium species had a prevalence of 5(1.6%), followed by *Rhizopus species*, *Trichophyton species* and *Penecillium viridicatum* with 4(1.3%), 3(3%) and 3(3%) respectively. The lowest prevalence of 1(0.3%) in *Malbranchea saccardo* and *Phoma saccardo* was also observed. These are the species isolated mostly from immunocompromised individuals. This agrees with the findings of Lane (1996). The mould infections are attributed to risk factors which are prime in compromising the integrity of the host defenses. Invasive fungal infections are an increasing problem in immunocompromised patients with candida, aspergillus and Zygomycetes being most frequent mycotic infections (St Georgegiev, 1997).

It was also noted that the Trichophyton species isolated which were originally superficial in their mycotic capabilities, was possibly due to the chronicity of the infections or serious ravage of the immunity status of those patients.

5. Conclusion

In conclusion, pulmonary fungal infections co – exist in tuberculosis infection according to this finding, although the prevalence rates of all the co – infections were low and statistically not significant, the presence of these infectious agents in TB patients poses great risk in making the patients more ravaged due to pathogenic synergism existing among the infections. It was also concluded that most of the fungi isolated were opportunistic in nature with no single dimorphic fungi isolated.

Recommendations

1. With the conventional Tuberculosis diagnoses, culture isolation of mycotic infectious agents is absolutely necessary in determining co – existing opportunistic infections for increasing the cure rate of tuberculosis patients.
2. A high degree of awareness and efforts for an early diagnosis are needed to improve on the poor prognosis.
3. The incidence of fungal infection can be reduced by minimizing risk factors such as poor hygiene, prolong antibiotic therapy, debilitating disease, corticosteroid therapy etc.
4. Although reducing the fungal infections is important by minimizing the risk factors, but reliable, prompt and effective treatment must be given to patients to prevent the risk of spread.

References

[1] Bensod, S. and Rai, M. (2008): "Emerging of Mycotic Infections in Patients Infected with *Mycobacterium tuberculosis" World J. Med. Sci.,* 3(2) Pp 74 – 8.

[2] Brooks, G. F., Butel, J. S. and Ornstol, L. N. (1991): *Medical Microbiology.* 19th Edition Pp 318-327.

[3] Chakrabarti, A. (2005): Microbiology of systemic fungal infection: *J postgrad Med* 51 (Suppll): S16 – S20.

[4] Chugh, T. D. and Khan, Z. U. (2000): Invasive fungal infections in Kuwait: A retrospective study. *Indian J. Chest Dis. and Allied Sci.,* 42(4):297-87.

[5] Collier, L., Balows, A. and Sussman, M. (1998): Topley and Wilson's Microbiology and Microbial Infections, 9th ed. London: Arnold

[6] Dabo, N. T. and Yusha'u, M. (2007): Incidence of mycoses in patients with suspected Tuberculosis in Kano, Nigeria. *International Journal of Pure and Applied Sciences.* 1(1):35-39. http://www.ijpas.com.

[7] Elizabeth, N. A. Yolanda L. V. Eva, H., Arcadio M. P., Steven, M., Miguel F. M. Ascenio, V. A. Jos´e L. S., Robert L. and Neil A. (2011): "Clustering of Mycobacterium tuberculosis Cases in Acapulco: Spoligotyping and Risk Factors". *Journal of Immunology Research* Volume 2011 Article ID 408375, 12 pages http://dx.doi.org/10.1155/2011/408375 Hindawi Publishing Corporation.

[8] Fluckiger, U., Marchetti, O., Bille, J., Eggimen, P., Zimmerli, S., Imhof, A. Garbino, J., Reuf, C., Pittet, D., Tauber, M., Glauser, M. and Calandra, T. (2006): Treatment options of Invasive fungal infections in adults. *Swiss Med. Wkly* 136: 447 – 463.

[9] Ganguly, D., (2000): Tuberculosis-triumphs and tragedies. *J. Indians Med. Assoc.* 14:96-98.

[10] Gupta, S.K., Chhabra, R., Sharma, B.S., Das, A., and Khosla, V. K. (2003): Vertebral Cryptococcus stimulating tuberculosis. *J. Indian Med. Assoc.* 17(6):556-559.

[11] Henderson, R. H. and Sundareshy, T. (1982): Cluster Sampling to assess Immunization Coverage: a Review of Experience in that simplified Sampling Method. Bulletin of the World Health Organization. 60 (2): 253-60.

[12] Hidalgo, J. A. and Vazquez (2004): Candidiasis. *e-Medicine Journal,* 5(3).

[13] Jasmer, R. M. Nahid, P. and Hopewell, P. C. (2002): Clinical Practice, latent tuberculosis infection. *The New England Journalof Medicine* 347(23): 1860 – 1866.

[14] John, H. (2002): The use of in vitro culture in the diagnosis of systemic fungal infection. http://www.bmb.leads.ac.uk, microbiology.

[15] Kuan – Yu Chen, Shiann – Chin, K., Po – Ren, H., Kwen – Tay, L. and Pan – Chyr, Y.(2001): Pulmonary Fungal infection: Emphasis on Microbiological Spectra, Patient outcome and Prognostic factors. *Chest,* 120(1): 177 – 184.

[16] Kuleta, J. K., Kozik, M.R. and Kozik A. (2009): Fungi Pathogenic to humans: Molecular basis of virulence of *Candida albicans, Cryptococcus neoformans* and *Aspergillus fumigatus*. *Acta Biochim Pol.* 56: 211 – 224.

[17] Lane, K..A.G.(1996): The Merck Manual of Diagnosis and Therapy. Merck Research Laboratories, Whitehouse Station (NJ).

[18] Murray, C. J. L. (1992): *Draft trip report, Geneva*. World Health Organization, CDC.

[19] Panda, B. N. Rosha, D. and Verma, M. (1998): Pulmonary TB: A predisposing factor for colonizing and invasive aspergillosisof lungs. *Indian Journal of TB*, 45: 221 – 222.

[20] Panda, B. N. (2004): Fungal infection of Lungs: the emerging scenario. *Indian J. Tuberc.* 51: 63 – 69.

[21] Reedy, J. L., Bastidas, R. J. and Heitman, J. (2007): The virulence of human pathogenic fungi: notes from the south of France. *Cell Host Microbe* 2:77 – 83.

[22] Shahid, M., Malik, A. and Bhargava, R. (2007): Secondary Aspergillosis in Bronchoalveolar lavages (BALS) of pulmonary TB patients from North –India. *Indian Journal of Medicine, Microbiology*, 20: 141 – 144.

[23] Steve, P., Pius, E. and Shitu, M. B. (2006): "Educational Sector Project: Institutional Assessment – Kano State" ESSPIN Retrieved 16 – 05 – 2010.

[24] St. Georgegiev, V. (1997): Infectious Diseases in Immunocompromised Host. *CRC,* 739 – 1148.

[25] Sunita, B. and Mahendra, R. (2008): Emerging of mycotic Infections in patients with Mycobacterium tuberculosis. *World Journal of Medical Sciences* 3(2): 78 – 80.

[26] USAID Report (2010): Building Partnerships to Control Tuberculosis: United State Agency for International Development, USA.

[27] Whalcn, C., Horsburgh, C.R. Horn, D. Lahart, C., Simberkoff, M. and Ellner, J.(2000): Accelerated course of Human Immunodeficiency Virus infection after tuberculosis. *Am. J. Respir Crit Care. Med.,* 151: 129.

[28] WHO, (2007): Tuberculosis (online). Available: http://who.int/mediacentre/factsheets/fs104/index.htm.

[29] WHO report (2008): Global tuberculosis control surveillance, planning, financing; 1514 – 1527.

[30] WHO Report (2012): Global tuberculosis control Geneva: World Health Organization.