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Research Article

Comparative Analysis of Anti-Fungal Activities of Itraconazole and Fluconazole against Blastomycosis (*Bilastomyces Dermatitidis*)

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Abstract

Bilastomyces dermatitidis is the causal agent of blastomycosis, that causes fungal infection found occasionally in humans and other animals in regions where the fungus is endemic. The samples were collected using clean container from different sites which were river rima Sokoto, river Goronyo and fadama soil Sokoto round. The concentration of (10^{-2}) and (10^{-3}) of each sample were inoculated using sterile wire loop into potato dextrose agar (PDA) media. The incubation was done at incubating room for growth to take place at 30°c for 7 days under aseptic conditions. The target organism (*Blastomyces dermatitidis*) was negative after isolation and identification. The non-target organisms are: *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Penicillium, Aureobasidium pullulans* and *Trichoderma harzianum* were isolated. The *Blastomyces dermatitidis* were not found as a threat form Sokoto metropolis. *Blastomyces* infection has also being described in Africa. *Blastomyces* dermatitidis most commonly infects the lungs, followed by skin, bone, prostate and the central nervous system. It occurs more commonly in men than women because of larger of men occupational exposure. The community should be frequently visiting clinic for medical check-up. Based on the above result, it was recommended that people should avoid exposure to endemic areas. The people that had gone to endemic areas should be tested for blastomycosis when returning. The communities or individuals should report to the clinic immediately when there are abnormalities in the body for early recognition and diagnosis for proper treatment.

Keywords: Anti-fungal; Fluconazole; Blastomyces dermatitidis; Sokoto

Introduction

Blastomyces dermatitidis is the causal agent of blastomycosis, and causes fungal infection found in humans and other animals in this part of the country [1]. The causal organism is a fungus living in soil and wet, decaying wood, often in an area close to a waterway such as a lake, river or stream [2]. Blastomycosis is generally readily treatable with systemic antifungal drugs once it is correctly diagnosed; however, delayed diagnosis is very common except in highly endemic areas [3].

Blastomycosis, a potentially very serious disease that typically begins with a characteristically subtle pneumonia-like infection that may progress, after 1 - 6 months, to a disseminated phase that causes lesions to form in capillary beds throughout the body, most notably the skin, internal organs, central nervous system and bone marrow [3]. *Blastomyces dermatitidis* is the name applied to the *Ascomycetous fungus, Ajellomyces dermatitidis*. Recently a second species has been described in the genus *Blastomyces, B. gilchristii,* which subsumes certain strains previously assigned to *Blastomyces dermatitidis* [2].

It was observed that the disease is most commonly misdiagnosed as tuberculosis or some other bacteria disease, and the doctor prescribes antibiotics. The bacteria are killed, and the fungus is left with no competition and the infection gets worse. When properly diagnosed, itraconazole can be used on mild cases. More severe cases begin with amphotericin B intravenous treatment for 4 - 10 days followed by itraconazole treatment for 2 - 6 months [4].

An uncommon but very dangerous type of primary blastomycosis manifests as acute respiratory distress syndrome (ARDS); for example, this was seen in 9 of 72 blastomycosis cases studied in northeast Tennessee [5]. Such cases may follow massive exposure, example, during bush clearing operations. The fatality rate in the ARDS cases in the Tennessee study was 89%, while in non-ARDS cases of pulmonary blastomycosis, the fatality rate was 10%. The organism has the potential to affect nearly every organ system during chronic disease [6].

Physical examination may reveal the abnormal breath sounds associated with pneumonia. Skin lesions, typically on the hands, arms, legs, or face, include papules, and later wart-like (verrucous) lesions, or ulcers with small pustules at the margins. Bone or joint infection will produce local tenderness and swelling. Inflammation of the prostate and testicle may be present. *Blastomyces dermatitidis* may be identified by fungal culture or microscopically from sputum or skin lesions. Chest x-ray may reveal infiltrates and pneumonia. X-ray of a bone infection can show areas of bone destruction. A tissue and/ or skin biopsy may reveal evidence of this condition [6]. The aim of this study was the comparative analysis of anti-fungal activities of itraconazole and fluconazole against *Blastomyces dermatitidis*, specifically, with the objectives of isolate and identify *Blastomyces dermatitidis* in wet soil samples and determination of antifungal activities of itraconazole and fluconazole against *Blastomyces dermatitidis.*

Materials and Methods

The materials used in this research include: autoclave, Bunsen burner, retort stands, weighing balance, wire loop, spatulas, hot air oven, beakers, conical flasks, Petri-dish, test tubes, ethanol, Potato Dextrose Agar (PDA), 5 ml syringe and needle, Itraconazole and Fluconazole.

Sample Collection

The wet soil samples were collected using clean container from three different sites which were River rima Sokoto, River Goronyo and fadama soil Sokoto round; into clean polythene bag labelled as A, B, C respectively. The samples were transported to microbiology research laboratory of Usmanu Danfodiyo University Sokoto

Serial dilution

Nine millilitres (9 ml) of distilled water was collected using syringe and needle and was poured into test tube. Another 9 ml of distilled water was poured into second test tube. It was continued up to 9 test tubes. The test tubes were sterilized along with distilled water using the autoclave operating at 121°c for 15 minutes. The test tubes were labelled as follow: A1, A2, A3, B1, B2, B3, C1, C2, C3. Then 1g of the wet soil from sample A was poured into the test tube A1 and it was mixed with 5 ml of syringe and needle. The 1 ml was collected from test tube A1 and was poured into the test tube A2. It was mixed with 5 ml of syringe and needle. Another 1 ml was collected from test tube A2 and was poured into the test tube A3. That is 10^{-1}, 10^{-2}, and 10^{-3} respectively. This was done the same with sample B and C accordingly [7].

Media Preparation

The 8g of PDA powder was weighed using weighing balance according to manufacturer instruction. The weighed PDA powder was introduced into the conical flask. The 200mls of distilled water was measured and poured into conical flask containing powder. The mixture was allowed to dissolve properly and covered with the cotton wool and aluminium foil. The flask was placed on the hot plate to heat the mixture and solubility was ensured. The PDA plate supplemented with 0.6g of streptomycin. The dissolved mixture was autoclave at 121°c for 15 minutes. The dissolved media was allowed to cool and was poured into six Petri-dishes (pour plate) after which were allowed to solidify [8].

Inoculation of Samples

The 0.1 ml of serial dilution of sample A2 (10^{-2}) was withdrawn using 5 ml of syringe and streaked (inoculated) into the Petri-dish labelled as A2 using sterile wire loop. Another 0.1 ml was withdrawn from serial dilution of sample A3 (10^{-3}) and streaked (inoculated) into the Petri-dish labelled as A 3. 0.1 ml was withdrawn from serial dilution of sample B2 (10^{-2}) and streaked (inoculated) into the Petri-dish labelled as B2. Another 0.1 ml was withdrawn from serial dilution of sample B3 (10^{-3}) and streaked (inoculated) into the Petri-dish labelled as B3. 0.1 ml was withdrawn from serial dilution of sample C2 (10^{-2}) and streaked (inoculated) into the Petri-dish labelled as C2. Another 0.1 ml was withdrawn from serial dilution of sample C3 (10^{-3}) and streaked (inoculated) into the Petri-dish labelled as C3. The Petri-dishes were kept in the incubating room for growth to take place at 30°c for 7 days under aseptic conditions. The growth appears in the form of a mould in one week. The colonies may be white to tan in colour, glabrous (smooth) or cottony in texture with varying number of aerial hyphae. The colonies of the yeast form develop within seven days and appear waxy and wrinkled, cream to tan in colour [7].

Processing and Maintaining of Isolates

The colonies that developed after 7days of incubation were continually subculture on PDA until pure culture was obtained. The isolate was maintained on PDA until when required [7].

Microscopic

The drop of iodine was placed on the slide. The small amount of pure growth colour was picked using sterile wire loop and placed on the slide near iodine drop. It was mixed with a drop of iodine. The light smear was made and covered with a cover glass. The wet smear was examined under x 10 and x 40 objective. The hyphae of the mould form are septate, and delicate was observed. In yeast form, the large, thick walled yeast cells with buds attached by a broad base were observed [8].

Sensitivity Test

With a sterile wire loop a loopful growth colours was pick and streaked on the PDA plate and antifungal disk was placed on the streaked plate and incubated at 30°C for few days. Zone of inhibition was used to report sensitivity of antifungal. The zone of inhibition was measured using ruler in millimetre. The larger the measurement, the greater the antifungal activity to the organism depending on the zone created [9].

Results

The table 1 showed that the sample (A) has a highest colony count of (6×10^{-2}) and sample (B) has a lowest colony count of (2.2×10^{-3}) in concentration of (10^{-2}). The sample(C) has a highest colony count of (9×10^{-3}) and sample (A) has a lowest colony count of (2×10^{-3}) in concentration of (10-3). The percentage concentration of (10^{-2}) and (10^{-3}) is 100% respectively. The overall percentage concentration is 200%.

Samples	Number colonies/ concentration CFU/g		Percentage of 10^{-2} %	Percentage of 10^{-3} %	Percentage total %
	10^{-2}	10^{-3}			
A	6×10^{-2}	2×10^{-3}	10.7	11.1	21.8
B	2.2×10^{-3}	7×10^{-3}	39.3	38.9	78.2
C	2.8×10^{-3}	9×10^{-3}	50	50	100
Total	5.6×10^{-3}	1.8500^{-4}	100	100	200

Table 1: showing the number of colonies per concentration.

Key A = Sample soil from River Goronyo; B = Sample Soil from River rima Sokoto; C = Sample soil from Fadama soil Sokoto round.

The table 2 showed non-target organisms and their morphology. The table 3 showed the frequency of occurrence of non-target organisms. The *Aspergillus niger* has a highest occurrence of 9 and follows by Penicillium of number 2 occurrence. The *Aspergillus fumigatus, Aspergillus flavus, Trichoderma harzianum* and *Aureobasidium pullulans* has lowest occurrence of 1. The table 4 shows target organism (*Blastomyces dermatitidis*) which was negative. The organism is not endemic in Sokoto metropolis, may be due to differences of geographical areas. This is because no history of blastomycosis. The only non-target organisms were isolated and identified. they are: *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus Penicillium, Aureobasidium pullulans* and *Trichoderma harzianum*. Therefore, the sensitivity test was not conducted.

S/n	Isolates	Morphology
	Aspergillus niger	Globular and elongated pellets, clumps and free mycelia trees
	Aspergillus flavus	Presence of sclerotia and cleistothecia
	Penicillium	Highly branched network of multinucleate, septate usually colourless.
	Aureobasidium pullulans	Smooth faunt pink, yeast-like colonies.
	Trichoderma harzianum	Concentric rings with green conidial production
	Aspergillus fumigates	Green echinulate conidia

Table 2: Showing the isolates and their morphology.

S/N	Fungal isolates		Frequency of occurrence	Percentage of occurrence (%)
1	Mould	*Aspergillus niger*	9	60.00
		Aspergillus flavus	1	6.67
		Aspergillus fumigatus	1	6.67
		Penicillium	2	13.33
2	Mould	*Trichoderma harzianum*	1	6.67
3	Mould	*Aureobasidium pullulans*	1	6.67
	Total		15	100

Table 3: Showing the distribution of isolates base on frequency of occurrence.

S/n	Samples	Isolated target organisms	Non-Isolated target organisms
	A	Negative	*Aspergillus niger*
			Aspergillus flavus
			Pennicillium
	B	Negative	*Aureobasidium pullulans*
			Trichoderma harzianum
	C	Negative	*Aspergillus fumigatus*

Table 4: Showing target and non-target organisms isolated.

Key A = Sample soil from River Goronyo;
B = Sample Soil from River rima Sokoto;
C = Sample soil from Fadama soil Sokoto round

Discussion

The table 4 shows target 0rganism (*Blastomyces dermatitidis*) which was negative. An uncommon but very dangerous type of primary blastomycosis manifests as acute respiratory distress syndrome [5]. The organism has the potential to affect nearly every organ system during chronic disease [6]. The non-target organisms are: *Aspergillus niger*, has been associated with otomycosis [10], cutaneous infections [11]. and pulmonary disease. *Aspergillus niger* is the most abundant species of *Aspergillus* in nature as it can grow on a large variety of substance [11].

Aspergillus flavus many strains produce significant quantities of toxic compounds known as mycotoxins which when consumed, are toxic to mammals (Agrios and George N 2005). The organism can cause liver cancer through consumption of contaminated feed or aspergillosis through invasive growth [12]. *Aspergillus fumigatus* primarily causes invasive infection in the lung and represents a major cause of these individuals [13]. Additionally, *Aspergillus fumigatus* can cause chronic pulmonary infection [13]. *Penicillium* cause generalized lemphadenopathy, hepatomegaly and splenomegaly. The respiratory system is commonly involved a well, cough, fever, dyspnea and chest pain [14-19].

Conclusion

Blastomycosis which is caused by *Blastomyces dermatitidis* is not endemic in Sokoto metropolis but endemic in the South central, South eastern and Midwestern United states, and Canadian provinces boarding the great lakes. *Blastomyces* infection has also being described in Africa. *Blastomyces dermatitidis* most commonly infects the lungs, followed by skin, bone, prostate and the central nervous system. It occurs more commonly in men than women because of larger of men occupational exposure. The community should be frequently visiting clinic for medical check-up. Based on the above result, it was recommended that people should avoid exposure to endemic areas. The people that had gone to endemic areas should be tested for blastomycosis when returning. The communities or individuals should report to the clinic immediately when there are abnormalities in the body for early recognition and diagnosis for proper treatment.

Bibliography

1. Di Salvo., *et al*. Ecology dermatitidis". Plenum (2010): 43-73.
2. Brown Elizabeth M., *et al*. "Phylogenetic Analysis Reveals a Cryptic Species Blastomyces gilchristii, sp. Nov. within the Human Pathogenic Fungus Blastomyces dermatitidis". *PLoS one* 8.3 (2013): E359237.
3. https://en.wikipedia.org/wiki/Blastomyces_dermatitidis
4. Peterson SW and Sigler L. "Molecular genetic variation in Emmonsia crescens and Emmonsia parva, etiologica gents of adiaspiromycosis, and their phylogenetic relationship to Blastomyces dermatitidis (Ajellomyces dermatitidis) and other systemic fungal pathogens". *Journal of Clinical Microbiology* 36.10 (2008): 2918-2925.
5. Vasquez JE., *et al*. "Blastomycosis in northeast Tennessee". *Chest* 114.2 (2010): 436-443.
6. Chapman SW and Sullivan DC. "Blastomyces dermatidis". In Madell GL, Benett JE, Dolin R, Editors (ed) Principes and practice of infectious diseases, 7th edition Churchill Livingstone, Philadelphia, PA (2010): 3319-3332.
7. Rippon John Willard. Medical mycology: The pathogenic fungi and the pathogenic actinomyctes (3rd Edition) (2008).
8. Chapman SW., *et al*. "Endemic Blastomycosis in Missisippi: Epidemiological and clinical studies". *Seminars in Respiratory Infections - Journals* 12.3 (2012): 219-228.
9. Baker FJ and Silverton RE. "Introduction to medical Laboratory Technology, (sixth edition), Butterularth and Company, London (2011): 51.
10. Araiza J., *et al*. "Otomycosis Clinical and Mycological study of 97 cases". *Revue De Laryngologie - Otologie - Rhinologie - Journals* 127.4 (2006): 251-254.

11. Loudon KW., *et al*. "Kitchens as a source of Aspergillus niger infection". *Journal of Hospital Infection* 32.3 (1996): 191-198.

12. Amaike Saori Nancy P and Keiler. "Aspergillus flavus". *The Annual Review of Phytopathology* 49 (2011): 107-133.

13. Hohi TM and Feldmesser M. "Aspergillus fumigatus, principles of pathogenesis and host defence". *Eukaryotic Cell* 6.11 (2007): 1953-1963.

14. Aftab BT., *et al*. "Itranazole inhibits angiogensis and growth in non-small cell being cancer". *Cancer Research* 71.21 (2011): 6764-6772.

15. Lippman M. "Size-selective health hazard sampling. Pp. In BS Cohen, CS McCammon (eds), Air Sampling Instruments, 9th edition. ACGIH Press (2001) 93-134.

16. Untereiner Wendy A., *et al*. "Naveau Onyegenales". *Mycologia* 96.4 (2004): 812-821.

17. WHO. Model list of essential medicines (PDF) World health organization (2014).

18. Watts EA., *et al*. "First reported case of intrauterine transmission of blastomycosis". *The Paediatric Infectious Disease Journal* 2.4 (1983): 308-310.

19. Xu J., *et al*. "Cholesterol trafficking is required for mTOR activation in endothelial cells". *Proceedings of the National Academy of Sciences* 107.10 (2010): 4764-4769.

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