Screening of Some Antifungal Drugs on Fungal Isolates from Barbering Equipment

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Abstract: This work was aimed at ascertaining the antifungal potency of some antifungal drugs on fungal isolates from barbering equipment. Two hundred (200) samples were collected from barbering equipment pre and post sterilization. Samples were inoculated onto Sabouraud Dextrose Agar and incubated at room temperature for 5 - 7days.Subcultures were carried out to purify the isolates. One hundred (100) isolates belonging to six (6) genera *Trichophyton, Microsporum, Aspergillus, Rhizopus, Candida* and *Scedosporium* were identified. Antifungal drugs fluconazole, ketoconazole, griseofulvin, itraconazole and nystatin of known concentrations were screened *in vitro* on the isolates using the Kirby-Bauer disc diffusion method. The isolates were resistant to fluconazole and griseofulvin. The Minimum Inhibitory Concentrations (MICs) of the effective drugs ranged from 0.15625 to lOmg/ml and Minimum Fungicidal Concentrations (MFCs) from 1.25 to >20mg/ml. Nystatin had different MIC and MFC values. The effective drugs exhibited little activities on the isolates proving their ineffectiveness in treatment.

Keywords: antifungal drugs, fungal isolates, barbering equipment, MIC and MFC

Introduction

Antifungal drugs are medicinal substances used to treat and prevent mycoses. They selectively eliminate fungal pathogens from a host with minimal toxicity to the host [1]. Examples of these drugs include polyenes (nystatin and amphotericin B), azoles (itraconazole, ketoconazole and fluconazole) etc. These drugs can be administered topically, orally or systemically depending on the type of the infection.

Mycoses are infections caused by fungal organisms. They can affect any part of the human body although fungal infections of the skin was the 4th most common disease in 2010 affecting 984 million people [2]. Fungal infection of the skin which extends into the outer layer of the skin is known as dermatophytosis often called ringworm. Dermatophytosis is caused by a variety of dermatophytes belonging to *Trichophyton, Microsporum* and *Epidermophyton* genera. Up to 20% of the population may be infected by ringworm at any given time [3].

All individuals (male and female) have approximately 300,000 hairs on their scalp with a growth rate of approximately half an inch per month [4]. Therefore, they are expected to visit a barbershop at least once a month for a haircut [5] often done without personal barbering equipment. Barbering equipment are tools used by barbers to cut, dress, groom, style and shave hair. This equipment includes electric clippers, scissors, combs, brushes etc.

From 2002 till date, there has been an increase in the establishment of barbering industry, the majority of which is controlled by people with little or no training/knowledge on infection controlled practices [5].

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This contributes to the health hazards associated with the barbering operations. An example of such hazards is the re-use of barbering clippers on a number of clients without appropriate disinfection or sterilization [5] since shared shaving equipment is commonly practiced. Thus, a barbershop could serve as a means of transmission of several communicable diseases of the scalp such as ringworm and dandruff from one client to another. These diseases have been reported in [5] to be fungal infections associated with barbering operations. Although the barbering industry is known for its aesthetic activity, research however shows the possibility of it making its patrons feel sick by acquisition of these contagious diseases according to [6].

Despite the use of antifungal drugs in the treatment of barbershop associated fungal infections, the infections still persist probably because of antifungal drug resistance. As the need to curb antifungal drug resistance cannot be over emphasized, it is therefore the aim of this research to determine the antifungal potency of some antifungal drugs on fungal isolates from barbering equipment, a novel approach and means for further research into antifungal drug resistance.

Materials and Methods

Sample size determination

Twenty (20) barbershops located within four villages (Amudo, Ifite, Umukwa and Umuogbunu) in Awka, Anambra State, Nigeria were randomly selected for this study.

Collection of samples

Sample collection was preceded after an oral assent from the study participants. A total of two hundred (200) samples were aseptically collected using sterile swab sticks from the barbering equipment and

worktops pre and post disinfection/sterilization. Samples were transported to the laboratory without delay for mycological analysis and maintained in the refrigerator at 4°C until they were ready to be analyzed.

Test isolates

Samples were each inoculated onto Sabouraud Dextrose Agar (SDA) (Micromaster Thane, Maharashta, India) plates incorporated with 0.05mg/ml ofchloramphenicol and incubated at room temperature for 3 – 7days.Fungal isolates were further purified by repeated subcultures on SDA and identified as *Trichophyton schoenleinii, Microsporum nanum, M. ferrugineum, Aspergillus niger, A. versicolor, A. nidulans, Rhizopus microsporus, A. glaucus, Candida albicans and Scedosporium apiospermum* based on their morphological and microscopic characteristics according to [7], [8] and [9]. Pure colonies were stored on slants at 4°C for further studies as described by [10]. The frequency of occurrence of the isolates was also calculated.

Preparation of McFarland turbidity standard

A 0.5 McFarland Standard was prepared by adding 0.5ml of 0.048M BaCl2 (1.17% w/v BaCl22H2O) to 99.5 ml of 0.18 M H2SO4 (1% v/v) with constant stirring. A barium sulphate precipitate was checked for optical density using matches curvettes with 1 cm path and distilled water as a blank standard. A UV-Vis spectrophotometer was used to measure the absorbance at 625nm. An absorbance of 0.1 was obtained which was in the accepted range of 0.08-0.13. The approximate cell density corresponding to 0.5 McFarland is 1×106 cells/ml [11].

Standardization of test isolates

Ten-fold serial dilution was carried out on the test organisms (molds) which were previously grown on SDA for 4 days. 0.1ml of the spore suspension of each isolate was taken from the stock culture and serially diluted. 0.1ml of the suspension was plated out by spread plate method for semi-confluent growth. Dilutions which gave working inoculums of 10^6CFU/ml were used. For the yeast isolates, the test organism was inoculated into Sabouraud Dextrose Broth (SDB) and incubated over-night. The resulting turbidity was standardized to 10^6 CFU/ml by matching it to 0.5 McFarland turbidity standard [12].

Antifungal disc preparation

Five antifungal drugs (fluconazole (F), ketoconazole (K), griseofulvin (G), itraconazole (I) and nystatin (N) were purchased from Joez pharmaceuticals at Awka, Anambra State, Nigeria. The antifungal potency of the drugs was established on the isolates. Each of 200mg of K, F, G and I and 500,000IU of N were dissolved homogenously in 200mls of dimethylsulphoxide (DMSO) to obtain 1mg/ml for K, F, G and I and 2,500IU/ml for N. Sterile discs (6mm in diameter) made from Whatman No 1 filter paper were impregnated with 1ml DMSO solution of each drug for 30minutes and allowed to dry at room temperature under aseptic conditions.

***In vitro* sensitivity screening**

This was done according to the method described by [13]. 0.1ml of the standardized test isolates were uniformly spread on labeled SDA Petri dishes with sterile swab sticks. Sterile forceps were then used to place the impregnated discs on the inoculated plates. Two replicates were made for each isolate. Water was used as negative control. The treated and control Petri dishes were incubated at room temperature for 3-7 days. The inhibition zone diameters were measured in (mm) and recorded.

Determination of Minimum Inhibitory Concentration (MIC)

The broth dilution method described by [14], [15] and [16] with slight modifications were employed. Two-fold serial dilutions of the drugs were made in sterile SDB. A total number of twelve test tubes were set up and labeled for each of the test organism. 1 ml of SDB was dispensed into test tubes 1 to 12 each except tube 11 that had 2ml of the broth. From drug stock solutions of 40mg/ml for K, F, G, and I and 100,000IU/ml for N obtained by dissolving 200mg of K,F,G and I and 500,000IU of N in 5mls of DMSO, 1 ml was dispensed into tube 1 and serially diluted up to the 10th tube from which 1ml was pipetted and discarded. The concentrations in the tubes ranged from 20mg/ml - 0.0390625mg/ml for K, F, G and I and 50,000IU – 97.656251U for N. After these dilutions were made, 0.1 ml of each of the standardized test organism was dispensed into tubes 1 to 12 with the exception of tube 11. Tube 11 which contained 2ml of SDB served as a control for the sterility of the medium. Tube 12 with broth cultures of the test organisms served as negative control and viability of the organisms. The tubes were incubated at room temperature for 3 - 7days. All tests were done in duplicates. Test tubes were observed for the presence or absence of growth (turbidity) from the 3rd day. The MIC was regarded as the lowest concentration (highest dilution) of the drug that inhibited visible growth (showed no turbidity).

Determination of the Minimum Fungicidal Concentration (MFC)

The method described by [17] was employed. The MFC was determined by inoculating a loop full of the test tube mixtures that showed no visible turbidity in MIC onto freshly prepared SD Aplates. The plates were incubated at room temperature for 7 days. A negative control with only SDA was also set up to confirm the sterility of the media. The MFC was regarded as the lowest concentration of the drug that did not permit any visible colony growth on the agar medium after the

period of incubation. The MFC test was also done in duplicates.

Experimental Results

A total of one hundred (100) fungal isolates belonging to six (6) genera were recovered. These included *Trichophyton schoenleinii* (20), *Microsporum ferrugineum* (14), *Aspergillus nidulans* (8), *Scedosporium apiospermum* (4), *Rhizopus microsporus* (6), *Aspergillus glaucus* (6), *Microsporum nanum* (15), *Aspergillus niger* (12), *Aspergillus versicolor* (10), *Candida albicans* (5).The frequency of occurrence of the organism was calculated. Molds occurred more than the yeast with a ratio of 9:1. Table 1 shows that *T. schoenleinii* has the highest percentage of occurrence of 20% as against *S. apiospermum* with the least percentage occurrence of 4%.

Table 1: Percentage frequency of occurrence of organisms

Microorganism	Percentage frequency of occurrence (%)
Trichophyton schoenleinii	20
Microsporum ferrugineum	14
Aspergillus nidulans	8
Scedosporium apiospermum	4
Rhizopus microsporus	6
Aspergillus glaucus	6
Microsporum nanum	15
Aspergillus niger	12
Aspergillus. versicolor	10
Candida albicans	5
Total	**100**

All the fungal isolates were resistant to fluconazole and griseofulvin. Ketoconazole, nystatin and itraconazole had effect on some of the isolates as seen in table 2.

Table 2: *In vitro* antifungal activity of drugs against test microorganisms (size of disc 6mm)

Microorganims	Mean Inhibition zone diameter of antifungal drugs (mm)				
	Ketoconazole (1mg/ml)	Fluconazole (1mg/ml)	Griseofulvin (1mg/ml)	Itraconazole (1mg/ml)	Nystatin (2,500IU/ml)
T. schoenleinii	-	-	-	8	-
M. ferrugineum	-	-	-	16	-
A. nidulans	20	-	-	-	-
S. apiospermum	26	-	-	-	14
R. microsporus	-	-	-	10	-
A. glaucus	-	-	-	10	12
M. nanum	18	-	-	-	10
A. niger	-	-	-	14	-
A. versicolor	24	-	-	12	10
C. albicans	28	-	-	12	14

Key

- = No inhibition zone was observed

The mean values of MICs and MFCs of the antifungal drugs are as shown in table 3. Generally, the MIC values of the effective drugs ranged from 0.15625 to 10mg/ml and their MFC from 1.25 to >20mg/ml except for nystatin which had a different unit and recorded its least MIC as 781.25IU and MFC as 1,562.5IU.

Table 3: Mean values of the minimum inhibitory and minimum fungicidal concentration of antifungal drugs on test organisms (mg/ml and IU/ml)

Drugs	Microorganisms									
	T. schoenleinii	*M. ferrugineum*	*A. nidulans*	*S. apiospermum*	*R. microsporus*	*A. glaucus*	*M. nanum*	*A. niger*	*A. versicolor*	*C. albicans*
Ketoconazole										
MIC	*	*	1.25	0.3125	*	*	0.625	*	0.3125	0.15625
MFC	*	*	5	1.25	*	*	2.5	*	2.5	1.25
Itraconazole										
MIC	10	2.5	*	*	5	5	*	5	5	5
MFC	>20	10	*	*	>20	>20	*	10	10	10
Nystatin										
MIC	*	*	*	781.25	*	781.25	781.25	*	1,562.5	781.25
MFC	*	*	*	3,125	*	3,125	6,250	*	25,000	1,562.5

Key * = MIC and MFC tests were not carried out

Discussion

The result of this study has shown that fungal organisms are associated with barbering equipment pre and post disinfection/sterilization. This is not arguable as [5] also isolated *Malassezia* and *Trichophyton* species from barbering clippers before and after the use of disinfectants. This could be attributed to the use of low quality disinfectants or improper sterilization methods. The organisms were identified and their frequency of occurrence determined. Molds occurred more than the yeast with a ratio of 9:1 which is in partial agreement with the work of [18] where the fungal isolates from barbering equipment were all molds with no yeast found. The reason for least occurrence of yeast is not too certain -but could be attributed to the fact that yeast grow typically in moist environments where there is a plentiful supply of simple, soluble nutrients such as sugars and amino acids hence are common on leaf and fruit surfaces, on roots and in various types of food [19]. From the result of this work also, *C. albicans* was the yeast associated with the barbering equipment sampled which is in accordance with the work of [20] who found *M. audouinii* and *C. albicans* to be associated with combs and scissors in barbershops. *T. schoenleinii* had the highest percentage frequency of occurrence of 20% (table 1) and as one of the causative agents of dermatophytosis, is an evidence of the disease presence and transmission among the clients.

Of the five drugs tested on the isolates, only three (K, I and N) had effects on some of the isolates. F and G were ineffective. Resistance of the isolates to F and G could be attributed to the concentrations used which if altered may yield a positive result. The 1mg/ml of the drugs used for the *in vitro* sensitivity screening was to ascertain if a maximum positive result could be achieved with lower concentrations. When compared to the concentration of fluconazole used in the work of [21] which had a lower unit (µg/ml) than the fluconazole used in this study, it was observed that the fluconazole in the work of [21] exhibited activity as it recorded MIC range of 0.25-1.0µg/ml on the *C. albicans* American Type Culture Collection (ATCC)® 90028 used as a reference organism. The resistance of the microorganisms isolated in this research to the tested drugs could also be attributed to the development of antifungal resistance by these organisms due to constant exposure to the antifungal agents. Other antifungal drug abuse could also constitute to the organism's resistance. Generally, the isolates showed poor sensitivity to the effective drugs as seen with some of the agents of dermatophytosis (table 2). This shows that the treatment of dermatophytosis caused by barbershop associated fungal organisms with the antifungal drugs used in this research could be difficult. The MICs and MFCs of the drugs on the isolates are high when still compared with the work of [21] (table 3).

Conclusion

This study has shown that fungal organisms are associated with barbering equipment. Furthermore, the fungal isolates were poorly susceptibility to the tested antifungal drugs showing that clients who could get infected by sharing barbering equipment may not be effectively treated with such drugs and may require further treatments which could be expensive.

It is therefore suggested that clients who patronize barbers should try their best in providing themselves with personal barbering equipment and programs be organized to educate barbers on the health hazard associated with their profession.

References

1. Dixon, D.M. and Walsh, T.J. (1996). Antifungal Agents. In: Medical Microbiology, 4th Edition. National Center for Biotechnology Information, U.S. National Library of Medicine 8600 Rockville Pike, Bethesda MD, 20894 U.S.A. Chp 76.
2. *Hay, R.J., Johns, N.E., Williams, H.C., Bolliger, I.W., Dellavalle, R.P., Margolis, D.J., Marks, R., Naldi, L., Weinstock, M.A., Wulf, S.K., Michaud, C., Murray, C.J.L. and Naghavi, M. (2013). "The Global Burden of Skin Disease in 2010: An Analysis of the Prevalence and Impact of Skin Conditions". The Journal of Investigative Dermatology,134 (6): 1527–34.*
3. *Mahmoud, A. G. and John, R. P. (2009). Antifungal Therapy. CRC Press. p. 258.*
4. Elewski, B.E. (1992). Cutaneous fungal infections. In: Topics in dermatology. Igaku-Shoin, New York and Tokyo.
5. Omoruyi, M.I. and Idemudia, M.I. (2011). Comparative analysis of the antiseptic properties of some disinfectants on bacteria and fungi of public health importance isolated from barbing clippers. *Journal of Asian Scientific Research,***1**(2): 65-68.
6. Kondo, M., Nakano, N. and Shiraki, Y. (2006). A Chinese-Japanese boy with black dot ringworm due to *Trichophyton violaceum*. *Journal of Dermatolology***33**(3):165-8.
7. St-Germain G, Summerbell R (1996). Identifying Filamentous Fungi – A Clinical Laboratory Handbook, 1st Ed. Star Publishing Co., Belmont, California.
8. Ogbo, F.C. (2005). Basic Microbiology: fundamentals and techniques. Cresco printing and publishers Enugu.
9. Ellis, D., David, S., Alexion, H., Handke, R. and Bartley, R. (2007). Description of Medical Fungi 2nd Edn. Women's and Children's Hospital North Adelaide 5006 Australia.
10. Cheesbrough, M. (2003). Distinct laboratory practical in tropical countries, part 2. Cambridge University press, UK.
11. 11. Kyrian-Ogbonna E.A., Nwobu R.A.U., Chidi-Onuorah L.C., Okafor U.C. and Ilikannu S.O. (2016). Crude and ethanol extracts of *Anacardium occidentale* and *Dennettia tripetala*: Effects on oral organisms and possible inclusion in toothpastes. *World Journal of Pharmaceutical Research,* 5(12): 1-11.
12. Riesselman, M.H., Hazen, K.C. and Cutler, J.E. (2000). Determination of antifungal MICs by a rapid susceptibility assay. *Journal of Clinical Microbiology* **38**(1): 333-340.
13. Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turck, M. (1996). Antibiotic susceptibility testing by standardized single method. *American Journal of Clinical Pathology,***45**(4):493-496.
14. Gbodi, T.A. and Irobi, O.N. (1992). Antifungal properties of crude extracts of *Aspergillus quardrilineatus*. *African Journal of Pharmacy and Pharmaceutical Science,***23**(1): 32-33.
15. Ngono Ngane, A., Biyif, L., Amvamzolo, P.H. and Bouchet, P.H. (2000). Evaluation of antifungal activity of extracts of two camerounian Ruthceae zanthoxy lep rieumii Guill and *Zanthoxylum xanthory* loides waterm. *Journal of Ethnopharmacology,***70**: 335-342.
16. Wokoma, E.C., Essien, I.E. and Agwa, O.K. (2007). The *in vitro* antifungal activity of garlic *(Allium sativum)* and onion *(Allium cepa)* extracts against dermatophytes and yeast. *The Nigerian Journal Microbiology,***21**: 1478-1484.
17. Shinkafi S. A. and Manga S. B. (2011). Isolation of dermatophytes and screening of selected medicinal plants used in the treatment of dermatophytoses. International *Research Journal of Microbiology***2**(1): 040-048.
18. Enemuor, S.C., Atabo, A.R. and Oguntibeju, O.O. (2012). Evaluation of microbiological hazards in barbershops in a university setting. *Scientific Research and Essays,*7(9): 1100-02.
19. Jim, D. (2014). The microbial world: yeasts and yeast-like fungi. *Saccharomyces, Crytococcus* and *C. albicans. http://archive.bio.ed.ac.uk/jdeacon/microbes/yeast.htm. Assessed on 26/03/2014.*
20. David, D.L., Edward, A., Zaruwa, M.Z. and Addass, P.A. (2010). Barbing saloon associated fungal disease infection in Mubi, Adamawa State-Nigeria. *World Journal of Medical Sciences,***5** (1): 17-21.
21. Pfaller, M.A., Bale, M. Buschelman, B. *et al.* (1995). Quality control guidelines for National Committee for Clinical Laboratory Standards-recommended broth macrodilution testing of amphotericin B, fluconazole, and flucytosine. *Journal of Clinical Microbiology.* **33**:1104-1107.