Distribution and Fluconazole Susceptibility Pattern of Fungi Isolated from Saloon Equipment in Rivers State, Nigeria

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ABSTRACT

The distribution and susceptibility pattern of fungal isolates from barbing equipment to Fluconazlole in barber shops distributed in three major cities; Bori, Omoku, and Port Harcourt in Rivers State, Nigeria were investigated. The fungal load including dermatophytes on the equipment (brush, clipper and comb) were determined using swab plate technique in which Sabouraud dextrose agar and dermatophyte test medium were used as the culture medium. Inoculated plates were incubated at 25°C. Antifungal Susceptibility testing was carried in which fluconazole was used as the antifungal agent at concentrations of 100mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml concentration using two-fold dilution. The mean range of the fungal counts for clipper, brush and comb were: 34.34x10^2 to 49.0x10^2 CFU/cm^2, 66.95 to 93.26 CFU/cm^2 and 40.09 to 74.61 x10^2 CFU/cm^2, respectively. Brush had the highest fungal counts followed by the comb while the clipper had the least fungal counts. The fungal counts recorded in brushes were significantly higher ($p \geq 0.05$) than those recorded for the clippers. Total of forty-four fungal isolates belonging to six genera and their percentage occurrence include; Aspergillus flavus (11.36%) Aspergillus terrus (11.36%), Fusarium solani (22.72%), Mucor indicus (15.91%), Rhizopus nigricans (15.91%), Trichophyton rubrum (4.54%) and Penicillium italicum (18.2%). The susceptibility pattern showed that except for Trichophyton rubrum, all fungal isolates were completely inhibited at 100mg/ml concentration of fluconazole. Also, except for Mucor indicus, the Minimal inhibitory Concentration of fluconazole was recorded at 12.5mg/ml. The occurrence of these organisms in barbing equipment calls for concern and the need for regulation of the barbing industry to prevent disease outbreaks is recommended.

Keywords: *Barbing Saloon equipment, Fungi, Dermatophytes, Fluconazole Susceptibility*

INTRODUCTION

Fomites are one major source of infectious pathogens. The environment also plays an important role in the transmission of infectious agents with environmental materials serving as vehicles (Stanley e*t al.*, 2019) Saloon equipment can be contaminated by pathogenic fungi and become a potential reservoir for these organisms. There is a high potential for abrasion of skin surfaces for recipients of barbing salon services. The infection that could arise from such abrasions could be transmitted from person to person if disinfection processes are not duly followed (Stanley e*t al.*, 2019).

There is a rise in the number of barbing saloons in Rivers State and it will continue to increase as new saloons are continuously established. Most of these saloon personnel have minimal knowledge on infectious diseases control. Saloons which are known for its aesthetic activity, has been shown by research that it has the the potential of causing illness by the acquisition of contagious diseases after visiting the saloon (Kondo *et al.*, 2006). Known for its aesthetic activity, research however shows the possibility of it making its patrons feel sick by the acquisition of contagious diseases after visiting the saloon (Kondo *et al.*, 2006). Most individuals visit the barbing saloon for a haircut at least once a month because all individuals (male and female) have approximately 300,000 hair on their scalp with a growth rate of approximately half an inch per month (Elewski, 2000). The advent of electric clippers for barbing operation replaced the traditional use of razor blades and other sharp objects following technological advancement (Mackenzie *et al.*, 2005). The inappropriate disinfection or sterilization methods used in many barbing saloons and the re-use of barbing equipment have heightened the concern regarding communicable diseases associated with the scalp.
The use of kerosene, diesel, ethanol, fuel, and other cleaning agents for the sterilization of clippers, combs, and, brushes is common practice among barbers in Nigeria (Kligman *et al.*, 2011). Bacterial and fungal infections such as ringworm, dandruff, other impetigo-like lesions, folliculitis decalvans and chronic scalp folliculitis have been reported to be infections associated with barbering operations. Opportunistic fungi are usually present in non-living cornified layers of the skin and its appendages (Kligman *et al.*, 2011). The health challenges posed by barbing saloons may vary depending on the equipment used, service rendered, and health status of both the patron and service provider. The use and reuse of saloon equipment such as brush, clipper, and comb can expose a client to infection from pathogens due to abrasions as this equipment has been implicated in disease establishment among those that receive saloon services (Elewski, 2000). This study was undertaken to bridge the gap in the information of the diseases associated with saloon equipment and the susceptibility to an antifungal agent.

Materials and Methods

Study Area

Three major towns were selected for this study in Rivers State: Port Harcourt (PHALGA), Omoku (ONELGA), and Bori (KHANA LGA). These towns represent the three senatorial districts in Rivers State and are highly populated in their respective districts. Port Harcourt is the capital and largest city in Rivers State. According to Demogrophia, 2016, the Port Harcourt urban area has an estimated population of 1,865,000 inhabitants. With a population of about

200,000 people, Omoku, a town located in the northern part of Rivers state and bordering with Delta state and Imo state, is the capital of the Ogba/Egbema/Ndoni Local Government Area (Demogrophia, 2016) while Bori is a city in Khana Local Government Area, Rivers State. It is the traditional headquarters of the Ogoni people and the second-largest city in Rivers State after Port Harcourt (Hamilton, 2003).

Sample Collection

One hundred and eighty **s**amples were collected from the barber's shop by swabbing the surface of the cutting edge of the clipper, combs, and brush using sterile moist swab stick (Michael *et al*., 2016). Samples were collected twice a month for two months. Samples were then transferred into sterile tubes containing 1mililitre of sterile distilled water to avoid drying and transported to the laboratory in ice pack containers. The name, source, and location were noted on the swab sticks and brought to the laboratory under sterile/aseptic conditions for microbiological analysis. Saloons that have at least 10 haircuts per day were taken for the study.

Isolation and Characterization of Fungal Isolates

Swab samples were dipped in 10ml of sterile normal saline and subsequently diluted into test tubes containing sterilized 9ml of normal saline to make 10^{-1} to 10^{-4} dilutions. Aliquots (0.1ml) of the dilutions were inoculated using sterile pipette onto Saboraud Dextrose Agar, and onto Dermatophyte Test Medium (DTM) for the isolation of dermatophytes (Elis *et al*., 2007). Plates were inoculated in duplicates and incubated at 25°C (Douglas and Robinson, 2018; 2019) for 2 to 5 days (Elis *et al*., 2007). After incubation, pure cultures of fungal isolateswere obtained by aseptically inoculating representative colonies of different morphological types on the culture plates onto freshly prepared Saboraud Dextrose Agar and Dermatophyte Test Medium plates and incubated at 25^{0}C for 2 to 5 days. The isolates were identified based on macroscopic characteristics (growth characteristics, pigment formation, texture) as well as microscopic morphology (formation of macroconidia and microconidia or other typical elements). The microscopical identification was done by lactophenol cotton blue mounts. In this method, a drop of lactophenol cotton blue was placed on a grease-free slide and the aerial mycelium of the investigated fungal isolates was cut and transferred into the drop of lactophenol cotton blue on the slide using a sterile inoculating needle. The slide was covered with a microscope coverslip and viewed under the x10 and x40 magnification lens of the compound microscope (Robinson, 2021). Characterization of fungal isolates was drawn from matching results with those reported by Mcdonald *et al.* (2000) and Elis *et al.* (2007).

Antifungal Susceptibility Test

The well in agar method on Saboraud dextrose Agar plates was used in evaluating the antifungal susceptibility pattern of the fungal isolates. Fluconazole (200mg) antifungal agent was used. Obire and Ogbonna (2017) method was adopted for antifungal testing. In this method, four concentrations of 100mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml of the drug were prepared using a two-fold dilution method. Plates were incubated at 25^{o}C for 72 hours. The reporting was done to indicate the presence or absence of fungal growth.

Results

The results obtained in the three locations and from clipper, brush and comb are summarized in Table 1. The mean fungal counts for clipper, comb and brush ranged from $3.43x10^3$-$4.90x10^3$, 4.01×10^3 - 7.51×10^3 and $6.75x10^3$ – 9.33×10^3CFU/cm^2 respectively. The fungal counts in the brushes were higher than the fungal counts in the comb and clippers but the clippers had the least fungal counts for all locations.

Results of the fungal distribution across the saloon equipment are presented in figure 1. Results showed 44 fungal isolates belonging to six genera; *Aspergillus*, *Fusarium*, *Mucor*, *Trichophyton*, *Penicillium* and *Rhizopus*. The percentage occurrence in the study locations includes; *Aspergillus flavus*(11.36%) *Aspergillus terrus* (11.36%), *Fusarium solani* (22.72%), *Mucor indicus* (15.91%), *Rhizopus nigricans* (15.91%), *Trichophyton rubrum* (4.54%), and *Penicillium italicum* (18.2%), respectively. Results also showed that in Bori, for brush, *Aspergillus flavus* and *Penicillium italicum* had the highest level of occurrence. For clipper, *Aspergillus terrus* had the highest and for comb, *Penicillium italicum* occurred more. In barbers shop at Omoku, *Aspergillus flavus* occurred more in the brush. For clipper, *Aspergillus terrus* occurred more and for comb, *Fusarium solani* occurred more. In barbers shop located in Port Harcourt, *Aspergillus flavus* and *Mucor indicus* had the highest occurrence. For clipper, *Fusarium solani* had the highest occurrence and for comb, *Rhizopus nigricans* had the highest occurrence.

Table 1: Mean Fungal Population (CFU/cm^2) in Saloon Equipment from Barbers' shop in Bori, Omoku and Port Harcourt respectively.

Saloon equipment	THF(x10^{3}Cfu/cm^2)	THF(x10^{3}Cfu/cm^2)	THF(x10^{3}Cfu/cm^2)
Clipper	3.43±5.4^a	4.90±2.46^a	4.57±1.46^a
Brush	6.75±1.50^b	9.33±1.30^b	7.98±3.22^a
Comb	4.01±5.4^b	5.30±1.31^a	7.51±2.49^a

*Means with the same superscript across the column shows no significant difference at $p \geq 0.05$
THF- Total Heterotrophic Fungi

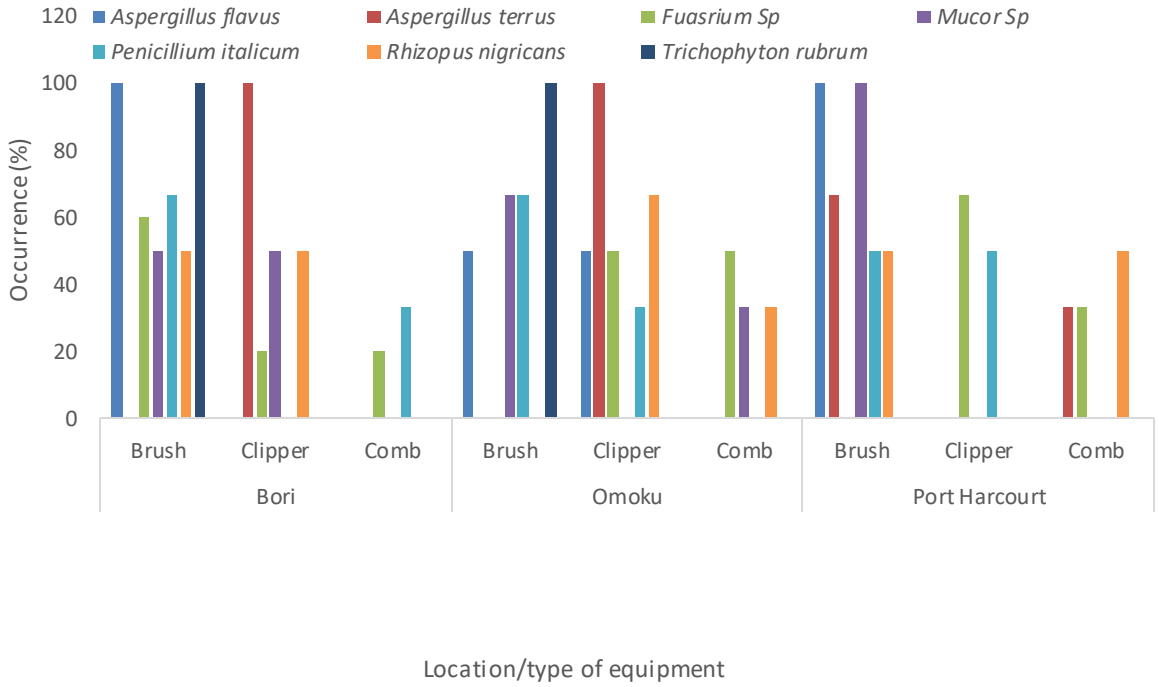

Figure 1: Percentage Occurrence of Different fungal isolates From Saloon Equipment from the Different Locations

Results of the antifungal susceptibility of fungal isolates from barbers' shop in Bori is presented in Table 2. Results showed that with the exception of *Trichophyton rubrum* which was resistant to the antifungal agent, *Penicillium italicum*, *Aspergillus* spp, *Fusarium solani*, *Mucor indicus*, and *Rhizopus nigricans* showed no growth at 100mg/ml concentration of the drug which means that they were susceptible to the drug. At 50mg/ml, only *Rhizopus nigricans*, *Aspergillus flavus*, and *T. rubrum* showed the presence of growth. Most of the isolates showed the presence of growth at 25mg/ml and 12.5mg/ml indicating a minimal inhibitory concentration of 50 percent for most of the isolates. Only *T. rubrum* could not be inhibited by any concentration of the drug

Table 2: Susceptibility pattern of Fungal Isolates from Bori to Different Concentrations of Fluconazole (200mg)

Isolates	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	MICmg/ml
Penicillium italicum	-	-	+	+	50
Penicillium italicum	-	-	+	+	50
Aspergillus flavus	-	-	-	+	50
Fusarim solani	-	-	+	+	50
Fusarium solani	-	-	+	+	50
Fusarium solani	-	-	-	+	25
Aspergillus terrus	-	-	+	+	50
Mucor indicus	-	-	-	+	25
Rhizopus nigricans	-	+	+	+	100
Fusarium solani	-	-	-	+	25
Aspergillus flavus	-	+	+	+	100
Fusarium solani	-	-	-	+	25
T. rubrum	+	+	+	+	-
Mucor indicus	-	-	-	+	25
Penicillium italicum	-	-	+	+	50
Rhizopus nigricans	-	-	+	+	50

N/B + = presence of growth, - = absence of growth

MIC- Minimal Inhibitory Concentration

Results in Table 3 show the sensitivity results for fungal isolates from Omoku. The result showed that with the exception of *Trichophyton rubrum*, other fungal isolates were completely inhibited at 100mg/ml concentration of the drug. At 50mg/ml, only *Rhizopus nigricans*, *Aspergillus flavus*, and *T. rubrum* showed the presence of growth. Only *Penicillium italicum*, *Mucor indicus*, *Fusarium solani*, and *Aspergillus terrus* isolates showed an absence of growth at 25mg/ml. Most of the isolates showed the presence of growth at 50 and 12.5mg/ml, indicating a minimal inhibitory concentration of 50 mg/ml for most of the isolates. Only *T. rubrum* could not be inhibited by any concentration of the drug.

Table 3: Susceptibility pattern of Fungal Isolates from Omoku to Different Concentrations of Fluconazole (200mg)

Isolates	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	MICmg/ml
Penicillium italicum	-	-	-	+	25
Fusarium solani	-	-	+	+	50
Rhizopus nigricans	-	+	+	+	100
Rhizopus nigricans	-	-	+	+	50
Mucor indicus	-	-	-	+	25
Aspergillus flavus	-	+	+	+	100
Penicillium italicum	-	-	+	+	50
T.rubrum	+	+	+	+	-
Aspergillus flavus	-	-	+	+	50
Mucor indicus	-	-	-	+	25
Fusarium solani	-	-	-	+	25
Aspergillus terrus	-	-	-	+	25
Rhizopus nigricans	-	-	+	+	50
Mucor indicus	-	-	+	+	50
Penicillium italicum	-	-	+	+	50

+ = presence of growth, - = absence of growth

MIC- Minimal Inhibitory Concentration

Results in Table 4 show the sensitivity results for fungal isolates from barbers' shops in Port Harcourt. The result showed that all the fungal isolates were inhibited at 100 and 50mg/ml of the drug concentrations. Only *Mucor indicus*, *Penicillium italicum* and *Rhizopus nigricans* isolates showed the presence of growth at 25mg/ml. Most of the isolates showed the presence of growth at 12.5mg/ml indicating a minimal inhibitory concentration of 25mg/ml for most of the isolates.

Table 4: Antifungal sensitivity of Fungal isolates from barbers' shops in Port Harcourt

Isolates	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	MICmg/ml
Mucor indicus	-	-	+	+	50
Aspergillus flavus	-	-	-	+	25
Rhizopus nigricans.	-	-	+	+	50
Penicillium italicum.	-	-	+	+	50
Aspergillus terrus	-	-	-	+	25
Fusariumsolani	-	-	-	+	25
Aspergillus terrus	-	-	-	+	25
Mucor indicus	-	-	-	-	25
Fusarium solani	-	-	-	+	25
Rhizopus nigricans	-	-	-	+	25
Penicillium italicum	-	-	+	+	50
Aspergillus terrus	-	-	-	+	25
Fusarium solani	-	-	-	+	25

+ = presence of growth, - = absence of growth

MIC- Minimal Inhibitory Concentration

Discussion

The study has shown the prevalence of opportunistic fungi in saloon equipment across the three sampling locations. Across the three locations, the brush had more fungal isolates than clipper and comb. This suggests that perhaps, more individuals share brushes than either comb and clipper. This outcome might also suggest that apart from the clipper, less attention is paid to other equipment. The isolation of species of *Rhizopus*, *Aspergillus*, *Mucor*, and *Fusarium* is in agreement with the study of Obire *et al.* (2010). These species of fungi are mostly opportunistic pathogens and could cause illness when they have access to the body through cuts or abrasions as is mostly the case for patrons of barbing saloons. Fewer isolates were gotten from comb which could be associated with its less useful in most saloons.

The low incidence of *Trichophyton rubrum* across the three locations compared to other fungal isolates is indicative that maybe most dermatophyte infections are not a result of sharing saloon equipment. However, necessary precautions must still be put in place to prevent the spread of infectious diseases. *Trichophyton rubrum* is a dermatophyte and has been implicated as one of

the leading causes of dermatophyte infectionin children (Elewski, 2000). Saloon operators in Rivers state need to pay more attention and increase the hygiene level in their various establishments. The thought that only clippers require attention should be discarded and appropriate disinfection measures should be applied on all equipment. The patrons of these establishments should also take precautions in getting their own equipment, especially brush and clippers as this would reduce their chances of getting infected after haircuts. The need for regulation in this industry by the government cannot be over-emphasized as the public deserve knowledgeable and well-trained personnel to man these saloons to avoid a public health crisis.

Due to the rising trend in fungal infections especially from barbers' shop, there is a need to assess the antifungal therapy to determine if commonly used antifungal agents are still potent in treating infections. The antifungal sensitivity of the fungal isolates associated with salon equipment from respective barbers' shop in Bori, Omoku and Port Harcourt showed varied responses to the fungal isolates to fluconazole at different concentrations. More so, the antifungal activity of fluconazole decreased with decreased concentration. This suggests that fluconazole was more potent to the fungal isolates at higher concentrations than at low concentrations. All the fungal isolates except *T. rubrum* (isolated from Omoku and Bori) were completely inhibited by the antifungal drug at 100% concentration despite the different locations. Fluconazole is among the azole antifungal agents that acts by inhibiting the enzyme CYP P450 14 α-Demethylase which converts lanosterol to ergosterol (Joanne *et al.*, 2011; Kathiravan *et al.*, 2012). They also affect the integrity of fungal membranes, altering their morphology and inhibiting growth (Kathiravan *et al.*, 2012; Tatsumi *et al.*, 2013). Thus, its antifungal activity on the fungal isolates in the present study could be attributed to the inhibition of the aforementioned enzymes thereby preventing the conversion of lanosterol to ergosterol. The antifungal activity of fluconazole and ketoconazole on *A. niger, A. flavus, Candida*, and *Mucor indicus* has been reported in a previous study (Robinson *et al.*, 2020). In this study, they reported that the antifungal agents were very effective against the fungal isolates at higher concentrations and that the effect decreased at lower concentrations, this corroborates with the present study. Although, the resistance observed in the present study by these fungal isolates does not agree with their findings. Resistance of fungal isolates to commonly used antifungal agents is increasing and is becoming a global problem (Ana *et al.*, 2015). The fungal isolates in the current study were resistant to fluconazole at 12.5% while some species of *Aspergillus* were resistant at 50% concentration. The mechanisms of resistance of *Aspergillus* sp to the azole antifungal agent has been attributed to alterations in the coding region of the cyp51A gene (positions G54, G138, M220, G448) or due to an insertion of a 34 to 36 base pair tandem repeat in the promoter region of the gene, including point (Ana *et al.*, 2015). This suggests that other fungal isolates which showed resistance to fluconazole could have altered their coding regions or modified their antifungal binding sites thereby preventing the expression of the antifungal activity. The exploitation of the azole antifungals in agricultural services has been reported to be the cause of resistance to the antifungal agents as well as resistance to triazole (Snelders *et al.*, 2009).

Conclusion

The study has shown that various species of fungi are associated and can be isolated from saloon equipment in Rivers State. The species of fungi isolated in course of the study are mostly non-

dermatophytes but could become opportunistic pathogens that could cause skin infections in humans. The diseases caused by these fungal species are contagious and can be spread from person to person, especially with the sharing of unsterilized and not properly sterilized saloon equipment. The only dermatophyte isolated from this study is *Trichophyton rubrum*. Since the fungal species isolated from this study can be transmitted from person to person and being that most individuals share saloon equipment, individuals should learn to use their equipment, especially brushes and clippers. Policy makers and government authorities must begin to consider the saloon industry a public health concern and take appropriate measures to forestall an outbreak of disease.

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