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Antifungal Activity Assessment of Selected Locally Sold Over-The-Counter Azole against Candida Isolates from Hospital and Community Settings of Rivers State, Nigeria

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Abstract
There seems to be multiple factors that could affect the performance of a drug which may range widely from measurements to packaging, storage, handling and standardization. *Candida* species are ubiquitous in nature and are found at different parts of the body, with some present as normal flora. However, drug abuse as mostly associated with the conscious intake of drugs without the guide or prescription of a physician, poses enormous challenge to personal and public health. Hence, this study was designed for comparative analysis of the antifungal activity of over the counter azole and standardized fluconazole disc on the *Candida* species isolated from community and hospital settings in Rivers State. All *Candida* isolates were inoculated onto a Sarbouraud dextrose agar plate to obtain a pure culture and then used for normal saline microscopy, germ tube test and carbohydrate assimilation tests to confirm *Candida* species. Antifungal susceptibility testing using the Kirby Bauer disc diffusion method was carried out and the data generated were type-set in Microsoft Excel version 2003, and transferred into statistical package for social sciences (SPSS), IBM version 21 for statistical analysis. The study results showed 59 *Candida* isolates with 11.9% *Candida* isolated testing negative to germ tube test while 88.1% of the tested positive to germ tube test. However, distribution of germ tube positive *Candida* species from hospital and community settings showed that the community setting had 90.6% germ tube positive *Candida* isolates and 9.4% germ tube negative *Candida isolates* while hospital settings showed 85.2% germ tube positive isolates and 14% germ tube negative isolates. In this study, 28.6% Non-albicans *Candida* (NAC) were susceptible to clotrimazole, 14.3% NAC were susceptible dose dependent while 57.1% were resistant to clotrimazole. However, fluconazole recorded 0% susceptibility dose dependence by Non-albicans *Candida*, 42.9% susceptibility and 57.1% resistance. Similarly, 63.5%, 15.4%, and 21.2% of *Candida albicans* were susceptible, susceptibility dose dependent and resistant respectively to fluconazole. While, 30.8%, 34.6% and 34.6% of *Candida albicans* were susceptible, susceptibility dose dependent and resistant to itraconazole. In conlusion, fluconazole had the best efficacy on non-albicans Candida while clotrimazole was best for killing *Candida albicans* It is believed that a change in attitude from self medication is very crucial as it remains a key factor that could be responsible for increased incidence of *Candida* species' resistance to azole therapy. Furthermore, change towards adherence to antifungal drug regimes when encouraged and cultivated as a positive habit for all patients, could enhance monitoring of drug efficacy and clinical/treatment outcomes.

Introduction

There are multiple factors that could affect the performance of a drug which may range widely from measurements to packaging, storage, handling and standardization. However, the actual consumer of the product bears the impact of any abnormality in performance of a drug product and so should remain the center of focus for any drug manufacture/ formulation strategy. Thus, antifungal resistance may be as a consequence of irregular intake of substandard drugs formulated with inappropriate quantity of the active ingredient sufficient to kill or inhibit the growth of the infecting fungus (Michael *et al*., 2008). Hence, quality control and quality assurance can play a major part in the internal quality monitoring and precision assessment of manufacturing, packaging and storage of azoles; bearing in mind their importance in the treatment of fungal infections (Anna and Brown, 2001).

Candida species are ubiquitous in nature (Pam *et al*., 2012) and are found at different parts of the body (Jumbo *et al*., 2010), with some present as normal flora (Abbey, 1995; Pam *et al*., 2012). *Candida species* belong to the order Saccharomyces (Abbey, 1995) and *Candida albicans* is considered the most frequently isolated fungal pathogen of the class blastomycetes (Abbey, 1995; Al-akeel *et al*., 2013). Nevertheless, we considered the fact that drug abuse mostly associated with the conscious intake of drugs without the guide or prescription of a physician, poses enormous challenge to personal and public health (UNICEF, 2008). Hence, azole, comprising of a five-member nitrogen heterocyclic ring compound containing at least one non-carbon attachment which could be either sulfur, nitrogen or oxygen (Eicher and Hamptmann, 2003), has been at the center of recent discuss on antifungal resistance by certain fungal pathogens including *Candida* species due to abuse.

In addition, previous research reports have recorded changing epidermiology of *Candida* infections

from different parts of the world (Pam *et al*., 2012). For instance, in Nigeria, Nwosu *et al*, (2001) in a study on patients with AIDS from 3 private medical laboratories reported vaginal candidiasis in 34.8% as the commonest genital infection. Also, Oyelese *et al*., (2005) in Ile-Ife from a review of findings from patients suspected to have sexually transmitted diseases, reported *Candida albicans* in 24% of the patients and also as the commonest agent of sexually transmitted infections. While, Akerele *et al*.,(2002) in Benin-city working on samples from antenatal women reported *Candida albicans* in 65% of the samples and it was also recorded as the commonest agent causing genital infection in antenatal women. However, the most common predisposing risk factors for candidiasis as outlined by Pam *et al*.,(2012) are pregnancy, age and parity, history of the use of broad spectrum antibiotics and high estrogen content of oral contraceptives used by women for birth control.

Materials and Methods

This study was designed for comparative analysis of the antifungal efficiency of locally sold azoles with a standard disc manufactured by Oxoid Ltd on *Candida* isolates from the University of Port Harcourt Teaching Hospital, Choba, Port Harcourt. / / All *Candida* isolates were inoculated onto a Sarbouraud dextrose agar plate to obtain a pure culture and then used for normal saline microscopy, germ tube test and carbohydrate assimilation tests to confirm *Candida* species and antifungal susceptibility testing using the Kirby Bauer disc diffusion method according to Ochei and Kolhartar, (2001). This research was carried out between July and December, 2015.

Exclusion Criteria

Candida isolates from patients who are using or have used antifungal drugs less than 14 days before submitting their samples for laboratory processing were excluded from being part of this study.

Inclusion Criteria

*Candida i*solates obtained from samples of persons who have not used antifungal drugs for a period of 14 days or more before submitting their samples for laboratory processing were included as part of this study.

Categorization Criteria for *Candida* Isolates into Settings

Candida isolates from samples gotten from persons who have not visited the hospital or are admitted into a hospital facility or employed as staff of a hospital/laboratory/biomedical facility for a period not more than 48 hours before sample collection, were classified as community settings *Candida* isolates. Whereas *Candida* isolates obtained from patient samples produced by patients who had been admitted into a hospital/biomedical facility or were staff working in a hospital/biomedical facility or patient relatives who were care givers to a patient admitted into a hospital/biomedical facility, for more than 48 hours were classified as hospital setting isolates.

Specimen Collection

Candida isolates from samples obtained from routine laboratory sample from the University of Port Harcourt teaching hospital form the core of this research.

Sample Processing

The inocula were prepared by growing the various *Candida* isolates on separate sarbouraud dextrose agar plates for purity which was then used for normal saline microscopy, germ tube test and carbohydrate assimilation tests to confirm *Candida* species and susceptibility testing using the Kirby Bauer disc diffusion method.

Preparation of Susceptibility Disc

The drugs used for this experiment were bought from pharmacy stores in Port Harcourt and dissolved in sterile distilled water in a sterilized test tube to the required concentration. These drugs include fluconazole 50mg capsule (Drugfield pharmaceuticals limited, Nigeria), ketoconazole 200mg tablet (Hovid Bhd-Malaysia), clotrimazole (Drugfield pharmaceuticals limited- Nigeria), and itraconazole 100mg (Hanmi pharmaceutical company limited-Korea). A Whatman filter paper 3 is perforated to 10mm diameter, placed in a glass Petri dish with lid closed, is sterilized by autoclaving at 15psi for 15 minutes. The Petri dish and the sterilized perforated Whatman filter paper were allowed to dry in a hot air oven at 45^{o}C for the azole drug soaked perforated Whatman paper to dry. When dried, they were then used as antifungal susceptibility disc for each azole having a known quantity of the antifungal agent. Each fluconazole disc contained 25µg/l, clotrimazole disc 25µg/l, ketoconazole disc 25µg/l, and itraconazole 20µg/l.

Susceptibility Testing Procedure (Kirby Bauer's Disc Diffusion Method)

Two to three colonies from the pure culture plate were transferred with a sterilized inoculating loop into 3ml of

sterile normal saline broth in a sterilized bijou bottle. The densities of these suspensions were adjusted to 0.5 McFarland standards. The surface of Sarbouraud dextrose agar plate was evenly inoculated with the organisms using a sterile swab dipped into the suspension and pressed against the side of the test tube to remove excess fluid. Using the wet swab inoculation was done in the Sarbouraud agar by evenly streaking across the surface of the plate with the swab three times, turning the plate at angle 60^{o} between each streaking. Then, the inoculated plates are allowed to dry for 10 minutes with the lid in place. By means of a sterilized forceps and different antifungal azole discs were applied onto the surface of the inoculated sarbouraud agar and the plates were incubated overnight at 37^{o}C. The zone of growth inhibition's diameter was observed and measured and compared to that of the control. All measurements are made with the unaided eye while viewing the back of the Petri dish and holding the plate a few inches above a black, non-reflecting surface illuminated with reflected light. The plate is viewed using a direct, vertical line of sight to avoid any parallax that may result in misreading the zone sizes are recorded on the recording sheet but growth up to the edge of the disk were reported as a zone of 0 mm.

Data Grouping and Analysis

The interpretive breakpoints for *Candida* species tested against control disc (25µg fluconazole) were based on the analysis of treatment outcomes of infections as determined in 2006 by Pfaller *et al*., (2006). From this study, however, the antifungal activity of azoles and the control were grouped into three; resistance (R≤14mm), and susceptible (S≥19mm) and medium susceptibility or susceptibility dose dependence (SDD>14mm<19mm) adopted in line with previous studies by Pam *et al*., (2012). The data generated from this study were type set in Microsoft Excel, version 2003, and transferred into statistical package for social sciences, IBM version 21, where the data were analyzed using different statistical tools like pie charts, graphs, bar charts and Chi-square tests all enshrined in SPSS IBM version 21.

Results

The study results for the 59 *Candida* species used showed in figure 1, the germ tube test result. 11.9% *Candida* isolates tested negative to germ tube test while 88.1% tested were positive to germ tube test. In this study, 28.6% Non-albicans Candida (NAC) were susceptible to clotrimazole, 14.3% NAC were susceptible dose dependent while 57.1% were resistant to clotrimazole. However, fluconazole recorded 0% susceptibility dose dependence by Non-albicans Candida, 42.9% susceptibility and 57.1% resistance. Also, 28.6%, 0% and 71.4% of non-albicans Candida were susceptible, susceptibility dose dependent and resistance respectively to ketoconazole. Nevertheless, itraconazole had 0% susceptibility by non-albicans Candida, 71.5% susceptibility dose dependent and 28.5% resistance by non-albicance Candida. Nevertheless, *Candida albicans* from this study showed 71.1% susceptibility to clotrimazole, 5.8% susceptibility dependence and 23.1% resistance to clotrimazole. Similarly, 63.5%, 15.4%, and 21.2% of *Candida albicans* were susceptible, susceptibility dose dependent and resistant respectively to fluconazole. While, 30.8%, 34.6% and 34.6% of Candida albicans were susceptible, susceptibility dose dependent and resistant to itraconazole. Also, the susceptibility of Candida albicans to ketoconazole was 53.8% while 23.1%, and 23.1% of *Candida albicans* used in this study were susceptibility dose dependent and resistant respectively. It further showed clotrimazole resistant group 16(27.1%), susceptible dose dependent 4(6.8%) and susceptible group 39(66.1%) while ketoconazole had a prevalence for resistant group as 27.1% (16), susceptible dose dependent group 25.4% (15) and susceptible group as 47.5% (28) itraconazole showed 50.8% resistance, 37.3% susceptibility dose dependence and 11.9% susceptible to tested *Candida* species. From this study, fluconazole showed 25.4% resistance, 20.3% susceptibility dependence and 54.2% susceptible to the 59 *Candida* species used for this study.

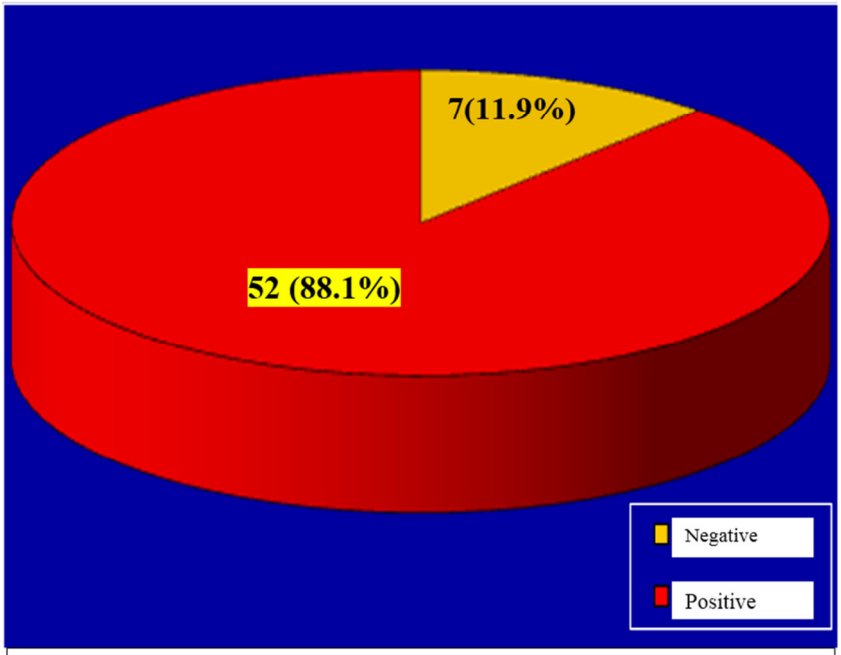

Figure 1: Germ Tube Test Result for the 59 Candida Isolates Used in this Study

Table 1: Frequency Distribution of Different Azoles and Control to *Candida* Isolales from Hospital and Community Settings of Rivers State

		Frequency (%)	Mean ± SEM
Control Group	Less than/Equal 14mm (Resistant)	2(3.4)	
	>14<19mm (SDD)	7(11.9)	2.8±0.1
	Equal/Greater than 19mm (Susceptible)	50(84.7)	
Clotrimazole	Less than/Equal to 14mm (Resistant)	16(27.1)	
	<19>14mm (SDD)	4(6.8)	2.4±0.1
	Greater than/Equal to 19mm (Susceptible)	39(66.1)	
Ketoconazole	Less than/Equal to 14mm (Resistant)	16(27.1)	
	<19>14mm (SDD)	15(25.4)	2.2±0.1
	Greater than/Equal to 19mm (Susceptible)	28(47.5)	
Itraconazole	Less than/Equal to 14mm (Resistant)	30(50.8)	
	<19>14mm (SDD)	22(37.3)	1.6±0.1
	Greater than/Equal to 19mm (Susceptible)	7(11.9)	
Fluconazole	Less than/Equal to 14mm (Resistant)	15(25.4)	
	<19>14mm (SDD)	12(20.3)	2.3±0.1
	Greater than/Equal to 19mm (Susceptible)	32(54.2)	

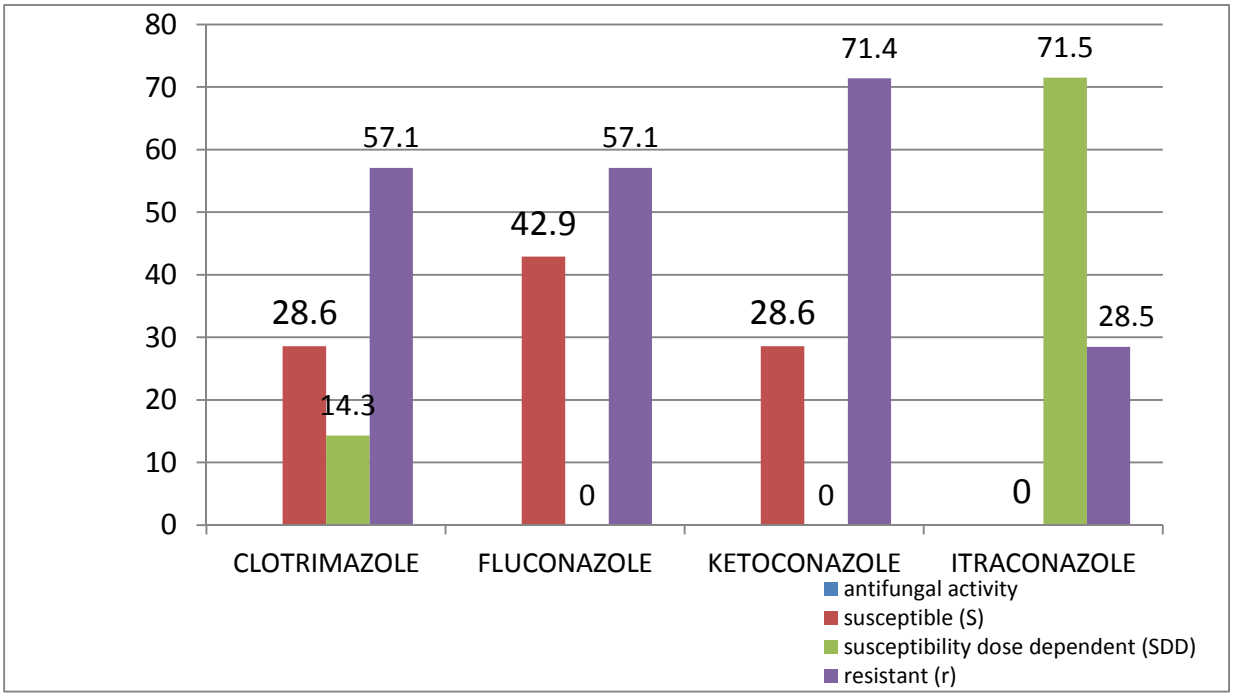

Figure 2: Cummulative Antibiogram of Non-albicans Candida (N=7)

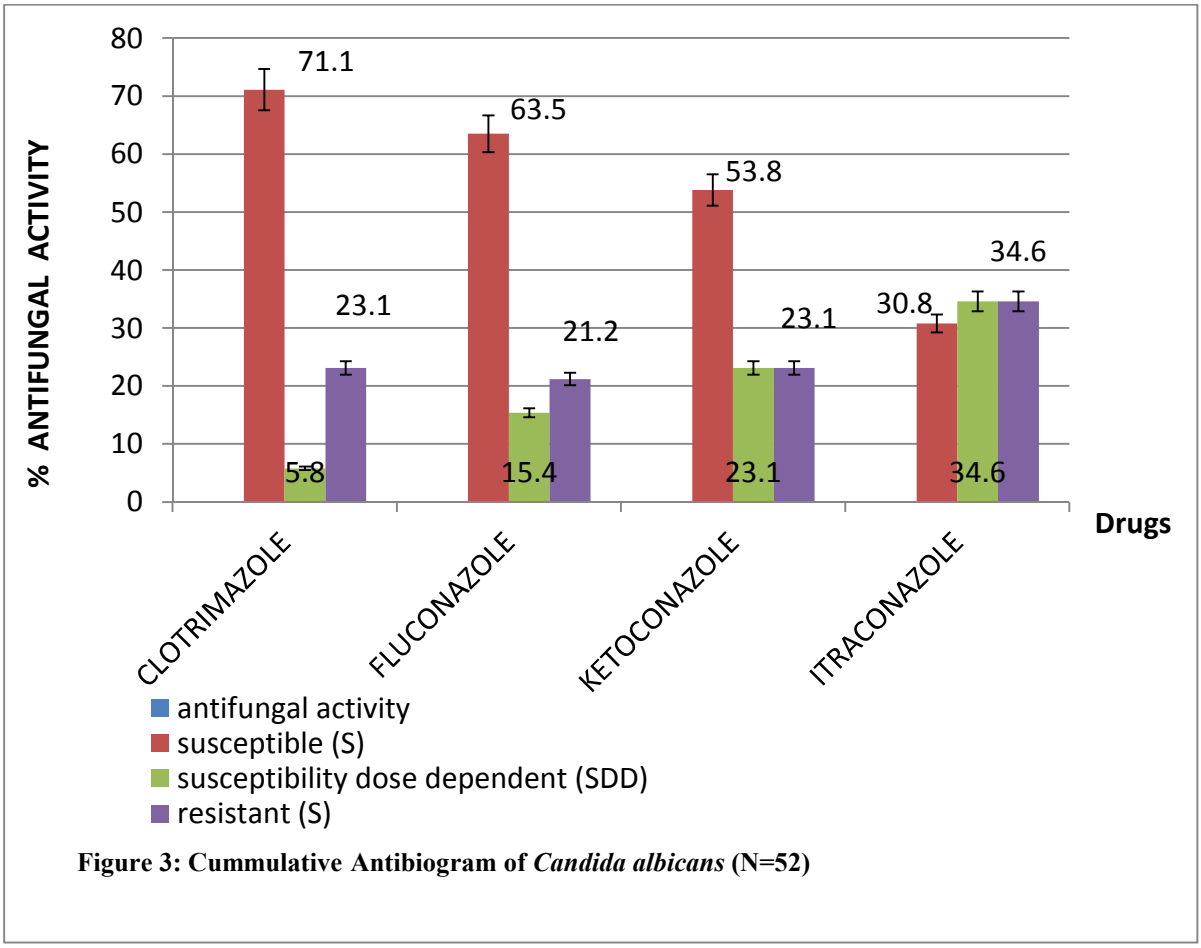

Figure 3: Cummulative Antibiogram of *Candida albicans* (N=52)

Discussion

Antifungal susceptibility testing as an important diagnostic procedure in the management and monitoring of *Candida* infections is the responsibility of a good medical mycologist or medical microbiologist; to determine

the susceptibility of any *Candida* isolate to antifungals. This study attempts to evaluate the antifungal efficacy of four azoles including two imidazoles (ketoconazole and clotrimazoles) and two triazoles (fluconazole and itraconazole), in relation to the activity of fluconazole disc prepared by Oxoid Limited and used as control and the standard for antifungal activity comparison. For control disc susceptibility, 84.7% of the 59 *Candida* species were susceptible while 11.9% were susceptible dose dependent and 3.4% became resistant to the control disc's active drug ingredient. Comparing the control disc antifungal activity with that of the prepared sensitivity disc from over the counter drugs shows that clotrimazole disc exhibited a greater antifungal activity against *Candida* species. That is, 66.1% of the 59 *Candida* species showed susceptibility to clotrimazole while only 47.5% were susceptible to ketoconazole. This shows that clotrimazole is more efficient than ketoconazole in the killing or inhibition of growth of *Candida* species and that imidazoles studies exhibited less susceptibility percentage compared with the control disc. Nevertheless, imidazoles used in this study showed a higher resistance percentage compared with the control disc's 3.4% resistance antifungal activity as 27.1% and 50.8% of the total 59 *Candida* species were resistant to clotrimazole and ketoconazole respectively. There was observed difference in both susceptibility and resistance percentages between the above described results and the research reported by Badiee and Alborzi (2011). Perhaps, medium susceptibility or susceptibility dose dependence of *Candida* species to azole appears to be increasing depending on the drug used as this implies that the *Candida* isolate would require a much higher dose of the drug in question before effective killing or growth inhibition can occur.

Similarly, this study showed different susceptibility percentage for both fluconazole and itraconazole as 54.2% of the total 59 *Candida* species used in this study were susceptible and a further 20.3% were susceptible dose dependent to fluconazole which was low compared to the 84.7% susceptibility and 11.9% susceptibility dose dependence observed for the control disc group and other researchers reported by Pfaller *et al.*,(2003), Bauters *et al*., (2002) and Citak *et al.,* (2005) which reported a susceptibility percentage of 87%, 79% and 87.5% respectively. However, itraconazole group showed that 11.9% and 37.3% of the total 59 *Candida* species tested were susceptible and susceptible dose dependent to itraconazole. This suggests a trend of increasing percentage of itraconazole antifungal activity against *Candida* species. This is comparable with published research report by Badiee and Alborzi (2011) and inconsistent with the control disc trend as shown in this study.

In this study, 28.6% Non-albicans Candida (NAC) were susceptible to clotrimazole, 14.3% NAC were susceptible dose dependent while 57.1% were resistant to clotrimazole. However, fluconazole recorded 0% susceptibility dose dependence by Non-albicans Candida, 42.9% susceptibility and 57.1% resistance. Also, 28.6%, 0% and 71.4% of non-albicans Candida were susceptible, susceptibility dose dependent and resistance respectively to ketoconazole. Nevertheless, itraconazole had 0% susceptibility by non-albicans Candida, 71.5% susceptibility dose dependent and 28.5% resistance by non-albicans *Candida*. This showed that itraconazole had the least efficacy in killing/growth inhibition of Non-albicans *Candida* species while fluconazole had the highest efficacy for killing/growth inhibition of Non-albicans *Candida.* Nevertheless, *Candida albicans* from this study showed 71.1% susceptibility to clotrimazole, 5.8% susceptibility dependence and 23.1% resistance to clotrimazole. Similarly, 63.5%, 15.4%, and 21.2% of *Candida albicans* were susceptible, susceptibility dose dependent and resistant respectively to fluconazole. While, 30.8%, 34.6% and 34.6% of Candida albicans were susceptible, susceptibility dose dependent and resistant to itraconazole. Also, the susceptibility of Candida albicans to ketoconazole was 53.8% while 23.1%, and 23.1% of *Candida albicans* used in this study were susceptibility dose dependent and resistant respectively. It is important to highlight here that, clotrimazole showed a better efficacy against *Candida albicans* than fluconazole, itraconazole and ketoconazole against Candida albicans.

Furthermore, this study results have shown that fluconazole among triazoles, was an effective drug for killing/growth inhibition of *Candida* species but with variation in the susceptibility percentage observed when comparing the antifungal susceptibility across hospital and community settings with itraconazole showing the highest susceptibility dose dependence among the community *Candida* isolates (41.9%) and the least susceptible community *Candida* isolates (25.8%) while hospital *Candida* isolates showed 35.7% of susceptibility and 35.7% susceptibility dose dependence. This was consistent with the report of Badiee and Alborzi (2011) which reported itraconazole as having the least susceptibility to *Candida* species. Moreover, there was observed a trend among the *Candida* species studied towards itraconazole resistance more than what was observed for fluconazole. This observation agreed with the findings of Pam *et al*.,(2012). While comparing fluconazole disc activity with that of the control disc, it could be argued that, since the standard control disc and the self prepared fluconazole disc obtained from the local pharmacy store were prepared to have the same quantity of the active drug fluconazole and they were used on the same set of *Candida* isolates, the inconsistency in results observed among the discs prepared from locally sold azoles and the control disc prepared by Oxoid ltd, may have arisen due to differences in standard quantity and quality of the active drug agent. However, it could also be argued that it could have been as a result of locally isolated *Candida* tolerance of the over-the-counter fluconazole. Also, this could be attributed to the growing trend of *Candida* species with genes that code for resistance against azoles in *Candida*

species due to prior exposure of these species to the locally sold brands of fluconazole. Nevertheless, it could have also been as a result of the effect of variations in manufacturing standards between the tests and control antifungal discs under comparison. While the Oxoid manufactured fluconazole disc high susceptibility percentage observed from this present study, could be attributed to the high standards, safety and precision in measurements during production, the same cannot be said of the manually produced azole discs as human error, obsolete equipment, epileptic power supply and the lack of conducive working environment could have contributed in no small measure to their declining susceptibility compared to that of the control.

Moreover, a comparative review of the susceptibility data of azoles against *Candida albicans* from this study showed that *Candida albicans* were more susceptible to clotrimazole than any other over-the-counter azole used in this study. Whereas, fluconazole showed the least percentage resistance by *Candida albicans* and itraconazole produced the highest percentage resistance by *Candida albicans*. Nevertheless, the reasons for the observed variations between individual azole disc antifungal activity in this study with those of Badiee and Alborzi (2011) and Pam *et al*.,(2012) was not known parse but could be attributed to difference in methodology and research protocol, measurements and the research environment.

Interestingly, Non-albicans *Candida* species showed a trend of high susceptibility dose dependence to azole antifungal drugs tested but increased resistance to azoles, with the highest seen among the ketoconazole group while statistical analysis of the antifungal activity of the azoles and the control disc using three different chi-square tests revealed that there was a significant likelihood ratio of clotrimazole antifungal activity to that of the control disc with P_{value}=0.003 at 95% confidence level. Whereas, there was also observed a significant probability (P_{value}=0.000) of associating the antifungal activity of the clotrimazole disc to that of the control disc used on the *Candida* isolates.

This study showed that antifungal manufactured in Nigeria (Drugfield pharmaceuticals limited- Nigeria) and clotrimazole (Drugfield pharmaceuticals limited- Nigeria) showed better efficacy against the tested *Candida* isolates than those azoles manufactured in Korea and Malaysia ; ketoconazole 200mg tablet (Hovid Bhd-Malaysia), and itraconazole 100mg (Hanmi pharmaceutical company limited-Korea). This discovery may have been a resultant effect of transportation or due to inferior standard of these drugs imported into Nigeria. It could also be due to the choice of azole selected from the products of these countries concerned showing their resultant variation in efficacy. Although, it was also observed that the Nigerian made drugs were more expensive than the foreign ones, they were still the best option in the treatment of candidiasis and related yeast infections going by the results of this study. Nevertheless, it is a known fact that business merchants in their bid to cut cost and make profit in a very competitive market, negotiate with foreign companies to lower their drug standards for their specific orders. This is an act of criminality, cowardice and genocide, as such; all parties who are involved in this devilish act should be tracked, halted, their goods constificated and punished by the law to serve as a deterrent for other dubious business minds. Also, stiffer policies on trans-border trades are needed to help arrest such menace especially in developing and third world countries with large volume of imports of consumable goods. This will help save lives and as well protect public health.

Furthermore, efficiency assessment of the four azoles used in this study using the Fisher's exact indicated that clotrimazole was the most efficient (P_{value}=0.000) in killing or growth inhibition of tested *Candida* isolates which could be as a result of their restricted usage in the treatment of superficial fungal infections due to its high degree of systemic toxicity (Pfizer, 2004). Our findings from this study showed that there was no significant impact of settings on the antifungal activity of azoles against *Candida* species. Also, there was an observed increase in the ability of *Candida* species to resist the antifungal activity of azoles with *Candida albicans* being less susceptible to triazoles than imidazoles. Thus, this study revealed a change in azole susceptibility towards resistance with increased susceptibility dose dependence observed from this study. This could have significant impact on the health of patients with candidiasis as treatment outcomes will suggest the prescription of higher azole doses for patients or change of therapy, which may predispose patients to multiple drug resistance and other undesirable side effects.

Conclusion

This study showed that antifungal manufactured in Nigeria, fluconazole (Drugfield pharmaceuticals limited-Nigeria) and clotrimazole (Drugfield pharmaceuticals limited- Nigeria), showed better efficacy against the tested *Candida* isolates than those azoles manufactured in Korea and Malaysia; ketoconazole 200mg tablet (Hovid Bhd-Malaysia), and itraconazole 100mg (Hanmi pharmaceutical company limited-Korea).Also, clotrimazole was best for killing/growth inhibition of Candida albicans than fluconazole, ketoconazole and itraconazole while fluconazole had the best efficacy against Non-albicans candida species. However, with the above situation of re-emerging data on antifungal resistance by *Candida* species and knowing the relevance of time kill assay in assessing antibacterial activity of certain antimicrobial agents, one can quickly conclude that, the time is ripe for innovative researches in time killing inhibitory properties of defined antifungal agents like frequently used azole drugs against *Candida* species. From this present study, clotrimazole was generally

adjudged the most efficient azole for killing or growth inhibition of *Candida* isolates obtained from different settings. However, while clotrimazole showed a better antifungal activity than fluconazole, ketoconazole and itraconazole respectively against community setting *Candida* isolates, fluconazole was best for killing/inhibiting growth of hospital settings *Candida* isolates while itraconazole drifted more towards resistance than any other azole used in this study.

Finally, the current trend in which empirical therapy is administered to patients with candidiasis without recurs for antifungal susceptibility testing should be reviewed as arguments from this study suggests that it could be a factor responsible for increased incidence of resistance to antifungal therapy by *Candida* species. Thus, antifungal susceptibility testing should be adopted as a policy in government and private health institutions to enhance patient drug monitoring and clinical outcomes. In the same vain, policies should be formulated, strengthened implemented on appropriate antifungal drug standards for manufacturing, storage and transport from one place to another. Such policies when formulated should make provision for appropriate laboratory equipment/facilities necessary for its implementation in accredited/affiliated government or private health institutions and corporate medical laboratories. Nevertheless, a change in attitude from self medication could also be very crucial in arresting this trend as it remains a key factor that could be responsible for increased incidence of *Candida* species' resistance to azole therapy. Therefore, a change towards adherence to antifungal drug regimes when encouraged by all stake holders (health personnel, patient relatives/ care givers and health institutions) and cultivated as a positive habit for all patients would enhance the monitoring of drug efficacy and clinical/treatment outcomes. Hence, the domestication of medicines around the globe will in no small measure impact positively on the national economics of individual countries and also improve research for new drugs, healthcare delivery, patient clinical outcome and reduce trade imbalance, most especially in favor of developing and third world countries.

Research Prospects/Recommendation for Further Study

No research is considered detailed enough to capture all variables necessary to explain a given phenomenon. As such, this study deems it necessary to make recommendations for further studies in molecular properties of *Candida* species' susceptibility and resistance against each tested azole in this study as well as comparative time killing properties of *Candida* species isolated from hospital and community settings based on their antifungal activity against frequently used azoles.

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