EMERGING AZOLE RESISTANCE AMONG *CANDIDA ALBICANS* FROM CLINICAL SOURCES IN NAIROBI, KENYA

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Abstract
In the recent years the number of serious opportunistic yeast infections, particularly in immunocompromised patients has increased significantly. The increased incidence of these infections has paralleled the emergence of HIV/AIDS pandemic that result in lowered resistance of the host. Azole antifungal agents has been extensively used in the management of these yeast infections. *Candida albicans* is one of the most frequently isolated yeasts in clinical laboratories and accounts for up to 80 % of the yeasts recovered from sites of infection. The study was set out to determine antifungal susceptibility of clinical isolates of *Candida albicans* and to establish the Minimum Inhibitory Concentrations (MIC) to Fluconazole, Clotrimazole, Nystatin and Amphotericin B. Laboratory based experiment were conducted at Mycology Laboratory, Kenya Medical Research Institute, Nairobi, Kenya. One hundred and thirty clinical isolates of *Candida albicans* were subjected to antifungal susceptibility testing. Susceptibility to Fluconazole, Clotrimazole, Nystatin and Amphotericin B was done using Broth Microdilution Technique with reference to Clinical laboratory Standard Institute (CLSI). The investigations showed that 16/130 (12.3 %) of the *Candida albicans* isolates were resistant (MIC ≥ 64 µg/ml) to fluconazole, 121/130 (93.1 %) of the isolates had an MIC ≤ 1 µg/ml to Amphotericin B. Of the isolates 51/130 (39.2 %) had MIC> 1 µg/ml to clotrimazole whereas 109/130 (83.8 %) of the isolates had MIC ≤ 1 µg/ml to nystatin. The study showed elevated MICs among *Candida albicans* isolates to fluconazole and clotrimazole and calls for constant antifungal resistance surveillance especially in the context of fluconazole maintenance treatment for opportunistic infections in HIV/AIDS.

Key words:

1.0 Introduction

The last two decades have seen a steady increase in the incidence of systemic opportunistic fungal infections especially in sub-Sahara Africa (Selik *et al.,* 1987). This rise is associated with Acquired Immunodeficiency Syndrome (AIDS) pandemic, prolonged antimicrobial therapy, invasive procedures and immunosuppressive therapy (Frye *et al*., 1988; Hazen, 1995; Reef and Mayer, 1995). Invasive fungal infections, particularly those caused by *Candida* and Cryptococcus species are emerging significant opportunistic organisms that have increased over the past few decades (Kremery and Barnes, 2002). These infections are major complications in immunocompromised patients as well as those with profound neutropenia due to hematological malignancies or chemotherapy usually associated with high mortality (Hikey, 1983; Harvey and Myres 1987).

The available treatment for fungal infections includes azoles (fluconazole, ketoconazole, voriconazole and clotrimazole) and polyene antifungals such as Nystatin, and Amphotericin B. In the recent past, there have been reports of emerging resistance among *Candida* species to some of the antifungal drugs (Bii *et al*., 2002; Seibold and Tintelnot 2003; Bii *et al*., 2006). This has made it necessary to isolate, identify yeasts from clinical sources and to determine and monitor antifungal resistance. The present study aimed at determining the current levels of MICs to the four commonly used antifungal drugs that are essentially used for clinical management of patients susceptible to opportunistic pathogens.

2.0 Materials and Methods

A total of 150 isolates of *Candida* species recovered from blood, sputum, swabs, urine and catheter tips isolated between 1997 - 2004 were used. The isolates were culture collections at Mycology Laboratory, Kenya Medical Research Institute from various health Institutions in Nairobi, Kenya. Azole resistance in yeasts is estimated at 11.0 % (Bii *et al.,* 2006). 1t was therefore necessary that a sample size expected to detect 89 % of non-azole resistant *Candida* be used. The formula according to Fischers *et al.*, (1998) was used to calculate and arrive at a sample size of 150 isolates.

Isolation of *Candida albicans* was done using Sabouraud Dextrose Agar (SDA, Oxoid LTD Basingstoke, Hampshire, England) supplemented with 2 % Chloramphenicol. The Germ Tube Test (GTT) was used for presumptive identification of *Candida albicans.* GTT negative yeasts were further identified on slide culture using chlamydospore formation on Corn Meal Agar incorporated with Tween 80 and ChromAgar Candida (Johnson and Nickson, 1970; Pfeller, 1996). Confirmation of the isolates was done using Analytical profile index (API 20 C aux). The procedures were done according to the manufacturers instructions. The susceptibility tests and Minimum Inhibitory Concentration (MIC) were determined as recommended by Clinical Laboratory Standards (CLSI) and (NCCLS, 2002). The MIC was scored as

the lowest concentration that significantly inhibited fungal growth. MIC_{50} was calculated as the minimum inhibitory concentration reached by 50 % and MIC_{90} as the manimum inhibitory concentration reached by 90 %.

3.0 Results

C. albicans susceptibility to fluconazole (MIC ≤ 8 µg/ml) was 95/130 (73.1 %), susceptible dose-dependent (MIC 16-32 µg/ml) 14.6 % and resistant (MIC ≥ 64 µg/ml) 12.3 %. The MIC_{50} and MIC_{90} to fluconazole were 4 µg/ml and 64 µg/ml respectively. At 1 µg/ml of Amphotericin B, 121/130 (93.1 %) of the isolates were inhibited by only 9/130 (6.9 %) showing MIC of ≤ 1 µg/ml. The MIC_{50} and MIC_{90} to Amphotericin B were 0.25 µg/ml and 1.0 µg/ml respectively. The MIC_{50} and MIC_{90} of clotrimazole were 1 and 16 µg/ml and nystatin 0.25 and 2 µg/ml respectively.

Table: Results of the response of 130 Clinical isolates of Candida albicans to common antifungals

Class of antifungal compounds	**Drug**	**Tested Concentration range (ug/ml) and number of isolates inhibited (%)**				
Azoles		0.03-0.25	0.5-1	2-8	16-32	≥64
	Clotrimazole	39(30)	40(30.8)	35(27.0)	16(12.2)	-
	Fluconazole	40()	35()	20()	19(14.6)	16(12.3)
Polyenes						
	Nystatin	76(58.3)	33(26.9)	14(10.8)	5(3.8)	-
	Amphotericin B	89(68.5)	32(24.6)	6(4.6)	3(2.3)	-

-Beyond tested concentration

4.0 Discussion

In the recent years the number of opportunistic yeast infections, particularly in immunocompromised patients has dramatically increased (Richardson and Warnock, 2003). Among them are *Candida* species especially *Candida albicans*. The higher incidence of fungal infections in hospitalized patients has resulted to the use of systemic antifungal agents especially fluconazole, which remains a first line antifungal agent of choice (Redding et *al*., 1994). Another reason for the increased Candida infections is due to more aggressive cancer and post-transplantation chemotherapy and the use of antibiotics, cytotoxins, immunosuppressives, corticosteroids and other macro disruptive procedures that result in lowered resistance of the host leaving the opportunistic *Candida albicans* to flourish.

Out of the 130 *Candida albicans* isolates sampled in this study, 73.1 % of *Candida albicans* were susceptible to fluconazole (MIC ≤ 8 µg/ml) and 12.3 % were resistant. This is in agreement with previous studies whereby fluconazole resistant *C. albicans* accounts for 9-12 % of the isolates from HIV-Positive patients (Newman, 1994; Wheat, 1998). Since *C. albicans* is a natural part of the intestinal and vaginal flora, drugs that keep candidiasis at its check may never completely eradicate it. The continued presence of the organism during treatment makes the emergence of drug resistant fungal organisms more likely. As the prophylactic use of azoles increases so is the likelihood of increased azole resistant *Candida* (Smith and Aronson, 1992; Hazen, 1995).

This study revealed that 39 % of *Candida albicans* had an MIC of ≥ 1 µg/ml to clotrimazole which is an indication of resistance to the drug. Clotrimazole has been shown to inhibit all the major fungi causing systemic infection, at a concentration of 1µg/ml, with efficacy against *Candida, Histoplasma and Aspergillus* species (Nyst *et al.*, 1992). Although favorable results from systemic treatment of candidiasis and aspergillosis have been described, most *C. albicans* still show high MICs to clotrimazole. Induction of low blood pressure and toxicity are some of the draw backs for its systemic use. Despite the high MIC to clotrimazole, the drug is extensively used in Kenya for management of vaginal candidiasis and for dermatological conditions. Azole antifungal agents have five membered organic rings that contain either two or three nitrogen molecules (imidazole and triazoles respectively) and are thought to inhibit cytochrome P_{450} dependent enzyme involved in the biosynthesis of all membrane sterols. The clinically useful imidazoles are clotrimazole, miconazole and ketoconazole. Two important triazoles are itraconazole and fluconazole. Similar mode of action implies that if *Candida* becomes resistant to one antifungal, cross resistance is possible with related antifungals with the same mode of action (Krcmery and Barnes, 2002).

Majority of the *Candida albicans* isolates were susceptible to Amphotericin B with only 6.9 % of the isolates having an MIC ≥ 1 µg/ml. Amphotericin B is still widely used as the drug of choice for most fatal disseminated fungal infections. However the high cost of the drug makes it unaffordable to the majority of patients especially in the developing world. It should also be noted that amphotericin B can only be administered in low doses due to its toxicity. Its perenteral administration in poor resource setting is also a challenge.

Most of the *Candida albicans* isolates tested were susceptible to nystatin with 80 % of isolates having an MIC range of 0.07-1.15 µg/ml. Nystatin has been considered effective against *Candida* oesophangitis. However a cure rate of only less than 10 % has been reported in Zaire and 21.6 % in Uganda (Nyst *et al.*, 1992; Ravera *et al.*, 1999). Amphotericin B and nystatin belong to polyene antifungal drugs. Polyene compounds interact with sterols to form channels through the membrane causing the cells to leak. However the toxicity of these compounds

limits their use and also the correlation between *in vitro* and *in vivo* studies (Seibold and Tintelnot, 2003).

5.0 Conclusion

The study revealed increased MICs to azoles among *Candida albicans* species which is an indication of resistance. This calls for regular monitoring of resistance levels of *Candida* species to the antifungals particularly azoles. This is particularly important given the increased prophylactic use of fluconazole among the rising numbers of HIV/AIDS infections. Additionally, management of *Candida albicans* using antifungals with different modes of action is recommended especially with HIV/AIDS patients.

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