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ABSTRACT

Objective: **To determine the susceptibility of clinical isolates of *Candida albicans* and to establish the minimum inhibitory concentrations (MIC) to commonly used antifungal drugs.**
Design: **Laboratory based experiment.**
Setting: **Mbagathi District Hospital, Nairobi, Kenya.**
Subjects: ***Candida albicans*** **isolated between 1998 and 2000 from the sputa of HIV/AIDS patients and throat swabs of children with acute respiratory infections (ARI).**
Methods: **Susceptibility to amphotericin B, clotrimazole, nystatin, and 5-fluorocytosine was done using agar dilution method (NCCLS 1997).**
Results: **Among the ARI isolates 29.3% and among HIV isolates 22.4% had MIC>0.5 µg/ml to amphotericin B. Over 80% of the ARI isolates had MICs>1 µg/ml to clotrimazole. The MIC range of most isolates to nystatin was 4-16 µg/ml while most isolates were susceptible to 5-fluorocytosine. There were no significant differences in susceptibility between ARI and HIV isolates to commonly used antifungal drugs.**
Conclusion: **Although fungal resistance has not been extensively studied, susceptibility tests showed some *Candida albicans* have increased MICs to commonly used antifungal drugs. The results call for further investigations on fungal resistance especially in the context of opportunistic infections in HIV/AIDS.**

INTRODUCTION

The world is currently faced with emerging and re-emerging infections and increased resistance to commonly used and inexpensive antibiotics(1). The HIV/AIDS pandemic has played a major role in the emergence of new opportunistic pathogens among which are those of fungal nature. Fungal infections particularly those due to yeast have significantly increased and the species diversity has been modified significantly(2). Approximately 10-15% cases of septicaemia seen in a tertiary hospital care are caused by *Candida* spp(3). Systemic candidosis is the commonest of the invasive mycoses with *C. albicans* being the main causative agent although other *Candida* spp are becoming increasingly significant(4). These have made it necessary to identify yeasts to species, and serotype level and to constantly monitor for antifungal drug resistance. Cases of endocarditis, meningitis and disseminated infections caused by amphotericin B resistant *Candida* spp have been reported(5).

Antifungal drug sensitivity profiles is not routinely carried out and therefore, the present status of fungal resistance to conventional antifungal drugs is unknown. Treatment of oropharyngeal candidiasis is by use of one per cent nystatin and clotrimazole. Though resistance to these drugs is becoming increasingly evident in clinical practice, the clinical efficacy of commonly used antifungal agents has not been studied in Kenya. The present study therefore aimed to determine the current MICs of commonly used antifungal drugs on *Candida albicans.*

MATERIALS AND METHODS

Candida albicans were isolated from the oropharynges of children aged under five years with acute respiratory infectionss (ARI) in Mbagathi District Hospital, Nairobi. *C. albicans* were isolated from the sputa of HIV positive adult patients in a cohort, established in Nairobi. Isolation was done using Sabouraud's dextrose agar (SDA) incorporated with two per cent 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 Tween 80 (6). Confirmation of the isolates was done using *Candida* Check (Iatron Laboratories, Tokyo, Japan) and Chrom agar medium (Sanofi diagnostic reagent, Paris)(7). The susceptibility tests and minimum inhibitory concentrations (MIC) were done as recommended by NCCLS(8). The Minimum Inhibitory Concentrations (MIC) was scored as the lowest concentration that significantly inhibited fungal growth. The MIC levels of oropharyngeal ARI isolates were compared with those of isolates from sputum of HIV positive patients.

RESULTS

From a total of 90 isolates investigated, 41 (45.6%) were from oropharynges of children under five years of age with acute respiratory infections and 49 (54.4%) were from the sputum specimens of HIV positive adult patients.

Table 1

Results of susceptibility of clinical isolates of Candida albicans to common antifungal drugs

Antifungal drug	No. of isolates in (a) ARI (n=41) (b) HIV (n=49)	No (%) with MIC ≤0.5 µg/ml	MIC ≥1 µg/ml
Amphotericin B	ARI	29 (70.7%)	12 (29.3%)
	HIV	38 (77.6%)	11 (22.4%)
Clotrimazole	ARI	7 (17.1%)	34 (82.9%)
	HIV	16 (32.7%)	33 (67.3%)
Nystatin	ARI	28 (68.3%)	13 (31.7%)
	HIV	30 (61.2%)	19 (38.8%)
		MIC ≤16 µg/ml	MIC ≤32 µg/ml
5-fluorocytosine	ARI	37 (90.2%)	4 (9.8%)
	HIV	46 (93.9%)	3 (6.1%)

Candida albicans isolates from oropharynges of children with ARI were 70.7% susceptible to amphotericin B (MIC ≤0.5 µg/ml). The isolates from HIV positive patients showed similar trend in MIC distribution as that from ARI specimens, with over 70% of the isolates having MIC ≤0.5 µg/ml (Table 1). There was no significant difference in susceptibility between isolates from ARI and those from HIV positive patients (p=0.62). Susceptibility of *C. albicans* isolates to clotrimazole indicated that isolates had MIC above the documented effective dosage by recording 82.9% for ARI and 67.2% for HIV isolates with MIC ≥1 µg/ml (p=0.15) (Table 1). Most of the isolates from both ARI and HIV patients (>60%) had MIC <0.5 µg/ml to nystatin, but only 31.7% and 38.8% of ARI and HIV isolates, respectively had MIC ≥1 µg/ml (Table 1). There was no significant difference between susceptibility of ARI and HIV isolates to nystatin (p=0.6). Out of all isolates from ARI and HIV patients, more than 90% of them had MICs below 16 µg/ml to 5-fluorocytosine (Table 1) while only 9.8% and 6.1% respectively, had MIC ≥32 µg/ml (p=0.81).

DISCUSSION

Multi-drug resistant micro-organisms are becoming a major challenge worldwide. The problem is attributed to irrational use of antibiotics and the increasing population of immuno-compromised individuals. Although multi-drug resistant bacterial pathogens has been the main focus in the past, infections by yeasts account for 10-15% of hospital infections(3,9) and represent an important proportion of opportunistic pathogens that may acquire resistance. Approximately 30% of ARI and HIV positive *C. albicans* isolates had MIC>0.5 µg/ml to amphotericin B, that is, the drug of choice for the management of fatal disseminated fungal infections. However, the high cost of the drug makes it unaffordable to the majority of the 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. The rise in MIC to amphotericin B is therefore a big challenge in management of life threatening infections caused by fungi. Some strains of *Candida* spp are naturally resistant to amphotericin B. The implication is that the isolation, identification and constant monitoring of resistance of the aetiological agent is critical for the clinical management of opportunistic mycoses.

Although the -azole group of antifungals has been known to be effective against *Candida* infection, azole resistance among *Candida* spp is increasing especially with increasing *Candida* infections in HIV/AIDS patients and the extensive use of broadspectrum antibiotics (10-12). Clotrimazole part of a long series of tritylimidazole derivatives has been shown to inhibit all the major fungi causing systemic infection, at a concentration of 1 µg/ml, with efficacy demonstrated against *Candida, Histoplasma* and *Aspergillus* species(13). Although favourable results from systemic treatment of candidiasis and aspergillosis have been realised, most of the *C. albicans* isolates show high MICs to the drug. Induction of low blood pressure and toxicity are some of the draw backs for its systemic use. However, a one per cent topical cream application for dermatomycoses and a 100 mg pessary for vaginal candidiasis are still widely used. In the present study, although clotrimazole is not prescribed for oral candidiasis, the MIC of *Candida albicans* isolated from the oropharynx is quite high. Despite the high MIC to clotrimazole, the drug is extensively used in Kenya for vaginal candidiasis and for dermatological conditions. From the present study, over 82.9% of ARI isolates and 67.3% of HIV isolates had MIC >1 µg/ml to clotrimazole which is an indication of resistance to the drug. Although clotrimazole is a commonly used drug for the management of vaginal candidiasis and *Candida* colonisation, the clinical efficacy has not been studied in Kenya.

Although oral nystatin has been considered effective against *Candida* oesophangitis(14), the MIC range of all *Candida albicans* isolates for nystatin was between 4-16 µg/ml, way beyond the recommended effective concentration. However, its clinical efficacy has not been studied, especially in immunocomprised individuals in Kenya. A cure rate of less than 10% in Zaire has been reported(14) and 21.6% in Uganda(15). Whether the low cure rates are due to increase in MIC to nystatin as shown in the current data is subject to further controlled *in-vitro* and clinical studies. Although standardised limits for antifungal drug susceptibility are yet to be set, over 90% of both oropharyngeal ARI and HIV isolates had MIC <16 µg/ml to 5-fluorocytosine. However, 21.2% of all the isolates had MIC >32 µg/ml (Table 1).

CONCLUSION

This study observed an increased MIC to commonly used antifungal drugs among clinical isolates of *C. albicans* which calls for more investigations and constant surveillance for fungal resistance in clinical practice.

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