Research Paper Medical Science

Antifungal Susceptibility Profiles of Candida Albicans Isolated from University of Nigeria Teaching Hospital.

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ABSTRACT

Candida albicans is the most common species causing candidiasis particularly in immune compromised individuals as opportunistic mycoses. Five hundred samples were obtained from patients with apparent symptoms of candidiasis and 252(50.4%) isolates were confirmed to be Candida albicans. Disk diffusion method was performed using seven antifungal agents: Ketoconazole (Keto), Itraconazole (Itrac), Voriconazole (Vori), Miconazole (Micoz), Ciclopirox (Ciclo), Terbinafine (Terbi) and Fluconazole (Flucz). The rate of susceptibility of isolates to each drug was as follows: Keto (58.0%), Itrac (50.0%), Vori (60.0%), Micoz (38.0%), Ciclo (24.0%), Terbi (12.0%) and Flucz (48.0%). Voriconazole showed greater activity than Cicloporox and terbinafine (P=0.022 and P=0.001), respectively. However, the activity of Vori was not significantly higher than those of Keto, Itrac, Micoz and flucz (P=0.138, 0.451, 0.193 and 0.459), respectively.

KEYWORDS Candida, albicans, antifungal, susceptibility

Introduction

There are several species of Candida capable of causing candidiasis (candidosis, moniliasis or oidiomycosis). Candida species are yeast like fungi that can form true hyphae and pseudohyphae and are confined to human and animal reservoirs and frequently recovered from the hospital environment (Brooks *et al*., 2004). Being that they exist as normal flora of the skin, mucus membranes and gastrointestinal tract, they colonize the mucosal surfaces of all humans during or soon after birth, and the risk of endogenous infection is ever-present (Kourkoumpetis *et al*., 2010). Candidiasis is the most common systemic mycosis, and the species causing infection include *Candida albicans, C. tropicals* among others (James *et al*., 2006). They are commonly encountered in immune-compromised individuals as opportunistic mycoses. Serious infections caused by *Candida albicans* are an increasing problem due to the immunosuppressive nature of surgery, Human Immunodeficiency Virus (HIV) infection, organ transplants and the treatment of malignancy (Guthrie *et al*., 2009). The susceptibility and incidence in patients with Acquired Immune Deficiency Syndrome (AIDS) are inversely correlated with the CD_4 Lymphocytic count and are likely to develop oropharyngeal candidiasis (Srikumar and Nagaraja, 2010). The main virulence factors enabling them to establish infection include: surface molecules that permit adherence of the organism, elaboration of acid proteases and phospholipases that involve penetration and ability to convert to a hyphae form (phenotypic switching) (Pfaller and Diekema, 2007). The organism establishes superficial infection (cutaneous or mucosal) by numerical invasion facilitated by damage to the epithelial surface (Kourkoumpetis *et al*., 2010). Systemic candidiasis occurs when there are inadequate host phagocytic defenses and dissemination of yeast can produce Candida infection almost anywhere in the body (Srikumar *et al*., 2010) Three of every 4 women have vulvovaginal candidiasis at least once in a lifetime (Jumbo *et al*., 2010). Patients that are critically ill and in medical or surgical Intensive Care Units are targets for opportunistic nosocomial candidiasis (Colombo *et al*., 2006). Usually, positive cultures from sterile sites are regarded as significant evidence of infection. In persons with systemic infections Candida species are now fourth most commonly isolated pathogen from blood cultures (Falcone *et al*., 2009). Large increase of Candida in the intestinal tract, often follow the administration of oral antibacterial agents and the organism can enter the circulation by crossing the intestinal mucosa (Kourkoumpetiset *et al*., 2010).The need for accurate and predictive susceptibility testing of fungi became a major issue, especially, in this AIDS era (Kourkoumpetis *et al*., 2010]. The use of antifungals at sub-therapeutic concentration and the emergence of resistant *C. albicans* and other fungi to antifungals *in-vitro*, and *in-vivo* have been documented (Fidel, 2003; Malani and Kouffinan, 2007). Broth based and Disc based susceptibility testing methods are available. Disc based is convenient, simple and economical as it produces easy to read sharp zones of inhibition (Pfaller *et al*., 2006). *Candida albicans* virulence and drug resistance requires the O- acyltransferase Gup1p primarily identified in *Saccharomyces cerevisiae* (Cowen *et al*., 2002). It is implicated in major cellular processes including plasma membrane shingolipid-sterol ordered domains assembly/integrity which had been associated with anti fungal resistance (Celia *et al*., 2010; Canovas and Perez-Martin, 2009). Candida invasive power include its ability to produce Candidapepsin (Monod, 2013) and the potential to exist in haploid state that mate efficiently to regenerate the diploid form, restoring heterozygosity and fitness (Hickman *et al*., 2013).

The introduction of new antifungal agents and recent reports of resistance emerging during treatment have highlighted the

need for *in-vitro* susceptibility testing (Lass-fiorl *et al*., 2009). However, susceptibility studies are not routinely carried out on Candida species isolated from our hospital, even on clinically demonstrated antifungal resistant isolates. Our objective in this study is to determine the susceptibility pattern of *Candida albicans* isolates from various patients specimens, to antifungal agents.

MATERIAL AND METHODS

Sample collection

Five hundred Samples obtained from suspected candidiasis patients attending University of Nigeria Teaching Hospital Clinics were received at the Microbiology laboratory. The specimens processed were high vaginal swab (HVS), endocervical swab, urine, sputum and blood (from a critically ill patient).

Cultural Methods: Standard methods for culturing as described by Brooks *et al* (2004) were used for all the samples except blood, and plating on chocolate agar, blood agar, macConkey agar and sabouraud dextrose agar (SDA). The blood sample was aseptically introduced into signal blood culture system (Oxoid batch No BC0102M, Lot NO 838224) following standard blood culture procedure. This was then inoculated unto SDA slants, blood agar and cystein lactose electrolyte deficient medium. The swabs that have been moistened with sterile nutrient broth were first spread on a position to seed the media (chocolate agar, blood agar, macConkey agar and sabouraud dextrose agar). The wire loop was sterilized and was used in streaking from the pool to four different areas of the medium for confluent growth. All the inoculated plates were incubated at 37^0C for 24 hours. Any positive fungal isolate was identified using standard methods (Gram stain,germ tube formation and biochemical assays) to the species level (Srikumar and Nagaraja, 2010) .

Antifungal Susceptibility Testing: All *Candida albicans* isolates were tested against various antifungal agents manufactured by Rosco Diagnostica A/S, Taastrupgaardsvej 30, DK-2630 Taastrup Denmark. Disc diffusion method for susceptibility testing was used. The test was performed as described by Pfaller *et al*., 2005 and National committee for clinical laboratory standard (Malani and Kouffinan, 2007). Sterile Mueller- Hinton agar supplemented with 2% glucose and 0.5µg of methylene blue per ml at a depth of 4.0mm in petri dishes were used. The agar surface was inoculated by using a swab dipped in a cell suspension adjusted to the turbidity of a 0.5 Mac Farland standard. Tablets of Ketoconazole (Keto) 15 µg, Itraconazole (Itrac) 8 µg, Voriconazole (Vori)1 µg, Miconazole 10 µg (Micoz), Ciclopirox 50 µg (Ciclo), terbinafine 30 µg (Terbi), fluconazole 25 µg (Flucz), were placed unto the surface of the plates and were then incubated at 37˚C for 24hr. Inhibition Zone Diameter (IZD) were read and Zones of ≥17 mm were taken to be susceptible(Pfaller *et al.,* 2005).

Statistical Analysis: This was carried out using SPPS package (version 15.0). Analysis of variants (ANOVA) was done and the values were considered at the 95% confidence limit and 0.05 probability level.

RESULTS

The 252 (50.4%) confirmed isolates of *Candida albicans* that were subjected to antifungal susceptibility tests using seven antifungal agents gave the following results.

The rate of susceptibility of isolates to each drug was as follows: Ketoconazole (58.0%), Itraconazole (50.0%), Voriconazole (60.0%), Miconazole (38.0%), Ciclopirox (24.0%), Terbinafine (12.0%) and Fluconazole (48.0%) (Figure1). Their respective resistance percentage rates were as follows: 42, 50, 40, 62, 76, 88 and 52, respectively. Figure 2 shows the mean inhibition zone diameters. The mean IZDs was as follows Keto 8.12 mm, Itrac 12.05 mm, Vori 12.26 mm, Micoz 8.28 mm, Ciclo 5.47 mm, Terbi 2.06 mm and Flucz 12.26 mm.

DISCUSSION

The percentage susceptibility of voriconazole (60%) was highest followed by ketoconazole (58%). This result is not consistent with the work of Pfaller *et al* (2005) where voriconazole was the most active (98.6%) against *C. albicans*. In this study voriconazole showed greater activity than cicloporox and terbinafine, P= 0.022 and P= 0.001, respectively. However, the activity of Vori was not significantly higher than those of Keto, Itrac, Micoz and flucz, P= 0.138, 0.451, 0.193 and 0.459, respectively. The antifungal activity of Ketoconazole, miconazole, and ciclopirox were all statistically significantly higher than that of terbinafine (P= 0.004, P= 0.002, P=0.036, respectively). Also Itraconazole and fluconazole each showed greater activity against terbinafine, (P= 0.0001).

Anti-fungal drug resistance is usually quantified using the minimum inhibitory concentration (MIC). The lowest drug concentration that results in significant reduction or complete lack of growth of the micro-organism is the MIC (Pfaller *et al*., 2006). Commercial kits like E-test directly quantifies anti fungal susceptibility in terms of discrete MIC values. The high rate of mortality from fungal infections and the relatively limited efficacies of current agents have produced a significant interest in the use of anti fungal combinations in these difficult to treat infections (Uludamar *et al*., 2010 Macphee *et al*., 2010; Jeddy *et al*.,2011; Gioglio *et al*., 2012]. The in vitro susceptibility testing method used has its own advantages and disadvantages. Some of the standard methods are cumbersome and not suitable for routine diagnosis. Others are relatively expensive, yet attractive, but MIC can be useful in the selection and monitoring of the best therapeutic agents (Guery *et al.,* 2009; Clancy and Ngugen, 2012; Andes *et al*., 2012). Prognostically, (Brooks *et al*., 2004; Kourkoumpetis *et al*., 2010) -B-D glucan(BG) is a biomarker for invasive Candidiasis.

A decrease in BG levels during therapy is associated with treatment success (Jaijakul *et al*., 2012). Recent methods for identification of species include, sequence analysis of the nuclear rRNA and internal transcribed spacer regions, coupled with multiple gene phylogenetic analysis (Eddouzi *et al*., 2013) and microsatellite length polymorphism and multi- locus sequence typing (Saghrouni, *et al*., 2013). Due to limited efficacies of current antifungal agents, there should be significant interest in the search for newer effective antifungals and the use of antifungal combinations to treat difficult infections.

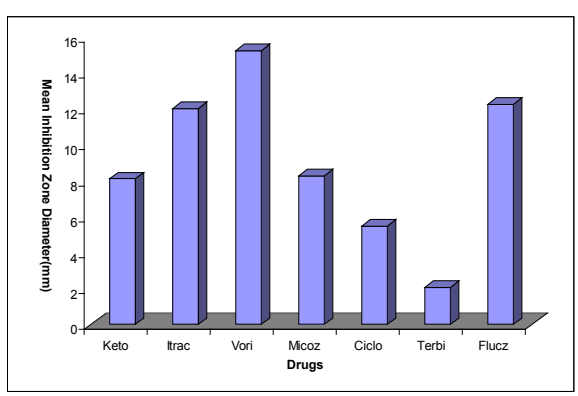

Figure 1: Mean inhibition zone diameter of the antifungals

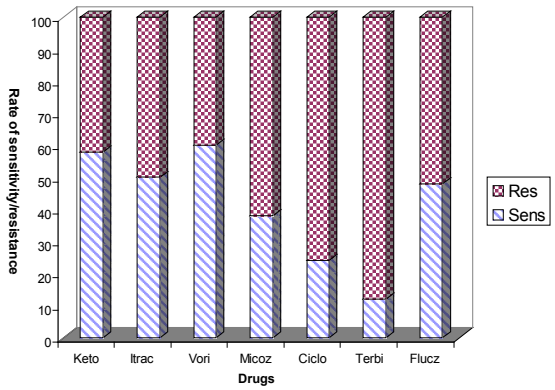

Figure 2: Susceptibility rates of the antifungal drugs against the *candida albicans* isolates.

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