GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Antimicrobial resistance among fungi from patients with urinary tract infections in Ojo, Lagos, Nigeria

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GSC Biological and Pharmaceutical Sciences, 2021, 14(03), 254–264

Publication history: Received on 12 February 2021; revised on 17 March 2021; accepted on 20 March 2021

Article DOI: https://doi.org/10.30574/gscbps.2021.14.3.0082

Abstract

The incidence of fungal urinary tract infections has risen gradually and has thus constituted a public health challenge. The aim of this study was to determine the prevalence of urinary tract infections by fungi in two health centres in Ojo, Lagos. A total of 200 patients attending the health centers constituting 160 males' urines and 40 females' vaginal swabs were recruited for this study. Midstream urine samples and vaginal swabs were aseptically collected and processed using standard mycological techniques. Fungal isolates were identified based on cultural characteristics, lactophenol blue stain, chlamydospore formation, colony colour on CHROM agar Candida medium and API yeast identification. Antifungal susceptibility testing of the isolates was performed by using the Broth dilution and Kirby-Bauer disk diffusion methods using two of the most commonly used antifungal agents. A total of 122 fungal isolates, of which 68 (55.7%) were *Candida* spp. and 54(44.3%) *Aspergillus* spp. were recovered. The *Candida* spp. included 64 (52.5%) *C. albicans* and 4(3.3%) *C. glabrata* while *Aspergillus* spp. included *A. flavus*, 20(16.4%), *A. fumigatus*, 24 (19.8%) and *A niger*, 10(8.2%). The most common fungal pathogens in the urinary tracts of the subjects were *Candida albicans* and *Aspergillus fumigatus*. Both *C. albicans* and *A. fumigatus* were highly susceptible to both fluconazole and amphotericin B in dimethyl sulphoxide and water (90-100%). Similarly, all *Aspergillus* spp. were susceptible to both antifungals except A. flavus which showed a slight resistance (10-15%), which appears to be emerging. Both fluconazole and amphotericin B still show high chances of therapeutic efficacy against fungal infections of the urinary tracts.

Keywords: Fungal Urinary Tract Infection; Aspergillus Spp.; *Aspergillus flavus*; *Candida albicans*; *Candida glabrata*; Fluconazole; Amphotericin B

1. Introduction

Fungal infections of the urinary tract are increasing in incidence, mostly due to the increasing use of antibiotics instrumentation and indwelling urinary catheters [1]. The incidence of fungal infection has increased significantly, so contributing to morbidity and mortality, which is caused by an increase in antimicrobial resistance and the restricted function of antifungal drugs, which retain many side effects. The incidence and prevalence of invasive fungal infections have increased since the 1980s, especially in the large population of immune compromised patients and/ or those hospitalized with serious underlying disease [2]. *Candida* species and in particular, *Candida albicans* is the most remarkable opportunistic fungal pathogen that causes nosocomial UTIs [3]. *Aspergillus* infection following ureteric stenting is very rare. Aspergillosis of the urinary tract may occur by 3 ways namely 1) by ascending infection from the lower tract, 2) from haematogenous dissemination or 3) due to *Aspergillus* cast in renal pelvis [4]. Renal aspergillosis due to haematogenous dissemination is the most common while localized infection is very rare [5]. Candiduria is the presence of *Candidia* species in urine, particularly in hospitalized patients, especially those in the intensive care units (ICUs) who often have multiple predisposing factors which include diabetes mellitus, indwelling urinary catheters, exposure to antimicrobial agents, cancer, long hospitalization, sex and age [6]. There are many types of oral and topical

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antifungal drugs that are commercially available. Depending on candidiasis, situation such as candiduria, cystitis, pyelonephritis, and vulvovaginal candidiasis, the mycoses therapies differ in antifungal medications. Among a vast range of antifungal drugs, the azole family is the largest one which inhibits lanesterol 14-B–demethylace activity, which leads to the disruption of the fungal cell membrane [7]. Echinocandins also prevent the creation of glucan in the fungal cell wall by inhibiting 1,3-beta-glucan synthase, which are administered intravenously particularly for the treatment of resistant *Candida* species. Other antifungal drugs include polyenes, allylamines aurines, benzoic acid, griseofulvin, crystal violet, coal tar, orotomide, sulphur etc. [8]. Antifungal drug resistance can be classified as microbiological either intrinsic or acquired while clinical resistance refers to the resistance of fungal infection despite treatment with adequate therapy [9]. Although microbiological resistance can contribute to the development of clinical resistance, other factors may also be involved, such as impaired immune function, underlying diseases reduced drug bio-availability and increased drug metabolism [9]. Resistance to available antifungal therapies is widespread and different fungal species have varying resistance patterns, which appear to be geographically determined. Resistance among fungal species causing UTIs especially *Candida* species to antifungals is a cause for concern in the case of immunocompromised patients, who are at a much higher risk of developing opportunistic complications especially in Sub-Saharan Africa with 70% of the global numbers of new HIV infections [10]. This study was carried out to determine the frequency of fungal urinary tract infections, the antimicrobial susceptibility pattern of the fungal pathogens among patients attending Health Centres, with a view to enhancing better treatment and management of fungiuria. In addition, the possible risk factors in relation to resistance profile were also considered.

2. Material and methods

2.1. Study Area

This study was conducted in two different locations around Ojo area in Lagos State which are Lagos State University Health Centre and Ojo Primary Health centre, Ojo. Ojo is one of the 20 Local Government Councils in Lagos State which is located in the western part of Lagos city centre with a population of 941,523, an area of 182 kilometer square, density of 6,869/ Km^2 according to the 2006 Nigerian Population Census.

2.2. Study Population

Both males and females between the ages of 15-50 years from these locations were studied. A total of 200 samples were collected, and out of these samples, 160 urine samples and 40 vaginal swab samples were collected aseptically. The mean age of the subjects was 28±2. The selection of patients was done with the support of attending physicians and nurses in the Health Centres used between September and December, 2019.

2.3. Criteria

Inclusion criteria were aged between 15 and 50 years, with signs and symptoms of urinary tract infections Exclusion criteria were those aged below 15 or over 50 years, diabetics, pregnant and menstruating women.

2.4. Ethical clearance and informed consent

Ethical clearance were sought and obtained from the health center where samples were collected for the study Lagos State University Health Centre and Ojo Primary Health Centre, all located in Ojo, Lagos. Specimens were collected from individual who gave consent to be part of the study. All data were analysed anonymously throughout the study.

2.5. Data collection

Urine and swab samples were carefully and appropriately collected into McCartney bottles aseptically by qualified laboratory personnel from the patient. The samples were kept in cooler boxes and taken to the laboratory for analysis. If samples were not immediately analysed, the samples were kept in a refrigerator at 4 ^{0}C within 24 hours of collection prior to analysis.

2.6. Mycological analysis

2.6.1. Culturing of specimens

Under aseptic conditions, the collected specimens were streaked directly on sabouraud dextrose agar (SDA) medium containing Chloramphenicol 10% (Plasmatic laboratory product LTD, UK) and culture plates were incubated for 24-48 h. Plates showing no growths were further incubated for 72 hours. On the other hand collected specimen swabs were directly placed into 90 mL sterile normal saline (0.85% NaCl, pH 7), vortexed, ten 11ml of the suspension was streaked

onto sterile Petri plates containing SDA, Difco supplemented with chloramphenicol (250 mg /L) Sigma, in replicate and incubated at 37 ^{0}C for 48-96 hours .

2.6.2. Macroscopic identification

After incubation, the plates were removed and observed for growth. The colonies were studied for their morphological characteristics such as colours, size, production of pseudohyphae and/or budding cells.

2.6.3. Direct Microscopic Examination (DME)

Smears from the collected sample stained with lactophenol cotton blue (LPCB) were prepared. Appearance of budding cells with or without pseudohyphae under macroscopic indicates positive results [11]. A 10% KOH wet mount was made from the swabs for the presence of pseudohyphae and/or budding suggestive of *Candida.*

2.7. Identification of fungal isolates

2.7.1. Germ tube test

A suspension of pure Candida isolate was made by inoculating a test tube containing 0.5 ml of human serum with a loopful of the organism. It was incubated in a water bath for 2-4 h at 37 ^{o}C. After incubation, a wet preparation was made by transferring an aliquot of the suspension onto a clean glass slide and covered with a coverslip. This was examined using x10 and x40 objectives respectively. The presence of elongated daughter cells from the mother cells without constriction at the origin of mother cells was noted as pseudohyphae, both were positive indication for *Candida albicans* [12, 13].

2.7.2. Chlamydospore formation test

Test colonies were stab-inoculated on cornmeal agar plate by slide culture technique and was incubated for 72 h at 25 ^{o}C. Chlamydospore formation was demonstrated by staining with LPCB. Yeas isolates found to be positive for chlamydospore formation were further confirmed as *Candida albicans* while those showing negative results were recorded as non-albicans *Candida* spp. [12]

2.7.3. Growth on HiCrome Candida Differential Agar

This selective and differential medium, allow differentiation of *Candida* species. *Candida albicans* appears as light green colored, smooth colonies, *C. tropicalis,* blue to metallic blue, *C. glabrata,* cream to white smooth colonies and *C. lustaniae*, pale to pink colonies (Mohammad et al., 2017).

2.7.4. Preparation of standardized inoculum

The Clinical and Laboratory Standards Institute guidelines, [14] were used to prepare $BaSO_4$ turbidity standard (0.5 McFarland's standard). Briefly, 99.5 Ml of solution A (1%v/v H_2SO_4) was added to 0.5 Ml of solution B (1.17% w/v $BaCl_2.2H_2O$) with constant stirring. Using matched curvette with a 1.0 cm path, the OD (625nm) was measured on the spectrophotometer. The 0.5 McFarland standard was distributed into disposable screw-capped universal bottle. From SDA plate, a discrete colony of test organisms were suspended in sterile distilled water and agitated briefly to homogenize. The yeast density which gave an OD (625nm) equivalent to that of 0.5 McFarland standard was referred to as the standardised inoculum.

2.7.5. API Candida (BioMereux)

A commercial Analytical Profile Index (API) yeast identification kit was used to carry out the biochemical test on the isolated *Candida* spp. The API Candida strip consists of 12 biochemical tests. Five carbohydrate–acidification tests (glucose, galactose, saccharose, trehalose and raffinose), and seven enzymatic tests (alpha-amylase, β-glucuronidase, β-galactosidase, β-maltosidase, β-xylosidase, N-acetyl-β-glucosaminidase and urease). Yeast inoculum suspension was prepared, inoculated to the strip and incubated for 24 h at a 35 ^{o}C and visual color reaction was observed. The results were compared with those given in the profile list in the package insert. One of the strips was used as negative control where sterile distilled water was used as inoculum.

2.7.6. Antifungal susceptibility testing

The antifungal susceptibility testing for fluconazole, amphotericin B was based on **CLSI** disc diffusion method [13]. Mueller Hinton glucose methylene blue agar surface was inoculated by using a sterile swab dipped in a standardized

Candida cell suspension, which was allowed to dry. The antifungal discs were dispensed on the inoculated SDA plates. Sensitivity plates were measured and interpreted according to CLSI interpretative break point.

2.7.7. Statistical methods

Data generated from questionnaires and results of the laboratory analysis were entered into Microsoft Excel and analysed using SPSS software (Version 20, IBM Corporation Armonk, NY, USA). Results obtained were reduced to percentages, tables and figures. The Pearson X2 test at a 95% confidence interval and 0.05 level of significance was used to determine the relationships between some socio-demographic data.

3. Results

The urine and vaginal samples cultured on SDA, HiChrome Candida Differential Agar and API Yeast identification kit had *Candida* spp. and *Aspergillus* spp. Of the 200 subjects examined, 34% had *C. albicans*, 27% had *Aspergillus* spp. and 39% had no growth (Figure1).

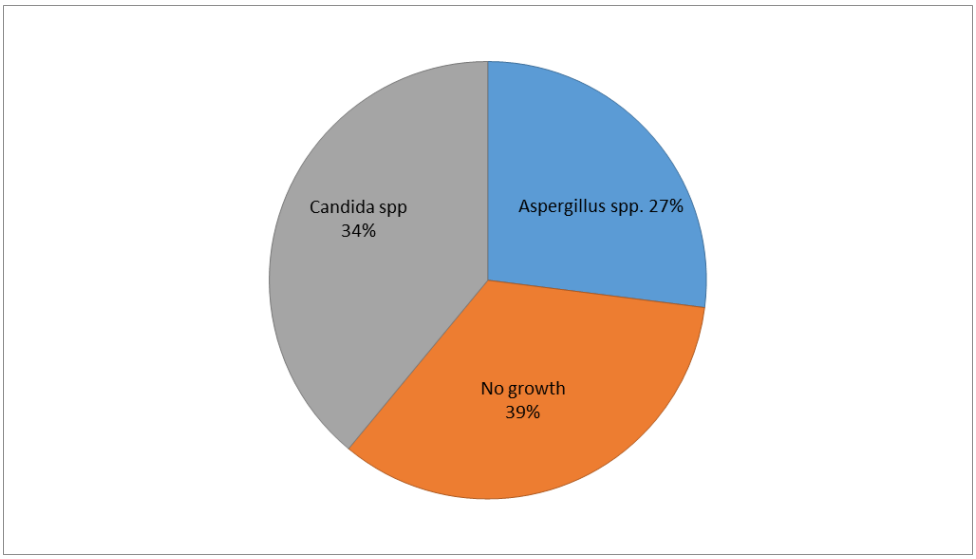

Figure 1 Percentage frequency of fungal isolates from urine and vaginal swabs.

The demographic characteristics of the subjects are recorded in Table 1. Results from this table revealed that individuals within the age group 20-30 recorded the highest occurrence of candidiasis and aspergillosis of 25% and 22.5% respectively. As regards occupation and marital status, candidiasis and aspergillosis were predominant among the businessmen/businesswomen as well as the married and single subjects (Table 2). Table 2 shows the characteristics of fungi after staining with Lactophenol blue, direct microscopy, growth on HiCrome Candida differential agar, chlamydospore formation test and API Candida test.

Sensitivity of fungi to the two major antifungal agents evaluated showed that *Candida* spp. are weakly resistant to fluconazole dissolved in dimethyl sulphoxide (Figure 2).

Water was also used as a diluent in the study of the susceptibility pattern of the fungi from UTI patients to fluconazole and amphotericin B. The zones of inhibition of the *Candida* spp.in water against fluconazole ranged from 10 -15 mm as concentration increased (Figure 3).

Aspergillus flavus and *A.fumigatus* ahowed 100% resistance to fluconazole in dimethyl sulphoxide at all concentrations (100 mg/l-300 mg/L) while *Aspergillus niger* were mildly susceptible at 250 mg/L and 300 mg/L cocentrations. Both Candida spp. were highly susceptible to amphotericin B when dissolved in dimethyl sulphoxide, with zones of inhibition ranging from 23 mm to 31 mm (Figure 4).

Candida spp. and *Aspergillus* spp. showed high susceptibility to amphotericin B in water. *Aspergillus flavus* however showed less susceptibility to the drug (Figures 5 and 7).

Susceptibility of the *Candida* spp. to amphotericin B studies showed varying degrees of susceptibility with little or differences at different concentrations in dimethyl sulphoxide (Figure 6).

Aspergillus spp. showed high a high susceptibility amphotericin B in water at various concentrations, thus suggests its therapeutic efficacy (Figure 7).

Table 1 Demographic characteristics of subjects in Ojo, Lagos

	No. examined	**No. candidate Spp positive(%)**	**No. positive Aspergillus (%)**	**P value**
<20	20	2(10)	1(5)	
20-30	80	20(25)	18(22.5)	
31-40	70	6(8.6)	5(7.1)	P>0.05
41-50	30	4(13.3)	3(10)	
Occupation				
Business	60	15(25)	10(16.7)	
Civil servants	65	10(15.4)	8(12.3)	
House wife	35	3(8.6)	4(11.4)	P>0.05
Students	40	4(10)	5(12.5)	
Marital status				
Married	110	18(16.4)	10(9.1)	
Single	90	14(15.5)	17(18.9)	P>0.05
Total	200	32(32)	27(28.0)	

Table 2 Fungal species isolated and their characteristics

Macroscopic characteristics	**Microscopic structure**
Green colour on Chrom agar	Circular rings attached together with septate tree like branches*. C. albicians*
Purple colour Chrom agar	Different septate crossing each other. *C. glabrata*
Light green colored powdery mass with white edges on SDA.	Septate to spikes extending from the top, appearing like sunflower. *A. flavus*
Dark green powdery mass with white scales	Septate with stone- like particles at the top like leaves falling off a tree. *A. fumigatus.*
Black coloured powdery mass with web-like edges on SDA	Ordinary septate with nothing at the front. *A. niger*

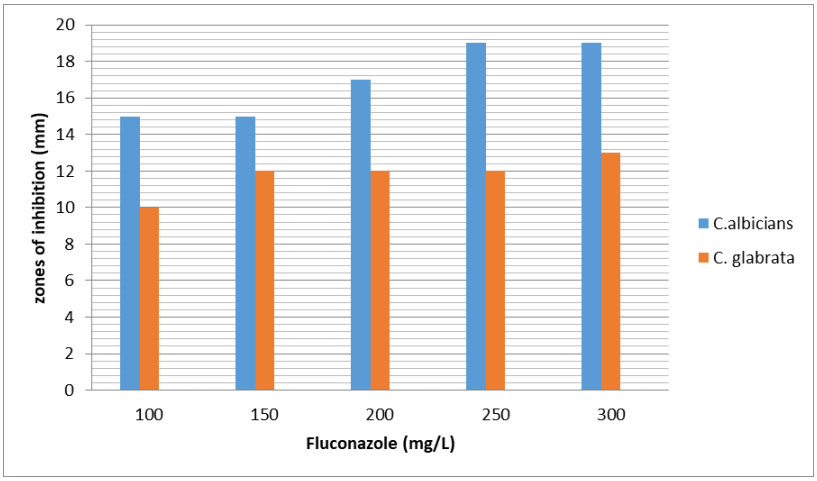

Figure 2 Zones of inhibition of *Candida* spp. to Fluconazole in dimethyl sulphoxide

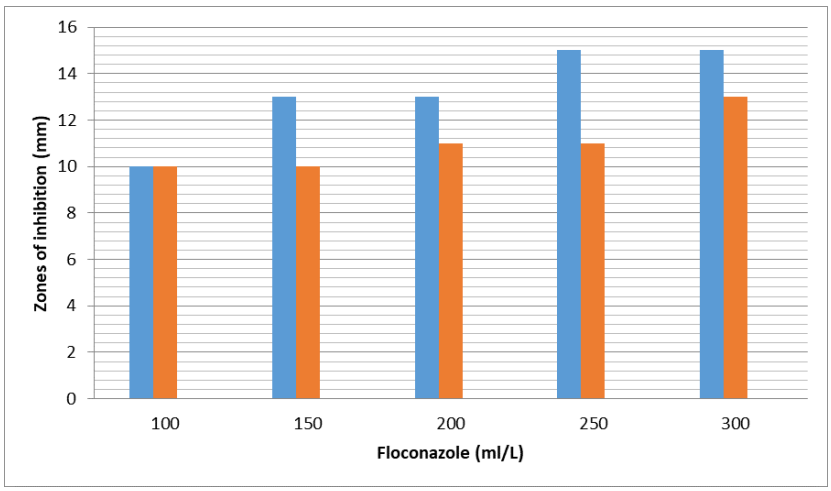

Figure 3 Zones of *Candida* spp. to different concentration of fluconazole in water.

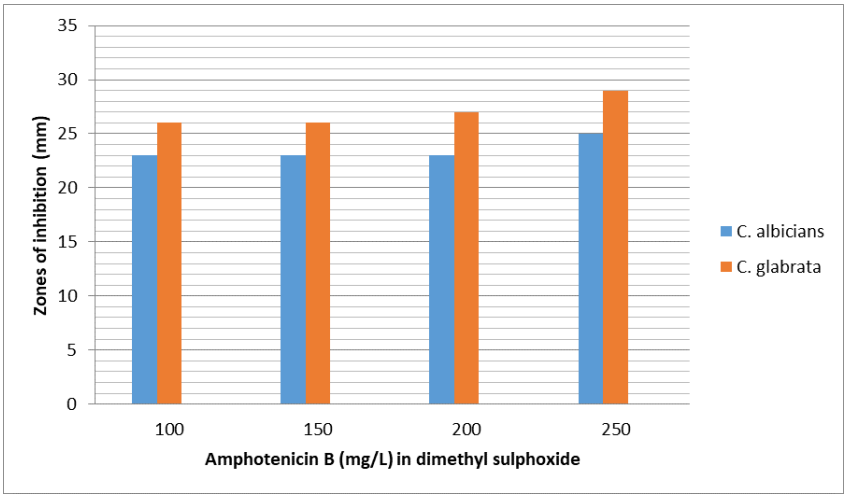

Figure 4 Zones inhibition of *Candida* spp. at different concentration of amphotericin B in dimethyl sulphoxide

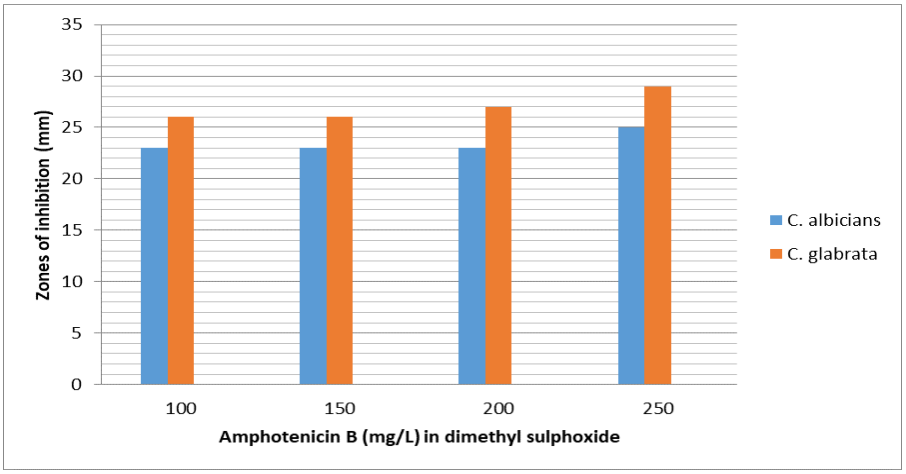

Figure 5 Zones of inhibition of *Candida* spp. to different concentrations of amphotericin B in water

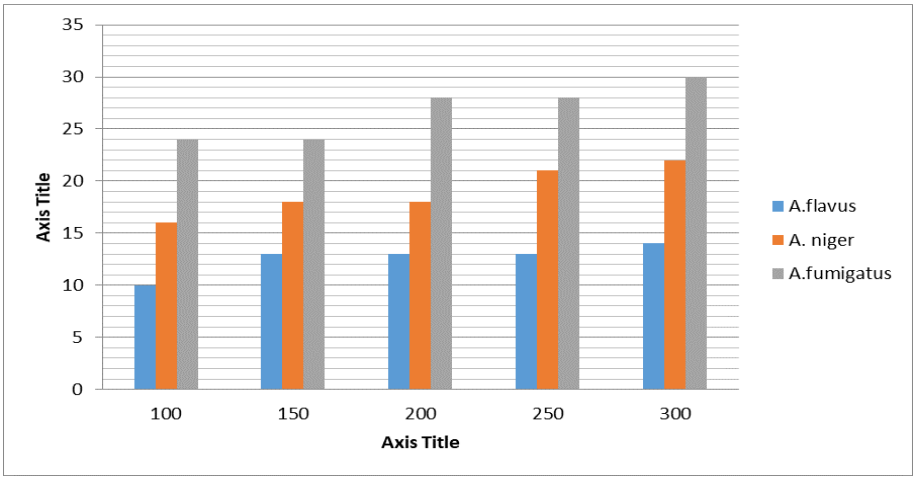

Figure 6 Sensitivity *of Aspergillus* spp. to amphotericin B in dimethyl sulphoxide**.**

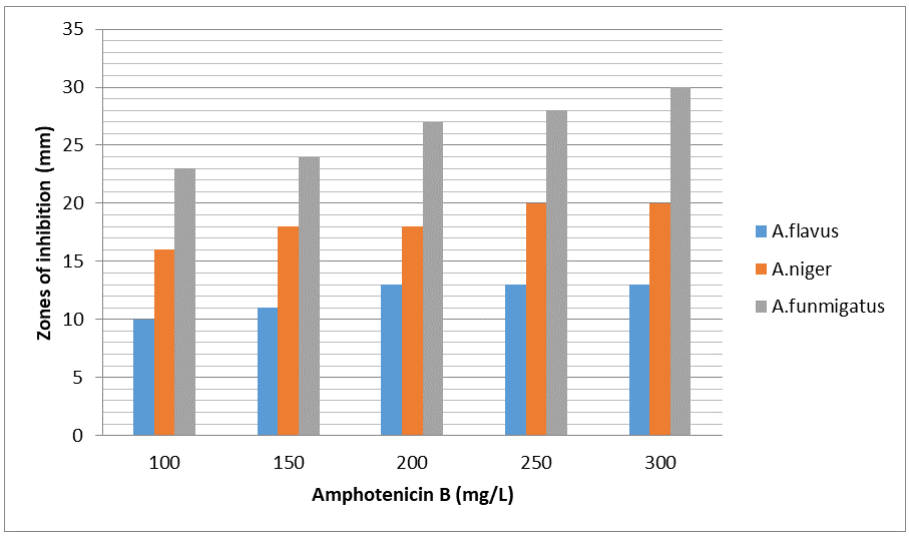

Figure 7 Zones of inhibition of *Aspergillus* spp. to different concentrations of amphotericin B in water.

4. Discussion

Vulvovaginal candidiasis is a common female genital infection affecting many women of child bearing age that are sexually active. Vaginal candidiasis is one of the commonest infections of women, second only to anaerobic bacterial vaginosis. *Candida* colonizes mucous membranes such as the vagina, urinary tract and the oral cavity [15, 16]. Studies on UTIs have focused on prevalence of fungi particularly *Candida* spp., optimal antibiotic treatment, the utility of urinary drainage, changes in causative microorganisms over time and in different regions and UTI categorisation, such as complicated and uncomplicated UTI [8, 15]. In this study, an overall prevalence of vaginal candidiasis of 34% and aspergillosis of 27% were recorded. This value is higher than 19% of vaginal candidiasis reported in Jos, Plateau State, Nigeria [15], but lower than 40.6% reported in Edo State, Nigeria by [17]. A high incidence of candiduria was recorded in this study. This study corroborates the 20.6% reported in Costa Rica [18]. The variation in prevalence can be due to the differences in patients, hospital settings, geographical regions and methodology. A high prevalence of urinary aspergillosis (27%) was found in this study. This is contrary to report by [19]. *Aspergillus* is not a common cause of fungal UTIs and that primary *Aspergillus* infection of the urinary tract is sufficiently rare, even moreso in immunocompetent patients. Other fungi cause UTI much less commonly than *Candida* spp. and in most cases, genitourinary tract involvement is only one manifestation of disseminated infection. Urinary aspergillosis is a rare infection in Nigeria, so its sudden emergence in this study calls for concern. The susceptibility of urinary aspergilli may be due to the fact that such A*spergillus* spp. are likely not to have been exposed to antimicrobials like other urinary fungi especially molds. None of the subjects had immunocompromising conditions, no case of invasive aspergillosis, which is a severe fungal infection in such patients was reported. Most of the positive cases with *Candida* spp. and *Aspergillus* spp. belong to the 21-30 age group, giving 20 (25%) and 18 (22.5%) respectively representing 47.5%. This finding is in line with the 73.02% prevalence reported by [20] and 71% by [20]. However, only one non-albicans *Candida* spp. which was *Candida glabrata* was recovered in this study. Studies have reported the occurrence of high incidences of non-albicans *Candida. W*omen who are younger and sexually active are more vulnerable to be infected with *Candida* spp. especially when they are immunocompromised. Business men and women (25%) and civil servants (15.4%) had more *Candida* spp. as well as married (16.4%) and single individuals (15.5%) respectively. Occupation and marital status were likely predisposing factors of urinary candidiasis and aspergillosis as there was statistically significant difference in their occurrence (P>0.05).

The antifungal sensitivity pattern of both *Candida* spp. and *Aspergillus* spp. to fluconazole and amphotericin-B dissolved in dimethyl sulphoxide and water showed that fluconazole in dimethyl sulphoxide enhanced the potency while those dissolved in water showed less antifungal effect with the concentration range of 100 t0 300 mg/L. This study shows that DSO increases the rate of susceptibility of *Candida* spp. or increases the potency of the antifungal agent-fluconazole. *Aspergillus* spp. showed low resistance to fluconazole as well as amphotericin-B. This result indicates that these species of *Aspergillus* had no intrinsic resistance to fluconazole but *Candida* spp. showed relatively low resistance to fluconazole with increased susceptibility as the concentration of the antifungal drug was increased, However, increase in the concentration of fluconazole could result in overdose which could in turn cause harm to the human system, become toxic or even increase fungal resistance to the drug totally [21]. *Candida glabrata* from this study showed a slight resistance to fluconazole than *Candida* as reported in Iraq by [21]. *Candida albicans* and *C. glabrata* were both susceptible to fluconazole but *C. glabrata* shows mild resistance to fluconazole. This shows that non-albicans *Candida* resistance to antifungal drug is increasing at an alarming rate as reported [22]. This is contrary to report by [20] with 61.2% resistance to fluconazole in Egypt. [23] reported 58.82% to fluconazole in Cameroon. However, no antifungal drug has been used for urinary tract aspergillosis due to low urine concentration [18]. Both *Candida* spp. and *Aspergillus* spp. showed high susceptibility to amphotericin-B except *A. flavus* which showed a mild resistance, thus less susceptibility. Even increasing the concentration of amphotericin-B did not show corresponding susceptibility. This is a pointer to the fact that amphotericin-B is not likely to be a drug of choice aspergillosis especially that due to *A. flavus,* thus other antifungal drugs should be used. [22], from their study in Belgium reported an alarmingly high fluconazole resistance rate of 21% in vaginal *C. albcans* isolates. Earlier findings showed varying degrees fluconazole resistance of 67% by [24], 61.2% by [20], and 2.2% by [25]. Different rates of fluconazole and itraconazole were reported in *Candida* strains especially the non-albicans strains [26]. Antifungal resistance has been demonstrated by *C. albicans, C. glabrata* and *C. krusei* as clearly the *Candida* species with the greatest potential to acquire resistance fluconazole and other azoles [27]. Fluconazole is widely used in public health setting in the African continent as it is empirically used in treatment of systemic or localized *Candida* infections. This is based on the fact that it is less toxic and much more effective than the imidazoles antifungal agents such as ketoconazole or amphotericin-B, even though it is a teratogenic drugs. Consequent on the above, the use of azoles as first line drugs systemic infections should be revisited in regions like West Africa, where their increasing inactivity is high. This study aligns with regional monitoring of Candida infections based on their prevalence and susceptibilities to antifungal drugs especially in rural areas devoid of microbiological laboratory tests [10]. From the foregoing, it is necessary for each to set up fungal infection monitoring institutions which would transform into the anticipated regional monitoring institutions. *Candida* infections treatments that fail to respond to

conventional antifungal drug treatment have become increasingly reported include the azole class including fluconazole, hence the need for several new antifungal agents. A combination of different antifungal agents is especially needed for serious infections to work in synergy [28]. Sale of antimicrobial medications remain un regulated in Africa and is aggravated by the penetration of fake and adulterated drugs with little or no active ingredients are readily available in pharmacies and on the streets. In addition, some practitioners often prescribe antimicrobial medications based on only clinical presentations, these among factors pose a serious public health threat, thus responsible for more and more antimicrobial drugs being rendered ineffective in treating microbial infections.

5. Conclusion

The most common fungal pathogens in the urinary tract system were *Candida albicans* and *Aspergillus fumigatus.* Fungiuria due to *Candida* spp. and *Aspergillus* spp. in the subjects indicated that there was a high prevalence of this infection in the study area. All the isolates showed high susceptibility to both fluconazole and amphotericin B, which are the most commonly used antimicrobials in Nigeria but also demonstrated mild resistance which appears to be emerging. Routine isolation and identification of fungal pathogens from the urinary tract with their antimicrobial susceptibility test can help in the successful treatment of fungiuria.

Compliance with ethical standards

Acknowledgments

The authors express their appreciation to the laboratory staff of the Department of Microbiology, Lagos State University, Ojo, Lagos, Nigeria for their assistance in carrying out the experiments. We also wish to express our thanks to the Physicians in the health centres used for their assistance in sample collection, Dr. K. A. Akinsinde of Nigerian Institute of Medical Research, Yaba, Lagos for his technical assistance and Mr. Fukpene Baitei of the Lagos State University International School, Ojo, Lagos, Nigeria who took time to carry out secretariat activities associated with this manuscript.

Authors' contributions

DDM conceived the experiment and both DDM and OMD designed and performed the experiments. DDM analysed the data and drafted the manuscript while both DDM and OMD jointly finalised the paper.

Disclosure of conflict of interest

The authors declare that they have no competing interest.

Author's declaration

The authors declared that there is no conflict of interest whatsoever**.**

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