Original Research Article

Antifungal Susceptibility Pattern in Otomycosis among Patients Attending a Tertiary Healthcare Institution in Port Harcourt, Nigeria

ABSTRACT:
Antifungal resistance is one of the factors considered to hamper effective treatment of otomycosis. Consequently, this study was aimed at isolating etiological agent for otomycosis among patients that visited University of Port Harcourt Teaching Hospital from June 2017 to June 2018 and carry out antifungal susceptibility testing with the isolates. Ear swab from 120 patients and 120 control group was aseptically collected. Nystatin, voriconazole and fluconazole were used for antifungal susceptibility testing by agar diffusion method. Isolation and identification of fungal isolates was by Standard microbiological methods. From our result, fungi were isolated from ear swab of 34 patients and 5 from the control. The isolates and their frequency of occurrence are *Candida* sp. (61.8 %), *Aspergillus* sp. (23.5 %) and *Penicillium* sp. (8.8 %), while *Candida albicans* was the only isolate seen among the control group. Susceptibility result revealed that *Candida* sp. and *Penicillium* sp. as being sensitive to voriconazole. (76.9 %) and fluconazole (66.7 %) respectively. Findings from this study underscore the importance of antifungal susceptibility testing before treatment of otomycosis in order to achieve high success rate thereby reduce cases of antifungal resistance.

Keywords: Otomycosis, Antifungal, Port Harcourt, Susceptibility pattern

1.0 INTRODUCTION

Otomycosis is a superficial fungal infection of the external auditory canal (EAC) which usually leads to otitis externa (Carney, 2008; Ontai and Osuji, 2016). This fungal infection has a global spread and its prevalence is largely dependent on different climatic conditions amongst other predisposing factors such as swimming, use of ear plugs, excessive cleaning of the ear, use of hearing aid, broad spectrum antibiotic agents, trauma to the ear cannal (Onotai and Osuji, 2016; Rao and Rao, 2016). Climatic conditions in the tropics and subtropics, hot humid, and dusty environment are some of the factors that predispose to a high prevalence of otomycosis (Agarwal and Devi, 2017). The studies on pattern of communication disorders in Nigeria are scarce. Knowing the pattern of communication disorders can assist in prevention, early detection, intervention and rehabilitation of children with the disorder (Ikenga and Oparaodu 2021),

Aspergillus and *Candida* are the commonest fungal genera implicated in otomycosis. Less frequently identified fungal species are *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida tropicalis* and *Candida parapsilosis* (Kiakojuri *et al.*, 2015). Mgbe *et al.* (2010) also identified *Penicillium* sp. and *Pitirosporum* sp. as aetiological agents implicated in otomycosis. According to Hydri *et al*. (2017), reoccurrence of otomycosis is high and medical treatment is often long term. Based on presumptive clinical diagnosis, Mgbe *et al.* (2010) reported 87.5 %, 89.4 % and 90.0 %, success rate in the treatment of otomycosis using genticin violet, locacerten viform and candibiotic drops respectively. Although otomycosis could be diagnosed clinically based on their symptoms, it is a common practice in some

hospitals not to identify etiologic agents for otomycosis using culture-based methods and also identify susceptibility patterns before commencement of medical treatment. This practice could result to improperly treated ear infections and complications (Kisembo *et al*., 2018; Mgbe *et al*., 2010). It could also contribute to increasing cases of antifungal resistance which is posing a threat in management of patients diagnosed with otomycosis (Panigrahi *et al.,* 2019). Therefore, this study seeks to identify fungal species implicated in otomycosis among patients attending a tertiary healthcare institution in Port Harcourt Rivers State Nigeria and determine its susceptibility pattern using common antifungal agents.

Oparaodu *et al* (2021)

2. MATERIALS AND METHODS

2.1 Duration and Study Area

This is a 1 year prospective study which was carried out in the Ear, Nose and Throat (ENT) clinic, of the University of Port Harcourt Teaching Hospital (UPTH) which serves as referrals for other states in South-South geopolitical zone in Nigeria.

2.2: Sample Collection

Total of Patients of all age groups who presented with features of otitis externa in ENT clinic of UPTH. The control group comprises of patients who were age (+/-5years) and sex matched presenting in the ENT clinics without otologic symptoms. Informed consent was taken and those who had commenced topical or systemic antibiotic or anti-fungal medications were excluded. The ear swabs were aseptically collected using sterile swab sticks and inoculated immediately.

2.3 Analysis

A detailed ear examination was done and findings noted. With the aid of an otoscope, the EAC and TM were examined according to methods adopted by Oparaodu *et al* (2021). Any discharge or debris in the EAC was noted and sample was aseptically collected using sterile swab. Two samples were taken from the affected ear of each subject. First swab was used for direct Gram stain and KOH mount and second swab for fungal culture; Swabs were inoculated on freshly prepared acidified Sabouraud Dextrose Agar (SDA) plates amended with 250mg Tetracycline then incubated at 25±2oC (room temperature) for 7 days.

2.4: Identification of the Fungal Isolates

The fungal isolates were identified based on morphological and microscopic characteristics such as colony growth pattern, conidial morphology, and pigmentation. The technique described by Odokuma and Okpokwasili (24), and adopted by Oparaodu *et al* (2021), was also employed for the identification of the isolated fungi using cotton blue in lactophenol stain. This was done by placing a drop of the stain on clean slide with the aid of a mounting needle, where a small portion of the aerial mycelia from the representative fungi cultures was removed and placed in a drop of lacto phenol. The mycelium was well spread on the slide with the needle. A cover slip was gently placed with little pressure to eliminate air bubbles. The slide was then mounted and viewed under the light microscope with ×10 and ×40 objective lenses. The morphological characteristics and appearance of the fungal isolates seen were identified in accordance with standard scheme for identification of fungi as adopted by Okerentugba and Ezereonye (27).

2.51: Antifungal sensitivity Testing

Susceptibility testing was done using disk diffusion method with susceptibility test disc of 1mcg voriconazole, 25mcg fluconazole, 100mcg nystatin. The inhibition zone diameter was recorded using clinical laboratory and standard institute (CLSI 2010) method

RESULTS

Ear swabs of 120 patients with otitis externa were evaluated during this period, which yielded fungal growth in 34 of them. Table 1 shows the distribution of fungal isolates in subjects with otomycosis and control. Antifungal agents which include voriconazole, nystatin and fluconazole were used to determine the antifungal susceptibility pattern of fungal isolates implicated in otomycosis. This is depicted in Table 2. Fungal cultures yielded 34 fungal isolates in 120 samples from a total of 120 clinically diagnosed cases of otomycosis. Fungal culture positivity rate is 28.3%. Significant association was observed between various predisposing factors and positive samples. Figure 1 shows that *Candida* spp. are the predominant fungi (n=34; 61.8%) among total fungal isolates, more frequent being *Candia albican* followed by *Candida tropicalis* and *Candida rugose*. Other fungi isolated were Candida spp *Candida krusei.*

Table 1: Distribution of fungal isolates among subjects with Otomycosis and Control

Fungal isolates	Subjects (34) n (%)	Control (5) n (%)
Candida	21 (61.8)	5 (100)
Candia albican	7	5
Candida tropicalis	3	0
Candida rugose	5	0
Candida krusei	6	0
Aspergillus	8 (23.5)	0 (0.0)
Aspergillus niger	4	0
Aspergillus fumigatus	4	0
Penicillium sp.	3 (8.8)	0 (0.0)
Candida albicans and *Penicillium* sp.	2 (5.9)	0 (0.0)
Total	34 (100.0)	5 (100.0)

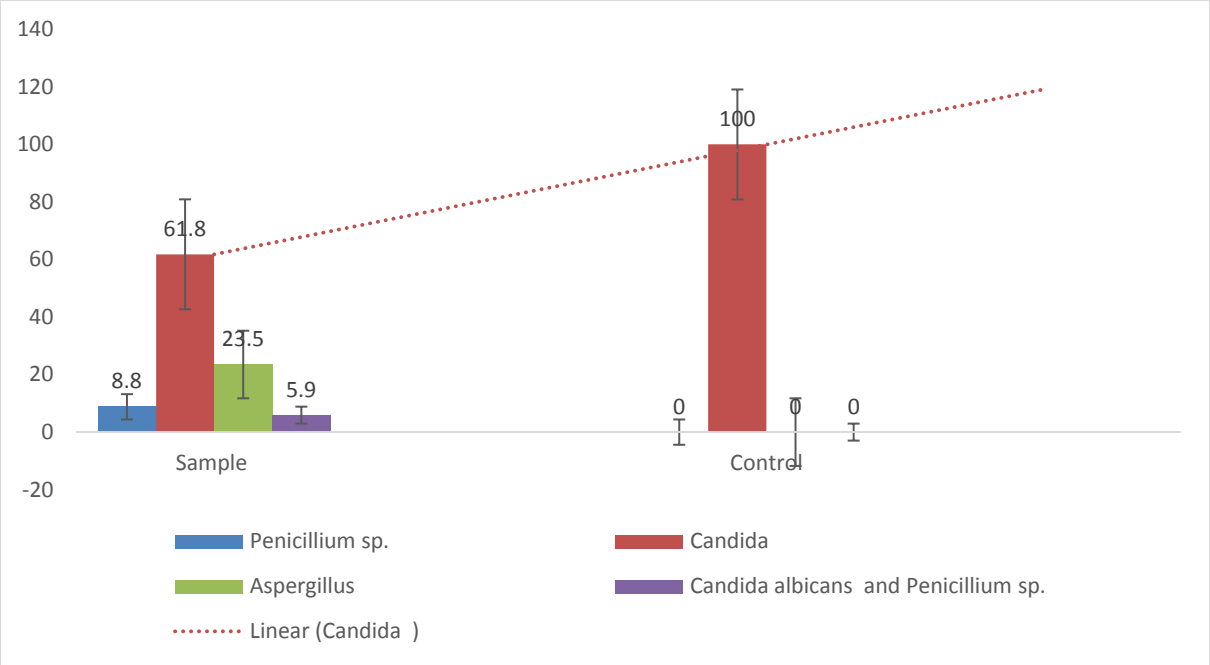
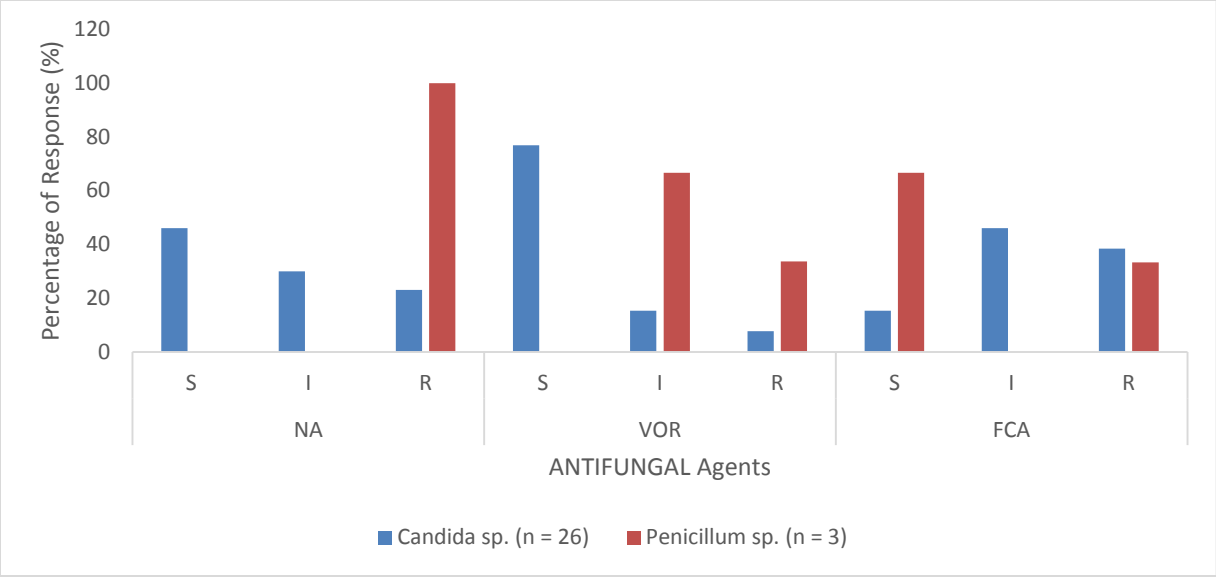


Figure 1: Percentage Occurrence of fungal isolates among subjects with Otomycosis and control

Table 2. Antifungal susceptibility pattern of fungal isolates implicated in Otomycosis

	Antifungal		
Isolates	NA	VOR	FCA
Candida sp. (n = 26)			
Sensitive	12 (46.1%)	20 (76.9%)	4 (15.4%)
Intermediate	8 (30.8%)	4 (15.4%)	12 (46.1%)
Resistant	6 (23.1%)	2 (7.7%)	10 (38.5%)
Yates corrected Chi square =	15.66 ; p value= 0.004		
Penicillum sp. (n = 3)			
Sensitive	0 (0.0%)	0 (0.0%)	2 (66.7%)
Intermediate	0 (0.0%)	2 (66.7%)	0 (0.0%)
Resistant	3 (100.0%)	1 (33.3%)	1 (33.3%)
Fishers exact p-value= 0.04			
n = number of isolates	NA – Nystatin	VOR – Voriconazole	FCA-Fluconazole



n = number of isolates NA – Nystatin VOR – Voriconazole FCA-Fluconazole

Figure 2: Percentage of Fungal Sensitivity to the Antifungal Agent

DISCUSSION

Otomycosis is a fungal infection that affects the external auditory canal. Although some antifungal agents are known to cure the disease, increasing cases of antifungal resistance is posing a challenge to medical practitioners and patients as well. Therefore, isolation and identification of etiologic agent involved in otomycosis followed by susceptibility testing of antifungal agents intended for use in medical treatment is most likely to result in high success rate. In a study by Pontes *et al*. (2009), *Candida* sp., with a frequency of 55% was the dominant fungi associated with ear swab of patients with otomycosis. This is in agreement with the result obtained from this study. However, Fasunla *et al*. (2007), Nazeer *et al*., 2015 and Singh *et al*. (2017) reported the dominance of *Aspergillus* sp. in the ear swab of otomycosis patients. In terms of frequency of occurrence in ear swab of otomycosis patients, Sarita *et al*. (2017) reported *Penicillium* spp. (1 %). *Candida* sp. is an opportunistic fungus. It is a harmless saprophyte but capable of being pathogenic when the host mechanism has been suppressed. *Aspergillus* sp. is also a saprophytic organism commonly seen in our environment. The temperature and humidity of external auditory canal is usually conducive for growth of *Aspegillus* sp. This could be one of the reasons for its dominance as etiologic agent for otomycosis (Singh *et al.*, 2017).

A striking result in this study was coinfection of *Candida albicans* and *Penicillium* sp. from the same ear swab. The reason is that previous studies on otomycosis rarely reported coinfection between two fungi genera. However, few

cases which involved co-infection between bacteria and fungi associated with otitis externa have been reported Ibiam et al 2013. Rare occurrence of co-infection between two fungi genera in the same ear swab of patients diagnosed with otomycosis is attributed to fungi involved usually inhibit growth of bacteria species. This medical condition is often difficult to treat with antifungal agents and chance of infection reoccurrence is high. Prolonged and often challenging medical treatment that involved coinfection could be as a result of biofilm produced by *Candida* sp. Recent studies suggest that *Aspergillus* sp. could also produce biofilm. According to Agarwal and Devi (2017), a case of otomycosis that involved mixed culture of *Aspergillus niger* and *Candida albicans* has been reported.

Susceptibility testing of *Candida* sp. isolated from ear swab of patients presenting with otomycosis to three antifungal drugs used in this study revealed that voriconazole was most sensitive 20 (76.9 %), followed by nystatin 12 (46.1 %) and lastly fluconazole 4 (15.4 %). High susceptibility of fungi implicated in otomycosis to voriconazole is in agreement with findings by Ali *et al*. (2017) that reported 93.48 % sensitivity. A related study by Jayachitra (2018) revealed that all *Candida albicans* associated with patients diagnosed with otomycosis were susceptible to voriconazole. Similarly, *Candida krusei* was also susceptible to voriconazole. Also, two isolates of *C. parapsilosis* were sensitive to voriconazole but only one of the isolates was resistant to fluconazole. Although voriconazole proved to be the most sensitive antifungal agent against *Candida* sp., its unavailability in topical form limits the practice of using it as a daily medication (Marta *et al.* 2013).

According to Ali *et al.* (2017), *Aspergillus niger, A. flavus, A. terreus, Rhizopus stolonifer* and *Pencillium duclauxi* implicated in otomycosis showed 100 % resistance to fluconazole. Meanwhile, the resistance for nystatin was also high among the five fungal isolates of which *A. terreus*, *R. stolonifer* and *P. duclauxi* was 100 %. High resistance of these isolates to fluconazole and nystatin is in agreement with our susceptibility results that involved *Candida* sp. Sarita *et al.* (2017) reported that 16 % and 25 % of *C. albicans* and *C. tropicalis*, respectively showed resistance to nystatin as well as fluconazole. Fluconazole is a common antifungal agent used in the treatment of patients diagnosed with otomycosis. Probably, indiscriminate use of fluconazole in treatment of fungal infections might have resulted in increased resistance of the drug to *Candida* sp. associated with otomycosis whereas the other two antifungal agents (voriconazole and nystatin) that are less frequently used in the treatment of otomycosis were more sensitive to *Candida* sp. This observation is similar to the research finding by Marta *et al.* (2013).

Among the three antifungal drugs tested against *Penicillium* sp., only fluconazole was sensitive (66.7 %), voriconazole showed intermediate resistance (66.7 %) and nystatin 100 % resistance. The report from Ali *et al*. (2008) in a related study showed one case of *Penicillium duclauxi* from ear swab of otomycotic patients which was

sensitive to nystatin but resistant to voriconazole and fluconazole.

CONCLUSION

Candida sp. was the dominant fungi isolated from the ear swab of otomycosis patients whereas the fungi with least frequency of occurrence involved coinfection between *Candida albicans* and *Penicillium* sp. Based on susceptibility testing of *Candida* sp. which is usually the dominant fungi implicated in otomycosis using three antifungal agents, voriconazole is best recommended for medical treatment. A situation *Penicillium* sp is also involved in otomycosis, fluconazole should be included in the medical treatment because *Pencillium* sp. was resistant to voriconazole.

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