

Clinical and Microbiological Epidemiology of Otomycosis in the Centre Region of Cameroon

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Abstract: Otomycosis is a superficial, sub-acute or chronic infection of the external auditory canal, characterized by pruritis, inflammation, pain and itching commonly seen in tropical and subtropical regions of the world. Various host and environmental factors can predispose a person to otomycosis. However, a clinical presentation along with otoscopic observations of the patients shows fungal and bacterial infections. Proper identification of causative agents is necessary in order to prevent recurrences and complications such as hearing lost. The aim of our study was to determine the fungi and bacteria pathogens causing otomycosis and to derive association of risk factors with otomycosis of the clinically diagnosed patients. A descriptive cross-sectional study was conducted in the otorhinolaryngology department at the University Teaching hospital and the Central hospital over a period of one year. A total of 250 clinically diagnosed patients of otomycosis of age above one year were included in the study. We evaluated age and sex distribution, predisposing factors and complaints of the clinically diagnosed patients for otomycosis. All samples collected were transported and evaluated by both direct microscopic examination and culture method for bacteria and fungi examination, which were identified by standard procedures. Among 250 samples, 46.22% yielded fungal growth, 21.33% grew bacteria only and 32.44% showed mixed growth of fungi and bacteria. Major fungal isolates were *Aspergillus* (n=121) including 75 isolates of *Aspergillus* section *Nigri*, 20 isolates of *Aspergillus* section *Flavi*, 13 isolates of *Aspergillus* section *Fumigati*, 8 isolates of *Aspergillus* section *Nudilante* and 5 isolates of *Aspergillus* section *Terrei*. 48 isolates were identified as *Candida* species. Major bacterial isolates were *Staphylococcus aureus* (n=45) followed by *Pseudomonas* species (n=26), *Klebsiella* species (n=21), *Escherichia coli* (n=7) and *Proteus* species (n=3). This study highlights the highest isolation of *Aspergillus* section *Nigri* in cases of clinically diagnosed otomycosis patients at the two reference hospital in Yaoundé, Cameroon with high prevalence seen in patients using antibiotic eardrops as a mean of treatment from pains and itching.

Keywords: Otomycosis, *Aspergillus* Species, *Candida* Species, Bacterial Pathogens, Predisposing Factor

1. Introduction

Otomycosis is a sub acute or chronic fungal infection of

the external auditory canal with some complications involving the middle ear [1]. It occurs because the protective lipid/acid balance of the ear is lost [2]. Otomycosis has a

worldwide distribution. It is estimated that approximately 25% of the total cases of ear infections are due to fungi, and the disease is more prevalent in warm and humid climates [3]. In the recent years there has been an increased in the incidence as a result of possibly huge number of immunocompromised patients [4]. The fungus may not be the primary cause but merely a secondary invader in cases of otitis externa. Therefore otomycosis can be seen in mixed fungal with bacterial infections [5].

Bacterial infections are the most common cause of otitis externa in subtropical areas, followed by fungal infections, which are responsible for 10–20 percent of otitis externa cases [6]. Although a wide spectrum of fungi is involved, *Aspergillus* spp and *Candida* spp are the most common species encountered [7, 8]. Dematiaceous fungi such as *Alternaria*, *Exophiala*, *Cladosporium* and *Nigrospora* have also been described as causative agents of otomycosis [8-10]. Fungi are abundant in soil or sand that contains decomposing vegetable matter. These materials are desiccated rapidly in tropical sun and blown in the wind as small dust particles. The airborne fungal spores are carried by water vapour, a fact that correlates the higher rates of infection with the monsoon, during which the relative humidity rises to 80% [11].

Factors that predisposed to otitis externa include absence of cerumen, high humidity, increased temperature, bacterial otitis externa, corticoid therapy, swimming [12] and local trauma caused by sharp objects like sticks or hearing aids. Cerumen has a pH of 4 to 5 and so suppresses bacteria but conducive for fungal growth. Aquatic sports, including swimming and surfing, are particularly associated with otomycosis because repeated exposure to water results in removal of cerumen and drying of the external auditory canal [13]. Otomycosis is predominantly unilateral [14], found in all age groups, but majority of the cases of otomycosis occur in patients aged 21-30 years with equal male – female distribution [15].

Yaoundé is a large town in the Centre Region of Cameroon. It is situated at the Southern plateau, the climate is equatorial consisting of 2 rainy season and 2 dry season with high temperature and high humidity throughout the year, a condition conducive for fungal and bacterial growth. Majority of patients attending the Central Hospital and University Teaching Hospital are agricultural labourers, in which their system of farming are bush fallowing and market gardening.

No epidemiological study has been done to identify the causative agents causing otomycosis in this geographical area. The present study has been undertaken to determine causative agents as well as the microbial distribution of pathogens involved in otomycosis in patients attending the ENT unit of Central Hospital and University Teaching Hospital with otitis externa.

2. Materials and Methods

2.1. Study Type

We carried out a descriptive cross sectional study on 250 patients clinically diagnosed to have otomycosis infection.

This was achieved by visiting the outpatient clinic of the otorhinolaryngology department weekly during a period of 12 months from March 2018 to February 2019 at the Central Hospital and University Teaching Hospital in Yaoundé, Cameroon. General information like age, sex, occupation, diabetic status, trauma, history of ear surgery or any fungal infection in other parts of body and laterality of symptoms were recorded. Any history of habits like use of oils/ear drops; wooden sticks or metal wax picks for removal of wax were also recorded.

2.2. Ethical Consideration

An Ethical clearance was obtained from the Centre Regional Committee for Human Health Research bearing no: 00842/CRERSHC/2018 and an authorization from the Directors of Central Hospital and University Teaching Hospital. Verbal informed consent was sought from individual patients from whom the ear samples were collected. To do this, it was explained to the patients in languages they understood that, the isolates that would be obtained from their samples would be used in this study. Samples from patients who consented were processed for bacteria and fungi growth at the Zion laboratory of Microbiology, Yaoundé. The ear samples from patients who disagreed were not included in the study.

2.2.1. Inclusion Criteria

All patients clinically diagnosed of otomycosis of age above one year presenting symptoms like itching, pain, feelings of blocked ear, tinnitus, deafness, discharge and in which otoscopic examinations reveals wet or dry masses of hyphae/spores were included in the study.

2.2.2. Exclusion Criteria

All patients presenting with symptoms of severe otitis media, tympanic membrane perforations, prior ear surgery were excluded in our study.

2.2.3. Collection of Samples and Processing

The samples were collected from the external auditory canal from patients that were clinically diagnosed to have otomycosis (figure 1) under aseptic conditions with the help of sterile cotton swab containing a preservation medium (Sigma transwab-liquid amies).

Samples were collected from either the right or left ear presenting signs or symptoms of otomycosis infection. All samples were evaluated by both direct examination and culture method. A portion of the sample was cultured on MacConkey agar, Chapman agar and Chocolates Agar plates at 37°C for 24hrs to 48 hrs for bacterial growth. Identification of the bacterial isolates was done by standard bacteriological procedures [16]. For mycological identification, direct microscopic examination was carried out by 10% KOH examination and inoculation of material was done on two prepared Sabouraud Dextrose Agar (SDA) plates supplemented with chloramphenicol. One plate was incubated at room temperature 25°C for 3-5 days and another at 37°C for 48 hours. Both plates were observed for fungal

growth daily. Fungal growth was identified by standard procedures [17]. Identification was done on the basis of colony morphology and lactophenol Cotton Blue (LPCB) mount microscopy. *Aspergillus* isolates were characterized by varying length of conidiophores and extent of coverage of vesicles by phialides. For characterization of *Candida* isolates, germ tube test was done by observing the production of germ tubes on isolates in serum after 2 hours of incubation at 37°C.

3. Results

3.1. Age-wise Distribution, Contributing Factors and Clinical Features of Otomycosis

Out of 250 cases, 121 cases were in 21-40 years of age group constituting maximum of (48.4%) of the cases clinically diagnosed of otomycosis (figure 2). We had 120 (48%) males and 130 (52%) females, giving a female predominance in otitis cases (52%).

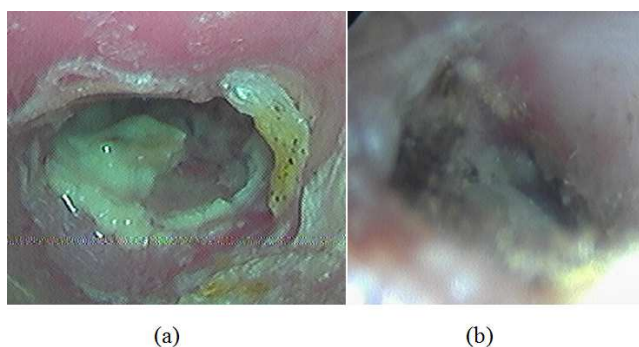


Figure 1. Otoscopic examination of fungi and bacteria isolates; (a) otoscopy of bacterial otitis (b) Otoscopy of *Aspergillus niger* mixed with *Candida* species otitis.

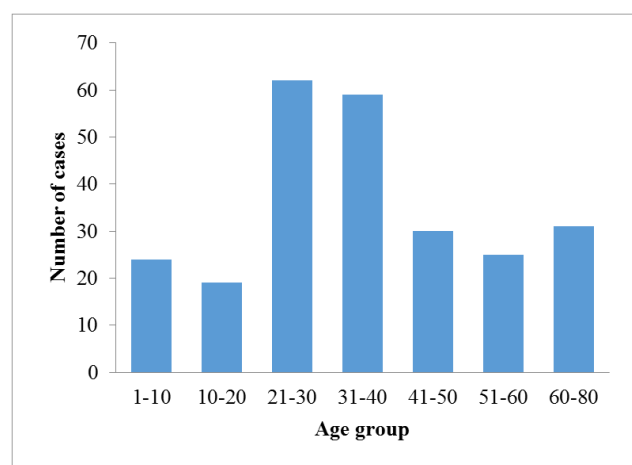


Figure 2. Age-wise distribution of the otomycosis cases.

Out of 250 patients 75 (30%) had right ear involvement and 175 (70%) had left ear involvement. In our study, 50 (22.2%) cases had bilateral infection and 175 (77.8%) cases had unilateral infection. There was a significant difference in right or left ear involvement. Majority of the

patients gave the practice of using of antibiotic ear drops as means of treatment (Table 1). Most common presenting complaint was itching of the ear, followed by hearing difficulty, otalgia, ear discharge, inflammation and tinnitus (Table 1).

Table 1. Symptomatology and predisposing factors of otomycosis.

Major symptoms	Number of cases	Percentage (%)
Itching	100	40
Earpains (otalgia)	50	20
Discharge (otorrhea)	30	12
Tinnitus (ring in the ear)	17	6.8
Inflammation	20	8
Hearing difficulty	33	13.2
Total	250	100

Predisposing factors	Number of cases	Percentage (%)
Antibiotic ear drops	60	24
Diabetes	40	16
Use of wooden object for cleaning the ear	30	12
Swimming	13	5.2
Earpiece	27	10.8
No cerumen	55	22
No predisposing factor	25	10
Total	250	100

3.2. Microbiological Findings

Among 250 samples, 110 (44%) showed only fungal elements, 81 (32.4%) showed both bacterial and fungal elements and 34 (13.6%) showed only bacteria. A total of 170 (68%) samples showed fungal elements and 80 (32%) showed bacteria. Among the 250 patients, 25 (10%) of the samples didn't show either fungal elements or bacteria on microscopy and culture. Among 170 samples showing fungal elements on KOH mount, 168 samples grew fungi in culture. Also among 80 samples showing bacteria on Gram stain, 78 samples grew bacteria in culture. There was very little difference in direct microscopy findings and culture results. Among 250 samples, 80 samples yielded single fungus and 24 samples yielded two fungi each, without any bacterial isolates. Forty-eight samples grew single bacteria and five samples yielded two bacterial isolates each, without showing any fungal growth. All other samples yielded mixed fungal and bacterial growth. A total of 179 fungi were isolated from the 250 samples and 102 bacterial isolates were grown from 250 samples. From the 250 samples, 225 cases yielded a positive growth for both fungi and bacteria, with 25 samples not showing any growth.

The various fungi isolated from study group are shown in (Table 2) the commonest isolate was *Aspergillus* section *Nigri* constituting 44.38% of fungal isolates. *Candida* species constituting 28.40% was the second commonest isolate. With 20 isolates of *Aspergillus* section *Flavi*, 13 isolates of *Aspergillus* section *Fumigati*, 8 isolates of *Aspergillus* section *Nidulante* and 5 isolate of *Aspergillus* section *Terrei*. *Aspergillus* spp. made up for 71.60% against 28.40% of

Candida species of the fungal isolated. *Aspergillus* species is the commonest fungus found in the atmosphere and a known contaminant everywhere. Conidia of these fungi spread through the air everywhere and wherever it finds suitable site for its growth it grows. When there is a predisposing condition for fungal growth in the external auditory canal, it is the commonest fungus to colonize and grow there. The various bacteria isolated from the study group is depicted in (Table 2) The major bacterial isolate was *Staphylococcus aureus* (44.12%), followed by *Pseudomonas* spp. (25.49%) and *Klebsiella* spp. (20.59%). *Escherichia coli* (6.86%) and *Proteus* spp. (2.94%), are the other isolates. A total of 102 bacteria were isolated from 121 samples.

Table 2. Distribution of aetiological agents of otomycosis.

Fungi species	Number of isolates	Percentage (%)
<i>Aspergillus</i> section <i>Nigri</i>	75	44.4%
<i>Aspergillus</i> section <i>Flavi</i>	20	11.8%
<i>Aspergillus</i> section <i>Fumigati</i>	13	7.7%
<i>Aspergillus</i> section <i>Nidulante</i>	8	4.7%
<i>Aspergillus</i> section <i>Terrei</i>	5	3.0%
<i>Candida</i> species	48	28.4%
Total	169	100

Bacterial species	Number of isolates	Percentage (%)
<i>Staphylococcus aureus</i>	45	44.12%
<i>Pseudomonas</i> species	26	25.49%
<i>Klebsiella</i> species	21	20.59%
<i>Escherichia coli</i>	7	6.86%
<i>Proteus</i> species	3	2.94%
Total	102	100

4. Discussion

Otomycosis is troublesome diseases encountered in the otorhinolaryngology department which affect the external auditory canal with infrequent complications involving the middle ear. This infection can be acute or sub acute, and is characterized by itching, earache, blocking sensation and discomfort. Outstanding to the varied microbiological flora of the ear, both fungi and bacteria are involved in otomycosis causing a mixed infection which can be bilateral or unilateral, early diagnosis is of paramount importance to guide proper treatment. In the present study; majority of the patients were in the age group 21 to 40 years and females were affected more than males which is corroborating with other studies showing higher incidence among 21-30 years of age [14, 18-21]. This may be because people of this age group are the active age group involved in different activities which expose them to the infectious agent present in the air [22]. We had a female predominance in otitis cases to be 52% against 48% in male. Similar findings have been reported by many authors [5, 23, 24]. This may be due to the fact that Female are highly involved in house hold work which exposed them to dusty air containing spores making them vulnerable to the otitis infection [22].

In our study, antibiotic ear drops was the most common

predisposing factor (24%) followed by no cerumen, diabetes, Ear pricking with hard objects, earpiece and swimming. This was in accordance with the study of Fasunla et al. [25], who found 42% of cases with the same predisposing factor. This study also corroborates with the studies conducted by Rawat Sarita et al. [21] which shows history of using wicks present in 35% of the cases, followed by association with diabetes in 7% and swimming history in 4% cases. Use of hard objects to clean the ear canal leads to trauma in which fungal spores settles and cause infection. Swimming in ponds and rivers is a common way of getting such infection because water trapped in ear canal after bathing in a humid climate can cause fungal infection of the ear [26] and use of antibiotic ear drops act as a risk factor because the antibiotic weakens the normal immunity of the ear and provides a suitable medium for the growth of the fungus [5].

In our study, common presenting symptom was itching in 24% of cases followed by ear pain (15.2%) which is similar to findings by other researchers [20, 27]. Inflammation of the ear canal leads to itching and pain and other symptoms like sensation of ear blockage, discharge and decreased hearing may be due to accumulation of fungal debris in the ear canal [27].

Among the 225 cases which showed culture positive, 104 cases showed fungal growth, 53 cases presented bacterial growth and 68 cases showed mixed growth. This our study corroborates with other studies carried by Kulal et al. [20], Rawat et al. [21]. This higher incidence of fungal growth can be attributed to hot and humid climate of the Centre Region of Cameroon.

Most of the fungal ear infections are caused by members of *Aspergillus* spp like *A. niger*, *A. fumigatus* and *Candida albicans* [20] which is seen in our study having *Aspergillus* section *Nigri* as the predominant pathogens in 47.48% of fungi growth. Species in the section *Nigri* can grow in cerumen, epithelial scales and debris deep in external auditory canal. This is in accordance with study by Joy et al. [28] and Paulose et al. [7]. Among bacteria, *Staphylococcus aureus* and *Pseudomonas* spp. were the predominant isolates. This finding is in accordance with study by Kulal et al. [20]. Bacterial infection of auditory canal may be one of the predisposing factors for development of otomycosis [5]. Mixed bacterial and fungal infections were also reported in a studies carried out in Indian [29, 26, 30].

5. Conclusion

The usage of antibiotic ear drops for treatment of otitis externa is the major predisposing factor seen in our study with Itching presenting the major symptom in majority of patients. *Aspergillus* and *Candida* species are the major fungal isolates and *Staphylococcus aureus* and *Pseudomonas* species are major bacterial isolates. Upon clinical suspicion, culture of both bacteria and fungi are required to identify the pathogen, as mixed infections are also seen in cases of otitis externa.

Conflicts of Interest

The authors declare no conflict of interest.

Author Contributions

CBT, JPD conceived and designed the experiments; IAE performed the experiments; JPD and IAE analyzed the data; IAE drafted the manuscript and JPD finalized the paper.

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