Original Article

Mycological Profiling in Children with Chronic Suppurative Otitis Media (CSOM) in Calabar, Nigeria

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ABSTRACT

Chronic suppurative otitis media (CSOM) is the inflammation of the middle ear and mastoid cavity. Fungal organisms have been implicated in most cases of CSOM. This study was conducted todetermine the fungal profile and antifungal susceptibility pattern of isolated fungal pathogens. Clinical samplescollected from 218 children with sterile disposable plastic Pasteur pipetteswere expressed onto swabs and were subjected to microscopic and culture analyses. The fungi were identified based on their cultural and morphological characteristics, before finally carrying out microscopic assessment using lactophenol blue staining procedure. The CLSI guideline was used to determine the susceptibility patterns against commonly used antifungal agents. The age group, 1-5 had the highest number of participants 68 (31.2%). The mean age of the participants was 8.69 ± 6.02 years. Out of the 218 participants, fungal pathogens were isolated from only 12participants, giving a CSOM prevalence rate of 5.5%. Of these 12 participants, 10 (83.3%) were female while only 2 (16.7%) were male. Aspergillus species followed by Candida albicans were the most common isolated fungi. A. niger was 100% sensitive to all tested drugs except clotrimazole to which it was only 66.7% sensitive. A. fumigatus was 100% sensitive to Nystatin, Fluconazole, and Ketoconazole. C. albicans demonstrated absolute susceptibility only to Ketoconazole and Voriconazole. The role of fungal infections in CSOM has been recognised in our setting. Mycology studies to determine commonly isolated fungal pathogens as well as their susceptibility patterns should be carried out in suspected cases of CSOM toimprove management and generate useful empiric treatment data.

Keywords: chronic suppurative otitis media, CSOM, fungal ear infections, otomycosis, SOM, suppurative otitis media.

INTRODUCTION

Chronic suppurative otitis media (CSOM) is the inflammation of the middle ear and mastoid cavity of six weeks duration or above, often associated with recurrent ear discharge through perforated tympanic membrane.[1] CSOM has also been defined as a non-healing disease condition due to perforation of tympanic membrane associated with chronic inflammatory changes of the mucoperiosteum of the middle ear cleft with or without mucopurulent otorrhoea of three months or more duration.[2,3] It is usually associated with multiple organisms and is well known for its persistence and recurrence. The inappropriate and widespread use of broad spectrum topical antibiotics, use of steroids, immunodeficient

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conditions, and presence of moisture in the ear canal predispose to fungal CSOM.[4,5] Also, a combination of common risk factors for acute otitis media (AOM) as well as factors associated with poor healthcare and low socioeconomic status such as living in crowded conditions, unhygienic lifestyle, living in a large family, day-care attendance, poor nutrition, low parental education, cotton bud cleaning of ears, unsterile ear piercing are all additional risk factors for CSOM.[6,7]

Chronicity of ear discharge is a crucial factor for otomycosis in CSOM, as it brings about humid condition in the ear, alteration of the pH to alkaline, and the accumulation of epithelial debris – all these providing optimal condition for the fungal organisms to thrive.[8]

The symptoms of CSOM seem to be non-specific, however, common symptoms include irritation, intractable itching, discomfort or restlessness, pain and discharge from ear. The most common isolated fungal pathogens in CSOM include *Aspergillus* and *Candida* species.[9-11] Complications of CSOM include brain abscess, septicaemia, meningitis, facial paralysis and mental retardation.[12-14] CSOM is one of the most frequently seen ear diseases among children in routine clinical practice, posing a high disease burden of up to 72 cases per 1000 inhabitants.[15] Among the enrolled children in nursery, primary and secondary school in a rural community in Kwara State, Nigeria, a prevalence rate of 7.3% has been reported.[16] The worldwide prevalence of CSOM has been put at 65-330 million people of which about half of them have hearing impairment.[17]

There is paucity of literature focusing on fungal CSOM with the fungal profile and susceptibility pattern in Nigeria in particular, and Africa in general. Therefore, this study was conducted to determine the profile of the fungal pathogens isolated from children with CSOM and the susceptibility pattern of these fungi to commonly used antimycotics.

MATERIALS AND METHODS

Study design

A cross-sectional prospective hospital-based study was conducted from September, 2019 to April, 2021 to determine the fungal profile and susceptibility pattern of the organisms to commonly used antifungal drugs for CSOM.

Study population

The participants comprised of children under the age of 18 years either admitted in the ENT wards or attending the Out-patients clinic of the University of Calabar Teaching Hospital, Nigeria, diagnosed clinically of having CSOM whose parents/guardians consented to be enrolled into the study.

Inclusion criteria:

Under 18-year-old children admitted in the ENT wards, or those attending the ENT out-patient clinic, General Out-patient clinic or the Paediatric Out-patient clinic with tympanic membrane perforation and active otorrhoea, clinically diagnosed of CSOM were recruited. The cut-off used for defining CSOM was ≥3 months.

Exclusion criteria

Those with non-suppurative ear disease, patients with otitis externa and patients with ear diseases with intact tympanic membrane, and those that refused to participate were excluded.

Sample size determination

The sample size was calculated using this formula $n = Z^2pq/d^2$ where n is the minimum required sample size in the population greater than 10,000, Z is standard normal deviate, p is prevalence from a previous study, q is proportion of failure, d is precision tolerable margin of error, and Z = 1.96, and prevalence rate was 14.7% based on a previous similar study.[18,19] A sample size of at least 196 was required for the study, however we used 218 considering a possible 10% attrition rate.

Study area

This study was conducted in Calabar city, Cross River State, Nigeria. Calabar is the capital city of Cross River State – one of the six states in the south-south geopolitical zones in Nigeria. Other major cities from which patients feed the major tertiary health facility (University of Calabar Teaching Hospital) include Akamkpa, Akpabuyo, Ikom, Obanliku, Obubra, Obudu, Odukpani, and Ogoja. Cross River State shares boundaries with Enugu and Abia States to the west, Republic of Cameroon to the east, Benue State to the north, and Akwa-Ibom and Atlantic Ocean to the south, spanning an area of over 23,074 square kilometres.[20] The State falls within the tropical rainfall belt and experiences an annual rainfall of about 1300 to 3000 mm, and mean annual temperature of 30°C.[20]

Sampling Technique

A consecutive sampling technique was deployed – this entailed recruiting into the study every subject meeting the criteria of inclusion until the sample size was achieved.

Data Collection Method and Tool

Information used in this study was obtained from thorough clinical examination, laboratory investigation, and well-structured interviewer-administered questionnaires. All study participants were subjected to sociodemographic and clinical information abstraction as per the structured questionnaire designed for the study. Biodata including child's age, gender, educational status, religion and tribe were abstracted. Clinical examination was carried out and assessment was made based on presentations and duration of symptoms for the disease and symptoms including ear discharge, ear pain, itching, restlessness, hearing loss, cough, recurrent nasal discharge, and fever were sought after. Also, possible risk factors for CSOM such as house-hold overcrowding, history of upper respiratory tract infection, none/incomplete immunization, absence of breast feeding, recurrent otitis media in a sibling, supine breast/bottle feeding position, parents' educational status, accommodation status, and use of local ototopics were obtained.

Clinical samples were collected from patients with sterile disposable plastic Pasteur pipettes. Negative pressure was first created in the plastic Pasteur pipette by compressing the pipette bulb before gently introducing it into the middle ear canal via the perforated tympanic membrane and the ear exudate aspirated by releasing the vacuum. This pipette for fungal culture and analysis had their content expressed onto a swab stick enclosed in a labelled amies transport media, which was enclosed in zip lock back for onward transport to the Medical Microbiology and Parasitology Laboratory within 30 – 120 minutes. Samples were collected separately from each discharging ear in cases of bilateral affectation.

Fungal study

Streaking was done on labelled Sabouraud's Dextrose Agar (SDA) plate with the swab sticks, then incubated at room temperature aerobically for 3 -14 days. Putting on appropriate personal protective equipment (mask, gowns and gloves), the growths were identified based on its cultural and morphological characteristics, before finally carrying out microscopic assessment using lactophenol blue staining procedure.[21] *Candida albicans* were differentiated from the other Candida species by carrying out Germ tube test.

The Clinical and Laboratory Standard Institute (CLSI) guidelines guided the conduct and interpretation of the antifungal susceptibility test using common antifungal drugs.[22] The Oxoid (Oxoid, UK) antifungal discs used included Nystatin (100U), Clotrimazole (10 µg), Fluconazole (25 µg), Ketoconazole (10 µg), Griseofulvin (2 µg), and Terbinafine (10 µg).

Ethical Consideration

The Helsinki Declaration medical research ethical principles were strictly followed.[23] Questionnaires were only self-administered to ensure anonymity and confidentiality. Ethical clearance was obtained from the Health Research Ethical Committee (HREC) of the University of Calabar Teaching Hospital, Calabar. Informed consent was obtained from children from the age of 12 years while for those below 12 years, their parents/guardians assented for their enrolment into the study. The procedure for sample collection was well-explained to parents/participants prior to collection.

Data Analysis

All statistical analyses were performed using IBM SPSS statistical software for Windows (version 20.0 SPSS Inc. Chicago, IL. USA). Continuous and categorical variables were summarised and reported as mean ± SD, and percent values respectively. Chi-square test was deployed to determine the relationship between categorical variables. Binary logistic regression model was built to predict the development of fungal CSOM, using forward LR method. Presence of Fungal CSOM was set as the outcome variable (predicted), while gender of the participants, age group, mother's education level, mother's occupation, history of previous discharge, classroom overcrowding, and use of traditional ototopic agents were set as the predictors (independent variables). The statistical significance was set at $p<0.05$.

RESULTS

Of the 218 participants in this study, 121 (55.5%) were male, and 97 (44.5%) were female. The age group, 1-5 had the highest number of participants 68 (31.2%), while less than 1 year and 11-15 age groups had the least number of participants of 26 (11.9%) respectively. The mean age of the participants was 8.69 ± 6.02 years. Eighty-four (42.4%) of the participants were in secondary school, while only 20 (10.1%) of them were in Day-care. About half of the participants' mothers had tertiary education, 103 (47.2%), while only 13 (6.0%) of the mothers had no formal education. (**Table 1**).Out of the 218 participants, fungal pathogens were isolated from 12 (5.5%) participants. Of these participants, 10 (83.3%) were female while only 2 (16.7%) were male (p=0.005, $X^2 = 7.8$). The distribution of fungal pathogens isolated is shown in **Figure 1** below, with 5 isolates coming from age group 1-5, while age group of less than 1 year had no fungal isolate. **Table 2** below shows that *Candida albicans* was the most isolated species (5/12, 41.7%), followed by *Aspergillusfumigatus* (4/12, 33.3%). However, Aspergillus species as a genus were the highest fungal pathogens isolated (7/12, 58,3%), while Candida was 5/12 (41.7%).

In 3 (25%) of the confirmed fungal CSOM cases, only the left ear was affected, in 8 (66.7%) cases, the right ear alone was affected, while in one case (8.3%), both ears were affected. Ear pain and itching were the most frequent symptoms presented by the participants, 7 (58.3%) respectively, while only once (8.3%) was cough and snoring presented as a complaint by the participants (**Table 3**). **Table 4** shows the sensitivity pattern of the isolated fungal organism to the commonly used antifungal agents in our hospital. *A. niger* was 100% sensitive to all tested drugs except clotrimazole to which it was only 66.7% sensitive. *A. fumigatus* was 100% sensitive to Nystatin, Fluconazole, and Ketoconazole, but displayed only 75% susceptibility to Clotrimazole, Voriconazole, and Terbinafine. On the other hand, *C. albicans* demonstrated absolute susceptibility only to Ketoconazole and Voriconazole, while showing varying resistance to other antifungals. **Table 5** shows the result of the binary logistic regression analysis. It showed that the odds of having a fungal CSOM is about 7 times for a female child versus when it is a male child (OR = 7.1; 95%CI = 1.508 - 33.213; $p<0.05$). Other independent variables were not included in the final equation, as such, they are not relevant as predictors.

Table 1. Showing Sociodemographic characteristics of the patients.

Sociodemographic Data	Frequency (%)
Mean age ± SD (in years)	8.69 ± 6.02
<1	26 (11.9)
1 – 5	68 (31.2)
6 – 10	51 (23.4)
11 – 15	26 (11.9)
> 15	47 (21.6)
Sex (n = 218):	
Male	121(55.5)
Female	97 (44.5)
Participants' Education (n = 198)	
Day Care	20 (10.1)
Nursery	43 (21.7)
Primary	51 (25.8)
Secondary	84 (42.4)
Mothers' Education (n = 218):	
No formal Education	13 (6)
Primary	4 (1.8)
Secondary	98 (45)
Tertiary	103 (47.2)
Fathers' Education (n = 218):	
No formal Education	6 (2.8)
Primary	2 (0.9)
Secondary	82 (37.6)
Tertiary	128 (58.7)
Mothers' Occupation (n = 218):	
Not gainfully Employed	17 (7.8)
Business	85 (39.0)
Civil Servant	73 (33.5)
Petty Trader	28 (12.8)
Farmer	11 (5.0)
Others	4 (1.8)
Fathers' Occupation (n = 218):	
Not gainfully Employed	3 (1.4)
Business	73 (33.5)
Civil Servant	81 (37.2)
Petty Trader	6 (2.8)

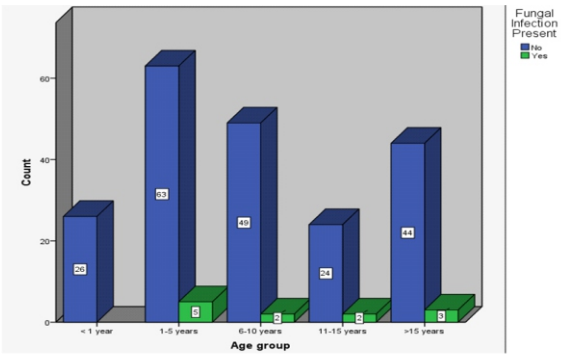

Figure 1. Distribution of fungal pathogen isolation among different age groups

Table 2. Fungal pathogens isolated from participants

Fungal Isolates	Frequency (%)	*p* - value
Aspergillus niger	3 (25)	0.766
Aspergillus fumigatus	4 (33.3)	
Candida albicans	5 (41.7)	
Total	12 (100)	

Table 3. Showing the distribution of symptoms among the participants with fungal CSOM

Presenceof Fungal CSOM (n)	Symptoms **Frequency (%)** Ear pain	Itching	Restlessness	Tinnitus	Hearing loss	Nasal discharge	Cough	Snoring	Fever
Yes (12)	7 (58.3)	7 (58.3)	4 (33.3)	3 (25.0)	2 (16.7)	3 (25 .0)	1 (8.3)	1 (8.3)	2 (16.7)
No (206)	125 (60.7)	117 (56.8)	72 (35.0)	49 (23.8)	44 (18.4)	42 (20.4)	54 (26.2)	44 (18.4)	56 (27.2)
P – value	0.87	0.90	0.90	0.92	0.87	0.70	0.17	0.28	0.42

Table 4. Antifungal susceptibility pattern of the isolated fungal pathogens

	Susceptibility pattern of the isolated Fungi (%)			**P - value**
Antibiotics	***A. niger***	***A. fumigatus***	***C. albicans***	
Clotrimazole	2 (66.7)	3 (75)	4 (80)	0.915
Nystatin	3 (100)	4 (100)	4 (80)	0.466
Fluconazole	3 (100)	4 (100)	3 (60)	0.186
Ketoconazole	3 (100)	4 (100)	5 (100)	a
Voriconazole	3 (100)	3 (75)	5 (100)	0.336
Terbinafine	3 (100)	3 (75)	(80)	0.657

Table 5. Binary logistic regression analysis for the prediction of fungal CSOM.

		B	S.E.	Wald	Sig.	OR	95% CI for OR Lower	Upper
	HistoryPrevDisch			.004	.998			
Step 1a	HistoryPrevDisch(1)	-24.045	40194.173	.000	1.000	.000	.000	
	HistoryPrevDisch(2)	-24.006	40194.173	.000	1.000	.000	.000	
	Gender(1)	1.852	.796	5.413	.020	6.373	1.339	30.333
	HistoryPrevDisch			.000	1.000			
Step 2b	HistoryPrevDisch(1)	-23.344	40193.885	.000	1.000	.000	.000	
	HistoryPrevDisch(2)	-23.359	40193.885	.000	1.000	.000	.000	
Step 3b	Gender(1)	1.957	.789	6.156	.013	7.078	1.508	33.213
	Constant	-3.998	.714	31.395	.000	.018		

a. Variable(s) entered on step 1: History of previous discharge; b. Variable(s) entered on step 2: Gender, B = coefficient of the model equation, S.E = standard error; Ward = ward statistics,

DISCUSSION

There has been rise in incidences of fungal infection of late owing to rise in the number of those that are immunocompromised and irrational and excessive use of topical antibiotics. Otomycoses are often associated with a decrease in host immunity and more often than not regarded as mere colonisation rather than invasion and as such does not require treatment. This assertion has been proven to be false. Reports of otomycoses involving *aspergillus* species with even intracranial complications suggest that fungal organisms can be pathogenic in ear infections as well.[24,25] Chronic secretions play a crucial role as a cause of otomycoses in CSOM.[26] The persistent otorrhoea cause a moist condition in the ear that alters the middle ear pH to a base, optimal for fungal growth.[27] Fungal diagnostics in most resource-constrained countries including Nigeria remains underdeveloped, fungal studies are only available in few tertiary health facilities. There is therefore need to have a good guess of commonly clinically encountered fungal pathogens and their antimicrobial profile.

In this present study, the overall prevalence of fungal infection in CSOM patients was 5.5%. Similar observation was made by Ogbogu *et al.* who reported a prevalence of 6.8% among children with CSOM attending a tertiary health facility in Edo State Nigeria, while a prevalence of 10.9% was reported by Jido *et al.*among patients with CSOM in Kaduna State, Nigeria.[28,29] However, a similar study in Abakiliki reported a higher prevalence of 21.3%.[30] On the other hand, Nwankwo *et al.* in Kano, Nigeria, in 2005 reported a rate (3.9%) lower than what was reported in this current study.[31] In contrast, much higher incidence of fungal infection in CSOM has been observed by Tawler *et al.* 49%.[32] FungalinfectionsinCSOM were more prevalent among females 83.3% than males 16.7%. This finding was similar to those by Akinjogunla *et al.*, Haneefa et al., and Panchal*et al.*, who also isolated more fungal pathogens in females than in males.[33-35] The reason for this disparity between the gender lines is not yet obvious, though there is a thinking that the persistent use of hijab or head ties by typical African women could be responsible for creating a more humid and moister environment required for fungal growth.On the contrary, studies conducted by Pradhan *et al*. and Vishwanatha *et al.*reported more cases of otomycosis in males than in females.[36,37]

Most fungal pathogens were isolated from the age group of 1-5 years in the index study. Afolabi *et al.* in their study also made a similar observation that under-5-year-old children had CSOM more thanany other age groups. Reason for this is not established, however, high incidences of upper respiratory tract infections in this group of children could predispose them to fungal CSOM.[38]

In this study, unilateral CSOM (91.7%) was more compared to bilateral infection (8.3%), and right ear more affected (66.7%) than left ear (25%). This finding is in congruent with the finding of studies by Haneefa *et al.*, and Ghosh *et al.*[34,39]Chauhan *et al.* reported a unilateral involvement that was as high as 92%.[40] Since most of these participants in this current study were right-handed, more frequent picking and rubbing of this side of the ear with the infected right hand could explain this preponderance of right ear affectation. Though, one previous study has reported equal or little variation in ratio of CSOM in both ears.[27]

Twelve fungal pathogens were isolated. The most frequently isolated fungus was *Aspergillus* species (58.3%), and the rest were *Candida* species (41.7%). Two species of *Aspergillus* were isolated - A. *fumigatus*in 33.3% while *A. niger* in 25%.A similar study by Kaur *et al.* has also reported *A. fumigatus* (41.1%) to be the most frequently isolated fungus followed by *A. niger* (36.8%).[41]This finding agrees with several other findings across the globe.[4,34,39]*C. albicans* has the ability to maintain synergistic relationship with skin bacterial flora such as *S. aureus* and the ecological niche of these skin flora, this most likely explains the findings.[42]

About the analysis of symptoms in this study, all the participants presented with otorrhoea, while 58.3% of them had ear pain and itching respectively. Hearing loss and fever were observed only in 16.7%. Haneefa *et al.* also reported ear pain and itching as the most common symptoms among his study participants in a tertiary care facility in India.[34]

Most of the fungal isolates displayed absolute susceptibility to the antifungal drugs tested. *A. niger* was 100% susceptible to all tested drugs – nystatin, fluconazole, ketoconazole, voriconazole, and terbinafine, except clotrimazole where susceptibility dropped to 66.7%. *A. fumigatus* displayed absolute susceptibility to nystatin, fluconazole, and ketoconazole but showed 25% resistanceto clotrimazole, voriconazole, and terbinafine. On the other hand, *C. albicans* were only 100% sensitive to ketoconazole and voriconazole, but showed varying levels of resistance to clotrimazole, nystatin, fluconazole, and terbinafine. This finding, especially with regard to ketoconazole is incongruent with findings from study by Akinjogunla *et al.*where the isolated species showed some resistance to ketoconazole.[33] In this study, all the fungal isolate were 100% sensitive to ketoconazole, a drug that has been recommended to be administered only topically because of its hepatotoxicity effect when given systemically. Terbinafine has an advantage over most other antifungal agents in that it can be given both topically and systemically.

CONCLUSION

An otolaryngologist should think of a possibility of fungal infections whenever handling a case of chronic suppurative otitis media. Obtaining specimen for fungal cultures should be a standard of care for this category of patients. In this study, *Aspergillus* species followed by *Candia* species were the most prevalent fungal pathogens isolated from children diagnosed of having CSOM. All isolated fungi showed absolute susceptibility to ketoconazole.

Recommendation

Each healthcare facility should conduct susceptibility testing of isolated fungal pathogens of CSOM against commonly prescribed antifungal agents within the healthcare facility vicinity. Empiric antifungal prescription for treatment of CSOM should be based on this antibiogram.

Limitation

The limitationof this study includes non-addition of cycloheximide and chloramphenicol to the general-purpose fungal culture media, SDA agar used in this study. Cycloheximide reduces environmental fungi while chloramphenicol is used to prevent bacterial contamination. Contamination could prevent the growth of pathogenic fungi.

Authors' Contributions: NUA and AAI conceptualized the study. NUA, AAI and GIO designed the study. NUA, PAI, DEE and BEE participated in fieldwork and data collection. AAI and NUA performed the data analysis and interpreted the data. AAI prepared the first draft of the manuscript, reviewed by NUA, GIO, PAI, DEE and BEE. All authors contributed to the development of the final manuscript and approved its submission.

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REFERENCES

1. Poorey V. Artilyer study of Bacterial flora in CSOM and its clinical significance. Indian J Otolaryngol Head Neck Surg 2002;54(2).
2. Berman S. Otitis media in children. N Eng J Med 1995;332(23):15601565.
3. Nwabuisi C, Ologe F. Pathogenic agents of chronic suppurative otitis media in Ilorin, Nigeria. East Afr Med J 2002;79:2025.
4. Kumar H, Seth S. Bacterial and Fungal Study of 100 Cases of Chronic Suppurative Otitis Media. J Clin Diagn Res 2011;5(6):12247.
5. Prasad S, Kotigadde S, Shekhar M. Primary Otomycosis in the Indian Subcontinent: Predisposing Factors, Microbiology, and Classification. Int J Microbiol 2014;2014:6 3 6 4 9 3.
6. Adoga A, Nimkur T, Silas O. Chronic suppurative otitis media: Socio-economic implications in a tertiary hospital in Northern Nigeria. Pan Afr Med J 2010;4(1).
7. Kamal N, Joarder A, Chowdhury A, Khan A. Prevalence of chronic suppurative otitis media among the children living in two selected slums of Dhaka City. Bangladesh Med Res Counc Bull 2004;30(3):95104.
8. Sengupta R, Kicker S. Otomycosis. Indian J Med

Sci 1978;32:57.

9. Osazuvza F, Osazuwa E, Osime C, Igharo E, Imade P, Lofor P. Etiologic agents of otitis media in Benin city, Nigeria. N Am J Med Sci 2011;3:95-98.

10. Shashikala B, Deepthi P, Viswanatha B. Fungal Flora in Chronic Suppurative Otitis Media: A Prospective Study in a Tertiary Care Hospital. Res Otolaryngol 2018;7(1):58.

11. Harahap M, Aboet A, Haryuna T. Pattern of fungal infections in Chronic Suppurative Otitis Media (CSOM) [Internet]. 2018 [cited 2023 Mar 3];Available from: https://www.taylorfrancis.com/chapters/oa-edit/10.1201/9781351190152-27/pattern-fungal-infections-chronic-suppurative-otitis-media-csom-harahap-aboet-haryuna

12. Ogisi F. Impedance screening for otitis media with effusion in Nigerian children. J Laryngol Otol 1988;102(11):9868.

13. Amedofu G, Opoku-Buabeng J, Ose-Bagyina A, Antwi B. Hearing loss among school children in Ghana. Ghana Med J 2003;37:148-152.

14. Okafor B. The chronic discharging ear in Nigeria. J Laryngol Otol 1984;98:1139.

15. Ologe F, Nwawolo C. Prevalence of chronic suppurative otitis media among school children in a rural community in Nigeria. Niger Postgr Med J 2002;9(2):636.

16. Acuin J. Chronic suppurative otitis media: a disease waiting for solutions. Comm Ear Hear 2007;4(6):1719.

17. Aarhus L, Tambs K, Kvestad E, Engdahl B. Childhood otitis media: a cohort study with 30-year follow-up of hearing (The HUNT Study). Ear Hear 2015;36:302308.

18. Ibrahim M. Research methodology and dissertation writing for health and allied health professionals. Abuja: Press Global Link Limited; 2009.

19. Amusa Y, Ijadunola I, Onayade O. Epidemiology of Otitis Media in a Local Tropical African population. WAJM 2005;24(3):22730.

20. About Cross River State [Internet]. 2023 [cited 2023 Mar 12];Available from: https://kekerete.tripod.com/CRSG/about.html#:~:text=The climate within Cross River,annual rainfall between 1300 3000mm.

21. Collins C, Lyne P. Microbiological Methods. 5th ed. Ibadan: Butterworth and Co-publisher Ltd; 2000.

22. Espinel-Ingroff A, Canton E, Fothergill A, Ghannoum M, Johnson E, Jones RN, et al. Quality Control Guidelines for Amphotericin B, Itraconazole, Posaconazole, and Voriconazole Disk Diffusion Susceptibility Tests with Nonsupplemented Mueller-Hinton Agar (CLSI M51-A Document) for Nondermatophyte Filamentous Fungi. J of Clin Microbiol 2011; 49(7):2568-2571 World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. J Int Bioethique 2004;15:1249.

24. Rowe-Jones J, Freedman A. Adjuvant itraconazole in the treatment of destructive sphenoid aspergillosis. Rhinology 1994;32:2037.

25. Kikuchi A, Funakubo T, Kohsyu H. A study of fungal infections in otorhinolaryngology. Acta Otolaryngol Suppl 1994;(suppl 511:2247.

26. Sauders J, Raju R, Boone J, Hales N, Berryhill W. Antibiotic resistance and otomycosis in the draining ear: Culture results by diagnosis. Am J Otolaryngol 2011;32:470476.

27. Dhingra R, Monga S, Kaur M, Brar R, Arora H. Role of fungal infections in CSOM: Prospective study. Indian J Basic Appl Med Res 2014;3(2):598608.

28. Ogbogu P, Eghafona N, Ogbogu M. Microbiology of otitis media among children attending a tertiary hospital in Benin City, Nigeria. J Public Heal Epidemiol 2013;5(7):280-284.

29. Jido B, Yahaya A, Yadock L. Isolation and

antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* in cases of Otitis media among patients attending Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. Eur J Biotechnol Biosci 2014;2(2):18-23.

30. Nnebe-agumadu U, Okike O, Orji1 I, Ibekwe R. Childhood suppurative otitis media in Abakaliki: Isolated microbes and in vitro antibiotic sensitivity pattern. Niger J Clin Pract 2011;14(2):15962.

31. Nwankwo E, Salisu A. Bacteriology of chronic discharging ear of patients in kano, Nigeria. J Med Lab Sci 2005;14(10):5762.

32. Talwar C, Kaur P, Pahwa R, Mittal A, Mehra Y. Fungal infections of ear with special reference to chronic suppurative otitis media. Mycopathologica 1968;104(1):4750.

33. Akinjogunla O, Eghafona N. Mycological Investigation in Patients with Acute Otitis Media. Sci J Microbiol 2012;1(1):1926.

34. Haneefa S, Bhama S, Rajahamsan J. Mycological Profile of Chronic Suppurative Otitis Media in a Tertiary Care Hospital in South India. IntJCurrMicrobiolAppSci 2017;6(2):12732.

35. Panchal P, Pethani J, Tatel D, Sanjay R. Analysis of various fungal agents in clinically suspected case of otomycosis. Indian J Basic Appl Med Res 2013;2(8):8659.

36. Pradhan B, Ratna N, Amaya R. Prevalence of otomycosis in OPD of ENT in Tribhuvan University of Teaching Hospital, Kathmnadu, Nepal. Ann Otol Rhinolaryngol 2003;112:3847.

37. Vishwanatha B, Vijayasree M. Otomycosis in immuno-competant and immune compromised patients; comparative study and literature review. Ear, Nose Throat J 2003;91:11421.

38. Afolabi O, Fadare J, Omokanye H, Olatoke F, Odi T, Saka M. Socioeconomic challenges of chronic suppurative otitis media management in state tertiary health facility in Nigeria. Egypt J Ear, Nose, Throat Allied Sci 2014;15:1722.

39. Ghosh A, Rana A, Prasad S. Prevalence of Fungal Infection in Chronic Suppurative Otitis Media A Study at Tertiary Care Hospital in Western Uttar Pradesh. Indian J Microbiol Res 2015;2(3):15962.

40. Chauhan S, Surender. Clinico-Mycological Profile of Otomycosis in Chronic Suppurative Otitis Media Patients Attending a Tertiary Care Teaching Hospital. J Pure Appl Microbiol 2021;15(2):8138.

41. Kaur R, Mittal N, Kakkar M, Aggarwal A, Mathur M. Otomycosis: A clinicomycologic