Sjournals
Scientific Journal of Microbiology (2012) 1(1) 19-26

Contents lists available at Sjournals
Scientific Journal of Microbiology
Journal homepage: www.Sjournals.com

Original article

Mycological investigation in patients with acute otitis media

O.J. Akinjogunla[a,*] and N.O. Eghafona[b]

[a]*University of Uyo, Uyo, Faculty of Science, Department of Microbiology, Akwa Ibom State, Nigeria*
[b]*Department of Microbiology, Faculty of Life Science, University of Benin, P.M.B. 1154, Benin City, Edo State, Nigeria*

[*]Corresponding author: University of Uyo, Uyo, Faculty of Science, Department of Microbiology, Akwa Ibom State, Phone No: +2348064069404

ARTICLE INFO

Article history:
Received 04 June 2012
Accepted 17 June 2012
Available online 30 June 2012

Keywords:
Acute otitis media
Deoxyribonuclease
Antifungal
Prevalence
Fungi

ABSTRACT

Middle ear swabbed samples from 272 patients with acute otitis media (AOM) attending Ear, Nose and Throat clinics were collected and cultured using standard mycological technique. Deoxyribonuclease and *In-vitro* susceptibility of the fungal isolates to Flucytosine (Flu), Nystatin (Nys), Voriconazole (Vor) and Ketoconazole (Ket) were evaluated using DNase agar and disc diffusion techniques, respectively. The results showed the highest prevalence of AOM in age group ≤ 10 years with 84 (30.9%) cases and lowest prevalence in age group ≥ 61 having 12 (4.4%) cases. Only 124 (45.6%) samples showed positive growth, while 148 (54.4%) showed no growth. Of the 124 samples with growth, 9(36.4%) samples showed growth of single fungal isolate, while 18 (6.6%) and 7 (2.6%) showed growth of two and three fungal isolates, respectively. *Aspergillus niger* was the predominant fungal isolate, followed by *Candida albicans* with 38 (24.4%), *Cryptococcus neoformans* 32 (20.5%), *Candida* spp 21 (13.5%) and *Aspergillus flavus* 14 (9.0%). Only 46 (29.5%) of fungal isolates were deoxyribonuclease producers, with 30/38 (78.9%) being *C. albicans* and 16/21 (76.2%) being *Candida* spp. The results of antifungal susceptibility showed that between 65.8 to 71.1% *C. albicans,* 64.3 to 85.7% *A. flavus,* 64.7 to 78.4% *A. niger*, 53.1 to 75.0% *C. neoformans*, and 52.4 to 57.1% *Candida* spp. were sensitive to Ket, Vor and Flu, while between 81.6-84.4% of *C. albicans* and *C. neoformans* were sensitive to Nys. In overall, 72.4%, 76.3%, 67.9% and 62.2% of the fungal isolates were sensitive to Flu, Nys, Vor and Ket, respectively. Consequently, Nystatin will be highly effective in treating AOM caused by fungi.

1. Introduction

Otitis media is the infection associated with the malfunctioning or inflammation of the middle ear due to pathogenic micro-organisms that are resident in the middle ear (Bluestone, 1998; Ekpo *et al*., 2009). There are two (2) types of otitis media: Acute Otitis Media (AOM) and Chronic Otitis Media (COM), of which each is subdivided into suppurative or non-suppurative, depending on the fluid present and also based on the complication (Bluestone, 1998). The aetiology of otitis media is multi-factorial including anatomical, infection, immunological, genetics and environmental factors (Bluestone and Klein, 2001; Casselbrant *et al*., 2004; Akinjogunla *et al*., 2012). Sources of infection in otitis media is solely dependent on the route by which infection reaches the middle ear and the chief route by which this occurs is through the eustachian tube (Daly,1997). Children are much more susceptible to acute otitis media since their eustachian tube is shorter and at more of a horizontal angle than in the adults and also because they have not developed the same resistance to bacteria and fungi as found in adults (Weiner and Collison, 2003). The patients with acute otitis media present the classic "earache", often accompanied by fever, possibly leading to insomnia, mild to moderate hearing loss, loss of balance, unresponsiveness to quiet sounds, unusual irritability, draining of fluid in the ear and eardrum perforations (Damoiseaux *et al*., 2000; Ehrlich *et al*., 2002). Symptoms of upper respiratory infections are often associated with acute otitis media in 94% cases (Arola *et al*., 1990).

In acute otitis media, the bacteria found in the middle ear include *Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae* and *Moraxella catarrhalis* (Akinjogunla *et al.,* 2012*)*. Occasionally, acute otitis media is usually caused by *Aspergillus* spp, *Candida* spp or other pathogens such as herpes virus (Kawo *et al*., 2010). Gulati (1997) and Loy *et al*. (2002) also reported that *Aspergillus niger, Candida albicans*, *Aspergillus fumigatus* and *Aspergillus* spp. caused acute otitis media. Treatment of fungal infections generally has been less successful than that of bacterial infections largely because eukaryotic fungal cells are much more similar to human cells than are bacteria (Prescott *et al*., 2011). Many drugs that inhibit or kill fungi are therefore quite toxic antibiotics for humans. In addition, most fungi have a detoxification system that modifies many antifungal agents (Hugo and Russell, 2007). Despite antifungal relatively low therapeutic index, a few drugs are useful in treating many major fungal diseases (Talaro and Talaro, 2002). A small battery of agents with special antifungal properties has been developed for treating systemic and superficial fungal infections. The main drug groups currently in use are the nystatin, the synthetic azoles and the flucytosine (Talaro and Talaro, 2002). The azoles are broad-spectrum antifungal agents with a complex ringed structure and the most effective azole drugs are; ketoconazole, fluconazole and itraconazole, which are used orally and topically (Talaro and Talaro, 2002). *Candida albicans* mutations in the ergosterol biosynthetic pathway and resistance to several antifungal agents such as fluconazole have been reported (Kelly *et al*., 1999; Sanglard *et al*., 2003). Fluconazole binds to and inhibit the activity of lanosterol 14 alpha-demethylase (erg 11p), a key player enzyme in the fungal ergosterol biosynthetic pathways (Kelly *et al*., 1993). Consequently, this study was aimed at investigating the fungal isolates associated with acute otitis media, determine their susceptibility to some commercially available antifungal drugs and also determine those that could produce deoxyribonuclease.

2. Materials and methods

2.1. Collection of samples

Middle-ear swabbed samples from 272 patients (aged ≤1 year to ≥ 60 years) attending Ear, Nose and Throat (ENT) clinics in Uyo and Ikot Ekpene in Akwa Ibom State, who had not received antibiotic therapy (topical or systemic) for the previous three days were collected aseptically using sterile swab sticks and transported in peptone water to maintain the swabs moist until being taken to the Microbiology Laboratory for mycological analyses. Eighty (80) samples were also collected from individuals without middle ear infection as control.

2.2. Isolation, characterization and identification of fungal isolates

The middle ear swabbed samples inoculated into the broth cultures for 4-6hrs were later streaked onto oven-sterilized plates of freshly prepared Sabourand Dextrose Agar (Composition: Peptone 10.0 g/l; Dextrose 40.0 g/l; Agar 15.0 g/l ; pH 5.6 ± 0.2). The plates were incubated aerobically at 25^{o}C for 3-7 days. After incubation, cultures were examined for significant growth. Subcultures were then made onto plates of freshly prepared Sabourand Dextrose Agar (SDA) supplemented with chloramphenicol (10µg) to act as a bactericidal agent and aerobically incubated for another 3-7days at 25^{o}C. Pure cultures of fungal isolates were characterized and identified based on their cultural and morphological features such as type of soma, nature of hyphae, pseudo-mycelium and asexual reproductive spore as discussed by Barnett and Hunter, (1987). Germ tube, sugar fermentation and assimilation tests were adopted for further characterization of yeasts

2.3. Germ tube test

Fungal isolates with cream-white colouration on Sabourand Dextrose Agar were presumptively identified as *Candida* spp. Colonies of the test isolates were picked and inoculated into 2mls of human serum in a small tube and incubated for 3hrs. After incubation, aliquot was removed using a loop and placed on a clean glass slide and overlaid with a cover slip for microscopical examination under high power for the formation of germ tube using 40x with the condenser, iris and diaphragm closed sufficiently to give a good constrast. Germ tubes present as slender tubes, with straight walls, without septum and constriction at the junction between cells. Presence of germ tube was an indicative of *Candida albicans.*

2.4. Detection of deoxyribonuclease (DNase) producing micro-organisms

Spot inoculation were done onto the surface of the DNase agar medium (Composition: Tryptose 20.0 g/l; Deoxyribonucleic acid 2.0 g/l; Sodium chloride 5.0 g/l; 12.0 g/l Agar; pH 7.3 ± 0.2) and incubated at 37^{o}C for 48hrs. After incubation, the growth on the surface of the agar was flooded with 1N hydrochloric acid. Polymerized DNA precipitated in the presence of 1N HCl and made the medium opaque. The fungal species that produced DNase enzymes, in sufficient quantity to hydrolyse DNA with clear zones around the colonies were evaluated as positive.

2.5. Antifungal drug susceptibility testing

In vitro susceptibility of the fungal isolates to four different antifungal drugs was determined using disk-diffusion technique. Sterile Petri dishes of Muller Hilton Agar or Sabourand Dextrose Agar were freshly prepared according to manufacturer's specifications. Distinct fungal colonies were emulsified in 5ml of sterile saline and 1 ml of each fungal isolates was inoculated into each of the Petri dishes containing Muller Hilton Agar for *Candida* species and Sabourand Dextrose Agar for other fungal species. These were allowed to stand for about 30 mins to enable the inoculated fungi to pre-diffuse. The commercially available discs containing the following antifungal drugs: Fluconazole (Flu, 25µg), Ketoconazole (Ket, 25µg), Voriconazole (Vor, 1µg) and Nystatin (Nys) were aseptically placed onto the surfaces of the agar plates with a sterile forceps and gently pressed to ensure even contact. The plates were incubated at 37°C for 24-48hrs for *Candida* species and 25°C for 48-72hrs for other fungal species. Zones of inhibition after incubation were observed and the diameters of inhibition zones were measured in millimeters (mm) using a ruler. The interpretation of the measurement as sensitive and resistant was made according to Pfaller *et al*. (2006) validated CLSL interpretative breakpoint for in-vitro antifungal susceptibility testing.

3. Results and discussion

Two hundred and seven-two (272) patient clinically diagnosed of Acute Otitis Media (AOM) attending Ear, Nose and Throat (ENT) clinics in Uyo and Ikot- Ekpene were obtained. Out of 272 cases, 124 (45.6%) were from males and 148 (54.4%) were from females. Table 1a shows that highest prevalence of AOM was observed in age group ≤ 10 years with 84 (30.9%) cases and lowest prevalence of AOM was observed in age group ≥ 61 having 12 (4.4%) cases. Of 84 cases having AOM in age group ≤ 10 years, 36 (29.0%) cases were males and 48 (32.4%) cases were females. Prevalence of AOM in other age groups is also shown in Table 1. The result showed that there was significant difference in the prevalence of AOM between age groups ≤ 10 years and other age groups at ($P < 0.5$). Of the 272 middle ear samples, 124 (45.6%)showed positive growth, while 148 (54.4%) samples showed no growth on Sabourand Dextrose

Agar . Among the 124 samples with positive growth, 99 (36.4%) samples showed growth of single fungal isolat e, while 18 (6.6%) and 7 (2.6%) showed growth of two and three fungal isolates, respectively (Table 2).

One hundred and fifty-six (156) fungal isolates were obtained from acute otitis media samples in which *Aspergillus niger* was found to be the most predominant fungi with 51 isolates (32.7%). Second highest fungal isolate was *Candida albicans* with 38 (24.4%). Other fungal isolates were *Aspergillus flavus* 14 (9.0%), *Cryptococcus neoformans* 32 (20.5%) and *Candida* spp 21 (13.5%) (Fig. 1). Seventy-two (72) fungal isolates were obtained from the male patients with AOM, while Eighty-four (84) fungal isolates were obtained from the female (Table 3). Highest numbers of fungal isolates were obtained among the age group ≤10 and the occurrences of fungal isolates associated with acute otitis media in relation to sex were statistically different at ($P < 0.5$). Extracellular deoxyribonuclease (DNase) production was tested in all the fungal isolates using DNase agar. Out of all the 156 fungal isolates obtained from patients with AOM, 46 (29.5%) fungal isolates were DNase producers, with 30/38 (78.9%) being *Candida albicans* and 16/21 (76.2%) being *Candida* spp. All the strains of *Aspergillus flavus*, *Aspergillus niger* and *Cryptococcus neoformans* were DNase negative (Table 4).

The results of antifungal susceptibility profiles of one hundred and fifty-six fungal isolates from acute otitis media showed that between 65.8 to71.1% *Candida albicans,* 64.3 to 85.7% *Aspergillus flavus,* 64.7 to 78.4% *Aspergillus niger*, 53.1 to 75.0% *Cryptococcus neoformans*, and 52.4 to 57.1% *Candida* spp. were sensitive to Ketoconazole, Voriconazole and Fluconazole, while between 81.6-84.4% of *C. albicans* and *C. neoformans* were sensitive to nystatin. In overall 72.4%, 76.3%, 67.9% and 62.2% of the fungal isolates were sensitive to Flu, Nys, Vor and Ket, respectively (Table 5).

The prevalence of acute otitis media has been reported to be higher in developing countries compared to advanced countries (Lasisi, 2008). This present study showed a high prevalence of acute otitis media in children within the age group of ≤ 10 when compared to patients of other age groups and this observation can be ascribed to physiological, anatomical and socio-cultural reasons (Li *et al.*, 2005; Akinjogunla *et al*.. 2012). The mycological studies of acute otitis media in this research revealed the occurrence of *A. niger*, *A. flavus, Candida* spp., *C. albicans* and *C. neoformans,* and this is an agreement with the reports of Henderson and Tsai (2001) where fungi such as *Candida albicans, Aspergillus niger, Candida* spp. were implicated as causes of acute otitis media. Loy *et al*. (2002) and Ekpo *et al* (2009) have also established *Aspergillus niger* and *Candida* spp. as aetiologic agents of acute otitis media. The most frequently isolated mould in this study was *A. niger*. The highest occurrence of *A. niger* obtained in this study is in conformity with the results of Loy *et al*. (2002) and Ekpo *et al*. (2009). *A. niger* is an opportunistic filamentous fungi, it has been identified as the cause of bilateral otomycosis and acute otitis media (Gugnai *et al*., 1989). *A. niger* grows on cerumen, epithelial scales and detritus deep in the external canal. The resulting accumulation of these inflammatory materials along with cerumen and fungal debris result in plug formation, which is extremely significant and usually leads to diminished hearing ability; pruritis, irritation of the surface layer of the external ear itself is a predisposing factor for bacterial colonization (Osazuwa *et al*., 2011).

The sensitivity of fungal isolates obtained from middle ear of patients with acute otitis media to fluconazole, voriconazole, ketoconazole and nystatin showed variable percentages of sensitivities. Azole has a broad spectrum of activity against *Candida* spp. and *Aspergillus* spp. It is absorbed orally and accumulate in organs which are frequent sites for systemic fungi infections and it can be therefore be used to treat and prevent a variety of systemic fungal infections. The high sensitivity of *C. albicans* and *A. niger* isolated from acute otitis media to fluconazole was reported in this research and this is in agreement with Hugo and Russell (2007) who reported that fluconazole as synthetic fluorinated pyrimidines demonstrated good activity against a range *C. albicans* and *Aspergillus* spp. Resistance of *C. albicans* and other *Candida* spp to ketoconazole in this study also correlates with the reports of Hugo and Russell (2007). The increasing resistance of *Candida* strain to azole might be due to the over-expression of efflux proteins which act by pumping the drug out of the cell at a rate faster than the drug enters the cell (Hugo and Russell (2007).

The widespread use of fluconazole has been accompanied by an increase in resistance and by which noticeable shift towards non albicans species with relative resistance to these antifungal agents (Cruenca-Estrella *et al*., 1999). Voriconazole is new extended spectrum triazoles, which is available in both oral and intravenous formulation. The increasing number and diversity of invasive infections, the expanding utilization of new and established antifungal agents, and the recognition of antifungal resistance as an important clinical problem have contributed to the need for the reproducible, clinically relevant antifungal susceptibility testing for *Candida s*pp. and filamentous fungi (Pfaller *et al*., 2006). DNase production was also found in fungal isolates such as *C. albicans*

and *Candida* spp and this agreed with Ewing (1986) and Finegold and Baron (1986) who reported that extracellular DNase production was a specific characteristic of genera of yeasts.

4. Conclusion

This study has provided and updated data on the incidence of the middle ear infection; the fungal isolates associated with acute otitis media and also revealed the actual therapy for acute otitis media.

Table 1
Age-wise and gender-wise distribution of acute otitis media and uninfected (control).

Age Range (Years)	Acute Otitis Media			Uninfected (Control)		
	Male	Female	Total	Male	Female	Total
	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
≤ 10	36 (29.0)	48 (32.4)	84 (30.9)	7 (17.5)	8 (20.0)	15 (18.8)
11-20	19 (15.3)	27 (18.2)	46 (16.9)	8 (20.0)	7 (17.5)	15 (18.8)
21-30	15 (12.1)	17 (11.5)	32 (11.8)	5 (12.5)	5 (12.5)	10 (12.5)
31-40	9 (7.3)	20 (13.5)	29 (10.7)	5 (12.5)	5 (12.5)	10 (12.5)
41-50	18 (14.5)	13 (8.8))	31 (11.4)	5 (12.5)	5 (12.5)	10 (12.5)
51-60	9 (7.3)	8 (5.4)	17 (6.3)	5 (12.5)	5 (12.5)	10 (12.5)
≥ 61	5 (4.0)	7 (4.7)	12 (4.4)	5 (12.5)	5 (12.5)	10 (12.5)
USP	13 (10.5)	8 (5.4)	21 (7.7)	-	-	-
Total	124 (45.6)	148 (54.4)	272 (100)	40 (50.0)	40 (50.0)	80 (100.0)

USP: Unspecified; Values in parenthesis are percentages
($P< 0.5$; X^2: 8.28; df: 7)

Table 2
Number and percentage of fungal isolates obtained from patients with acute otitis media and uninfected (control) in relation to sex.

Number of Isolates	Acute Otitis Media (AOM)			Control (Uninfected)		
	Male	Female	Total	Male	Female	Total
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
0	66 (53.2)	82 (55.4)	148 (54.4)	33 (82.5)	35 (87.5)	68 (85.0)
1	47 (38.0)	52 (35.1)	99 (36.4)	4 (10.0)	5 (12.5)	9 (11.3)
2	8 (6.5)	10 (6.8)	18 (6.6)	3 (7.5)	-	3 (3.7)
3	3 (2.4)	4 (2.7)	7 (2.6)	-	-	-
Total	124 (100)	148 (100)	272 (100)	40 (100)	40 (100)	80 (100)

Values in parenthesis are percentages

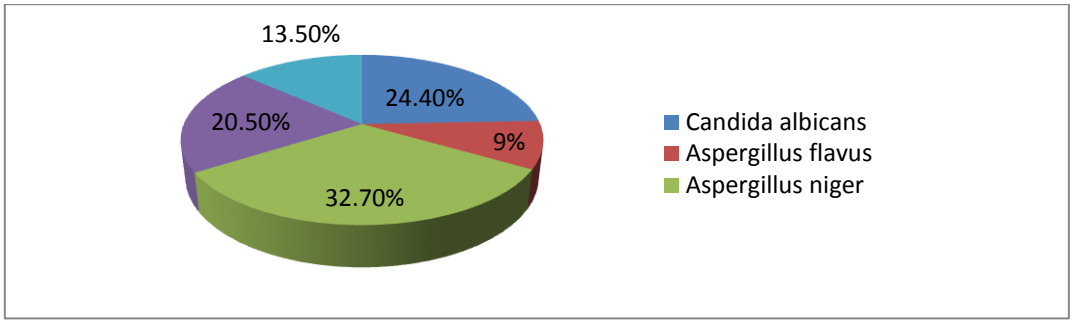

Fig. 1. Frequency of distribution of fungal isolates associated with acute otitis media.

Table 3

Age and gender specific distribution of fungal isolates among patients with acute otitis media.

Fungal spp. Isolated	Gender		Age Group								Total
	Male No. (%)	Female No. (%)	≤10 No. (%)	11-20 No. (%)	21-30 No. (%)	31-40 No. (%)	41-50 No. (%)	51-60 No. (%)	≥ 61 No. (%)	USP No. (%)	No. (%)
C. albicans	16(22.2)	22(26.2)	12 (26.1)	7 (25.0)	4 (25.0)	4 (20.0)	6 (31.6)	2 (20.0)	1 (14.3)	2 (20.0)	38 (24.4)
A. flavus	7 (9.7)	7(8.3)	4 (8.7)	2 (7.1)	4 (25.0)	1 (5.0)	2 (10.5)	--	1 (14.3)	--	14 (9.0)
A. niger	22 (30.6)	29(34.5)	23 (50.0)	7 (25.0)	3 (18.8)	6 (30.0)	2 (10.5)	4 (40.0)	2 (28.6)	4 (40.0)	51 (32.7)
C. neoformans	19 (26.4)	13(15.5)	5 (10.9)	10 (35.7)	5 (31.3)	3 (15.0)	3 (15.8)	4 (40.0)	1 (14.3)	1 (10.0)	32 (20.5)
Candida spp.	8 (11.1)	13(15.5)	2 (4.3)	2 (7.1)	--	6 (30.0)	6 (31.6)	--	2 (28.6)	3 (30.0)	21 (13.5)
Total	72 (100)	84(100)	46 (29.5)	28 (17.9)	16(10.3)	20(12.8)	19(12.2)	10(6.4)	7 (4.5)	10(6.4)	156(100)

Values in parenthesis are percentages; USP: Unspecified

Table 4

Prevalence of extracellular deoxyribonuclease (DNase) producing fungal isolates from acute otitis media (AOM)

Source	Fungal spp.	Number of Occurrence	No / (%) of DNase Producers	No / (%) of DNase Non Producer
AOM				
	C. albicans	38	30 (78.9)	8 (21.1)
	A. flavus	14	-	14 (100)
	A. niger	51	-	51 (100)
	C. neoformans	32	-	32 (100)
	Candida spp.	21	16 (76.2)	5 (23.8)
	Total	156	46 (29.5)	110 (70.5)

Values in parenthesis are percentage

Table 5

In-vitro susceptibility of fungal isolates from acute otitis media to antifungal drugs.

Source	Fungal spp.	Number of Occurrence	Flu^s No. (%)	Flu^r No. (%)	Nys^s No. (%)	Nys^r No. (%)	Vor^s No. (%)	Vor^r No.(%)	Ket^s No.(%)	Ket^r No.(%)
AOM	*C. albicans*	38	27(71.1)	11(28.9)	31(81.6)	7(18.4)	25(65.8)	13(34.2)	25(65.8)	13(34.2)
	A. Flavus	14	12(85.7)	2(14.3)	10(71.4)	4(28.6)	12(85.7)	2(14.3)	9(64.3)	5(35.7)
	A. niger	51	40(78.4)	11(2 1.6)	35(68.6)	16(31.4)	33(64.7)	18(35.3)	35(68.6)	16(31.4)
	C. neoformans	32	22(68.8)	10(31.2)	27(84.4)	5(15.6)	24(75.0)	8(25.0)	17(53.1)	15(46.9)
	Candida spp	21	12(57.1)	9(42.9)	16(76.2)	5(23.8)	12(57.1)	9(42.9)	11(52.4)	10(47.6)
	Total	156	113(72.4)	43(27.6)	119(76.3)	37(23.7)	106(67.9)	50(32.1)	97(62.2)	59(37.8)

Values in parenthesis are percentages; Flu: Fluconazole; Nys: Nystatin; Vor: Voriconazole; Ket: Ketoconazole r: resistance; s: sensitive

Acknowledgment

The authors sincerely appreciate the assistance of all the staff of Ear, Nose and Throat (ENT) of the hospitals used, the patients who co-operated for this study and Dr. J.P. Essien of Microbiology Department, University of Uyo, Akwa Ibom State.

References

Akinjogunla, O.J., Eghafona, N.O., Enabulele, I.O., 2012. Aetiologic agents of acute otitis media (AOM): prevalence, antibiotic susceptibility, β-lactamase (βL) and extended spectrum β-lactamase (ESBL) production. Journal of Microbiology, Biotechnol. Food Sci. 1 (3), 333-353.

Arola, M., Ruuskanen, O., Ziegler, T., Mertsola, J., Nanto-Salonen, K., Putto-Laurila, A., Viljanen, M.K., Halonen P. (1990). Clinical role of respiratory virus infection in acute otitis media. Pediatrics. 86, 848-55.

Barnett, H.L., Hunter, B.B., 1987. Illustrallated General of Imperfecti fungi, (5th edition). Burges Publishing company, Minnesota, p: 241.

Bluestone, C.D., 1998. Otitis Media; to treat or not to treat/ consultant. pp. 1421-1433.

Bluestone, C.D., Klein, J.O. 2001. Microbiology. In: Bluestone CD, Klein JO, (3rd edn). Otitis Media in Infants and Children. Philadelphia, PA: W.B. Saunders; pp. 979-1014.

Casselbrant, M.L., Mandel, E.M., Rockette, H.E., Kurs-Lasky, M., Fall, P.A., Bluestone, C.D., Ferrell, R.E. (2004). The genetic component of middle ear disease in the first 5 years of life. Arch Otolaryngol Head Neck Surg. 130(3), 273-278.

Cruenca-Estrella, M., Ruiz-Diez, B., Martinez-Suarez, J.V., Monzon, A., Rodriguez-Tudela, J.I., 1999.Comparative In-Vitro Activity of Voriconazole (Uk-109, 496) and Six Other Antifungal Agent Against Clinical Isolates Of Scedosporium prolificans and Scedosporium apiospermum. J. Antimicro Chem. 43, 149-151.

Daly, A., 1997. Knowledge and attitude about otitis media risk: Implication for prevention. J. Paediatrics. 100 (3), 93-96.

Damoiseaux, R., Balen, F., Hoes, A., Verheij, T., de-Melker, R., 2000. Primary care based randomized, double blind trial of amoxicillin for acute otitis media in children aged under 2 years. BMJ. 320 (7231), 350-354.

Ehrlich, G.D., Costerton, J.W., Hayes, J.D., Daigle, B.J. and Ehrlich, M.D., 2002. Mucosal Biofilm Formation on Middle-ear Mucosa in the Chinchilla Model of Otitis Media. JAMA. 287 (13): 1710-1715.

Ekpo, M.A., Akinjogunla, O.J., Idiong, D.F., 2009. Microorganisms associated with acute otitis media diagnosed in Uyo City, Nigeria. Sci Res Essay. 4 (6): 560-564

Ewing, W.H. (1986). Edwards and Ewings Identification of Enterobacteriaceae (4th Edition) Elsevier Science Publishing, New York.

Finegold, S.M. and Baron, F.A., 1986. Baily and Scott's Diagnostic Microbiology. (8th edn). Saunders co. Philadelphia.

Gugnai, H.C., Okafor, B.C., Nzeilb, F. and Njoku-Obian, M., 1989. Mycoses, etiological agent of Otomycosis in Nigeria. Mycoses. 32: 224-229.

Gulati, S.K., 1997. Investigative profile in patients of chronic suppurative otitis media. Indian J Otol. 3 (2), 59-62

Henderson, S.O., Tsai, J. 2001. Pediatrics, Otitis media. Emedicine J. 2,7.

Hugo, W.B., Russell, A.D. 2007. Pharmaceutical Microbiology, (7th Ed). Blackwell. Science. 481.

Kawo, A.H., Daiyah, B.M., Yusha'u, M., 2010. Susceptibility patterns of bacterial pathogens associated with otitis media at Murtala Muhammed Specialist Hospital, Kano, Nigeria. Int. J. Pharm. App. Sc. 1, 74–78.

Kelly, S.L., Lamb, D.C., Kelly, D.E., 1999. Y132H substitution in Candida albicans sterol 14alpha-demethylase confers fluconazole resistance by preventing binding to haem. FEMS Microbiol Lett. 180, 171-175.

Lasisi, A.O., 2008. Otolayngological Practice in Developing Country: a profile of met and unmet needs. East Central Afri. J. Surg., 13(2), 101–104.

Li, Y.H., Anne, T., Tse, H.K., Bes, M., Jerome, E., Asok, K., Tong, T.T., Ban, H.T., 2005.Community associated methicillin resistant Staphylococcus aureus, Singapore. Emerg. Infect Dis. 11, 341-342

Loy, A., Tan, A.L., Lu, P.K., 2002. Microbiology of chronic suppurative otitis media. Singapore Med. Jour. 43(6):296-299.

Osazuwa, F., Nwofor, Q. and Ibeh, I.N., 2011. Aetiologic agent of otitis media in Benin City. Res. J. Med. Sci. 5(2), 112-115

Pfaller, M.A., Diekema, D.J., Sheehan, D.J., 2006. Interpretive breakpoints for fluconazole and Candida revisited: A blueprint for the future of antifungal susceptibility testing. Clin. Microbiol. Rev., 19, 435-444.

Prescott, L.M., Harley, J.P., Klein, D.A., 2011. Microbiology. (8th edn). McGraw-Hill companies, Inc, New York. Pp. 993.

Sanglard, D., Ischer, F., Parkinson, T., Falconer, D., Bille, J., 2003. Candida albicans mutations in the ergosterol biosynthetic pathway and resistance to several antifungal agents. Antimicrob. Agents Chemother. 47, 2404-241

Talaro, K.P., Talaro, A., 2002. Foundations in Microbiology. (4th edn). McGraw-Hill companies, Inc, New York. Pp 899.

Weiner, R., Collison, P.J., 2003. Middle Ear Pathogens in Otitis Prone Children, South Dakota J. Med. 56, 103-107.