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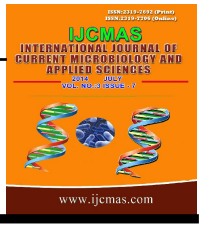

Original Research Article

Microorganisms Associated with Chronic Suppurative Otitis media

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Keywords

Chronic Suppurative Otitis media; *Staphylococcus aureus; Streptococcus pyogenes, Staphylociccus epidermidis, Pasturella multocida, Staphylococcus haemolycos and Manheimia haemolitica*

A B S T R A C T

A research was carried out on chronic suppurative otitis media. Bacteriological and mycological examination were carried out on twenty (20) ear swab samples collected from three different hospitals within sokoto metropolis. The hospitals include Usmanu Danfodiyo University Teaching Hospital, Maryam Abacha Hospital Sokoto and Specialist Hospital Sokoto. A total number of eleven (11) bacterial isolates were identified with the following percentage frequency of occurrence, *Pseudomonas aeruginosa* had the highest frequency of occurrence of (32.2%), *Staphylococcus aureus* (21.6%), followed by *Klebsiella pneumonia* with (13.5%), *Streptococcus pneumonia and Yersiania enterocolitica* had (10.8%), *Proteus mirabilis* had (8.1%). *Streptococcus pyogenes, Staphylociccus epidermidis, Pasturella multocida*, *Staphylococcus haemolycos* and *Manheimia haemolitica* had the least frequency of occurrences of (4.5%) each. A total of five (5) fungal isolates were identified *Candida albicans* had the highest frequency of occurrence of (60%), followed by *Aspergillus fumigatus* with (3.3%), *Candida paraplosis* (20%), *Asperfillus niger* (10%), and *Aspergillus flavus* (6.7%). *Pseudomonas aeruginosa* and *Candida albicans* have been found to be the most frequent organisms associated with chronic suppurative otitis media. Personal hygiene as well as using clean object in cleaning ears are very important to avoid the entry of micro-organisms.

Introduction

Chronic suppurative otitis media is defined as chronic inflammation of the middle ear and gmastoid cavity, which presents with recurrent ear discharges or otorrhoea through a tympanic perforation. The disease usually begins in childhood as a spontaneous tympanic perforation due to an acute infection of the middle ear, known as acute otitis media, or as a sequel of less severe forms of otitis media (e.g. secretory otitis media).

The infection may occur during the first 6 years of a child's life, with a peak around 2 years (Monica, 2004).The point in time when acute otitis media becomes chronic suppurative otitis media is still controversial. Generally, patients with tympanic perforations which continue to discharge mucoid material for periods of from 6 weeks to 3 months, despite medical treatment, are recognized as chronic suppurative otitis media cases (Anthony, 2003).

The world health organisation definition requires only 2 weeks of otorrhoea, but otolaryngologists tend to adopt a longer duration, e.g. more than 3 months of active disease. The ultimate fate of the tympanic perforation is still largely undocumented. Thus, both the start and the end of the disease process are difficult to define. Although healing is often observed over prolonged periods, there are more patients who develop either recurrent bouts of otorrhoea (active chronic suppurative otitis media) or a dry but permanent tympanic perforation (inactive chronic suppurative otitis media) (Anonymous, 2000). Inactive otitis media refers to a previously discharging ear that has apparently ceased [discharging] without probability of resumption in the near future; the term is common among Asian colleagues. Often, the perforation heals imperfectly with areas of retraction and scarring in the eardrum which do not vibrate in response to sound, as well as normal areas. The episodes of otorrhoea are often provoked by upper respiratory infections. This is particularly common in children. Soiling of the middle ear from swimming or bathing also leads to intermittent and unpleasant discharges. A decidedly smaller group of patients, particularly those who have not been treated, develop life-threatening complications (Barry *et al.,* 2005*)*.Several systems of nomenclature have been developed to distinguish between different types of otitis media, reflecting the lack of complete understanding of the processes responsible for the inflammation and healing of the middle ear. For the purpose of this report, the presence of a persistent tympanic perforation and middle ear discharge differentiates chronic suppurative otitis media from other chronic forms of otitis media (Reichler *et al,* 2002).A subset of chronic suppurative otitis media may have cholesteatomas or other suppurative complications. The non-chronic suppurative otitis media group includes such entities as chronic non-suppurative otitis media, chronic otitis media with effusion, chronic secretory otitis media, chronic seromucous otitis media, chronic middle ear catarrh, chronic serous otitis media, chronic mucoid otitis media, otitis media with persistent effusions, and glue ear. All these are recurrent or persistent effusions in the middle ear behind an intact tympanic membrane in which the principal symptom, if present at all, is deafness and not ear discharge (Henderson *et al.,* 2002).

Most common micro-organisms found in are *Pseudomonas aerugenosa, staphylococcus aureus, Proteus mirabilis, klebsiella pneumonia, Escherichia coli, Aspergilus spp and Candida spp.* but these organisms vary in various geographical area.Many authors have focus their attention on the bacterial flora of chronic suppurative otitis media, but very little is known about the mycological aspect of these, the importance of which has been increasing in the recent years because of the excessive use of broad spectrum antibiotics, corticosteroids and cytotoxic chemotherapy and increase in the number of immune deficiency condition (Anthony *et al.,* 2003).The wide spread use of antibiotics has precipitated the emergence of multiple resistance strain of the bacteria which can produce both the primary and the post operative infections. The indiscriminate haphazard and hay-hearted use antibiotics and poor follow up of the patient have resulted in the persistent of low grade infections. The changes in the microbiological flora following the advent of sophisticated synthetic antibiotics have increase the relevance of the reappraisal of the modern day flora in chronic suppurative otitis media and their invitro antibiotics pattern is very important for the clinician to

plan a general outline of treatment for patient with chronically discharged ear (Henderson *et al.*,2002).

Many researchers have focus their attention on the bacterial flora of chronic suppurative otitis media, but very little is known about the mycological aspect of it, the importance of which has been increasing in the recent years because of the excessive use of broad spectrum antibiotics, corticosteroids and cytotoxic chemotherapy and increase in the number of immune deficiency condition. This research work was aimed at determining the microorganisms responsible for causing chronic suppurative otitis.

Materials and Methods

Sample collection

A total number of twenty (20) samples were obtained from three different hospitals. The hospitals include: Usmanu Danfodiyo University Teaching hospital Sokoto, Maryam Abacha hospital and Specialist hospital Sokoto. The samples were collected from both male and female patients, by carefully inserting a sterile swab stick inside the outer ear canal and gently removed. The lid was immediately closed and the swab sticks were placed back into the container. The samples were immediately transported to the microbiology laboratory of the Usmanu Danfodiyo Sokoto for analysis.

Identification of the Bacterial isolates

Ear swabs were inoculated directly into the already prepared nutrient agar plates, by using streaking method of inoculation. The plates were then incubated at 37^0C for24 hours. The colonies obtained were subcultured using freshly prepared media to obtain pure colonies (Cowan and Steel, 2002).

Gram's staining

A smear was fixed at the centre of a grease free slide and allowed to dry, then washed off with clean water. The smear was flooded with Lugol's iodine for 30 seconds after which it was washed off with clean water. Acetone was then added and immediately washed off. Neutral red was added for about one minute, and then washed off with clean water. The back of the slides were wiped off and then allowed to air dried after which microscopy was conducted.

Biochemical tests

Biochemical tests were conducted to identify the bacterial isolates of to specie level.

Catalase test

A drop of hydrogen peroxide was put on a glass slide with the use of a sterile wire loop. Colonies of the test isolates were emulsified using Hydrogen peroxide. Catalase positive reaction was observed by the immediate production of bubbles (Cheesbrough, 2006).

Coagulase test

A drop of normal saline was put on a glass slide; colonies of the test organisms were emulsified within the saline. A drop of human plasma was added and mixed gently. After about 10 seconds it was observed for clotting which indicated a positive result (Cheesbrough, 2000).

Citrate test

This was carried out by inoculating the test isolates into Simon's citrate agar slopes which were then inoculated at 37^0C. A change in colour of the medium from green to blue which indicated a positive result was observed (Cheesbrough, 2000).

Indole test

This was carried out in accordance with the procedure of Cheesbrough, (2000). The test organisms were inoculated inside Bijou bottles containing 3ml of sterile tryptone water. The organisms were incubated at 35-37^{0}C for 48hous. This was followed by the addition of 0.5ml Kovac's reagent. A red color on the surface layer was observed within 10 minutes, which indicated positive test for indole.

Carbohydrates fermentation test

The sugars fermentation media were formulated for glucose, lactose, manitol, sucrose in the basal medium containing peptone. If the organisms inoculated did not ferment sugars then, peptone is metabolise and the breakdown of amino acid yield ammonium this results in the medium becoming alkaline. If the sugars were fermented, acids are produced and the medium is acidic. After period of incubation, few drops of phenol red were added. A yellow color was observed which indicated a positive result (Perkin, 2007).

Methyl red voges prosecauer test (mr-vp)

5ml of methyl red voges proscauer was inoculated with the isolates. The MR-VP test was carried out after incubation at 37^{0}C for 48 to 72 hours. After incubation, 1ml of broth was added. A red colour which indicated a positive result was observed.
To the rest of the broth in the original tube, 5 drops of 40% potassium hydroxide was added, followed by the addition of 15 drops of nephthol in ethanol. The cap of the tube was loosened and the tube was shaken and placed in a sloppy position. The development of a red colour starting from the liquid air interface within an hour indicated a positive result (Broby, 2002).

Double sugar iron test

The surface of the slant was streaked and the butt stabbed 2-3 times. The cap was closed loosely and incubated at 35^{0}C for 24 hours. The medium was not dried and there was an evidence of water condensation at the slant butt junction (Cronin, 2004).

Isolation and identification of fungal isolates

The ear swabs were inoculated on plates containing sterilised potato dextrose agar media by streaking method. The PDA plates were incubated at room temperature for 72 hours. After incubation the growth of fungi was observed and growth was sub cultured to obtain pure culture (Cowan and Steel, 2002).

The conial morphology of the fungal isolates was observed. The colour and type of growth either woolly or cottony was observed. Microscopic identification was carried out by preparing wet mount using lacto phenol cotton blue to observe for the characteristics of fungi, such as hyphae, whether septate or non septate (Cowan and Steel, 2002).

Results and Discussion

A number of eleven (11) bacterial species and five (5) fungal species were isolated from the ears of twenty individuals comprising of both males and 8 females. The bacterial species includes; *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Streptococcus pneumonia, Proteus mirabilis, Streptococcus pyogenes, Yesinia enterocolitica, Pasturella multicoda, Staphylococcus epidermidis, Staphylococcus haemolyticos,* and the fungal species were *Candida albicans, Aspergillus fumigates,*

Aspergillus niger, *Aspergillus flavus,*and *Candida paraplosis.*

Table.1 indicated the results of gram reactions and biochemical tests conducted on bacterial isolates. From the result there was no difference between gram positive and gram negative bacteria in association with human ears from both male and female samples. Table 2 showed Results of macroscopic and microscopic characteristics of fungi isolated.

Table 3 indicated the results of bacteria isolated from ear swabs of individuals. Among the gram positive bacteria *Staphylococcus aureus* was the most frequent bacterial isolate detected. followed by *Streptococcus pneumonia,* other gram positive isolate detected ware *Staphylococcus epidermidis, Streptococcus pyogenes,* and *Staphylococcus haemolyticu.* Among the gram negative bacteria isolated *Pseudomonas aeruginosa* was the most frequent bacteria isolated.

It is followed by *Klebdiella pneumonia* and *Yersinia enterocolitica, others are Proteus mirabilis, Pasturella multicoda, manheimia haemolytica,* and *citrobacter.* Table 4 showed the results of fungi isolated. *Candida albicans* is the most frequent isolate, it is then followed by *Aspergillus fumigatus, Candida paraplosis, Aspergillus niger* and *Aspergillus flavus* which appeared less frequent.

Table 3 indicated the percentage frequency of occurrence of bacterial isolates. From the results, it was found out that *Pseudomonas aeruginosa* had the highest percentage frequency of occurrence of 10 (32.3%), followed by *Staphylococcus aureus* with 8 (21.6%) then *Klebsiella pneumonia* with 5 (13.5%), then followed by *Yesinia enterocolitica* and Strreptococcus pneumonia with 4 (10.8%), then *Proteus mirabilis* with 3 (8.1%), and then followed by *Streptococcus pyogenes, Staphylococcus epidermidis, Pasturella multocida, Staphylococcus haemolycos, and manheimia haemolytica* with 2 (5.4%) each.

Table 4 indicated the percentage frequency of occurrence of fungal isolates.the results indicated *Candida albicans* with the highest frequency of occurrence of 9 (60%), followed by *Aspergillus fumigatus* account with 5 (3.3%) and then *Candida paraplosis with* 3 (20%), then followed by Aspergillus niger with percentage frequency of 2 (10%), the fungal species with least percentage frequency was Aspergillus flavus with 1 (6.7%).

From the research conducted the following species of bacteria and fungi were isolated *pseudomonas spp, Staphylococcus spp, Streptococcus spp, Candida spp and Aspergillus spp.* Various studies by different researchers have been carried out, establishing the significance of bacteria like *Streptococcus pneumoniae, Pseudomonas aeruginosa, Proteus species, Staphylococcus aureus, Klebsiella species, Streptococcus pneumoniae* and fungi like *Candida albican, Aspergillus fumigates, Candida paraplosis, Aspergillus flavus, Aspergillus niger,* in their connection with human ears (Harold and Fancis 2002, Mackie and Mc Courtney, 2008).

Table 3: Results of bacteria isolated from ear swabs of individuals

SAMPLE	BACTERIAL ISOLATES
Sample A(M)	*Pseudomonas aeruginosa, Klebsiella pneumonia, Streptococcus pyogens, Staphylococcus aureus*
Sample B (M)	*Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis, Yersinia enterocolitica*
Sample C (F)	*Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus haemolyticus, Streptococcus pneumonia, Yersinia enterocolitica*
Sample D (F)	*Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus mirabilis, Staphylococcus aureus*
Sample E (M)	*Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes, Staphylococcus epidermidis, klebsiella pneumonia, Pasrurella multocida*
Sample F (M)	*Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis, Yersinia enterocolitica, Pasturella multocoda*
Sample G (F)	*Staphylococcus aureus, Manheimia haemolytica, Proteus mirabilis, Streptococcus epidermidis, Klebsiella pneimonia*
Sample H (F)	*pseudomonas aeruginosa, Staphylococcus aureus, Yersinia enterocolitica, Manheimia haemolytica, Proteus mirabilis*
Sample I (M)	*Streptococcus epidermidis, Klebsiella pneumonia, Manheimia haemolytica, Pasturella multocida, Proteus mirabilis*
Sample J (M)	*Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus mirabilis, Staphylococcus aureus*
Sample K (F)	*Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella Manheimia haemolytica, Proteus mirabilis*
Sample L (F)	*Klebsilla pneumonia, Staphylococcus haemolyticus, Streptococcus pneumonia, Staphylococcus aureus,*
Sample M (M)	*Pseudomonas aeruginosa, Staphylococcus aureus, Pasturella a multocida, Streptococcus epidermidis, Proteus mirabilis*
Sample N (M)	*Streptococcus epidermidis, Klebsiella pneumonia, Manheimia haemolytica, Pasturella multocida, Proteus mirabilis*
Sample O (F)	*Pseudomonas aeruginosa ,Klebsiella pneumonia, Streptococcus epidermidis, Staphylococcus aureus, Proteus mirabilis*
Sample P (F)	*Streptococcus pneumonia, Manheimia haemolytica, Pasturella multicida, Yersinia enterocolitica, Proteus mirabilis*
Sample Q (M)	*Klebsiella pneumonia, Staphylococcus eidermidis, Streptococcus pyogenes, Pseudomonas aeruginosa, Proteus mirabilis*
Sample R (M)	*Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumonia*
Sample S (F)	*Pseudomonas aeruginosa, Staphylococcus epidermidis, Yersinia enterocolitica, Pasturella multocida*
Sample T (F)	*Pseudomonas aeruginosa,Klebsiella pneumonia, Staphylococcus aureus, Streptococcus pyogens, Streptococcus pneumonia*

Key: M = Male F= Female

Table.4 Results of fungi isolated from ear swabs of individuals

SAMLES (GENDER)	FUNGAL ISOLATES
Sample A(M)	*Candida albican, Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus*
Sample B(M)	*Candida albican, Candida paraplosis, Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus*
Sample C (F)	*Candida paraplosis, Aspergillus flavus, Aspergillus niger*
Sample D (F)	*Aspergillus niger, Aspergillus fumigatus, Candida albican*
Sample E (M)	*Candida albican, Aspergillus flavus, Aspergillus fumigatus,*
Sample F (M)	*Candida albica, Aspergillus fumigatus, Aspergillus niger*
Sample G (F)	*Aspergillus fumigatus, Aspergillus flavuus, Aspergillus niger*
Sample H (F)	*Candida albican, Candida paraplosis, Aspergillus fumigatus*
Sample I (M)	*Candida albican, Aspergillus niger, Aspergillus flavus, AspergIllus fumigatus*
Sample J (M)	*Aspergillus fumigatus, Aspergillus flavus,Candida paraplosis*
Sample K (F)	*Candida albican, Aspergillus flavus, Aspergillus niger*
Sample L (F)	*Candida albican, Aspergillus flavus, Aspergillus niger*
Sample M (M)	*Candida albican, Aspergillus fumigatus, Candida albicans*
Sample N (M)	*Candida albican, Aspergillus fumigatus,Aspergillus niger*
Sample O (F)	*Candida albican, Candida paraplosis, Aspergillus flavus*
Sample P (F)	*Candida paraplosis, Aspergillus flavus, Candida albican*
Sample Q(M)	*Candida albican, Aspergillus niger, Aspergillus flavus*
Sample R (M)	*Candida albican, Candida paraplosis, Aspergillus fumigatus*
Sample S (F)	*Candida albican, Aspergillus niger, Aspergillus fumigatus*
Sample T (F)	*Candida albicanAspergillus flavus, Aspergillus fumigatus*

Key: M=Male, F= Female

Table.5 Percentage frequency of occurrence of bacteria isolated from ears of individuals

BACTERIAL ISOLATES	FREQUENCY OF OCCURRENC(%)
Pseudomonas aeruginosa	10 (32.2%)
Staphylococcus aureus	8 (21.6%)
Klebsiella pneumonia	5 (13.5%)
Streptococcus pyogens	2 (5.4%)
Staphylococcus haemolytic	2 (5.4%)
Proteus mirabilis	3 (8.1%)
Streptococcus pneumonia	4 (10.8%)
Pasturella multocida	2 (5.4%)
Yersinia enterocolitica	4 (10.8%)
Streptococcus epidermidis	2 (5.4%)
Manheimia haemolytica	2 (5.4%)

Table.6 Percentage frequency of occurrence of fungi isolated from ears of individuals

FUNGAL ISOLATES	FREQUENCY OF OCCURRENCE (%)
Candida albican	9 (60%)
Aspergillus fumigatus	5 (3.3%)
Candida paraplosis	3 (20%)
Aspergillus niger	2 (10%)
Aspergillus flavus	1 (6.7%)

According to Harold *et al*, (2002) bacteria such as *Staphylococcus aureus*, *Yersinia enterocolitica*, *Streptococcus pneumoniae*, *Proteus Species*, *Pasturella multocida*, *Pseudomonas* species and fungi such as *Candida* species and *Apergillus* species are usually associated with contamination of both internal and external ear leading to majority of ear infections. The findings of the present study agree with the works of Senuturia *et al.*, (2007), Harold and Francis (2002) and Mackie and McCartney (2008) who isolated similar groups of bacteria and fungi from human ear swab.

The present findings shows that there is no greater difference in frequency of occurance between gram positive and gram negative bacteria in association with human ears. Among gram positive bacteria *Staphylococcus aureus* were the frequent bacterial isolates detected, it is then followed by *streptococcus pnuemoniae* which occur in exactly half of the samples used in this study. Other gram positive bacterial isolates detected are *Streptococcus pyogens*, *Staphylococcus epidermidis and Staphylococcus heamolyticus* which also occur in exactly half of the samples collected. Also, among the gram nagetive bacterial isolates are *Pseudomonas aeruginosa* which is found occurring in all the samples collected. It is followed by *Klebsiella pneumonia*, *Yersinia enterocolitica*, *Proteus mirabilis* which are detected thirteen times each among the samples collected. Other Gram negative bacteria isolated are *pasturella multicoda*, *Manheimia heamolytica* with less occurance in the samples collected. Among fungal isolates *Candida albican* is the most predominant isolates that is found

in the entire sample collected. It then followed by *Aspergillus fumigates, Candida paraplosis*, which is found sixteen times in all the samples collected. Other fungal isolates detected are *Aspergillus flavus, Aspergillus niger* with less occurrence in the sample collected.

The results obtained in this research were in agreement with Brook and Yocum (2005), when they isolates these organisms in a pure culture of ear swabs samples of human. The findings of this study was also in conformity with the findings of Ibekwe *et al.*, (2009), who found that *Pseudomonas aeruginosa* and *Candida albicans* were the most frequent organism associated with human ear swabs samples. The occurrence of these organisms may be due to the geographical difference and socio-economic factors. Many of these bacterial isolates detected inhibits the ear canal and become secondary opportunistics invaders when conditions are favorable and it probably ascended from the pharynx through the auditive tubes tympanic cavities.

This study determined the micro-organisms associated with chronic suppurative otitis media. Both the isolates and frequency of occurrence enables analysis of the scale problem. The pattern of isolates reported in this study were consistent with the usually reported pattern reported by Harold and francis (2004), with *Pseudomonas aeruginosa*, and *candida albican* been the most prevalent isolates found to be associated with human ears.

References

Atlas., R.M (2008) *Microbiology Fundamentals and Applications*. Second edition Macmillan Publishing Company Pp 669, 638

Acuin., J, Smith., A, Mackenzie., I (2000). *Treatments for chronic suppurative otitis media.* Cochrane Database of Systematic Reviews, pp 4.

Acuin., J (2004*). Chronic suppurative otitis media among children in a slum area*. (unpublished).

Adair-Bischoff., CE, Sauve., RS (2008). *Environmental tobacco smoke in middle ear disease in preschool age children*. Arch Pediatr Adolesc Med, 152 (2): 127-133.

Alberti., P (2006). *Thailand outreach project. Hearing International*, 5 (1): 17.

Alho., OP, Jokinen., K, Laitakari., K, Palokangas., J (2007). *Chronic suppurative otitis media and cholesteatoma.* Vanishing diseases among Western populations? Clin Otolaryngol, 22 (4): 358-361.

Alho., OP (2000.) *The validity of questionnaire reports of history of acute otitis media*. *Am J Epid.*, 132 (6): 1164-1169.

Antarasena., S, Antarasena., N, Lekagul., S, Lutadul., D (2008). *The epidemiology of deafness in Thailand*. Otolaryngol Head Neck Surg, 3 (1): 9-13.

Brooby., G.W (2002). *the discharging ear in the tropics, a guide to diagnosis and management in the district hospita*l, tropical Doctor, 22:10-33.

Brook.,I. and Yocum., P. (2008). *Quantitative bacterial cultures* and Cheesbrough., M.(2000), District *Laboratory practice in tropical Countries* part 2 Cambridge University Pres Pp 132 – 36

Cowon., S.T and Steel., K.J. (2002), Manual *for the identification of medical bacterial* 2nd edition, Cambridge University Press Pp 51-60

Cohen., D, Tamir., D (2009). *The prevalence of middle ear pathologies in Jerusalem school children*. *Am J Otol.*, 10 (6): 456-459.

Cooke., E, Raghuvaran., G (2004). *Clindamycin in conjunction with surgery of the chronic suppurative ear*. Br J Clin Practice, 28 (2): 57-59.

Cronin., J, Dogra., TS, Khan., IA (2004).

Conservative management of chronic ear disease. J Laryngol Otol., 88 (2): 113-118.

Cummings., C (2003). *Otolaryngology–Head and Neck Surgery*. St Louis,Mosby-Year Book, 2nd ed,Vol. 4: 2830

Devan., P.P. Donalson., J.D, John., L.L (2002), *middle ear mastoids otolaryngology and facial plastic surgery middle ear*, Edition Macmillan publishing company Pp 669, 638

Harold., P. L. and Francis., W.O. G. (2002), *Antibiotic and Chemotherapy.* 6th edition by Tim Horne Edinburgh London Pp 213 – 26

Kanai., M.I (2008), *Medical Laboratory Technology* McGraw – Hill company, New Delhi India Vol 3 Pp 612.

Kumurya., A. S., Kawo., A. H. and Uba., A. (2010), *Prevalence and in-vitro susceptibility studies of Bacteria isolated from Hospital Patients Presenting with otitis media in Kano*, Nigeria. Biological and Environmental Sciences Journal for the Tropic *BEST* 7 (1) 37-39 *lactamase activity in chronic suppurative otitis media*. Ann. Otol.

Mackie., T.J and McCartney., J.E (2008), Medical *Microbiology; A Guide to microbial Infections; Pathogenesis, Immunity, Laboratory diagnosis and control* 15th edition. Pp 175 – 78, 309 – 11 189, 406 and 464.

Micheal., J. leslie., E, Divid., M (2002). otitis media article medicine pead, j. otolamogology Edited oral brown 3(7) 1-49

Mongkolrattanothai., K, Oram., R, Redleaf., M, Bova., J, and Englund., J. (2003), *Tuberculous otitis media with mastoiditis and central nervous system involvement* (21); 208 – 11

National Institute on Deafness and Other Communication Disorders, (2002)

Ogisi and osamor., Y.Y. Bacteriology F (2002). *choronic otitis media in Benin Nigeria. Med J*. 12:187-190.

Pichichero., M.E (2001), *Dialogues in acute otitis media Abott Laboratory* 1:1 Senuturia., B.H, Gessert., C.F, Carr., C.D, Baumann., E.S (2008), *studies concerned with tubotympanitis*. 67: 440 – 67. Rhinol. Laryngol. 98: 293-297.

Perkins., BA et al, (2007). *Evaluation of an algorithm for integrated management of childhood illness in an area of Kenya with high malaria transmission*. Bull World Health Organiz, 75 (suppl 1): 33-42.

Picozzi., G, Browning., G, Calder., I (2003). *Controlled trial of gentamicin and hydrocorisone ear drops in the treatment of active chronic otitis media.* Clin Otolaryngol, 8: 367-368.

Picozzi., G, Browning., G, Calder., I (2004). *Controlled trial of gentamicin and hydrocortisone ear drops with and without systemic metronidazole in the treatment of active chronic otitismedia.* Clinical Otolayngology, 9: 305.

Pisacane., A, Ruas., I (2006). *Bacteriology of otitis media in Mozambique.* Lancet,

Watson., D.A *chronic otitis media otolaryngology* pg 12.

Wiet., R, DeBlanc., G et al, (2001). *Natural history of otitis media in the American native*. Ann Otol Rhinol Laryngol, 89: 14-19.

Wiet., RJ (2009). *Patterns of ear disease in the South-western American Indian*. Arch Otolaryngol, 105: 381-385.

Wilde., AD, England J, Jones AS (2005). *An alternative to regular dressings for otitis externa and chronic suppurative otitis media?* J Laryngol Otol. 109 (2): 101-103.

Wilson., SJ (2005). *Deafness in developing countries*: approaches to a global program of prevention. Arch Otolaryngol, 111: 2-9.

Yuen., P, Lau., S, Chau., P, Hui.,Y (2004). *Ofloxacin eardrop treatment for active chronic suppurative otitis media:* prospective randomized study. *Am J Otolaryngol*. 15: 670-673.

Zorita., C,Villar., J, Bosch., J (2004). *Long-term results of cholesteatoma surgery in children, Acta Otorrinolaringol* Esp, 45(4): 233-236 (in Spanish).