Asia Pacific Environmental and Occupational Health Journal (ISSN 2462 -2214), Vol 3(1): 20 – 27, 2017
Published online © 2017 Environmental and Occupational Health Society

Bacteriology of Otitis Media and Its Host-Environmental-Infection Factors

Aliyu IA[1*], Kumurya AS[1], Bala JA[1] , John OC[2]

[1]*Department of Medical Laboratory Science, Faculty of Allied Health Sciences, College of Health Sciences, Bayero University Kano, Nigeria*

[2]*Federal Collage of Veterinary and Medical Laboratory Science, National Veterinary Research Institute, VOM, Jos Plateau State, Nigeria*

***Corresponding author**: Aliyu IA; isahaa97@gmail.com; Department of Medical Laboratory Science, Faculty of Allied Health Sciences, College of Health Sciences, Bayero University Kano, Nigeria; +2348065882020.

ABSTRACT

Objectives: Otitis media is one of the most prevalent infection causing serious complication and sequelae. The present study was design to isolate bacteria associated with Otitis media and to establish a possible host-environmental-infection factors that might interact to influence the incidence of Otitis media and infecting organism.

Methods: Middle ear swabs and aspirates were aseptically collected from 71 patients who fulfil the study inclusion criteria for bacterial culture and antimicrobial susceptibility testing after administration of consent form and questionnaire.

Results: Bacterial pathogens were isolated from 63(88.7%) of the subjects, while 8(11.3%) of the subjects were bacteriologically sterile. *Staphylococcus aureus* was the most abundant isolate identified in 25(36.2%) of the total subject studied. This is followed by *Pseudomonas aeruginosa* with incidence of 20(28%), *Proteus mirabilis*, *Klebsiella pneumonia* and *Escherichia coli* have similar frequency each with 7(10%), *Streptococcus pyogenes* was the least isolates with the frequency of 3 (4.3%).

Conclusion: Swimming in ponds and river, traumatic inoculations as well as poor personal and environmental hygiene were identified as the likely predisposing environmental factors which contribute to otitis media in children and adolescent. It was observed that most isolates are enteric bacteria which indicate a concern in personal and environmental hygienic.

Key words: *otitis media, bacteria, personal hygiene, environmental hygiene, infection*

1. Introduction

The ear is a sensory, sensitive organ for hearing divided into three regions, the external ear, the middle ear and the internal ear. Its functions are; to receive sound of a certain frequency range and translate it into nerve impulses that are interpreted in the auditory center of the brain. It also senses linear and rotational acceleration of the head, and generates nerves impulses that include the corrective body movement necessary to restore a state of equilibrium (Julie, 2005). Otitis media generally refers to infection of the ear affecting the middle ear, the ear canal and the eardrum. Otitis media is also defined as the inflammatory disease of the mucosal lining of the middle ear cavity with it effusion which tends to be of serous nature in adult and of a mucous nature in children giving rise to glue ear otitis (Steele, 1998, Hoberman et al., 2011).

Otitis media is a serious health care concern worldwide, not only because of the distress it causes to the patient and the family, but also because of the substantial economic burden it imposes on the health care system (Alwotayan and, Alabdulhadi, 2003). During the year 1990, almost 25 million otitis media related hospital visit were reported in United States, with almost 809 out of 1000 visit been prescribed antibacterial drug (McCaig et al., 2002). Otitis media account for the second most prevalent infection among children and adolescent (Monasta et al., 2012). It also account for 30% of Doctors visitation (MacIntyre et al., 2010). It is the commonest ear pathology in otorhinolaryngological practice. It is also the commonest pediatrics otorhinolaryngological presentation (Iseh and Adegbite, 2004).

1.1 Risk Factors of Otitis Media

Breast-feeding has been suggested as an important factor in the prevention of respiratory tract infections and middle ear disease in infancy (Bowatte et al., 2015, Bluestone and Klein, 1983). Many studies have shown an inverse relationship between the incidence of middle ear disease and the duration of breast-feeding. But the mechanisms of the protective effect of breast milk remain obscure, although some studies shows that it is as a result of some factors present in milk (Bowatte et al., 2015). Children in group day care have been shown to be more likely to have otitis media as complication of an upper respiratory tract infection when compared to those in home care (Niemela et al., 1994). Contributing incidence of upper respiratory infections with resultant frequent examination by Physician system (Alwotayan and, Alabdulhadi, 2003). Passive smoke exposure has come under increased scrutiny as a risk factor for respiratory tract infection, including Otitis media. This is because of pathological and physiological changes in the respiratory tract, goblet cell hyperplasia, mucus hyper secretion, ciliostasis, decreased mucociliary transport, and alternation of the immune defenses associated with exposure (Shurin et al., 1979, Jones et al., 2012). There is also an increased incidence of chronic and recurrent Otitis media and Otorrhea in children whose mothers smoked (Jones et al., 2012). Many associated medical conditions predispose children to Otitis media. Cleft palate and craniofacial anomalies, especially if the midface is involved, appear to be related to an increased risk of otitis media (Alwotayan and, Alabdulhadi, 2003).

An increased incidence of Otitis media in children with congenital or acquired immune dysfunction (lgG subclass deficiency, AIDS, medication) have also been reported, ciliary dysfunction was also found to frequently predisposes children to Otitis media (Tasker et al., 2002). Edema of the nasopharynx and Eustachian tube from prolonged nasotrachel intubation or nasogastric tube placement may lead to Otitis media and Sinusitis. Nasal obstruction from enlarged adenoids, sinusitis, and malignancy also lead to Otitis media (Okur et al., 2004).

The sinuses and Eustachian tube are lined with the ciliated respiratory epithelium, which pushes mucous out of these structures and any trapped bacteria are removed. In middle ear and sinus infection it is likely that viruses such as RSV, influenza and Rhinovirus might invade this epithelium destroy the cells and compromise the mucocialliary function allowing bacteria to enter this sterile area (Struthers and Westran, 2003). Otitis media may be caused by any of many species of microorganism (Niemela et al., 1994). *Pseudomonas aeruginosa, Staphylococcus aureus,* Proteus species Streptococcus species were on various occasion been reported to have caused otitis media (Giebink, 1997, Burke, 1989). Previous studies have not precisely defined the host-environment-infection factors that interact to influence the incidence of Otitis media or manner in which such interaction takes place. Moreover there is paucity of data in human studies to have adequately addressed this issue, hence the need to carry out this work. This work was design to isolate bacterial pathogens associated with Otitis media and to establish a possible host-environmental-infection factors that interact to influence the incidence of Otitis media

2. Materials and Method

2.1. Study Area Subject and Inclusion Criteria

The study area for this research was Jos University Teaching Hospital (J.U.T.H), Jos Plateau. Patients who were clinically diagnosed to have Otitis media by the clinician at ear nose and throat (ENT) department of JUTH, (characterized with otalgia followed by Otorrhoea, and/or perforated tympanic membrane) who are attending ENT clinic of JUTH, and who have not received any antimicrobial/antibacterial chemotherapy for the preceding week and have accepted to participate in this research were employed as the subject of research.

2.2. Ethical Clearance, Consent Form and Questionnaire

The ethical clearance was obtained from the ethical committee of Jos University Teaching Hospital after ethical defense and following due ethical process before the commencement of the research. Consent forms were given to each subject to seek their consent after which the Questionnaire was administered.

2.3. Samples Collection

Sample were collected aeptically according to (Cheesbrough, 2006) briefly, purulent materials were collected from the subject. The Patients studied were known to have received no antibacterial medication during the preceding week. The surrounding ear canal was sterilized with 70% alcohol and sterile normal saline was used to irrigate the canal to remove any remaining alcohol solution. The saline was also cleaned with sterile absorbent cotton wool. Specimen were collected by carefully inserting a sterile thin swab sticks deeply into the auditory canal, by pulling the pinna of patients upward and back-wards or outwards so that further access were created into the auditory canal. The swab was gently rotated, the sample was collected and quickly placed into the Amies transport media. In patients with enough purulent discharge aspirate was taken using sterile pasture pipettes, and also quickly transferred into Amies transport media before transporting to laboratory for further analysis.

2.4. Method of Isolation

The samples were routinely gram stained and plated directly onto 2 chocolate agar plates, 2 blood agar plates and one of MacConkey Agar plates. One blood Agar and one chocolate Agar plate each were incubated at 5-10% carbon dioxide in a candle jar. And the other two with MacConkey Agar plate were incubated aerobically all at 37°C for 24 to 48 hours. The resultant colonies were used for identification, and plates which showed no growth after 48 hours of incubation were reported as negative (No bacterial growth) (Cheesbrough, 2006).

2.5. Identification

The resultant colonies were identified by their colonial appearances, gram staining and biochemical tests. All gram positive cocci were further subjected to basic test such as catalase and coagulase using 3% hydrogen peroxide and rabbit plasma respectively. While gram positive cocci, catalase and coagulase positive isolates were identified as *Staphylococcal aureus* and beta hemolytic, catalase negative and bacitracin susceptible were identified as *Streptococcus pyogenes* (Cheesbrough, 2006). *Pseudomonas aeruginosa, Proteus mirabilis Klebsiella pneumoniae and Escherichia coli* were all identified biochemically according to (Cheesbrough, 2006).

3. Results

Table 1 list the distribution of patients in this study. Different species of bacteria were isolated and identified from 63 (88.7%) out of the total sample studied. While 8 (11.3%) samples were negative for bacterial growth. Bacterial identification shows that 25 (36.2%) of total isolates were confirmed to be *Staphylococcus aureus,* while 20 (28.9%) of the isolate were identified to be *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniaea* and *Escherichia coli* were each confirmed to be 7 (10.1 %) isolates each respectively. The least common isolates was *Streptococcus pyogenes* with the frequency of 3 (4.3%) (Table 2). Mixed bacterial infection were identified in 6 (8.5%) of the samples. *S aureus* and *P aeruginosa* mixed culture were frequent then others. (Table 3).

Table 1. Frequency distribution of age group and gender among the patients studied.

Sex	1- 5	6-10	1-15	1-20	20-above	Total
Female	6	3	4	6	10	29 (40.8%)
Male	4	4	13	9	12	42 (59.2%)
Total	10	7	17	15	22	71 (100%)
%	14.1	9.9	23.9	21.0	30.9	

Table 2. Frequency distribution of Bacterial Pathogens Isolated

S/N	Pathogen isolated	No. of isolates	%
1	*Staphylococcus aureus*	25	36.2
2	*Pseudomonas aeruginosa*	20	28.9
3	*Proteus mirabilis*	7	10.1
4	*Klebsiella pnuemoniaea*	7	10.1
5	*Escherichia coli*	7	10.1
6	*Streptococcus pyogenes*	3	4.3

Table 3. Samples with mixed bacterial growth

S/N	Isolates	Incidence
1	*S aureus* and *P aeruginosa*	2
2	*P mirabilis* and *E coli*	1
3	*P aeruginosa* and *E coli*	1
4	*S aureus* and *K pneumoniae*	1
5	*P mirabilis* and *E coli*	1

3.1. Antimicrobial susceptibility testing

To determine antimicrobial susceptibility pattern of the isolate, antimicrobial sensitivity testing was undertaken. Commercially available antimicrobial susceptibility disc were used. Gram positive drugs were selected and applied for gram positive isolates, while gram negative drugs were selected and applied for gram negative isolate both at tissue level concentration. The results showed that For gram negative bacteria streptomycin was the most effective drug which 33 (76.2%) of the total gram negative isolate sensitive. This is followed by chloramphenicol, where 32 (59.5%) of the total isolate are sensitive. And gentamycin in which 25 (35.7%) of the total isolate were sensitive. The least effective drug was Ceporex in which only 13 (30.1%) of the total gram negative isolate were sensitivity (Table 4).

On the other hand, erythromycin was found to be most effective drug against gram positive isolate *in vitro* with 23 (82.1%) of total gram positive isolate sensitivity, followed by streptomycin in which 19 (67.9%) of the isolate were sensitive and gentamycin with 18 (64.3%) of the total gram positives isolate sensitive. Ciprofloxacin was the least sensitive drug *in vitro*, with only 12 (42.9%) sensitive isolate (Table 5).

Table 4. Antimicrobial susceptibility testing for Gram negative isolates, the number of susceptible isolate and their corresponding percentage for each drug.

Gram positive isolates	Total No. of isolates	S	CPX	CN	OFX	SXT	AU	PEF	CEP
P. aeruginosa	21	15	17	10	5	6	6	4	4
P mirabilis	7	6	5	6	3	3	2	2	3
K pneumoniae	7	6	4	4	3	3	3	2	2
E coli	7	6	6	5	4	3	4	5	4
Total	42	33	32	25	15	15	15	13	13
%	78.6	76.2	59.5	35.7	37.7	35.7	35.7	30.1	30.1

Note: S = Stremptomacin, PN = Penicillin, SXT = Septrin, CEP = Ceporex, CN = Gentamicin, NA = Nalidixic acid, CPX = Chloramphenicol, OFX = Tarivid, AU = Augmenti, PE P= Peflacin

Table 5. Antimicrobial susceptibility testing for Gram positive isolates, the number of susceptible isolate and their corresponding percentage for each drug.

Gram positive isolates	Total No. of isolates	APX	E	S	CN	CH	CPX	NB	RD	LC	FLX
S aureus	25	8	20	17	16	14	10	11	12	12	17
S pyogenes	3	1	3	2	2	3	2	2	1	3	3
Total	28	9	23	19	18	17	12	13	13	16	20
%		32.1	82.1	67.9	64.3	60.7	42.9	46.4	46.4	57.1	71.4

Note: APX= Ampiclox, CN= Gentamycin, NB= Norbactin, E= Erythromycin, CH= Chloramphenicol, RD= Rifanpin , S= Streptoneycin, CPX= Ciproflox, LC= Lincocin, FLX= Flaxapen

4. Discussion

Environmental contribution in the pathology of otitis media cannot be overemphasis, several studies have investigated risk factors associated with the otitis media, some of these work were reviewed by (Jones et al., 2012). Other risk factors have also been demonstrated by a number of researchers (Baraibar, 1997, Kong, 2009, Abou-Halawa and Alhumaid, 2004). Moreover, data on the patients-environmental-infection factors, which associate and influence infection of middle ear were not fully elucidated. The present study was design to investigate Host-pathogen-environmental factor in relation to otitis media, effort has been placed to try to find environmental link between bacterial pathogen isolated and identified from the patients with the possible environmental factor that likely predisposes an individuals to that particular pathogens. Studies on the bacteriology of otitis media from different parts of the world shows an interesting varying nature of outcomes, the pattern of isolates, species types and occurrence was clearly shown to be influence by the hygienic status of the region (developed versus poor regions), climatic or geographical variation of the regions as well as normal flora of upper respiratory tract and common pathogen in the particular environment (Rashid et al., 2014, Pumarola et al., 2013, Afolabi et al., 2013, Friedel et al., 2014).

Haemophilus influenza Streptococcus pneumoniaea and *B catarrhalis* have consistently been reported as important bacterial agents of otitis media in US and elsewhere in developed countries (Celin et al., 1991, Coker et al., 2010). On the other hand, in tropical coun tries, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and other enteric bacteria were frequently reported to be associated with otitis media (Iseh and Adegbite, 2014, Aduda et al., 2013, Abera and Kibret, 2011).

In the present study, 71 ear swabs samples were collected and was bacteriologically examined, 63(88.7%) of the samples contained at least one bacterial agent, mixed bacterial growth were obtained in 8 (11.3%) of the studied subjects, while 6 (8.5%) were found to be bacteriologically sterile.

Staphylococcus aureus, was the most abundance organism among the isolates, with the relative frequency distribution of 25 (35.2%), this finding were in concordance with what others found in this region and elsewhere (Iseh and Adegbite , 2004, Yeo et al., 2007). *S aureus* is coagulase positive gram positive cocci, which mostly live as a commensals in the nasopharynx (Bogaert et al., 2004), they are reported to have ascended from the nasopharynx during acute otitis media to the middle ear via Eustachian tube (Kurono et al., 1998) where they become pathogens, *S aureus* usually is transferred by air, and reach the ear as droplet from the respiratory tract as dry particles or from the body surface (aerosols) (Liu, 2009). Mouth has been reported to be the chief source, (Struthers and Westran, 2003), identified the possible mechanism of bacterial infection in middle ear to be as a result of previous infection with virus like RSV, influenza, CMV and Rhinovirus which invade the middle ear epithelium, cause inflammation of tympanic membrane destroy the cells and compromise the mucocialliary function of this cells allowing bacteria to enter this sterile area. Poverty level of the community, poor hygienic environment, overcrowded living environment (Massa, 2009) might contribute to the exposure of the subject to the possible viral infection which subsequently result in bacterial invasion of the middle ear. The abundance of *S aureus* might be as result of the close proximity of the external ear where they live as normal flora and middle ear.

Next to *Staphylococcus aureus* was *pseudomonas aeruginosa* which has relative frequency of 20 (28.9%), *P aeruginosa* has also been found to be one of the commonest bacterial isolate in the otitis media in many studies (Iseh and Adegbite, 2004). These are Gram negative rods that inhabit human gut and body as saprophyte and can cause otitis media as a result of secondary trauma or surgery (Cheesbrough, 2006). Even though this isolated is far from the middle ear, they are found to be transferred to the ear mostly through environmental and personal unhygienic practices. It was observed that, 17 (85% n=20) of the patients with this isolate have history of swimming in pond or rivers, and of ear scratching. Suggesting possible traumatic inoculation of the organism to the ear.

Other isolates includes *Proteus mirabilis*, *Escherichia coli* and *Klebsiella pneumoniae* each with relative frequency distribution of 7 (10.1%). other researchers have also reported the isolation of each of these organism in various location especially in the developing country (Afolabi et al., 2004, Elmanama et al., 2014, Habibu , 2015). These organisms were found as the normal habitant of human and animals intestinal tract (Jawetz et al., 2007). They both got access to ear by trauma or as secondary pathogens. Patients studied with history of cleaning or scratching ear with broom sticks 5(71.4% n=7) for *P mirabilis* and *E coli* and 6(85.7% n=7) for *K pneumoniae* were harboring the organism respectively, and 6(85.7%) in each case have history of dirty ponds or river swimming. The least in occurrence was *Streptococcus pyogenes* with relative frequency of 3(4.3%). This result was also in concordance with the finding of Segal et al., in which incidence of 3.1% was reported (Segal et al., 2005) and the finding of (Kakuta et al., 2014) I Japan. This is a gram positive cocci *beta* hemolytic organism, otherwise known as group A beta-hemolytic streptococcus. They are known to be important bacterial agents of pharyngotonsilitis, soft tissue, skin and invasive infections (Segal et al., 2005). The result showed that, all of the isolates are from children under 5 years. The more reason or most likely source of this organisms may be from upper respiratory tract where in the children it exist as a normal flora (Cheesbrough, 2006), and because the Eustachian tube of the ear is joint with pharyngotympanic tube of the nose (upper respiratory organ) which is a passage between the tympanic membrane of the middle ear and the connective-tube of the Eustachian that connect the nose. It is very easy now for this organisms to move down to tympanic and perforate the membrane, and because of the more horizontal Eustachian compare to adult, and juvenile immune system it make it easy for this bacteria to colonized middle ear of this category of subject (Iseh and Adegbite, 2004).

The result of Antimicrobial sensitivity test using disc diffusion method show higher level of resistance in most isolates, *Pseudomonas aeruginosa* which is the second most prevalent isolate has been found to be notoriously resistant to almost all the drug. Chloramphenicol was found to be most effective drug for this isolate, higher resistance to Augmentin, peflacin and ceporex was observed in all gram negative isolates, this are most effective empherical therapy in the study area during the study time (Iseh and Adegbite, 2004). this result was in concordance with other findings (Orji and Dike, 2015), but anti-microbial pattern is different from what was reported by (Elmanama et val., 2014). This suggest the need to carry out antimicrobial sensitivity testing for each individual isolate from each patients. For gram positive isolates erythromycin was found to be most sensitive drug *in vitro*, this finding was also reported elsewhere (Iseh and Adegbite, 2004). Though, ciprofloxacin was observed to be less sensitive drug, this finding was not in concordance with many finding in which ciprofloxacin was reported to be the most effective drug of choice for treatment of bacterial agent of otitis media (Afolabi et al., 2013). However, in the present study, a substantial increase resistance has been observed even to Augmentin, third generation cephalosporin, quinolones as well as the frequently used anti-microbial such as Gentamycin, Septrin, Erythromycin, and Chloramphenicol. A high level of resistance in both Gram Negative and Gram positive isolate was observed with Taravid (oflaxicin), Penicillin, Ceporex, Peflacin, Ampiclox and Rifanpin.

5. Conclusion

The result of the present study shows that, *Staphylococcus aureus* was the most prevalent organisms isolated from cases of otitis media in the study area. Followed by *Pseudomonas aeruginosa*, the remaining isolates except *Streptococcus pyogenes* were all members of bacterial family known as Enterobacteriacea, which signifies hygienic implication. The sources of these organisms are most likely from contaminated environmental source which got access to ear by trauma or by accidental contact in susceptible individuals. The result also shows that the infection with enteric organism is more prevalent in adolescent (10-20 years old) who are more exposed to environment and can on their own use contaminated object in their ears, and can swim in ponds or rivers with or without their parent awareness. The result also shows high resistance of most isolate to virtually all the drugs commonly use in empherical treatment of Otitis media. Finally we report that demography, personal as well as environmental factors can contributes toward development of otitis media or may worsen already established otitis media. And recommend good personal and environmental hygiene, avoidance of the risk factor/behavior as well as antimicrobial testing for each individual case, in order to avoid infection and development of drug resistance in already established cases.

Acknowledgement

Authors want to thanks and appreciate staffs of ear nose and throat (ENT) department of Jos university teaching hospital, Jos, Plateau state, Nigeria, for their un-tiring co-operation throughout the duration of this study. And to the voluntarily participating patient who consented to be enrolled in this study for their kind cooperation and response.

References

Abou-Halawa, A. S., & Alhumaid, H. I. (2014). Prevalence and Risk Factors of Otitis Media with Effusion Among School Children in Saudi Arabia. *Otolaryngology--Head and Neck Surgery*, 151(1 suppl): P107-P.

Abera, B., & Kibret, M. (2011). Bacteriology and antimicrobial susceptibility of otitis media at dessie regional health research laboratory, Ethiopia. *Ethiopian Journal of Health Development*, 25(2), 161-7.

Aduda DS, Macharia IM, Mugwe P, Oburra H, Farragher B, Brabin, B, et al. (2013). Bacteriology of chronic suppurative otitis media (CSOM) in children in Garissa district, Kenya: A point prevalence study. *International journal of pediatric otorhinolaryngology*, 77(7), 1107-11.

Afolabi, O., Salaudeen, A., Ologe, F., Nwabuisi, C., & Nwawolo, C. (2013). Pattern of bacterial isolates in the middle ear discharge of patients with chronic suppurative otitis media in a tertiary hospital in North central Nigeria. *African health sciences*, 12(3), 362-7.

Alwotayan, R., & Alabdulhadi, K. Otitis media (2003): a review for the family physician. BULLETIN OF THE KUWAIT INSTITUTE FOR MEDICAL SPECIALIZATION, 2:83-9.

Baraibar R. (1997). Incidence and risk factors of acute otitis media in children. Clinical Microbiology and Infection, 3:3S13-3S22.

Bluestone, C., & Klein, J. (1983). Intratemporal complications and sequelae of otitis media. *Pediatric otolaryngology*, 1:583-635.

Bogaert D, Van Belkum A, Sluijter M, Luijendijk A, de Groot R, Rümke H, et al. (2004). Colonisation by Streptococcus pneumoniae and Staphylococcus aureus in healthy children. *The Lancet*, 363(9424), 1871-2.

Bowatte G, Tham R, Allen K, Tan D, Lau M, Dai X, et al. (2015). Breastfeeding and childhood acute otitis media: a systematic review and meta-analysis. *Acta Paediatrica*., 104(S467):85-95.

Burke P. (1989). Otitis media with effusion: is medical management an option? *JR Coll Gen Pract*., 39(326):377-82.

Celin, S. E., Bluestone, C. D., Stephenson, J., Yilmaz, H. M., & Collins, J. J. (1991). Bacteriology of acute otitis media in adults. *JAMA*, 266(16):2249-52.

Cheesbrough M. (2006). District laboratory practice in tropical countries: Cambridge university press.

Elmanama, A. A., Tayyem, N. E. A., & Allah, S. A. N. (2014). The bacterial etiology of otitis media and their antibiogram among children in Gaza Strip, Palestine. *Egyptian Journal of Ear, Nose, Throat and Allied Sciences*,15(2):87-91.

Friedel, V., Zilora, S., Bogaard, D., Casey, J., & Pichichero, M. (2014). Five-year prospective study of paediatric acute otitis media in Rochester, NY: modelling analysis of the risk of pneumococcal colonization in the nasopharynx and infection. *Epidemiology and infection*., 142(10):2186-94.

Giebink G. S. (1997). Vaccination Against Middle-ear Bacterial and Viral Pathogens. Annals of the New York Academy of Sciences., 830(1):330-52.

Habibu A. (2015). Bacteriology of otitis media among patients attending general hospital Bichi, Nigeria.

Hoberman A, Paradise JL, Rockette HE, Shaikh N, Wald ER, Kearney DH, et al. (2011). Treatment of acute otitis media in children under 2 years of age. *New England Journal of Medicine*, 364(2):105-15.

Iseh, K, & Adegbite, T. (2004). Pattern and bacteriology of acute suppurative otitis media in Sokoto, Nigeria.

Jawetz, M., Joseph, L. & Adelberg. (2007). Herpesvirus, Jawetz, Melnick, and Adelberg's Medical Microbiology, 163-6.

Jones, L. L., Hassanien, A., Cook, D. G., Britton, J., & Leonardi-Bee, J. (2012). Parental smoking and the risk of middle ear disease in children: a systematic review and meta-analysis. *Archives of pediatrics & adolescent medicine*, 166(1):18-27.

Kakuta R., Yano H, Hidaka H, Miyazaki H, Irimada M, Oda K, et al. (2014). Severe acute otitis media caused by mucoid streptococcus pyogenes in a previously healthy adult. *The Tohoku journal of experimental medicine*, 232(4):301-4.

Kong. K., & Coates, H. L. (2009). Natural history, definitions, risk factors and burden of otitis media. *Medical Journal of Australia*,191(9):S39.

Kurono, Y., Tomonaga, K., & Mogi, G. (1988). Staphylococcus epidermidis and Staphylococcus aureus in otitis media with effusion. Archives of Otolaryngology–Head & Neck Surgery, 114(11):1262-5.

Liu G. Y. (2009). Molecular pathogenesis of Staphylococcus aureus infection. *Nature Publishing Group*.

MacIntyre EA, Karr CJ, Koehoorn M, Demers P, Tamburic L, Lencar C, et al. (2010). Otitis media incidence and risk factors in a population-based birth cohort. *Paediatr Child Health*, 15(7):437-42.

Massa, H. M., Cripps, A. W., & Lehmann, D. (2009). Otitis media: viruses, bacteria, biofilms and vaccines. *Medical Journal of Australia*, 191(9):S44.

McCaig, L. F., Besser, R. E., Hughes, J. M. (2002). Trends in antimicrobial prescribing rates for children and adolescents. *Jama*, 287(23):3096-102.

Monasta L, Ronfani L, Marchetti F, Montico M, Brumatti LV, Bavcar A, et al. (2012). Burden of disease caused by otitis media: systematic review and global estimates. *PloS one*, 7(4):e36226.

Niemela, M., Uhari, M., JOUNIO-ERVASTI, K., Luotonen, J., Alho, O-P, & Vierimaa, E. (1994). Lack of specific symptomatology in children with acute otitis media. *The Pediatric infectious disease journal*, 13(9):765-8.

Okur, E., Yildirim, I., Kilic, M. A., & Guzelsoy, S. (2004). Prevalence of otitis media with effusion among primary school children in Kahramanmaras, in Turkey. *International journal of pediatric otorhinolaryngology*, 68(5):557-62.

Orji, F., & Dike, B. (2015). Observations on the current bacteriological profile of chronic suppurative otitis media in South eastern Nigeria. *Annals of medical and health sciences research*, 5(2):124-8.

Pumarola F, Marès J, Losada I, Minguella I, Moraga F, Tarragó D, et al. (2013). Microbiology of bacteria causing recurrent acute otitis media (AOM) and AOM treatment failure in young children in Spain: shifting pathogens in the post-pneumococcal conjugate vaccination era. *International journal of pediatric otorhinolaryngology*, 77(8):1231-6.

Rashid, M., Joshi, M., Saha, R., & Pagrani, M. (2014). Bacteriological Profile and Antimicrobial Susceptibility Pattern among Patients with Chronic Suppurative Otitis Media in a Hospital Based Study in India. *The Indian Practitioner*, 67(12):775-8.

Segal, N., Givon-Lavi, N., Leibovitz, E., Yagupsky, P., Leiberman A, & Dagan, R. (2005). Acute otitis media caused by Streptococcus pyogenes in children. *Clinical infectious diseases*, 41(1):35-41.

Shurin, P. A., Pelton, S. I., Donner, A., & Klein, J. O. (1979). Persistence of middle-ear effusion after acute otitis media in children. *New England Journal of Medicine*, 300(20):1121-3.

Steele R. (1998). Management of otitis media. *Infections in Medicine*, 15(3):174-8+ 203.

Struthers, J. K., & Westran, R. P. (2003). Clinical bacteriology: CRC Press.

Tasker, A., Dettmar, P. W., Panetti, M., Koufman, J. A., P, Birchall, J., & Pearson, J. P. (2002). Is gastric reflux a cause of otitis media with effusion in children? *The Laryngoscope*, 112(11):1930-4.

Yeo, S. G, Park, D. C., Hong, S. M., Cha, CI, & Kim, M. G. (2007). Bacteriology of chronic suppurative otitis media–a multi-center study. *Acta oto-laryngologica*, 127(10):1062-7.