A field study to isolate bacterial pathogens from otitis media

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■ **Abstract:**

Otitis media is an inflammation of the middle ear and has a worldwide distribution, particularly among children. A total of 68 patients between the ages of 1-42 years who presented with signs and symptoms of otitis media The study was conducted at the Poly Clinic in Ajdabyia City. Samples were transported to the microbiology lab in Aben Naphaes Lab. Middle ear discharge was collected, processed, and bacterial isolates identified using standard microbiological techniques. Disc susceptibility tests were performed on bacterial isolates. Results indicated that there was a high frequency of occurrence of bacteria incriminated in otitis media in the 1–5 and 6–11 year age groups in both sexes. Male patients in the two age groups were more infected than their female counterparts. The sensitivity pattern obtained in this study shows a progressive increase in the emergence of strains that are resistant to many chemotherapeutic agents. Drug resistance among bacterial isolates is a common phenomenon that poses a challenge to both medical practitioners and genetic scientists. A routine check on sensitivity patterns among these pathogens is vital to regulating the prescription measures to be employed. We recommend, according to the result of this study that after swimming or bathing, dry the external canal with a blow dryer on a low setting or by instillation of acidifying or alcohol drops, and avoid overzealous cleansing and scratching (trauma) of the ear.

Keywords: Bacterial infection, ear infection, Otitis media.

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■ المستخلص:

التهاب الأذن الوسطى هو التهاب يصيب الأذن الوسطى وله انتشار عالمي، خاصة بين الأطفال. ما مجموعه 68 مريضا تتراوح أعمارهم بين 1-42 سنة ظهرت عليهم علامات وأعراض التهاب الأذن الوسطى. أجريت الدراسة في إحدى العيادات المجمعة بمدينة اجدابيا.و تم نقل العينات إلى مختبر الأحياء الدقيقة في مختبر ابن النفيس. وتم جمع إفرازات الأذن الوسطى ومعالجتها وتحديد العزلات البكتيرية باستخدام التقنيات الميكروبيولوجية القياسية. وتم إجراء اختبارات حساسية المضادات الحيوية بطريقة الأقراص على العزلات البكتيرية. أشارت النتائج إلى وجود تواتر عال لحدوث بكتيريا التهاب الأذن الوسطى في الفئتين العمريتين 1-5 و 6-11 سنة في الجنسين. كان المرضى الذكور في الفئتين العمريتين أكثر إصابة من نظرائهم من الإناث. يظهر نمط الحساسية للمضادات الحيوية الذي تم الحصول عليه في هذه الدراسة زيادة تدريجية في ظهور سلالات مقاومة للعديد من عوامل العلاج الكيميائي. مقاومة الأدوية بين العزلات البكتيرية هي ظاهرة شائعة

تعد مقاومة الأدوية بين العزلات البكتيرية ظاهرة شائعة تشكل تحديا لكل من الممارسين الطبيين وعلماء الوراثة. يعد الفحص الروتيني لأنماط الحساسية بين مسببات الأمراض هذه أمرا حيويا لتنظيم مقياس الوصفة الطبية الذي سيتم استخدامه. نوصي وفقا لنتيجة هذه الدراسة أنه بعد السباحة أو الاستحمام، قم بتجفيف القناة الخارجية باستخدام مجفف شعر على درجة حرارة منخفضة أو عن طريق تقطير قطرات الحمض أو الكحول كذلك تجنب التطهير المفرط والخدش (تلف) لقناة الأذن

الكلمات المفتاحية: العدوى البكتيرية، عدوى الأذن، التهاب الأذن الوسطى

■ INTRODUCTION

Otitis media is defined as the inflammation of the middle ear and is a common cause of children's visits to physicians. While the term otitis media commonly denotes an inflammation of the middle ear, the disease can be more accurately described as acute, sub-acute, chronic, suppurative, or non-suppurative. Previous studies have reported otitis media to have a worldwide prevalence, affecting all age groups but being more common in children. A study carried out indicated that that; Eustachian tube abnormalities are a primary determinant of otitis media. Other determinants include viral infections and systemic immune deficiencies, which predispose to superimposed bacterial disease. [1,2] Thus, sources of infection in otitis media are dependent on the middle ear, and the principal route by which this

occurs is the Eustachian tube. [3]The lower immunity of children as compared to adults, the shorter and more horizontal Eustachian tube in children, which permits easier access of microorganisms from the nasopharynx, and the fact that bacteria adhere better to epithelial cells in children than adults have been suggested as possible reasons for the higher prevalence in children. Other risk factors for otitis media include exposure to smoke, crowded living conditions, and low socioeconomic class. [3] The preceding conditions are very common in resource poor countries like Nigeria. As these diseases can be prevented and treated, public awareness of personal hygiene should be increased among the general population to decrease morbidity due to the disease. Treatment is also very necessary and urgent to prevent complications such as meningitis and septicemia.Otitis media is an infection or inflammation of the middle ear. This inflammation often begins when infections that cause sore throats, colds, or other respiratory or breathing problems spread to the middle ear. These can be viral or bacterial infections. Seventy-five percent of children experience at least one episode of otitis media by their third birthday. Almost half of these children will have three or more ear infections during their first 3 years. It is estimated that medical costs and lost wages because of otitis media amount to $5 billion a year in the United States. Although otitis media is primarily a disease of infants and young children, it can also affect adults. [1, 3] Acute otitis media (AOM) is an infection of abrupt onset that usually presents with ear pain. In young children, this may result in pulling at the ear, increased crying, and poor sleep. Decreased eating and a fever may also be present. OME is typically not associated with symptoms. Occasionally, a feeling of fullness is described. It is defined as the presence of non-infectious fluid in the middle ear for more than three months. [4]Chronic suppurative otitis media (CSOM) is middle ear inflammation of longer than two weeks that results in episodes of discharge from the ear. It may be a complication of acute otitis media. Pain is rarely present. All three may be associated with hearing loss. This progresses to a prolonged inflammatory response causing mucosal (middle ear) oedema, ulceration, and perforation. The middle ear attempts to resolve this ulceration through the production of granulation tissue and polyp formation. This can lead to increased discharge and failure to arrest the inflammation.[5] Worldwide, approximately 11% of the human population is affected by AOM every year, or 709 million cases. About 4.4% of the population develops (CSOM). According to the World Health Organization, CSOM is a primary cause of

hearing loss in children. The prevalence in low-income countries may be up to three times as high as in developed countries.[5]Acute otitis media is very common in childhood under five years of age in the United States (US) *and* affects 11% of people each year (709 million cases), with half occurring in those below five years. Chronic suppurative otitis media affects about 5%, or 31 million, of these cases, with 22.6% of cases occurring annually under the age of five years. Otitis media resulted in 2,400 deaths in 2013, down from 4,900 deaths in 1990.[6]

In otitis media, the etiologic bacteria may be aerobic (e.g., *Pseudomonas aeruginosa*, *Escherichia coli, Staphylococcus aureus, Streptococcus pyrogens, Proteus mirabilis, Klebsiella species) or* anaerobic (e.g., *Bacteroides)*. [7,8] Otitis media not only causes severe pain but may also result in serious complications if it is not treated. An untreated infection can travel from the middle ear to the nearby parts of the head, including the brain. Untreated otitis media may lead to permanent hearing impairment. Frequent ear infections are likely to have speech and language disabilities. [9,10]. Age is a significant predictor of otitis media frequency, severity, and responsiveness to treatment. Infants and toddlers are more severely affected, may take longer to respond to treatment, and can be more difficult to diagnose accurately. [10, 11]

Risk factors such as season, allergy predisposition, and the presence of older siblings are known to be determinants of recurrent otitis media and persistent middle ear effusions (MEE). History of recurrence, environmental exposure to tobacco smoke, use of daycare, and lack of breastfeeding have all been associated with increased risk of development, recurrence, and persistence (MEE). Pacifier use has been associated with more frequent episodes of AOM. Long-term antibiotics, while they decrease rates of infection during treatment, have an unknown effect on long-term outcomes such as loss. This method of prevention has been associated with the emergence of antibiotic-resistant otitis bacteria. They are thus, not recommended. Evidence does not support zinc supplementation as an effort to reduce otitis rates, except maybe in those with severe malnutrition such as marasmus [12]

Oral and topical painkillers are effective in treating the pain caused by otitis media. Topical agents shown to be effective include antipyrine and benzocaine ear drops. Decongestants and antihistamines, either nasal or oral, are not recommended due to their lack of benefit and concerns regarding side

effects. Half of cases of ear pain in children resolve without treatment in three days, and 90% resolve in seven or eight days. The evidence for acute otitis media does not support the use of steroids. [13] It is important to weigh the benefits and harms before using antibiotics for acute otitis media. As over 82% of acute episodes settle without treatment, antibiotics should be prescribed for severe bilateral or unilateral disease in all infants and children with severe signs and symptoms. The first-line antibiotic treatment, if warranted, is amoxicillin or another penicillin derivative, and a beta-lactamase inhibitor is recommended.[14] Complementary and alternative medicine is not recommended for otitis media with effusion because there is no evidence of benefit. Homeopathic treatments have not been proven to be effective for acute otitis media in a study with children. An osteopathic manipulation technique called the Gal breath technique was evaluated in one randomized controlled clinical trial; one reviewer concluded that it was promising, but a 2010 evidence report found the evidence inconclusive. [15]

■ MATERIALS AND METHODS

● Study population

The study was conducted at the Polyclinic in Ajdabyia City**.** Samples were transported to the microbiology lab in Aben Naphaes Lab. A total of 68 (39 males and 29 females) patients with signs and symptoms of otitis media attending ear, nose and throat clinics in the hospital were enrolled for this study in July 2018.

● Sample collection and processing

Pus or purulent discharges from cases of otitis media were collected from patients with the disease using a sterile swab. The ear swabs were used to inoculate chocolate agar and MacConkey agar plates by streaking. The chocolate agar plates were incubated at 5% carbon dioxide in a candle jar, and the MacConkey plates were incubated aerobically at 37 ^{o}C for 24 hours. The resulting bacterial colonies were identified by standard bacteriological techniques and using an API 20E strip**.** The reactions are read according to the reading table, and the identification is obtained by referring to the analytical profile index or using the identification software. Laboratory antimicrobial susceptibility testing is performed using disc diffusion susceptibility tests**:** Disc diffusion techniques are used by most laboratories to routinely test for antimicrobial susceptibility. A disc of blotting paper is impregnated with a

known volume and appropriate concentration of an antimicrobial, and this is placed on a plate of susceptibility test agar uniformly inoculated with the test organism. Antibiotics Augmentin, ampicillin, streptomycin, gentamicin, cefotaxime, erythromycin, ciprofloxacin, chloramphenicol, ofloxacin, and azithromycin were used in this study[16].

■ RESULTS

Table 3.1 shows the distribution of bacterial agents of otitis media according to sex and age groups at the general hospital. The result indicated that there was a high frequency of occurrence of bacteria incriminated in otitis media in the 1–5 and 6–11 year age groups in both sexes. Male patients in the two age groups were more infected than female patients, while in the 12–17 year age group the male and female had an equal rate of infection. It was also observed from Table 2 that the frequency of infection decreased with age.

Table1: Distribution of bacterial agent of otitis media in relation to age

(Age group (years	Male	Female	Sample size
1-5	14	5	19
6-11	12	9	21
12-17	8	8	16
18-23	1	4	5
24-29	3	2	5
30-35	0	1	1
36-41	0	0	0
41<	1	0	1
Total	39	29	68

Table 3.2 shows the distribution of bacterial agent of otitis media in relation to gender. Out of the fourteen (14) isolates of Escherichia coli recorded, nine (9) were from male patients and five (5) from female patients. Out of the sixteen (16) isolates of *Pseudomonas aeruginosa* recorded, ten (10) were

from male patients and six (6) were from female patients. Of the eleven (11) isolates of *Proteus species* recorded, six (6) were from male patients and five (5) from female patients. Of the fourteen (14) isolates of *Staphylococcus aureus* recorded, eight (8) and six (6) were from male and female patients, respectively. In addition, of the thirteen (13) isolates of *Streptococcus species* recorded, six (6) and seven (7) were from male and female patients, respectively. Table 2 further indicates that of the overall total bacterial agents isolated from sixty-eight (68), thirty-nine (39) were from male patients and twenty-nine (29) were from female patients.

Table2: Distribution of bacterial agent of otitis media in relation to gender

Sex	*Escherichia coli*	*Pseudomonas aeruginosa*	*Proteus species*	*Staphylococcus aureus*	*Streptococcus Species*	Total
Male	9	10	6	8	6	39
Female	5	6	5	6	7	29
Total	14	16	11	14	13	68

Antibiogram of the bacterial agents incriminated in otitis media against ten (10) antimicrobial agents illustrated in Table **3. 3**

Fourteen (14) *Escherichia coli* isolates were tested against each antimicrobial. Out of the fourteen isolates, ten (71.4%) were sensitive to Augmentin, 9 (64.3%) to ampicillin, 7 (50.0%) to streptomycin, 7 (50.0%) to gentamicin, 5 (35.7%) to cefotaxime, 3 (21.4%) to erythromycin, 7 (50.0%) to ciprofloxacin, 3 (21.4%) to chloramphenicol, 6 (42.6%) and 5 (35.7%) to ofloxacin and azithromycin, respectively.Fourteen (14) *Escherichia coli* isolates were tested against each antimicrobial. Out of the fourteen isolates, 10 (71.4%) were sensitive to Augmentin, nine (64.3%) to ampicillin, 7 (50.0%) to streptomycin, 7 (50.0%) to gentamicin, 5 (35.7%) to cefotaxime, 3 (21.4%) to erythromycin, 7 (50.0%) to ciprofloxacin, 3 (21.4%) to chloramphenicol, 6 (42.6%) and 5 (35.7%) to ofloxacin and azithromycin, respectively. A total of sixteen *Pseudomonas aeruginosa* isolates were tested. Nine (56.3%) were sensitive to Augmentin, 4 (25.0%) to ampicillin, 6 (37.7%) to streptomycin, 8 (50.0%) to gentamicin, 8(50.0%) to cefotaxime, 8 (50.0%) to ciprofloxacin, 6 (37.7%) to chloramphenicol, and 4 (25.0%) to ofloxacin. A total of eleven (11) isolates of Proteus spp. tested, seven 7 (63.6%) were

sensitive to Augmentin, 6 (54.4%) to ampicillin, 4 (36.36%) to streptomycin, 3 (27.27%) to gentamicin, 1 (9.09%) to cefotaxime, 3 (27.27%) to erythromycin, 6 (54.4%) to ciprofloxacin, 5 (45.45%) to chloramphenicol, 6 (54.4%) and 6 (54.4%) to ofloxacin and azithromycin respectively. Fourteen isolates of *Staphylococcus aureus* were tested. Nine (96.4%) were sensitive to Augmentin, 4 (28.6%) to ampicillin, 7 (50.0%) to streptomycin, 6 (42.6%) to gentamicin, 1 (7.1%) to cefotaxime, 6 (42.6%) to erythromycin, 4 (28.6%) to ciprofloxacin, 7 (50.0%) to chloramphenicol, 6 (42.6%) and 5 (35.7%) to ofloxacin and azithromycin, respectively. Thirteen (13) isolates of *Streptococcus* species were also tested against ten antimicrobials. Ten (10.76%) were sensitive to Augmentin, 5 (38.46%) to ampicillin, 35 (38.46%) to streptomycin, 5 (38.46%) to gentamicin, 2 (15.38%) to cefotaxime, 5 (38.46%) to erythromycin, 4 (30.77%) to ciprofloxacin, 6 (46.15%) to chloramphenicol, 2 (15.38%) and 4 (30.77%) to ofloxacin and azithromycin, respectively.

Table 3: Antibiogram used against bacteria isolates

Antibiotics	*Escherichia Coli* (n=14)	*Pseudomonas aeruginosa* (n=16)	*Proteus species* (n=11)	*Staph aureus* (n=14)	*Strep species.* (n=13)
augmentin	10 (71.4%)	9 (56.3%)	7 (63.6%)	9 (64.3%)	10 (76.92%)
ampicillin	9 (64.3%)	4 (25.0%)	6 (54.4%)	4 (28.6%)	5 (38.46%)
streptomycin	7 (50.0%)	6 (37.7%)	4 (36.36%)	7 (50.0%)	3 (23.07%)
gentamicin	7 (50.0%)	8(50.0%)	3 (27.27%)	6 (42.6%)	5 (38.46%)
cefotaxime	5 (35.7%)	4 (25.0%)	1 (9.09%)	1 (7.1%)	2 (15.38%)
erythromycin	3 (21.4%)	-	3 (27.27%)	6 (42.6%)	5 (38.46%)
ciprofloxacin	7 (50.0%)	8(50.0%)	6 (54.4%)	4 (28.6%)	4 (30.77%)
chloram-phenicol	3 (21.4%)	6 (37.7%)	5 (45.45%)	7 (50.0%)	6 (46.15%)
ofloxacin	6 (42.6%)	4 (25.0%)	6 (54.4%)	6 (42.6%)	2 (15.38%)
azithromycin	5 (35.7%)	-	6 (54.4%)	5 (35.7%)	4 (30.77%)

■ DISCUSSION

There are many reasons why children are more likely to suffer from otitis media than adults are. First, children have more trouble fighting infections; this is because their immune systems are still developing. Another reason is that the Eustachian tube is a small passageway that connects the upper part of the throat to the middle ear. It is shorter and straighter in a child than in an adult. Bacteria reach the middle ear through the lining or passageway of the Eustachian tube and can then produce infection. In this process, the white cells accumulate, often killing bacteria and dying themselves, leading to the formation of pus, a thick yellowish-white fluid in the middle ear. As the fluid increases. [10]. The bacterial agents encountered in the present study were Streptococcus species, *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli, and Proteus* species. This corroborates the works of Egbe, C., and Mordi, R. (2010)[2], who isolated the same organisms from patients with bacterial otitis media. *Pseudomonas aeruginosa* (23.5%) was the most prevalent cause of bacterial otitis media followed by *Staphylococcus aureus* and *Escherichia coli* (20.6% each), and the least prevalent was *Proteus* species (16.2%). Most of these organisms are part of the normal flora of the skin and are opportunistic pathogens, implying that if they gain entrance into the human body, they cause infection in tissues and mucous membranes. Ekpo, M.A., and Akinjogunla (2009)[17] also reveal that bacteria causing otitis media have a higher prevalence among male patients than their female counterparts do. These observations agree with those of Oyeleke, S.B. (2009)[13], who reported the occurrence of otitis media being more prevalent among males than females; the reason for this is unclear. Similarly, the results of this study reveal that children less than six years old are more prone to otitis media; this is in agreement with previous studies such as Habibu, U.A., and Takalmawa (2002)[1]. Children usually have fewer recurrent episodes of otitis media than older adults do. This is due to so many factors, such as developmental differences between children and adults with respect to the anatomical positioning of the Eustachian tube; children have a shorter and more horizontal Eustachian tube.

The sensitivity pattern obtained in this study is similar to those reported by Derkey, C. S., and Bluestone (1989),[18] Belfield K et al(2015)[19] by showing a progressive increase in the emergence of strains that are resistant to many

chemotherapeutic agents. However, in the present study, a substantial increase in resistance has been observed even to augmentin, third-generation cephalosporins, quinolones, and frequently used antimicrobials such as gentamicin, erythromycin, azithromycin, and chloramphenicol. The overuse and misuse of antimicrobials have been reported to lead to the death of sensitive strains to survive, multiply, and infect new hosts. Richard J Fair and Yitzhak Torv (2014)[20]. , Belfield K. *et al*. (2015) [19].These factors probably account for the pattern of resistance observed in this study.

■ CONCLUSION& RECOMONDATIONS

Otitis media is an ear infection of the middle ear, the area just behind the eardrum. It happens when the Eustachian tubes, which connect the middle ear to the nose, are blocked with fluid. Mucus, pus, and bacteria can also pool behind the eardrum, causing pressure and pain. *Pseudomonas aeruginosa* was the predominant bacterial isolate causing otitis media (23.5%), and otitis media incidence was more common among children younger than six years. In the present study, Augmentin and ciprofloxacin were found to be effective in the treatment of otitis media. Drug resistance among bacterial isolates is a common phenomenon that poses a challenge to both medical practitioners and genetic scientists. A routine check on sensitivity patterns among these pathogens is vital to regulating the prescription measures to be employed. Avoid overzealous cleansing and scratching (trauma) of the ear canal. Avoid cotton-tipped swabs. Earplugs and bathing caps may be used to keep the ears dry; however, there is little evidence to guide recommendations. Frequent use of earplugs may also act as a local irritant and promote infection. Long-term antibiotics, while they decrease rates of infection during treatment, have an unknown effect on long-term outcomes such as hearing loss. This method of prevention has been associated with the emergence of antibiotic-resistant otitis bacteria. Some things can be done that may reduce the child's risk of developing the condition. These include making sure your child is up-to-date with their routine vaccinations, particularly the pneumococcal vaccine, avoid exposing the child to smoky environments (passive smoking),do not give the child a dummy once they are older than six to 12 months old. Breastfeed infants for at least 6 months to help prevent the development of early episodes of ear infections. If a baby is bottle-fed, hold the baby at an angle instead of lying the child down with the bottle. Parents and kids should wash their hands well and often to stop the spread of germs that can cause colds and, therefore, ear infections.

■ REFERENCES

[1] Habibu, U.A. and Takalmawa, H.U. The Epidemiology and Bacteriology of middle Ear infection: A Comparative Study of two hospitals in Kano metropolis. Journal of Life and Environmental Science, University of Maiduguri, 2002, vol. 4 (1), 198-203

[2] Egbe, C., Mordi, R., Omoregie, R. and Enabulele, O. Prevalence of otitis media in Okada community, Edo state, Nigeria, Maced. J. Med. Sci., 2010, 3(3):299-302

[3] Alo, M.N., Anyim, C., Okonkwo, E. C. and Orji, J. O. Prevalence, Antibiogram of bacterial pathogens associated with otitis media among primary school children in Ebonyi state, Journal of Pharmacy and Biological Sciences, 2012, 1: 17-20

[4] Klein, J. O. Otitis externa, Otitis media, mastoiditis. In: Mandell, G. L., Douglas, R. G. and Bennette, J. E. (eds): Principles and practice of infectious diseases, 3rd ed. New York, Chuchilliving stone, 1990, pp: 505-510

[5] Tarlow, M. Otitis media. Pathogenesis and medical sequel, Ear, Nose and Throat J., 1998, 77 (suppl. 6): 3-6

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

[7] Shimanura, K., Shigemi, H., Kurono, Y., Mogi, G. The role of bacterial in otitis media with effusion, Arch. Otolaryngol. Head Neck Surg., 1990, 116(10): 1143–1146

[8] Bluestone, C.D. and Klein, J.O. Microbiology. In: Bluestone CD, Klein JO, eds. Otitis Media in Infants and Children. 3rd ed. Philadelphia, P A: W. B. Saunders., 2001, PP. 79-1014

[9] Aich, M.L., Biswas, A.C., Ahmed, M., Joarder, M.A.H., Datta, P.G., Alauddin, M. Prevalence of otitis media with effusion among school going children in Bangladesh, Bangladesh J Otorhinolaryngol, 2009, 15(1):31–34

[10] Brook, I., Frazier, E. Microbial dynamics of persistent purulent otitis media in children. J. Pediatr., 1996, 128(2): 237-240.

[11] Saunders, J.E., Raju, R.P., Boone, J., Berryhill, W. Current Bacteriology of Suppurative Otitis: Resistant Patterns and outcomes Analysis, Otology & Neurotology, 2009, 30(3): 339-343.

[12] Koksal, V., Reisli, I. Acute otitis media in children. J Ankara Med Sch. 2002, 56(1):19–24.

[13] Oyeleke, S.B. Screening for bacteria agents responsible for otitis media and their antibiogram, African Journal of Microbiology Research, 2009, 3(5): 249-252.

[14] Nwabuisi, C., Ologe, F.E. Pathogenic agents of chronic suppurative otitis media in Ilorin, Nigeria. East Afr Med J. 2002, 79(4):202-5.

[15] Bluestone, C. D. Epidemiology and pathogenesis of chronic suppurative otitis

media: Implications for prevention and treatment. Int. J. Peadiatr. Otorhinolaryngol, 1998, 42 (3), 207-23

[16] Andrews, J.M. BSA standardized disc susceptibility testing method (version 8). J. Antimicrob. Chemother, 2009, 64(3):454–489

[17] Ekpo, M.A., Akinjogunla, O.J. and Idiong, O.F. Microorganisms associated with acute otitis media diagnoized in Uyo City, Nigeria. Science Research and Essay, 2009, 4(6): 560-564.

[18] Derkey, C. S., Bluestone, C. D., Thompson, A. E. and Kardatske, D. Otitis media in pediatric intensive care unit: a prospective study. Otolaryngol. Head Neck Surg., 1989, 100 (4): 292-9

[19] Belfield K., Bayston R., Birchall J.P., Daniel M. Do orally administered antibiotics reach concentrations in the middle ear sufficient to eradicate planktonic and biofilm bacteria? A review. *Int. J. Pediatr. Otorhinolaryngol.* 2015; 79:296–300

[20] Richard J Fair and Yitzhak Tor. Antibiotics and Bacterial Resistance in the 21st Century. *Perspect Medicin Chem*. 2014; 6: 25–64.