

Identification of Microorganism Associated With Otitis Media among Children in Ganawuri Area of Plateau State, Nigeria

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Abstract: Out of the 182 samples of middle ear exudates examined 154 specimens contained bacterial pathogens and 18 specimens were sterile. Table 4.1 shows the total Isolation rate of bacterial pathogens from the 182 samples. A total of 53 of the isolates were confirmed as *Staphylococcus aureus* by Gram's Method, catalase and coagulase tests. And 34 of the isolates produced some greenish pigmentation on macConkey agar medium and also gave a fishy or musty odour. They were also found to be motile at 37°C strongly oxidase positive and were confirmed as *Pseudomonas aeruginosa*. Of the 154 total isolates, 25 showed some swarming in several cases at 37°C after 24 hours of incubation on blood agar and were confirmed as *proteus mirabilis*. Apart from *Haemophilus Infuenzae* and *Streptococcus Pyogenes* which were identified, using X and V factors, Satellitism test and bacitracin disc respectively, others such as *Escherichia coli* were identified biochemically to species level. Of all the six different bacterial pathogen isolated *staphylococcus aureus* was the most prevalent accounting for 34.42%, followed by *Pseudomonas aeruginosa* with 22.08% and *Proteous mirabilis* with 16.23%, *Streptococcus pyogenes* 13.64%, *Escherichia coli* 8.44%, while the lowest prevalent rate was recorded in *Haemophilus influenza* with 5.19%. Total positive sample recoded in female was 81 which is higher than 71 positive samples recorded in male but statistically there was no significant difference ($p>0.05$). out of 154 positive samples, Children between age group 1 – 3 were statistically significantly affected ($p>0.05$) with total number of 59(38.31%) positive samples followed by age group 4 – 6 with 47(30.52%), age group 7 – 9 with 30(19.48%) and age group 10 – 12 with 18(11.69%).

Key words: otitis media, organism, ear discharge.

I. Introduction

The inflammation of the middle ear cavity in man is referred to as Otitis media. Otitis media can be caused by infections, allergy, anatomic or functional deviations of the middle ear or Eustachian tube, Jawez [1]. The ear is a sense organ concerned with hearing and balance. Sound waves transmitted from outside enters the ear. It is made up of three parts, the outer, middle and inner ear [2]. The ear infection can be acute (acute otitis media) or chronic (chronic or suppurative otitis media). Acute otitis media is a middle ear effusion (fluid) associated with symptoms of pain, fever and irritability. Some children may also suffer from loss of appetite and even vomiting. Recurrent otitis media is when there are three or more separate episodes of acute otitis media in a six months period. Otitis media with effusion, often referred to as glue ear, describes fluid in the middle ear with no sign of fever or inflammation of the ear drum [3].

The middle ear has no natural flora, normally sterile. The members of the normal flora are opportunists and can cause disease when host defenses are impaired [1]. Children are much more susceptible to otitis media since the Eustachian tube is short and at more of a horizontal angle than in the adult ear [4]. They also have not developed the same resistance to bacteria, viruses and fungi as adults. The Eustachian tube is usually closed but opens regularly to ventilate or replenish the air in the middle ear. This tube also equalizes middle ear air pressure in response to air pressure changes in the environment. However, Eustachian tube that is blocked by swelling of the lining or plugged with mucous from a cold or from other reason cannot open to ventilate the middle ear [5].

The most common bacteria causes for acute otitis media are *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*. The incidence of penicillin resistant *Streptococcus pneumoniae* has risen, but rates vary between countries. Others are *Escherichia coli*, *Staphylococcus epidemidis* etc. while the fungal likely agents are; *Aspergillus niger* and *Candida* species. Viruses such as respiratory syncytial virus (RSV) and those that cause common cold may also result in otitis media by damaging the normal defenses of the epithelial cells in the upper respiratory tract [6]. Animals and vectors have no role in transmission of the agents responsible for otitis media. Environmental factors are important in patients in whom allergy is the precipitating

events [7]. 75% of children experience at least one episode of otitis media by the third birthday. Almost one-half of these children will have three or more ear infections during their first three years of life [8].

Otitis media is often difficult to detect because most young children do not yet have sufficient speech and language skills to tell someone what is bothering them. Common signs to look for are:

- unusual irritability
- difficulty sleeping
- tugging or pulling at one or both ears
- fever
- fluid draining from the ear
- not respond to soft sounds
- turn up the television or radio
- talk louder [4].

Acute bacteria otitis media can cause pain that lead to sleeplessness for both children and parents, can also cause eardrum perforation, not all of which heals. It can spread to cause mastoiditis and or meningitis, brain abscesses and even death. If a severe infection goes untreated long enough, high fever can occur and cause febrile seizures. Appropriate antibiotic administration prevents most of such complications [9]. When the middle ear becomes acutely infected, pressure builds up behind the eardrum (tympanic membrane), frequently causing pain. It may result in bullous myringitis in which the tympanic membrane is inflamed and blistered. In severe or untreated cases, the tympanic membrane may rupture, allowing the pus in the middle ear space to drain into the ear canal. Though the rupture of the tympanic membrane suggests a traumatic process, it is relief of pressure and pain [10].

How the defense molecules and cells involved in immunity respond to bacteria and viruses that often lead to otitis media is also under investigation. Scientists are evaluating the success of certain drugs currently being used for the treatment of otitis media and are examining new drugs that may be more effective, easier to administer and better at preventing new infections. Most importantly, research is leading to the availability of vaccines that will prevent otitis media [8]. The Ad hoc committee on definition and classification of otitis media in 1980 strictly defined otitis media as inflammation of the middle ear that may or may not be infectious and may or may not be associated with effusion. Effusion when present may be serious, purulent or mucoid [11]. The acute inflammatory response to the initiating viral infection or allergic reaction changes the thin cuboidal lining of the middle ear into a structure that is two or three times its normal thickness. As the swelling involves the orifice of the Eustachian tube, the human becomes occluded and exudates accumulate. Pus that did not drain out of the middle ear becomes a focus for organization leading to mucosal thickening. Round cell infiltration and fibrosis of the middle ear mark chronic infection [12]

Aspiration of the middle ear fluid in the syndrome of acute otitis media yields a bacteriological sterile fluid in about one of the cases. Respiratory syncytial virus, Influenza A, Coxsackie, Parainfluenza and Adenoviruses have on occasions been isolated from this fluid but most attempt to isolate Mycoplasma have been unsuccessful [13].

There are many reasons why children are more likely to suffer from otitis media than adults. Children have more trouble fighting infection, this is because their immune system are still developing [14]. Infants in whom otitis media with effusion develop in the first year of life have an increase risk of recurrent middle ear infection. The overall prevalence of the disease tends to decrease with age especially after the age of 6 years. The incidence is higher in males, lower socioeconomic groups, Eskimos, Americans, Indians, children with cleft palate and is also higher in winter and early spring when viral respiratory diseases are frequent. Use of pacifiers beyond age two is associated with substantial increase in otitis media. Other conditions that cause inflammation of the nasal mucosa beside viral infections appear to play a role in some cases [15]. According to a study on the correlation between otitis media and breast feeding infants by [16], they found out that short duration of breast feeding is a risk factor for otitis media in early childhood and that feeding while lying down and early entry into child care centers increase the duration of otitis media infection in the first few years of life. Other factors such as overcrowding, poor nutrition, poor hygiene, and lack of attention to symptoms may also increase the incidence, type and severity of otitis media.

The present study is aimed at isolating the prevalent bacterial agents responsible for otitis media among children in Plateau Specialist Hospital Jos.

II. Objectives

To identify the possible causative agents to specie level.

To determine the most common bacterial organism that affects the population.

To access the level of otitis media among children and to suggest ways that should be used to minimize the problems.

III. Sample Collection

Samples were collected from 182 children with signs and symptoms of the Otitis media from Ganawuri district of Riyom local government area in Plateau state, which comprises children between the ages 1 to 12.

The eardrum and surrounding ear canal of the subjects were sterilized with 70% alcohol. Normal saline was used to irrigate the canal and removed any alcohol remnant, after which the ear was clean and dried with sterile absorbent cotton wool. The specimen was collected by inserting and gentle rotation of sterile swab stick into the middle ear.

IV. Isolation

Each case of otitis media was sample on two sterile swabs sticks one for culture and the other for direct staining by Gram's Method. The ear swabs were plated directly into two chocolate agar plats, two blood agar plates, one Macconkey agar plate and one phenyl ethyl alcohol agar. One blood agar and chocolate agar plates each ere incubated with about 10% carbon dioxide in a candle jar and others incubated aerobically at 37^oc for 48 hours. The plates were read and the resultant colonies Grams stained were examined under the microscope using the x100 objective. All agar plates that showed no growth after 48 hours of incubation were considered as negative.

V. Identification

All colonies on chocolate agar, blood agar and Macconkey agar plates were identical by colonies appearances. Those on the chocolate and blood agar plates were stained by grams method. Catalase test were performed on all the colonies that were gram positive cocci using Hydrogen peroxide.

The catalase positive colonies were further subjected to coagulase test using human plasma. The slide and tube coagulase test were performed for confirmation of result. Those that were both catalase and coagulase positive were identified as *Staphylococcus aureus* and the catalase negative as *Staphylococcus* species. Identification of *Staphylococcus* species were done by using commercially prepared bacitracin and optochin discs, and the types of haemolysis on the blood agar plate. Those that produced Beta-haemolysis and sensitive to bacitracin were regarded as *Staphylococcus pyogenes*.

The following tests were carried out on the gram's negative organisms. Oxidase test, using 1% solution of tetraethyl P-Phenylene diamine dihydrochloride, motility tests, by the hanging drop technique and biochemical test. Set of one percent sugar solutions of glucose, maltose, lactose, salicin, dulcitol, inositol, were used. Peptone water and glucose phosphate peptone water were also used for indole and methyl red test respectively. Christensen's medium, koser's citrate and potassium cyanide media were also used in the biochemical reactions.

All the sugar solution were prepared in sterile Bijou and McCartney bottle and sterilized by tantalization. The other media used in biochemical reaction were sterilized by autoclaving at 121C^o for 15 minutes. Durham tubes were placed in each of the McCartney bottles containing glucose for the detection of gas production after fermentation.

Typical representative colonies were then inoculated in different peptone water bottles and incubated aerobically at 37^oc for 3-5 hours. A drop from each peptone water culture was aseptically inoculated into sets of sugar solutions with sterile Pasteur pipettes. A straight wire used in inculcating Koser's citrate and potassium cyanide. After overnight incubation at 37^oc, all the sugars were examined for fermentation by observing for colour change of the Andrade's indicator and for gas production. Others were examined for growth in them and the results recorded. Those suspected to be *Haemophilus* species were identified by using x and v factor discs. Those organisms sensitive to both x and v factors were identified as *Haemophilus influenzae* satellitism test were also carried out on the organisms susceptible to the x and v factors.

VI. Some Characterization Test Used For Screening Catalase Test

A drop of three percent hydrogen peroxide (H₂O₂) was added to the centre of clean slide. The test organism was mixed with it using a wire loop. Catalase positive was identified by immediate or active bubbling of gas and negative when there was no release of bubbles or gas.

VII. Coagulase Test

Slide method (To detect bound coagulase). A drop of normal saline was placed at the centre of 2 slides. The test organism was emulsified on each of the drops of saline, after which a drop of plasma was added to one, with the other as control for granular appearance of the organism from true coagulase clumping. Coagulase positive was identified by the clumping of the organism and negative when there was no clumping.

Tube method (To detect free coagulase).

1. ¹/₁₀ dilution of the human plasma was prepared using normal saline (0.2ml of plasma with 1.8ml of normal saline).

2. Three test tubes were taken and labeled test, positives and negative controls.
3. 0.5ml of the diluted plasma was pipette into each tube.
4. 5 drops of the test organism in broth was then added to the tube labeled test, 5 drops to the positive and negative control respectively.
5. They were mixed and incubated at 37^o for 1-6 hours, examined at time interval for 30 minutes after one hour duration. Fibrin clot was recorded as positive and no fibrin clot as negative. That is no free coagulase produced.

Gram Staining Technique

1. A smear was made, dried and fixed with gentle heat.
2. The fixed smear was covered with crystal violet stain for 60 seconds after which it was washed in running tap-water,
3. The smear was again covered with lugol's iodine for 60 seconds and washed in running tap water.
4. Using alcohol, the preparation was decolorized until no more colour appears to ooze out of the smear and washed with water.
5. The smear was counterstained with neutral red stained for one minute, after which it was washed, blot dried with filter paper and examined under the microscope. Gram positive bacteria were purplish or dark blue in colour. Gram negative bacteria were pinkish or reddish in colour.

Urease Activity

A slope of urea agar was heavily inoculated and incubated at 37^o overnight. Red colour in the medium indicated a positive reaction.

Motility Test

A bottle of peptone water was inoculated with the test organism and incubated for about 2-3 hours at 37^o after which was examined for motility by the hanging drop method using a high power dry objective and reduced illumination motile organisms dashed across the field of view.

Satellitism Test

A loopful of the suspected Haemophilus growth in about 2ml sterile peptone water was mixed, and inoculated on a nutrient agar and blood agar plates using a sterile swab stick. A pure culture of staphylococcus aureus was stricked across each of the inoculated plates. Both plates were incubated at increased carbondioxide (5-10%) environment at 37^oc overnight after which the plates were examined for growth and satellite colonies.

Indole Test

The suspected organism was inoculated in peptone water and incubated at 37^oc for 24hours; 1ml of xylene was added followed by addition of 1ml of Ehrlich's reagent after thorough shaking. A pink or red colour indicates the presence of indole.

VIII. Result

Out of the 182 samples of middle ear exudates examined 154 specimens contained bacterial pathogens and 18 specimens were sterile. Table 4.1 shows the total Isolation rate of bacterial pathogens from the 182 samples.

A total of 53 of the isolates were confirmed as Staphylococcus aureus by Gram's Method, catalase and coagulase tests. And 34 of the isolates produced some greenish pigmentation on macConkey agar medium and also gave a fishy odour. They were also found to be motile at 37^oC strongly oxidase positive and were confirmed as Pseudomonas aeruginosa. Of the 154 total isolates, 25 showed some swarming in several cases at 37^oc after 24 hours of incubation on blood agar and where confirmed as proteus mirabilis. Apart from Haemophilus Infuenzae and Streptococcus Pyogenes which were identified, using X and V factors, Satellitism test and bacitracin disc respectively, others such as Escherichia coli were identified biochemically to species level. Of all the six different bacterial pathogen isolated staphylococcus aureus was the most prevalent accounting for 34.42%, followed by Pseudomonas aeruginosa with 22.08% and Proteous mirabilis with 16.23%, Streptococcus pyogenes 13.64%, Escherichia coli 8.44%, while the lowest prevalent rate was recorded in Haemophilus influenza with 5.19%. Total positive sample recoded in female was 81 which is higher than 71 positive samples recorded in male but statistically there was no significant difference (p>0.05). out of 154 positive samples, Children between age group 1 – 3 were statistically significantly affected (p>0.05) with total number of 59(38.31%) positive samples followed by age group 4 – 6 with 47(30.52%), age group 7 – 9 with 30(19.48%) and age group 10 – 12 with 18(11.69%).

Table 1: Distribution of pathogen isolated from samples

Pathogen isolated	Number of sample
Staphilococcus aureus	53
Psudomonas aeruginosa	34
Proteous mirabilis	25
Streptococcus pyogenes	21
Escherichia coli	13
Haemophilus influenza	8
TOTAL	154



Figure 1: Children with ear discharge

Table 2: Age distribution of bacterial isolates

Age group	Number of Bacteria isolated	Percentage of Bacteria Isolated (%)
1 - 3	59	38.31
4 - 6	47	30.52
7 - 9	30	19.48
10 - 12	18	11.69
TOTAL	154	100

Table 3: Sex distribution of bacteria isolates and their percentage

Microbial agents	Male(%)	Female(%)	Total(%)
Staphilococcus aureus	23(32.39)	30(36.14)	53(34.42)
Psudomonas aeruginosa	15(21.13)	19(22.89)	34(22.08)
Proteous mirabilis	14(19.72)	12(14.46)	26(16.23)
Streptococcus pyogenes	10(14.08)	10(12.05)	20(13.64)
Escherichia coli	6(8.45)	7(8.43)	13(8.44)
Haemophilus influenza	3(4.23)	5(6.02)	8(5.19)
TOTAL	71(46.10)	83(53.90)	154(100)

Figure 1: Pie chart showing percentage prevalent rate of microbial agents.

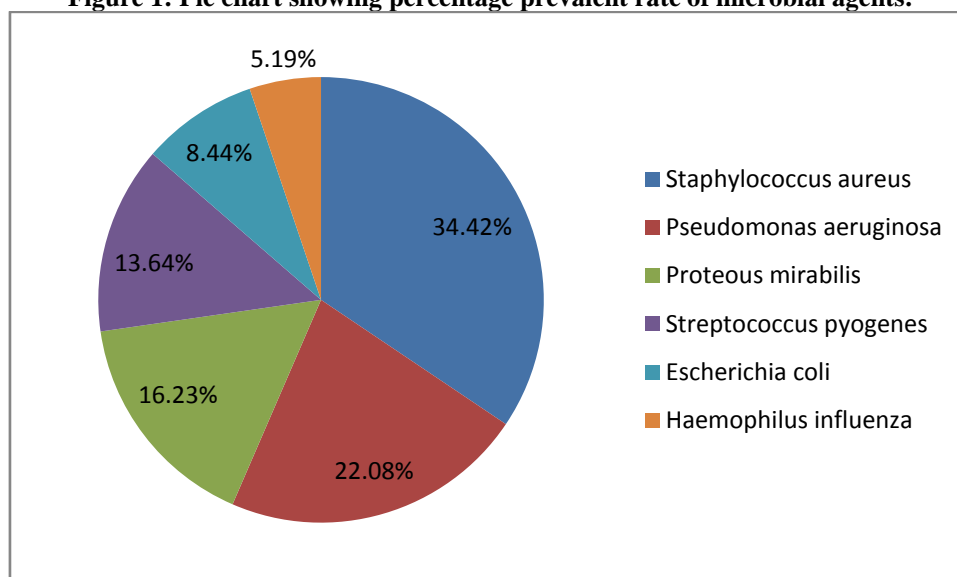


Figure 2: Pie Chart showing the percentage rate of the organisms isolated

IX. Discussion

Out of 154 bacterial pathogens isolated from 182 samples of the middle ear aspirated fluid, *Staphylococcus aureus* was the most frequently isolated (34.42%). The prevalent rate of other pathogens isolated in decreasing frequency were *Pseudomonas aeruginosa* with 22.08% and *Proteus mirabilis* with 16.23%, *Streptococcus pyogenes* 13.64%, *Escherichia coli* 8.44%, while the lowest prevalent rate was recorded in *Haemophilus influenza* with 5.19%, this is contrary to the findings of Philomena et al., 2013 who reported *Pseudomonas aeruginosa* as the most prevalent aetiological agents of otitis media with 33.33% followed by *Staphylococcus aureus* with 23.19%. Generally the findings from this study is higher compare to the findings in United States of America reported by Maharjan et al., 2006 [17]. It has been reported that the prevalence of otitis media is higher in developing countries when compared to developed countries and the possible reason may be due to local customs and beliefs harmful traditional practices, poor care facility and treatment of acute cases by the first contact personel [18]. Anaerobic bacteria, *Mycoplasma pneumonia*, viruses and *Chlamydia trachomatis* have been reported as possible pathogens of the middle ear [19],[20]. Specimens were not processed to recover these agents. All the patients examined in this study had symptoms (fever, irritability, pain, poor feeding) and the majority were between one to nine years (both sexes) with suppurative otitis media. It is obvious from this finding that females (with total percentage isolates of 54.16%) were more susceptible to infection of the middle ear than the males (45.84%) although statistically, there was no significant difference ($p>0.05$), this is in agreement with the research conducted in Kupa medical centre with prevalence rates of 47.1% for males and 52.9% for females [21].

The peak incidence was seen in infants than in children and may possibly be as a result of the fact that the immune system of the infants are still developing. The disease is known to be a childhood disease with high incidence in the first three years of life [21], this is supported by the findings of a higher incidence in children between 1 – 3 and 4-6 years of age in this work.

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