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BACTERIAL AGENTS OF OTITIS MEDIA AND THEIR SENSITIVITY TO SOME ANTIBIOTICS IN AMINU KANO TEACHING HOSPITAL, KANO STATE

***Shamsuddeen, U., Usman A. D., Bukar, A. and Safiya, I. A.**
Department of Biological sciences, Bayero University, Kano Nigeria
*Correspondence author

ABSTRACT
Fifty ear swab samples were examined from pediatric patients attending Aminu Kano Teaching Hospital, Kano. The samples were collected from ENT department, pediatric out patients department (PODP) and General out Patients Department (GOPD). The swabs were tested by culturing for bacterial pathogens, where 47 (94.0%) of the samples yielded growth. The most predominant isolate was Staphylococcus aureus, with a total occurrence of 26 (55.32%) followed by Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae and Streptococcus pneumoniae, with total occurrences of 12 (25.53%), 7 (14.89%), 1(2/13%) and 1 (2.13%) respectively. Based on age group, 0-2yrs age group were more infected (16 infected). Of the two sexes, males were 28 (56%) while females were 22 (44%) and all were within the age range of 0-12 years. Antimicrobial sensitivity test showed that the bacterial isolates were susceptible to Augmentin, Gentamycin, Tetracycline, Amoxicillin and ofloxacin but resistant to Ampicillin, Streptomycin, Cotrimoxazole, cefixime and Cloxacillin.

Key Words: Bacterial agents, otitis media, sensitivity, antibiotics, AKTH.

INTRODUCTION

Otitis media is an inflammatory disease of mucosal lining of the middle ear, Otitis is a Greek word meaning inflammation of the ear while media meaning middle. Bacterial infection of the middle ear normally originates from an upper respiratory tract infection with the bacteria entering the auditory (Eustachian) tube, which is the principal portal of entry of pathogens to the ear (Atlas, 1998). Otitis media simply means an inflammation of the middle ear (the space between the external auditory canal and the inner ear), it can be associated with an infection where it is caused by bacteria that migrate into the middle ear via the Eustachian tube.

Occasionally, otitis media may be caused by fungi (*Aspergillus* or *Candida*) or other pathogens such as herpes virus, in this situation usually, either there is a problem with immune function or there is a hole (perforation) in the ear drum (Mongkolrattanothai *et al,* 2003). Otits, media affects approximately half of all children between the ages of six months and three years and approximately one-third of cases are caused by *streptococcus pneumoniae* (Mackie and Mc cartney 1998).

Over 50 percent of the cases of Otitis media involve *Streptococcus pneumoniae* as the aetiological agent, *Haemophilus infeuenzeae* and *streptococcus pyogenes* are also frequently involved (Atlas, 1998). *Moraxella catarrhalis* has been incriminated in otitis media and may be isolated on occasion from blood culture in patients who are immunocompromised (Mackie and Mc cartney 1998). It is the aim this work to investigate the bacterial agents of otitis media and determine their sensitivity to some antibiotics so that recommendations may be made accordingly.

MATERIALS AND METHODS

Sample Collection

Aminu Kano Teaching Hospital (AKTH) Kano was the area selected for the study because of large number of patients attending the hospital. Pus or purulent discharges from cases of otitis media were collected from patients having the disease using sterile swab sticks (Kumurya *et al*., 2010).

Methodology

After collection of sample, the ear swabs were used to inoculate directly unto chocolate and Mac Conkey agar plates by streaking. The chocolate agar plates were incubated at 5% carbon dioxide in a candle jar and the Mac Conkey plates incubated aerobically at 37^0C for 24hours. After which plates were read and resultant colonies were subcultured on agar slants for subsequent identification.

Identification of bacterial Isolates

Identification and characterization of the bacterial isolates were carried out as described by Cowon and Steel (2002) after they have been examined using such biochemical tests as Grams reaction, Catalase test, Oxidase test, Indole test, Coagulase test, Urease test, Optochin Inhibition and Citrate utilization test.

BIOCHEMICAL CHARACTERIZATION OF ISOLATES

Gram's Staining

A smear was fixed at the centre of a grease-free slide and allowed to dry, then heat fixed. The fixed smear was flooded with crystal violet stain for 30 seconds, and 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 2 minutes, then washed off with clean water. Back of the slide was wiped, and then the glass drained and allowed to air dry, after which microscopy was done (Cheesbrough 2000).

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 organism were emulsified in the hydrogen peroxide. Catalase positive reaction was seen by their immediate production of bubbles (Cheesbrough, 2000).

Coagulase Test A drop of normal saline was put on a slide; a colony of the test organism was emulsified in the saline. A drop of human plasma was added and mixed gently. After about 10 second it was observed for cloting. (Cheesbrough, 2000).

Indole Test
This was carried out according to Cheesbrough, (2000). The test organism was inoculated into Bijou bottle containing 3ml of sterile tryptone water, incubated at 35-37°C for 48hrs. This was followed by the addition of 0.5ml Kovac's reagent. Red color on the surface layer within 10 minutes indicated positive test for indole.

Oxidase Test
Filter paper was soaked with 2 drops of freshly prepared oxidase reagent. Colony of the test organism was smeared on the filter paper using glass rod. Positive oxidase was indicted by the production of a deep purple/blue colour within 10 seconds (Cheesbrough, 2000)

Citrate Utilization Test
This test was done by inoculating the organism into Simon's citrate agar slopes which were then incubated at 37^0C. A change in colour of the medium from green to blue is considered positive (Cheesbrough, 2000)

Urease Production Test
This was carried out by inoculating the urea slopes with colonies of the organism and incubated at 37^0C for 24 hours. A change in colour of the medium from yellow to pink/red was indicative of a positive result (Kanai, 1998)

Optochin Susceptibility Test
Optochin is an antimicrobial available in commercial discs. Optochin disc like other sensitivity discs, was placed on the surface of the agar inoculated with the test organism. The antimicrobial diffuses from the disc into the medium, following overnight incubation at 37^0C the plates were observed for susceptibility of the organism to the optochin disc.

Preparation of Turbidity Standard
One per cent (1% v/v) solution of sulphuric acid was prepared by adding 1ml of concentrated (H_2SO_4) into 99ml of water. One per cent (1% w/v) solution of barium chloride was also prepared by dissolving 0.5g of dehydrated barium chloride in 50ml distilled water. Barium chloride solution (0.6ml) was added to 99.4ml of sulphuric acid solution to yield 1.0% w/v barium sulphate suspension. The turbid solution formed was transferred into a test tube as the standard for comparison (Cheesebrough, 2000).

Standardization of inoculum
Using inoculation loop, enough material from an overnight culture of test organisms (*Staphylococcus aureus, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Streptococcus pneumoneae*) was transferred into a tube containing 2.0 ml normal saline, until the turbidity of the suspension matched the turbidity of the standard (1% barium sulphate) (Cheesebrough 2000).

Sensitivity testing of the isolates to the antibiotics
Two loopfuls of the standard inoculum were evenly streaked on to the plates of nutrient agar in duplicates. Sensitivity discs were placed at intervals on the agar plates after which the plates were incubated at 37^0C for 24 hours and sensitivity patterns to various isolates were observed. Discs that showed greater than 25mm zone diameter of inhibition, the organism was considered sensitive, while those with less than 25mm were considered resistant (Cheesbrough, 2000).

RESULTS
Of the 50 swabs tested 47 (94%) yielded bacterial growth. Of the 47 that yielded growth, 26 (55.32%) yielded *Staphylococcus aureus*, 12 (25.53) yielded *Proteus mirabilis*, *Pseudomonas aeryginosa, Streptococcus pneumoneae* and *Klebsiella pneumoneae* 7(14.49%), 1(2.13%) and 1(2.13%) respectively (Table 2). Table 3 shows the number of bacterial isolates based on age group and their percentage occurences, where the age group 0-2years had the highest number of isolates. Table 4 shows Susceptibility profile (pattern) of the organisms screened..

Table 1: Distribution of subjects according to sex.

SEX	NUMBER OF PATIENTS	PERCENTAGE (%)
Males	28	56
Females	22	44
Total	**50**	**100**

Table 2: Percentage occurrences of the bacterial isolates

ORGANISMS ISOLATED	NUMBER	PERCENTAGE (%)
Staphylococcus aureus	26	55.32
Proteus mirabilis	12	25.53
Pseudomonas aeruginosa	7	14.89
Streptococcus pneumoniae	1	2.13
Klebsiella pneumoniae	1	2.13
Total	**47**	**100**

Table 3: Distribution of bacterial isolates according to age group

AGE	Number of isolates	PERCENTAGE (%)
0 – 2	16	34.04
2 – 4	10	21.28
4 – 6	10	21.28
6 – 8	5	10.64
8 – 10	4	8.51
10 – 12	2	4.26
Total	**47**	**100**

Table 4: Tested Antibiotics

Antibiotics tested	Diameter of of zone of inhibition (mm)
Augmentin	>25
Gentamycin	>25
Tetracycline	>25
Amoxicillin	>25
ofloxacin	>25
Chloramphenicol	>25
Erythromycin	>25
Ampicillin	<25
Streptomycin	<25
cefixime	<25
Cotrimoxazole	<25
Cloxacillin	<25

DISCUSSION

Various studies by different researchers have been carried out, establishing the significance of *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Proteus* species*, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*, *Klebsiella* species and *streptococcus pneumoniae* in the causation of otitis media (Harold and Fancis 1992, Mackie and Mc Cartney, 1998). According to Harold *et al.,* (1992) *Staphylococcus aureus*, Enterobacteria and *Pseudomonas* species are usually associated with contamination in external meatus. The findings of the present study agree with the works of Senuturia *et. al.* (1958), Harold and Francis (1992) and Mackie and McCartney (1998) who isolated similar groups of bacteria from patients with otitis media.

Many antimicrobial agents such as augmentin ampicillin, erythromycin, chloramphenicol, tetracycline and chloramphenicol are normally prescribed for the treatment of bacterial otitis media (Pichichero, 2001).

The sensitivity patterns obtained in this study show resistance to some antimicrobial agents. A high level resistance was observed with ampicillin, cotrimoxazole, cefixime and ciprofloxacin, and sensitivity was seen to augmentin, aentamycin, ofloxacin, amoxicillin, tetracycline, chloramphenicol and erythromycin among the antibiotics.

Resistance shown to antibacterial agents by the bacteria might be due to various reasons. Organisms are innately resistant to certain antibiotic as seen in mycoplasma that lack a cell wall, Mycoplasma tends to be resistant to Penicillin and other drugs that target peptidoglycan. Minor alteration in the target so that it is no longer bound by the drug can cause resistance. Other reasons include over production of target sites, inactivation of the antibiotic, alterations in membrane permeability, spontaneous mutation through DNA transfer all can contribute to the development of resistance to antimicrobial agents as opined by Eugene *et al,* 1998. Contributions of the above reasons or factors might have accounted for the resistance observed in this study

Conclusion and Recommendation

The bacteria associated with otitis media include *Staphylococcus aureus*, by *Proteus mirabilis, Pseudomonas aeruginosa, Streptococcus Pneumoniae* and *Klebsiella Pneumoniae.* They have been found to be sensitive to Gentamycin, Tetracycline, Augmentin, Ofloxacin, Amoxicillin and Erythromycin and Chloramphenical.

It is therefore recommended that culture and sensitivity should be done in order to determine suitable antibacterial agent to which the causative organisms are susceptible. Observation of personal hygiene as well as using clean objects in cleaning the ear is also recommended since unclean objects will serve as a of entry of microorganism into the ear.

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