Antibiotic Susceptibility of Bacterial Pathogens Associated With Otitis Media Among Patients in Specialist Hospital Sokoto

[1]Abdullahi H., [2]Okwor, I.E.A.J. and [3]Manga, S.B.
[1]Pathology Department, Specialist Hospital Sokoto
[2]Department of Medical microbiology, Federal College of Veterinary and medical Laboratory Technology, Jos.
[3]Department of Microbiology, Usmanu Danfodiyo University, Sokoto

Abstract: A total of 120 ear swab samples were examined from patients of different age groups attending Specialist Hospital, Sokoto, with ear infection. The swab samples were cultured on Chocolate agar, Blood agar and MacConkey agar media. Identification of the isolated bacteria was carried out using standard biochemical tests. The results show an overall infection rate of 76.68% (92 out of 120).And the age group mostly affected is the 0-5 years with infection rate of 54.17%. The results of the identification showed the following bacteria in decreasing order of prevalence as *Staphylococcus aureus* 30 (30.61%), *Pseudomonas aeruginosa* 17 (17.35%), *Proteus mirabilis* 16 (16.33%), *Citrobacter freundii* 14 (14.29%), *Escherichia coli* 6 (6.12%), *Serratia mercescens* 6 (6.12%), *Staphylococcus albus* 5(5.10%) and *Enterobacter species* 4(4.08%). Antibiotic Sensitivity test was carried out. The results indicated that Ciprofloxacin (10µg), Tarivid (10µg) and Levofloxacin (5µg) are the best antibiotics with mean zones of inhibitions of 10.70mm, 12.25mm and 12.25mm respectively, whereas Ampicillin (25µg)(4.00mm) Cotrimoxazole (25µg) (0.00mm) and Tetracycline (10µg) (1.63mm) did not show promising result.

INTRODUCTION

Otitis media also known as glue ear is an inflammation of the middle ear (Wikipedia 2006). Different ear conditions caused by viruses, bacteria, Fungi or the presence of exudative ear lesion are collectively called Otitis media (TPSA, 2006; Mongkolrattanothai *et al*; 2003). However bacteria are the major aetiological agents of Otitis media (Kathy, 2005)Otitis media is a common infection of children; particularly those aged 6 months to 3 years. Complications and sequelae of otitis media persist in some individuals into the adult years and they include loss of hearing, acquired Cholesteatoma, Labyrinthitis, Mastoiditis, Loss of verbal intelligence, vertigo, ear ache, anorexia, vomiting and irritability (Bluestone and Klein, 1983; Micheal, 2000).In

Nigeria, there have been cases of Otitis media and their identification in some part of the country (Okwori *et al.*, 2005). There is however the need to investigate and document cases in the north, particularly Sokoto and its environment

With the exception of *Moraxella* species the list of bacteria causing Otitis media has not appreciably changed for many years and appears to be similar worldwide (Katzenmeyer *et al.*, 1999). In treating Otitis media infection, it is important to differentiate between different forms of Otitis media because it affects treatment options. Not all forms of Otitis media need antibiotics treatment (Barbara and Joel, 2005). An estimated 3-4 billion dollars is spent each year in united state of America on care of patients with acute Otitis media and related complications (Kathy, 2005). Although few cases have been documented in Nigeria, the economic loses in terms of cost of medication and the morbidity associated with it has never been estimated. The present study was undertaken to establish bacterial causative agents of Otitis media in Sokoto as well as to determine the antibiogram pattern of the isolates.

MATERIALS AND METHODS

Purulent materials from the cases of Otitis media were collected from patients suffering from the infection using sterile swab sticks. The patients had not received antibacterial medication during the preceding week. The eardrum and the surrounding ear canal were disinfected with 70% alcohol solution. Normal saline (0.85%) was used to irrigate the canal and to remove any remaining alcohol, after which the saline itself was subsequently cleared out with sterile absorbent cotton wool. The specimens were obtained by insertion and gentle rotation of sterile swabs in the middle ear. Two swab samples were collected for each patient sample. A total of 120 samples were collected, 67 from females and 53 from male patients.

Isolation of Bacteria

Each of the swabs collected was inoculated aseptically in replicates onto Chocolate agar, Blood agar and MacConkey agar media, while the other swabs were processed and examined by Gram's Method of staining. One of chocolate agar culture and blood agar culture were incubated in 10% CO_2 in a candle jar while the other Blood agar, Chocolate agar and MacConkey agar plates were incubated aerobically at 37°C for 24 hours. The resultant colonies were identified from their colonial appearances and Gram staining. Plates showing no growth after 48 hours were discarded and were reported as no bacterial growth. The same procedures were repeated for the other samples (Cowan and Steel, 2002). Further identification and characterization of the bacterial isolates were carried out as described by Cowan and Steel (2002) using biochemical tests including Catalase, Coagulase, Motility, Oxidase, Indole, Citrate utilization, Urease tests and Growth on TSI

Antibiotic Sensitivity Testing

The disk-diffusion method was used. The following antibiotic sensitivity discs were used. Ampicilline (10g), Cotrimoxazole (1.25g), Cephalexin (30g), Ciprofloxacin (10g), Tarivid (10g), Penicillin, Tetracycline(30g) and Levofloxacin (5g). Culture containing appropriate concentration of test organisms (10^6 cfu/ml) corresponding to 0.5 opacity standard was streaked by Stokes method (Duguid et al 1978 and Rotimi et al 1994), onto nutrient agar medium. These standard antibiotic disks were then placed carefully but firmly by the help of a scalpel onto the surface of the Nutrient Agar medium. The plates were incubated for 24 hours at 37°C. Zones of inhibition were seen and recorded as areas of clearing around the antibiotic disk in which the bacteria failed to grow. The zones are measured in millimeter (Gould and Bowie, 1952).

RESULTS AND DISCUSSION

The results on the occurrence of Otitis media infection among the age and sex groups of patients attending Specialist Hospital Sokoto is presented on Table 1. The overall infection rate for both sexes 76.68% i.e. 92 infected out of 120 patients. Out of the 67 females and 53 male patients examined, infection rate in female is 71.64% while that of the male is 83.01%. Among the age groups, 0-5 year's group was heavily infected (54.17%) as compared to other age groups. The age groups 6-10 and 16-20 year's had 6.67% infection rate each. The least infected age group was 11-15 years (4.17%). However, statistical analysis using't' test did not show any significant difference in the number of infected subject between male and female. The organism isolated and

identified by the biochemical methods (Table 2) includes; *Staphylococcus aureus* 30(30.61%), *Pseudomonas aeruginosa 17* (17.35%), *Proteus mirabilis 16* (16.33%), *Citrobacter freundii* 14 (14.29%), *Escherichia coli* and *Serratia mercescens* 6(6.12%) each, *Staphylococcus albus* 5(5.10%) and *Enterobacter Species* 4(4.08%). All the pathogens isolated were sensitive to Tarivid and levofloxacin (Table 3). The most sensitive bacteria (zone of inhibition greater than 12mm) to these two drugs are *Staphylococcus aureus*, *Pseudomonas aeruginosa, Proteus mirabilis* and *Citrobacter freundii*. A great number were sensitive to Ciprofloxacin and Cephalexin. The most resisted antibiotics are Ampicillin, Tetracycline and Cotrimoxazole (total resistance or lack of activity was recorded with Cotrimoxazole). Analysis of variance conducted on the zones of inhibition for the various antibiotics using the completely randomized design at 5% level of significance showed that significant difference exists. Post experimental contrast using Least significant difference test (LSD=3.39) show that Tarivid and Levofloxacin had higher zones of inhibition as compared to other antibiotics except Ciprofloxacin. Closely following these drugs in activity are the Cephalexin and Penicillin.

From the findings of this research, the age group of 0-5 years was heavily infected (70.65%) as compared with the other age groups. This agrees with reports made by NIDCD (2002); Kathy (2005) and Wikipedia (2006) that the infection is more common in children than adult because their immune systems are still developing and their Eustachian tubes are shorter. And from the eight different pathogens isolated, *Staphylococcus aureus* was the most prevalent accounting for 30.61 and *Enterobacter Species* was the least having occurrence rate of only 4.08%. The present finding agrees with that of Ogisi and Osammor (1992); Oni *et al.*, (2002); and Okwori *et al.,* (2005) who reported *Pseudomonas aeruginosa, Proteus mirabilis* and *Staphylococcus aureus* as being the commonest cause of Otitis media. Similarly it was reported that Otitis media is also caused by *Staphylococcus aureus, some* gram-negative bacteria (*Proteus mirabilis* and *Escherichia coli)* (AOMC, 2006; Block 1997; Jacob *et al.*, 1998; Gilson and Kurpep, 2004).However, the present findings differs from reports of AOMC (2006); and Wikipedia (2006) that *Streptococus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis* are the commonest bacterial pathogens that causes Otitis media infection. These differences could be attributed to different types of Otitis media infection being acute, serious or chronic. *Staphylococcus aureus* is well documented as the causative agent of acute Otitis Media (AOM) and Chronic Otitis media, *Streptococcus pyogenes, Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis* are more associated with AOM (Rosenfield *et al.*, 1994; Bluestone and Kessener, 1994 and AOMC, 2006). *Pseudomonas aeruginosa* and Coliforms are prominent gram-negative bacteria documented as causative agents of chronic otitis media (Mackie, 1998).

Although there have been reports on drug-resistance of bacterial pathogens causing otitis media, many antimicrobial agents such as augmetin, amoxicillin, ampicillin, septrin, gentamycin, cephalexin, tarivid, floxapen and rifampicin amoung others are normally prescribed for the treatment of bacterial causing otitis media infection (Thurburn *et al.*, 2003; Tomson *and* Torlow, 2004). In the present research, the pathogens are highly sensitive to tarivid, levofloxacin, Cephalexin and Ciprofloxacin, but some were less sensitive to Ampicillin, Tetracyclin and Penicillin G while others are completely resistant to them. All the pathogens were completely resistant to Cotrimoxazole. Alot of factors

have been attributed to the resistance of this pathogens, including genetic mutations, bacteria producing enzymes that destroy or in activate antimicrobials, bacteria changing to other metabolic systems not affected by the antimicrobial being used and bacteria altering the permeability of their cell membrane, making it difficult for antimicrobials to enter (Cheesbrough, 2000).

CONCLUSION

From this study, an overall infection rate of 76.68% from males and females of different age groups has been determined. The age group heavily affected is the 0-5 years with an infection rate of 54.17%. The most common bacterial pathogens isolated in decreasing order are *Staphylococcus aureus*(30), *Pseudomonas aeruginosa*(17), *Proteus mirabilis*(16), *Citrobacter freundii*(14), *Escherichia coli*(6) , *Serratia mercescens*(6), *Staphylococcus albus*(5) and *Enterobacter* species(4). Similarly the most effective antibiotics to most of these bacteria are: Tarivid, Levofloxacin, Cephalexin and Ciprofloxacin.

Table 1: Occurrence of Otitis media infection among the age and sex groups of patients

Age group (yrs)	Male Patients			Female Patients			Both Sexes (Total)		
	No. examined (n)	No. infected (x)	% infection. Rate (x/n x 100)	No. examined (n)	No. infected (x)	% infec. Rate (x/n x 100)	No. examined (N)	No. infected (X)	% infec. Rate (X/Nx100)
0 – 5	39.00	34.00	64.15	42	31	46.27	81.00	65.00	54.17
6 – 10	3.00	2.00	3.77	8	6	8.96	11.00	8.00	6.67
11 – 15	0.00	0.00	0.00	6	5	7.46	6.00	5.00	4.17
16 - 20	3.00	3.00	5.66	7	5	7.46	10.00	8.00	6.67
> 20	8.00	5.00	9.43	4	1	1.49	12.00	6.00	5.00
Total	53.00	44.00	83.01	67	48	71.64	120.00	92.00	76.68

Table 2: Results of Antibiotics sensitivity test

Bacteria Isolated	Number and (% isolated)	Zone of inhibition in mm/ Antibiotics							
		Amp	Cot	Cep	Cip	Ofl	Pen	Tcn	Lev
Staphylococcus aureus	30(30.61)	12	R	25	30	30	10	9	30
Pseudomonas aeruginosa	17(17.35)	R	R	4	15	17	R	R	17
Proteus mirabilis	16(16.33)	16	R	8	7	16	16	R	16
Citrobacter freundii	14(14.29)	R	R	14	14	14	R	R	14
Escherichia coli	6(6.12)	R	R	6	6	6	2	R	6
Serratia marcescens	6(6.12)	4	R	2	6	6	5	4	6
S. albus	5(5.10)	R	R	5	5	5	2	R	5
Enterobacter species	4(4.08)	R	R	4	4	4	R	R	4
Total	98(100)	32	R	68	87	98	35	13	98

Key:
Amp= Ampicillin, Cot= Cotrimoxazole, Cep= Cephalexin, Cip= Ciprofloxacin Ofl= Tarivid, Pen = Penicillin G, Tcn= Tetracycline and Lev= Levofloxacin,
R= zero inhibition zone, ( ) = Percentage

REFERENCES

Acute Otitis Media in Children, AOMC (2006). Acute otitis media: Current Concepts*www.kidshealth.org/parent/infections /ear/otitis_media.html.*1-2.

Barbara P.H. and Joel K. (2005). Middle Ear Infections *Nemour Foundation.* 1-6.

Block S.L. (1997). Causative pathogens, Antibiotic resistant and therapeutic considerations in acute Otitis Media. *Pediatric Infectious Disease Journal* **16**:499-56.

Bluestone C.D. and Kessener (1994). Epidemiology and Pathogenesis of Chronic Suppurative Otitis Media. *Pediatric otoryhinolarygol.* **42(3)**: 207-223.

Cheesbrough (2000): *District Laboratory Practical In Tropical Countries Part 2.* P.2-19.

Cowan S.T. and Steel K. J. (2002). Manual for the Identification of Medical Bacteria. 2nd Edition *Cambridge University Press.* P.51-120.

Gilson and Kurpep, P. (2004). Otitis media with effusion: Is medical Management an option. *J.R. Cul. Gen. Pract.* **39(326):** 377-82.

Gould J.C. and Bowie J.H. (1952). The determination of bacterial Sensitivity to antibiotics. *Edinburgh Medical Journal* **59:**178.

Jacobs M.R., Dagan R., Appelbaum P.C. and Burch D.J. (1998). Prevalence of antimicrobial Resistant Pathogens in middle ear Fluid: Multinational study of 917 Children with acute otitis media. *Antimicrob. Agents Chemotherapy* **42**: 589-595.

Kathy A.C. (2005). Otitis media. *www.emedicine.com/EMERG/ Otitis Media 351.html.* 2-11.

Michael E.P. (2000). Acute Otitis media: Part I. Improving Diagnostic Accuracy. *American Accademy of Family Physicians, AAFP.* **61(7)**: 2051-6.

Mongkolrattanothai K., Oram R., Red Leaf M., Bova J. and Englung J.A. (2003). "Tuberculosis Otitis Media with Mastoiditis and Central Nervous System Involvement". *Pediatric Infectious Disease Journal* 22(5): 453-6.

National Institute On Deafness and other Communication Disorders, NIDCD (2002). Otitis Media (Ear Infection). *www.nidcd.nih.gov/health/hearing/otitis media.asp.* 4-7.

Ogisi F.A. and Osammor J.Y.(1992). Bacteriology of Chronic Otitis Media in Benin. *Nigerian Medical Journal* **12:**187-90.

Okwori A.E.J., Olabode A.O. and Chukwu O.O.C. (2005). Spectrum of Bacterial Isolates from Cases of Otitis Media among Children in Vom and Jos, Plateau State. *Nigerian Journal of Microbiology* **19(1-2)**: 437-443.

Oni A.A., Nwaorgu O. G.B., Bakare R.A., Ogunkunle M.O. and Toki R.A., (2002). The discharging ears in Adults in Ibadan, Nigeria: Causative agents and antimicrobial Sensitivity Pattern. *African Journal Cliical. Experimental Microbiology* **3(1)**: 1-5.

Rosenfield R.M., Vetrees J.E., Carr J. *et al.,* (1994). Clinical Efficacy of Antimicrobial Drugs for Acute Otitis Media: Meta Analysis of 5400 Children from Thirty Three Randomized Trials. *Journal Pediatrics* 124(3): 355-67.

Texas Pediatric Surgical Associates, TPSA (2006). Chronic and Recurrent Otitis Media (Ear Infections). **832:**325-7234.

Thurburn C.K., Knutt S.J. and Edwards D.J. (2003). Invitro Activities of Oral Beta Lactamase that causes Achieved in Human against Penicillin Susceptible and Resistant *pneumococci* and Potentials to Select Resistant. *Antimicrobial Agents Chemotherapy* 42(8): 1973-1978.

Tomson and Torlow M. (2004). Otitis Media Pathogenesis and Medical Sequence. *Ear, Nose and Throat Journal* **79 (Suppl. 6)**: 3-6.

Wikipedia, the free encyclopedia (2006). Otitis Media. *"Www.en.wikipedia.org/wiki/otitis_media."*