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Isolation and antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* in cases of Otitis media among patients attending Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.

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Abstract

Patients with Otitis Media were evaluated for bacterial pathogens and their susceptibility status to available anti-bacterial drugs. A total of 110 ear swab samples from cases of Otitis media among patients attending Ahmadu Bello University Teaching Hospital, Zaria were collected between February to July 2011 and cultured on blood chocolate and MacConkey agar, incubated at 37 °C both aerobically and anaerobically. The isolates were then subjected to the standard bacteriological techniques and sensitivity test using Kirby Bauer Disc Diffusion method. Out of the 110 samples examined, 30 (27.3%) were *Pseudomonas aeruginosa*, 25 (22.7%) *Staphylococcus aureus*, 10 (9.0%) *Streptococcus pyogenes*, 18 (16.3%), *Proteus* specie and 12 (10.9%) *Candida* specie, while 15 (13.6%) showed no growth at all and the difference between the various pathogens was not significant ($P > 0.05$). Patients of age group between 0-5 years are most susceptible to Otitis media than any other age group and the difference in the prevalence of the infection in relation to age was not statistically significant ($P > 0.05$). Otitis Media conditions were higher in the female (56.7%) than the male patients (43.3%). Amikacin was found to be the most effective drug amongst other antimicrobials in the treatment of Otitis media caused by *Pseudomonas aeruginosa*, but the difference between the various antibiotics used was not statistical significant ($P > 0.05$). This study had showed that *Pseudomonas aeruginosa* to be the pathogen most incriminated in the causation of Otitis media. Therefore, it is essential that all stakeholders both in primary, secondary and tertiary healthcare facilities to support and educate the general public on various preventive measures against Otitis media infection.

Keywords: Antibiotics, Eustachian tube, Otitis media, *Pseudomonas aeruginosa*, Susceptibility, Zaria.

1. Introduction

Otitis media (OM) is a prevailing and notorious infection in developing and under developing countries causing serious damage and threatening complications. Otitis media is an inflammation of the middle ear or a middle ear infection, usually caused by bacteria, that occurs when fluid buildup behind an eardrum. Occasionally, Otitis media may be caused by fungi, viruses, *mycoplasma pneumoniae* and *Clamidia trachomatis* [1]. It occurs in the area between the tympanic membrane and the inner ear, including a duct known as the Eustachian tube. It is one of the two categories of the ear inflammation that can underlie what is called an earaches, the other being otitis external. Disease other than ear infection can also cause ear pain, including cancers of any structure that shares nerves supply with the ear and shingle which can lead to herpeszoster oticus. The health-economic burden of this disorder is severe especially in Africa and other developing nations where the disease prevalence could be as high as 11% with severe economic implications [2-5].

Otitis media has been classified into three main types such as Acute Otitis Media (AOM), Otitis media with Effusion (OME) and Chronic Otitis Media with Effusion (COME) [6]. The four main causes of otitis media are allergy, infection, blockage of the Eustachian tube and nutritional deficiency. *Pseudomonas aeruginosa* is an aerobic, motile, gram- negative rod and is a leading cause of opportunistic nosocomial infections. It is responsible for 10% of all

hospital-acquired infections [7]. It has been implicated in diverse infections such as pneumonia, urinary-tract infection, skin and soft-tissue infections, in severe burns and in Infections among immunocompromised individuals. Infections caused by *P. aeruginosa* are often severe, life-threatening and are difficult to treat because of limited susceptibility to antimicrobial agents and high frequency of emergence of antibiotic resistance during therapy [8].

Antibiotics remain to be the basis for treating Otitis media and its complications, but the growing bacterial resistance to antibiotics has altered the clinical picture of Otitis media and its complications. Bacterial resistance to antibiotics may emerge and contribute to the latent mastoiditis with subtle local and general symptoms and potentially disastrous consequences [9]. *Pseudomonas aeruginosa* and other *Pseudomonad's* are resistant to many antimicrobial agents and therefore become dominant and important when more susceptible bacteria of the normal flora are suppressed [10]. A number of studies have reported on the prevalence of Otitis media [11-12]. However, majority of these studies have focused on clinical diagnosis with little or no report on bacteriological etiology of Otitis media. There exists a dearth of information on a study carried out in the study area on the prevalence of Otitis media incorporating bacteriological and antimicrobial resistance. Therefore, the present study was undertaken to isolate and identify the bacteriological pathogens of Otitis media as well as to determine the antibiotic susceptibility patterns of pathogenic *P. aeruginosa* isolated from various clinical samples from patients attending Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.

2. Materials and methods

2.1. The Study Area

Zaria is a city in Kaduna State in northern Nigeria as well as a Local Government Area (formerly known as Zazzau), it was one of the original seven Hausa city states. It is also home to Ahmadu Bello University, the largest university in Nigeria and second largest university in African continent [13]. Ahmadu Bello University Teaching Hospital, Zaria is a tertiary hospital with referral status and serves the many private hospitals and Government Specialist Hospitals in the State and neighboring States.

Zaria city possesses a tropical continental climate with pronounced dry season lasting up to seven months (October-May). The rainy season lasts from May to September/October. It covers an area extending between latitude 11° 04'N and latitude 11° 067'N as well as between longitude 7° 42'E and 7° 00'E; and has a population of 408,198 [14], with majority of its population using farming as

a source of income. Major crops produce are; Guinea corn, Cotton, Groundnuts and Tobacco [15]. Some few people are artisans; from traditional crafts like leather work, dyeing and cap making, to tinkers and furniture makers [16].

2.2. Sample Collection

110 ear swab samples from cases of Otitis media were collected between February to July 2011 from patients attending Ahmadu Bello University Teaching Hospital, Zaria. Samples of both unilateral and bilateral samples ear swabs were collected using sterile swab sticks which were properly labeled for each patient and brought to the Medical Microbiology Laboratory for analysis within 4-6 hours after collection.

2.3. Isolation of Bacteria

The samples were inoculated onto blood chocolate and MacConkey agar plates. All plates were incubated both aerobically and anaerobically at 37 °C overnight. Emergent colonies were identified according to standard bacteriological methods as described by [17]. The Biochemical tests that were carried out include; gram stain, catalase, oxidase, urease, methyl red test, coagulase, citrate utilization and indole production.

2.4. Antimicrobial Susceptibility Test (Kirby Bauer Disk Diffusion Method)

All detected *P. aeruginosa* isolates were tested for antimicrobial susceptibility test by the standard Kirby-Bauer disc diffusion method according to [18]. The test organism was picked up with a sterile loop, suspended in peptone water and incubated at 37 °C for 2 hours. The turbidity of the suspension was adjusted to 0.5 McFarland's standard (1.5×10^8 CFU/mL). It was then spread on the surface of Mueller-Hinton agar (MHA) plate using sterile cotton swab.

A total of 9 antibacterial agents in the following concentrations: Amikacin (30 µg), Ampicillin (10 µg), Ceftazidime (30 µg), Ceftriazone (30 µg), Ciprofloxacin (10 µg), Erythromycin (15 µg), Gentamycin (10 µg), Imipenem (10 µg), Streptomycin (30 µg) were used at to perform disc susceptibilities test on the isolated pathogens. The zones of inhibition were measured and interpreted according to Clinical and Laboratory Standards Institute guidelines [19].

2.5. Statistical Analysis

Data were analyzed using Chi Square (X^2) test using the statistical software SPSS Version 19. P value of less than 0.05 was considered significant.

Table 1: Prevalence of various isolates from Patients with Otitis media attending Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.

Organisms Isolated	No. positive	Prevalence (%)
<i>Pseudomonas aeruginosa</i>	30	27.3
<i>Staphylococcus aureus</i>	25	22.7
<i>Streptococcus pyogenes</i>	10	9.0
<i>Proteus specie</i>	18	16.3
<i>Candida specie</i>	12	10.9
Number showing no growth	15	13.6
Total	110	100

2.6. Ethical Consideration

Permission to carry out this study was granted by Ahmadu Bello University (ABU) Teaching Hospital Management

and informed consent was obtained from the parents/guardians of the participating patients prior to sample collections.

Table 2: Distribution of *Pseudomonas aeruginosa* in relation to age groups among Patients attending Ahmadu Bello University, Zaria Teaching Hospital, Nigeria.

Age group (Years)	No. of samples	No. of positive samples	No. of negative samples	Prevalence (%)
0-5	61	19	42	63.3
6-10	14	7	12	23.3
11-15	5	4	2	13.4
>16	6	0	7	0.0
Total	110	30	80	100

Table 3: Antimicrobial susceptibility (Sensitivity) pattern of *Pseudomonas aeruginosa* from Patients with Otitis media attending Ahmadu Bello University, Zaria Teaching Hospital, Nigeria.

Antibiotics	No. of isolates sensitive	No. of isolates resistant	Sensitivity of drug (%)
Amikacin	29	1	96.7
Ceftazidime	28	2	93.3
Ceftriaxone	1	29	3.3
Ciprofloxacin	26	4	86.7
Erythromycin	2	28	6.7
Gentamycin	24	6	80.0
Imipenem	22	8	73.3
Streptomycin	9	21	30.0
Ampicillin	0	30	0.0

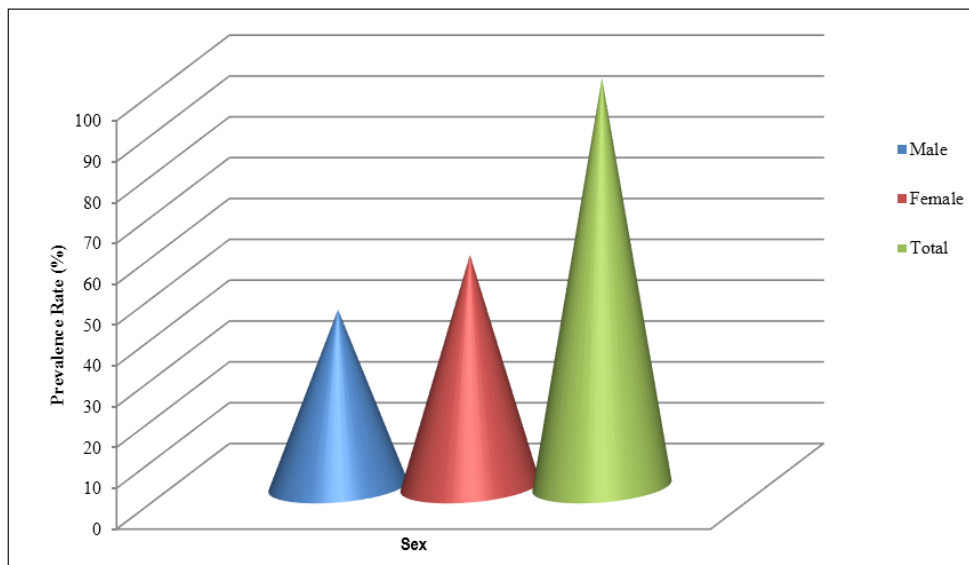


Fig 1: Prevalence of *Pseudomonas aeruginosa* in relation to gender of Patients with Otitis media attending Ahmadu Bello University, Zaria Teaching Hospital, Nigeria.

3. Results

Organism isolated were *Pseudomonas aeruginosa* (27.3%), *Staphylococcus aureus* (22.7%), *Streptococcus pyogenes* (9.0%), *Proteus specie* (16.3%), *Candida specie* (10.9%), while (13.6%) shows no growth at all (Table 1). There was no significant difference between the various species isolated ($P>0.05$). Since the work was primarily aimed at isolation of *Pseudomonas aeruginosa* from cases of Otitis media, those positive for *Pseudomonas aeruginosa* were

further classified based on age group and gender. Based on age group, patients within the age group 0-5 years had the highest prevalence (63.3%) followed by age group between 6-10 years (23.3%), while age group between 11-5 years had the least prevalence of 13.3% (Table 2). However, the difference in the prevalence of *P. aeruginosa* in relation to various age groups was not significant ($P>0.05$). The result in fig. 1 showed that Otitis media conditions were higher in the female (56.7%) than the male patients (43.3%), but this

was not found to be significant ($P>0.05$). Antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* against some selected antibiotics is indicated in table 3 and the result showed that 29 (96.7%) were sensitive to Amikacin, 28 (93.3%) were sensitive to Ceftazidime, 26 (86.7%) were sensitive to ciprofloxacin, 24 (80.0%) were sensitive to Gentamycin, 22 (73.3%) were sensitive to Imipenem, 9 (6.7%) sensitive to Erythromycin, 1 (3.3%) sensitive to Ceftriaxone and none was sensitive to Ampicillin, and the difference between the various antibiotics used was not statistically significant ($P>0.05$).

4. Discussion

Otitis media is frequently encountered in tropical and subtropical areas [20]. This study revealed that Otitis media was found in 27.3% of cases from total number of 110 patients attending Ahmadu University Teaching Hospital, Zaria, Nigeria. It should be noted that bacterial agents of Otitis media were only considered in this study where culture method was used as a means of isolation of the agents. Other agents of disease like viruses and fungi also cause Otitis media. *Pseudomonas aeruginosa* is the most common non-fermenting bacterium specie isolated from clinical samples in the present study and presents a serious therapeutic challenge for the treatment of both community-acquired nosocomial infections.

The preponderance of *P. aeruginosa* observed in this study is in agreement with the previous studies in Nigeria [20-26] and elsewhere such as in Karachi [27], Basrah, Iraq [28], Rawalpindi [29], South India [30] among others but differs from studies in other areas where *Streptococcus pneumoniae*, *Haemophilus influenza*, and *Moraxella catarrhalis* predominate [31, 32] as well the higher prevalence of *Staphylococcus aureus* in Jordan [33] and in north India [34]. Moreover, the relatively high prevalence rate of 27.3% of *P. aeruginosa* isolates obtained in this study is in close conformity with the prevalence rate of 20.0% and 32.1%, 37.5% reported by [35, 36] and [26] in Gujarat, India, respectively. Lower prevalence of 2.1% was obtained by [37] in Northeastern Nigeria, 9.7% by [30] in South Odisha, India, 9.3% was reported by [38] in Andhra Pradesh, India. The varied prevalence rates of *P. aeruginosa* from different places by various researchers may be attributed to the variation in microorganisms in different regions, type of clinical specimen received for examination, studied population, type of hospitals as well as geographical locations. A similar view shared by [30].

It was observed in this study that children between the age group 0-5 years are more prone to Otitis media than any age group. This is result in agreement with others [24, 25]. This may be due to their poor personal hygiene in addition to other environmental factors. Moreover, this trend could well be explained to the fact that children at this age had shorter and more horizontal position of their Eustachian tube, lower immunity of children compared to their adult counterpart; as well as due to the fact that bacterial pathogens adheres well to epithelial cells of children than adults [39, 40, 23]. According to [27] Otitis media (OM) affects about 2/3 of youngsters at least once before they reach their second birthday. Another evidence is that children have poor immune response as they are in their immunological developmental stage; hence they have not developed the

same resistance to viruses and bacteria as their adult counterpart. [41] Also proved that breast feeding for the first twelve month of life is associated with a reduction in the number and duration of all Otitis media infections. Age group greater than 16 were found with no prevalence of *Pseudomonas aeruginosa* at all due to the fact that at this age their immune system adequately function well which enable them to overcome any foreign antigen that might get into the body and causes the stimulation of antibody to each specific antigen [41]. It has been reported that exposure to smoke, crowded living conditions and low socio-economic class are among the risk factor of Otitis media [42].

Although not significant, the higher prevalence of Otitis media among female patients observed in this study is in conformity to the findings of [24, 34, 42]; but in disagreement with the reports of many researchers such as [26, 33, 43, 44] among others. The reason for this disparity in the prevalence of Otitis media in relation to gender is incomprehensible.

In this study low sensitivity of Ampicillin against *P. aeruginosa* may be attributable to many factors like their relatively low costs and their availability leading to frequent misuse. This correlates with the findings of some researchers in the region but different settings [25, 44, 45, 46]. Similarly, the poor activities of Ampicillin were also reported in Basra, Iraq [28] and in Tanzania [47]. Moreover, the high resistance of *P. aeruginosa* isolates observed in the present study is not surprising because prescriptions of antibiotics without laboratory guidance as well as over the counter sales of antibiotics without prescription is common in Nigerian setting and have been suggested as possible reasons for increase resistance observed in the country [48,49]. Contrarily, the resistance of these organisms was at variance with the results of [50-52], who reported that these organisms were moderately susceptible to commonly used antibiotics. However, antimicrobial susceptibility pattern showed Amikacin to be very effective against *Pseudomonas aeruginosa* in this study possibly due to its less vulnerability to misuse in the community.

5. Conclusions

The result of this study had thus, revealed that *Pseudomonas aeruginosa* was found to be the most prevalent bacterial pathogen causing Otitis media (ear infection). It was observed from this study that Amikacin was the most suitable drug in the treatment of Otitis media caused by *Pseudomonas aeruginosa*. Moreso, the resistance against Ampicillin, Ceftriaxone and Erythromycin were found to be very high as observed from the result. This emergence of multidrug resistance calls for judicious use of antibiotics so as to prevent the possible development of further resistance to pathogenic agents for Otitis media. Furthermore, drugs such as Ceftazidime and Ciprofloxacin should be considered as reserve drugs for the treatment of severe nosocomial *P. aeruginosa* infections.

Due to the debilitating effect of the disease, it is therefore essential that all stakeholders both in primary, secondary and tertiary healthcare facilities to support and educate the general public on various preventive measures against Otitis media infection. The Federal Government should support the national and state agencies for the control of Otitis media infection, such as National Institution on

Deafness and other communicable Disorders (NIDCD), World Health Organization (WHO), Centre for Disease Control (CDC) and other Non-Governmental Organizations, so as to create more awareness to the general public on prevention and control of Otitis media. Breast feeding mothers should always avoid giving their babies breast milk or bottle feeding while the babies are horizontally supine. Vaccines should be given to the children at the appropriate time especially vaccine against causative agent of Otitis media infections like anti-*Haemophilus influenzae* vaccine and patient should always rush to the nearest hospital when any sign or symptom of Otitis media is noticed.

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