

Multi-Drug Resistant Acute Otitis Media amongst Children Attending Out-Patient Clinic in Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka, South-East Nigeria

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Abstract

Objective: The study assessed microbial etiology and multi-drug resistant profile of the pathogens isolated from children diagnosed of acute otitis media (AOM) at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka. **Method:** Ear swab samples were collected aseptically from 40 children that visited the children out-patient clinic of the hospital. The samples were cultured within 20 minutes of collection and the isolates identified microbiologically. Antimicrobial susceptibility testing was done by Kirby-Bauer's disc diffusion method. **Results:** A total of 2025 children visited the hospital during the study period and 40 (1.98%) had clinical diagnosis of acute otitis media. Ten (25%) of the 40 samples did not yield any microbial growth. Children aged ≤one year had the highest prevalence of AOM (55%). *Staphylococcus aureus* (34.9%) was the predominant bacteria isolate followed by *Pseudomonas aeruginosa* (30.2%) while *Candida krusei* (14%) was the predominant fungi isolate. All the bacterial isolates were resistant to ampicillin and co-amoxiclav. *Staphylococcus aureus* isolates were marginally susceptible to gentamicin (60%) and sparfloxacin (73.3%). *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* were resistant to ceftriaxone, ciprofloxacin, streptomycin, cefuroxime, erythromycin and azithromycin. Nystatin and voriconazole had comparable effect (60%) against the fungal isolates and flucona-

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zole was ineffective. Conclusion: The incidence of multiple antibiotic resistant bacteria associated with acute otitis media in the pediatric patient was very high. Empiric treatment of the infection in the community should be discouraged. AOM preventive strategies should be targeted on the very young children to reduce the risk of recurrence.

Keywords

Acute Otitis Media, Antimicrobial Resistance, Tertiary Health Institution, Paediatric Infection, Low-Income Country

1. Introduction

Otitis media in children accounts for great number of visits to paediatric infection clinics globally and especially in many developing countries [1] [2]. It is worsened by increase in day-care centres outside the home and unhygienic environments [3]. Acute otitis media is most common among children between the ages of three months and three years with a peak incidence between 6 and 9 months [1]. By one year of age, at least 60% of children have experienced an episode and 17% have suffered at least 3 episodes of AOM [4]. It is an inflammation of the middle ear usually caused by bacteria, but sometimes by fungi, virus, *Mycoplasma pneumoniae* and *Chlamydia trachomatis* [5]. Otitis media is characterized by fluid build up behind the ear drum and occurs in area between the tympanic membrane and the inner ear, including the duct called Eustachian tube [6]. Children are more affected by acute otitis media than adults because their Eustachian tubes are shorter and more horizontal and their immune functions are not yet fully developed to resist pathogens [7] [8]. 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) [9].

Otitis media is prevalent among Nigerian children and it is found to occur more in children of mothers with lower educational status and in children sleeping more than 4 in the same room [2]. In a study carried out in Ilesha, Osun state of Nigeria over one year period from 2004 to 2005, the prevalent of AOM was 1.01% [10].

Drug resistant is a common phenomenon among children with infectious disease in our environment owing to irrational use of antimicrobial agents. This study therefore, assessed the microbial etiology and multi-drug resistant profile of the pathogens isolated from children diagnosed of acute otitis media in the children's outpatient clinics of Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH), Awka. The hospital serves as a referral center to a lot of surrounding village communities of over 2 million persons.

2. Methodology

2.1. Study Population/Center

The study was done at COOUTH Awka, which is a tertiary Health centre located at Awka, the capital of Anambra State. It is a 150 bedded Hospital established in 2011 to train medical personnel and cater for the health needs of surrounding communities of over 2 million population. The study population comprised children aged one month to 9 years, who presented at the paediatric out-patient clinic of the hospital from January to April 2015. Participants were recruited consecutively after a verbal informed consent was obtained from their legal care givers.

2.2. Samples Collection

Recruitment of study participants and sample collection were done by the paediatrician. Diagnosis was based on clinical manifestation of acute otitis media on the children attending the paediatric out-patient clinic of the hospital. Forty samples of ear swab were collected using sterile swab sticks and were labeled for age and sex of patients.

2.3. Ethical Considerations

The study was explained to the parents of the children and a verbal consent was obtained from them. The study

protocol was approved (Approval number: ANSUTH/AA/VOL.XI/004) by the ethics committee of COOUTH, Awka.

2.4. Isolation and Identification of Organisms and Antibigram Study

The swab sticks used for the collection of the samples were streaked directly on well labeled MacConkey, mannitol salt, cetrinide and chocolate blood agar, incubated at 37°C for 24 h to enable for bacteria isolation. After incubation, cultures were examined for significant growth. Subcultures were prepared in nutrient agar plates, and incubated for another 24 h. Grams staining and Biochemical test (Indole production, Oxidase, catalase and Coagulase) was carried out in accordance with standard methods [11]. To enable for fungal isolation and identification, the swab sticks were streaked directly on the well-labeled Sabouraud Dextrose Agar (SDA) plates and incubated at room temperature for 48 hours. Each fungal growth was identified based on their morphological and cultural characteristics. All yeast isolates were identified with CHROMagar. The Antimicrobial susceptibility testing was done by Kirby-Bauer's disc diffusion method and interpretation was in according with European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (<http://www.eucast.org>) for these drugs: azithromycin, erythromycin, ciprofloxacin, sparfloracin, gentamicin, streptomycin, ceftriaxone, cefuroxime, ampicillin, co-amoxiclav, nystatin, fluconazole and voriconazole.

2.5. Data Analysis

This was done using Graphpad Prism Version 5.0 statistical package. Tests of significance were done using One-way analysis of variance, Chi-square test and Mann Whitney test and P-value of <0.05 was considered significant. Results were presented in tables.

3. Results

Out of 2025 children who attended the children's out-patient clinic during the period, 40 of them were diagnosed of having acute otitis media giving an incidence rate of 1.98%. Their ages ranged from 1 month to 9 years comprising 25 males and 15 females with male-female ratio of 1.67:1 (Table 1). AOM was significantly more prevalent in males than in females ($P < 0.05$). The age group ≤ 1 year were most affected, having about half the total number the AOM patient.

Table 2 depicts the different causative organisms isolated. Thirty (75%) out of the 40 samples collected were culture positive, while the remaining ten yielded no organism. Thirty three bacteria isolates were recovered in the study with *Staphylococcus aureus* (34.9%) being the predominant isolate followed by *Pseudomonas aeruginosa* (30.2%). These were not statistically significant $P > 0.05$. A total of 10 fungi isolates were obtained as shown in Table 2 with *Candida krusei* accounting for the highest (14%) of AOM of *Candida* aetiology while *Candida glabrata* and other *Candida* spp. were equal in occurrence (4.7%). Mann Whitney test of significance showed that bacteria, although more commonly isolated in the study, are not significantly the more common etiology than fungi ($P > 0.05$). Out of the 40 samples collected, 30 (66.67%) yielded positive single microbial cultures while 10 (33.3%) had mixed microbial growth.

Table 3 shows the resistance profile of the isolates. The most promising drug for the management of AOM of bacterial origin in these patients appears to be ciprofloxacin, a drug not commonly recommended for use in

Table 1. Age and sex distribution of patients.

Age (years)	Males	Females	Total	% Total
≤ 1	18	4	22	55
2 - 4	1	7	8	20
5 - 7	1	4	5	12.5
8 - 9	5	0	5	12.5
Total	25	15	40	100.0

P value = 0.0003 (Chi square test).

Table 2. A profile of the isolates.

Bacteria	Number (%)	Fungi	Number (%)
<i>Staphylococcus aureus</i>	15 (34.9)	<i>Candida glabrata</i>	2 (4.7)
<i>Pseudomonas aeruginosa</i>	13 (30.2)	<i>Candida krusei</i>	6 (14)
<i>Escherichia coli</i>	4 (9.3)	Other <i>Candida</i> spp.	2 (4.7)
<i>Streptococcus</i> spp.	1 (2.3)	Total	10 (23.4)
Total	33 (76.7)		

P value = 0.6286 (Mann Whitney test: Two-tailed).

children, followed by gentamicin. They demonstrated the least resistance profile. On the other hand, the β -lactamase antibiotics were totally useless having a resistance profile range of 90.91% - 100% (**Table 3(a)**).

Out of the three antifungal agents tested, voriconazole and nystatin had comparable result (**Table 3(b)**) while fluconazole was not very effective. One-way analysis of variance P value = 0.8503. There was, therefore, no significant difference in the effectiveness of the three agents.

The incidence of multi-drug resistant isolates (**Table 3(c)**) was calculated as

$$\begin{aligned} &\text{The incidence of multi-drug resistant isolates} \\ &= \frac{\text{Number of isolates with MARI} > 20\%}{\text{Total number of Isolates}} \times 100 = 33/33 * 100 = 100\% \end{aligned}$$

3.1. Abbreviations and Acronyms

AOM = Acute otitis media.

EUCAST = European Committee on Antimicrobial Susceptibility Testing.

COOUTH = Chukwuemeka Odimegwu Ojukwu University Teaching Hospital.

OME = Otitis media with effusion.

COME= Chronic Otitis media with effusion.

3.2. Equations

$$\text{The incidence of multi-drug resistant isolates} = \frac{\text{Number of isolates with MARI} > 20\%}{\text{Total number of Isolates}} \times 100$$

4. Discussion

This study showed that children ≤ 1 year were most prone to AOM as they had the highest incidence rate (55%). This is age most prone to day-care outside the home. The finding corresponds with some previous studies [8] [12]. Other workers inside and outside Nigeria had also reported high incidence of AOM in day-care centers [3] [9]. Children, generally, are more predisposed to having more incidence of AOM than the adults. This is attributed to their having shorter and more horizontal Eustachian tube, lower immunity compared to adults and the fact that bacteria adhere better to epithelial cells of young children than adults [7]. The males had a significantly ($P < 0.05$) higher prevalence than females and this consistent with reports of Nwankwo and Okeke [13] and Wang *et al.* [14]. The reason for this observation is still unknown.

The present study showed that *Staphylococcus aureus* were the predominant isolate causing acute otitis media followed by *Pseudomonas aeruginosa*. All the identified bacterial isolates correspond to similar study done by Nwankwo and Okeke [13] who also reported the preponderance of *S. aureus*. In contrast however Osazuwa *et al.* [12] and Ogbogu *et al.* [15] reported *Pseudomonas aeruginosa* as the most frequent isolate of otitis media followed by *S. aureus* and *Proteus* spp. In developed countries, the commonest bacterial pathogens of acute otitis media were *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* [3] [5] [16]. Possible reasons for the difference in isolated organisms might be related to the effect of climate and pattern of antibiotic use. The remaining 10 samples

Table 3. Resistance profile of the isolates to conventional antimicrobials.

(a)						
Antibacterial agents						
S/N	Antibiotics	Number of the isolates resistance to commercial antibiotics (%)				
		<i>P. aeruginosa</i> N = 13	<i>E. coli</i> N = 4	<i>Streptococcus</i> spp. N = 1	<i>S. aureus</i> N = 15	Total N = 33
1	Azithromycin	13 (100)	0 (0)	1 (100)	15 (100)	29 (87.88)
2	Erythromycin	13 (100)	0 (0)	1 (100)	13 (86.7)	27 (81.82)
3	Ciprofloxacin	6 (46.15)	2 (50)	0 (0)	7 (46.7)	15 (45.45)
4	Sparfloxacin	12 (92.3)	3 (75)	0 (0)	4 (26.7)	19 (57.58)
5	Gentamicin	5 (38.5)	4 (100)	1 (100)	6 (40)	16 (48.48)
6	Streptomycin	12 (92.3)	3 (75)	1 (100)	15 (100)	31 (93.94)
7	Ceftriaxone	12 (92.3)	4 (100)	1 (100)	13 (87.7)	30 (90.91)
8	Cefuroxime	13 (100)	4 (100)	1 (100)	12 (80)	30 (90.91)
9	Ampicillin	13 (100)	4 (100)	1 (100)	15 (100)	33 (100)
10	Co-amoxiclav	13 (100)	4 (100)	1 (100)	15 (100)	33 (100)

(b)					
Antifungal agents					
S/N	Antifungal agent	Number of the isolates resistance to commercial antifungal agents (%)			
		<i>Candida glabrata</i> N = 2	<i>Candida krusei</i> N = 6	<i>Other Candida</i> spp. N = 2	Total N = 10
1	Nystatin	2 (100)	0 (0)	2 (100)	4 (40)
2	Fluconazole	2 (100)	2 (33.33)	2 (100)	6 (60)
3	Voriconazole	1 (50)	2 (33.33)	1 (50)	4 (40)

(c)					
Multi-Antibiotics Resistance Index (MARI) analysis of the bacterial isolates					
S/N	Bacterial Isolates	Number of Isolates	Number of isolates resistant to ≥ 3 antibiotics	Number of antibiotics to which the isolates were resistant (a)	MARI (a/b) in %
1	<i>P. aeruginosa</i>	13	13	10	100
2	<i>E. coli</i>	4	4	8	80
3	<i>Streptococcus</i> spp.	1	1	8	100
4	<i>S. aureus</i>	15	15	10	80
	Total	33	33		

that were culture negative, suggested that the parents or caregiver may have given some antibiotics to these children before presenting to the hospital, and this could have inhibited the growth of the pathogens.

This study showed that mixed microbial infection are less than single microbial infection which correlates and correspond to similar study carried out in India by Saraswati *et al.* [17]. *Candida krusei* and *C. glabrata* were the commonest fungal organisms isolated in this study and contrasted with previous study done in Benin City by Ogbogu *et al.* [15] which isolated *C. albicans*. Possible reasons for this observation may be related to environment influence.

The isolates showed a high level of multi-antibiotic resistance (100%). This corresponds with similar study carried out at by Nwankwo and Okeke [13] at Umuahia, Abia State of Nigeria. This might be due to misuse of the antibiotic and if not corrected may lead to loss of the drug efficacy in treating infections. The only *Streptococcus spp* isolated in the present study did not show any resistance to ciprofloxacin. This corresponds to the report in similar study by Nwankwo and Okeke [13] where 1 out of 8 isolates of *Streptococcus* were resistant to ciprofloxacin. In consonant with our study, *Pseudomonas spp.* had been shown to be resistant to ciprofloxacin [18]. Similar drug resistant pattern was also observed on cefuroxime and other beta-lactam antibiotics when used against *Escherichia coli*, *Pseudomonas aeruginosa* and *Streptococcus species* [18]. The resistance of *Staphylococcus* and *Streptococcus* to sparfloxacin, although low in our study (26.7% and 0% respectively) and of *Pseudomonas* and *E. coli*, observed to be high, (92.3% and 75% resistance respectively) could be due to point mutation in the DNA gyrase of the bacteria leading to the loss of the organisms binding affinity to the drugs, hence loss of activity [19].

The resistance profile of the fungal isolate to different antifungal agents (nystatin, fluconazole and voriconazole) used in this study is a serious concern. This could be as a result of misuse of fluconazole in the environment and increased use in the management of HIV infection. The drug, although a prescription—only medicine is often purchased by patients without prescription. Similar report exists in the northern part of the country [20] and calls for the speedy implementation of the policy on rational drug use in the country.

5. Conclusion

The incidence of multiple antibiotic resistant bacteria associated with acute otitis media in the paediatric patient was unacceptably very high. Empirical treatment of the infection in the community should be discouraged. Instead thorough microbiological investigations should be done before commencement of treatment to avoid treatment failure and spread of resistant organisms. AOM preventive strategies should be targeted on the male and very young children to reduce the risk of recurrence.

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