PREVALENCE AND ANTIBIOTIC-RESISTANCE INDICES OF BACTERIAL PATHOGENS OF OTITIS MEDIA AMONG PATIENTS ATTENDING A TERTIARY HOSPITAL IN CALABAR, NIGERIA

Abstract

Background: Otitis media (OM) is a common childhood disease frequently associated with severe complications and sequelae, often in absence of timely and appropriate antibacterial interventions.

Aim: To investigate the identity and antibiogram of bacterial agents of OM among patients attending a tertiary health facility in Calabar, Nigeria.

Methods: Ear specimens collected from a total of 70 subjects were processed by conventional bacteriological methods for isolation and identification of pathogens. Antibiotic susceptibility testing was carried out on all the isolates using the Kirby-Bauer disk diffusion method. Multiple antibiotic resistance (MAR) indices of the isolates were evaluated.

Results: The prevalence of bacterial OM was 72.9%. A total of 51 bacterial isolates including *Pseudomonas aeruginaosa* (26)*, Staphylococcus aureus* (17)*, Proteus mirabilis* (6), and *Klebsiella pneumoniae* (2) were found. Isolates of *P. aeruginosa* and *S. aureus* showed high susceptibility to amoxicillin-clavulanate (92.3%) and ciprofloxacin (88.3%), respectively. The prevalence of multidrug resistant (MDR) isolates was 25.4%. MDR percentages were 100%, 33.3%, 29.4% and 15.3% for *K. pneumonia*e, *P. mirabilis*, *S. aureus*, and *P. aeruginosa*, respectively. MAR indexing revealed high values ranging from 0.4 to 0.87 for all the isolates.

Conclusion: High susceptibility of the predominant isolates to amoxicillin-clavulanate and ciprofloxacin may underscore the drugs' potential as antibiotics of choice for prompt treatment of OM in the population. However, the overall high drug-resistance indices of the isolates would suggest dire implications for empiric antibacterial therapy in the population. Choice of antibiotics for treatment of bacterial infections in this population should be driven by results of microbiological drug-susceptibility tests.

*Keywords: Otitis media, bacterial pathogens, antibiotic-resistance, prevalence***.**

INTRODUCTION

Otitis media (OM) is a set of inflammatory disease of the eardrum and middle ear, typically classified into acute otitis media (AOM), otitis media with effusion (OME), and chronic suppurative otitis media (CSOM).[1] Whereas the disease can affect persons of all ages, children under the age of 5 years are most susceptible to OM, often due to underdeveloped immunity

and Eustachian tube dysfunction.[2,3] Although rarely associated with mortality any longer, the morbidity associated with otitis media is significant with around 1.2 billion people affected by the disease worldwide[4]. Ranked second and fifth in the list of leading causes of hearing loss and global burden of diseases,[5] respectively, otitis media is a major recurring disease in low and middle income countries, with the highest incidence rate from sub-Saharan Africa and South Asia.[6]

OM often occurs following bacterial infection of the upper respiratory tract, but can also be caused by allergy as well as functional and anatomical changes of the Eustachian tube or middle ear.[2] *Streptococcus pneumoniae,* known to colonize the nasopharynx of 27-65% children[7], is the primary pathogen of OME. Although viruses and fungi are also associated with the disease,[8] the major etiological agents of otitis media are pathogenic bacteria, including *Haemophilus influenza, Moraxella cartahallis, Staphylococcus aureus, Pseudomonas aeruginosa, Eschericia coli, Proteus mirabilis, Klebsiella species,* and anaerobic bacteria such as *Bacteriodes, peptostreptococcus, Propionibacterium* and to a lesser extent *Streptococcus pyogenes*.[9,10,11,12]

OM is the most common disease of childhood that frequently necessitates emergency visits to hospitals[13]. Although the disease can sometimes resolve without antimicrobial drug treatment, it often requires clinical intervention with antibiotics for relief and remission of symptoms and complications[4]. While the use of broad-spectrum antibiotics for treatment of AOM has been known to reduce the frequency of ear infections and produce overall decline in the incidence of complications of OM, it is also associated with development and spread of drug-resistant bacteria[14]. Whereas most studies on OM have focused largely on its prevalence and clinical diagnosis, contemporary data on microbial etiology of the disease is scanty, particularly in relation to antimicrobial drug resistance in this epidemiological setting. This study was aimed at determining the identity and antibiotic-resistance profiles of bacterial pathogens of OM in the

population. This knowledge may be crucial for prompt antimicrobial interventions of OM, particularly in emergency situations, in this locality.

METHODOLOGY

This cross sectional study was carried out between November 2020 and April 2021 in Calabar, a densely populated capital of Cross River state, Nigeria. The study participants were patients of all ages, except those aged below 6 months, who presented with ear drainage or discharge at the Ear, Nose, and Throat (ENT) Clinic of University of Calabar Teaching Hospital (UCTH), Calabar. A nonprobability (convenience) sampling technique was used. A total of 70 patients were recruited into the study, following submission of a signed or thumb-printed written informed consent by the participants (a parent or guardian for underage children). All the study participants were clearly informed of the purpose and potential benefits of the study, with firm guarantee to maintain their personal information with strict confidentiality for the research purpose only. Ethical clearance for the study was sought and obtained from the Ethical Committee of UCTH, Calabar, while permission for use of the hospital facilities was also obtained from the Hospital management.

Collection and processing of specimens

Samples of ear discharge/drainage were collected by the consulting Medical Officers in the clinic using a sterile swab stick. Prior to collecting the samples, a sterile pipette was used to aspirate the drainage to avoid stirring the discharge. The specimens were placed into an AMIES transport medium and transported to the microbiology laboratory for processing within an hour of collection.

Ear specimens were inoculated onto bacteriological media including chocolate agar, blood agar, cysteine lactose electrolyte deficient agar (CLED). The inoculated plates were incubated at 37^0C in a 5-10% carbon dioxide enriched atmosphere and examined for growth of bacteria after 24-

48 hours' incubation. The isolates were identified by conventional bacteriological and biochemical procedures.

Antimicrobial susceptibility testing of ear isolates

Antimicrobial susceptibility test was carried out on each bacterial isolate using the disk diffusion method on nutrient agar plates. Three to five pure colonies of each bacterium were picked and transferred to a tube containing 5ml sterile peptone water. The preparations were mixed thoroughly to make the suspension homogenous. The bacterial suspension was incubated at 37 ^{0}C until its turbidity matched the opacity of 0.5 McFarland Standard (equivalent to bacterial concentration of 1.5x10^8 colony forming unit/ml). A sterile swab was dipped in the suspension and used to inoculate nutrient agar plate by spreading it uniformly on the agar surface, and allowed to dry for about 15-30 minutes. Commercially available antibiotics susceptibility test discs were placed on the inoculated plate using sterile forceps, with adequate spacing (at least 24 mm) between discs to avoid overlapping zones of inhibition; the antibiotics used included amoxicillin-clavulanate (30µg), Erythromycin (15µg), Zithromax (30µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Gentamicin (10µg), Ceftriaxone (30µg), and Ceftazidime (30µg). The plates were kept to dry at room temperature for 30 minutes, then incubated overnight at 37^{0}C, and examined for zones of inhibition. Susceptibility pattern was interpreted by comparison of the zone of inhibition according to Clinical and Laboratory Standards Institute guidelines (CLSI).[15]

Determination of Multidrug Resistance

Multidrug resistance (MDR) was defined by resistance of an isolate to three or more classes of antibiotics tested against the isolate[16].

Determination of Multiple Antibiotic Resistance Index

Multiple antibiotics resistance (MAR) index for each isolate was calculated as a ratio of the number of antibiotics which an isolate showed resistance to the total number of antibiotics used for susceptibility testing against the isolate [17].

Data analysis

The data obtained in this study were analyzed using IBM SPSS Statistics 20 computer software (SPSS for Windows 20.0; SPSS Inc, Chicago, Illinois). Proportions were presented in frequencies and percentages. Pearson's chi-square analysis was used to test the statistical significance of bacterial etiology and other variables on the prevalence of otitis media. P-values were set at ≤ 0.05 level of significance.

RESULTS

Prevalence of bacterial otitis media among the study participants

The prevalence of bacterial ear-infection in the study population was 72.9%. Prevalence of infection was slightly higher in males (75.9%) than females (70.7%). Subjects aged 1-10 years had the highest prevalence of infection (91.7%), followed by those aged 31-40 years (77.8%), while those aged 21-30 years had the least (50.0%). Neither the age nor sex of the study participants was found to be significantly associated with the prevalence of infection ($p>0.05$) [Table 1].

Distribution of bacterial pathogens of otitis media in the study population

Out of a total of 70 ear specimens analyzed in this study, 51 (72.9%) yielded growth of bacterial pathogens (Table 2). A total of 51 isolates comprising of *Pseudomonas aeruginosa* (26),

Table 1: Prevalence of bacteria-associated OM among the Subjects

Variables	No. tested	No. infected (%)	P- value
Age			0.22

1-10	24	22 (91.7)	
11- 20	13	10 (76.9)	
21- 30	8	4 (50.0)	
31- 40	9	7 (77.8)	
41- 50	4	3 (75.0)	
>50	12	8 (66.7)	
Total	70	51 (72.9)	
Gender			
Male	29	22 (75.9)	0.63
Female	41	29 (70.7)	
Total	70	51 (72.9)	

Table 2: Bacterial isolates of otitis media in the study population

Pathogens	No. isolated (%)
NBG	19 (27.1)
P. aeruginosa	26 (37.1)
S. aureus	17 (24.3)
P. mirabilis	6 (8.6)
K. pneumonia	2 (2.9)
Total	70 (100)

Staphylococcus aureus (17)*, Proteus mirabilis* (6), and *Klebsiella pneumonia* (2) were obtained in the study.

Antibiotics susceptibility and resistance patterns of bacterial isolates

The susceptibility and resistance of bacterial isolates to commonly used antibiotics is shown in Table 3.

P. aeruginosa isolates were more susceptible to amoxicillin-clavulanate (92.3%), ceftriaxone (76.9%), and ceftazidime (69.2%). In decreasing order, the isolates showed high resistance to erythromycin (100%), gentamicin (69.2%), azithromycin (61.5%), levofloxacin (34.6%), and ciprofloxacin (34.6%).

The susceptibility of *S. aureus* isolates was 88.2%, 82.4%, 76.5%, 70.5%, and 64.7% to ciprofloxacin, ceftriaxone, amoxicillin-clavulanate, azithromycin and ceftazidime, respectively. The isolates were more resistant to erythromycin (82.3%), gentamicin (64.7%), and levofloxacin (41.2%) compared to other antibiotics.

The sensitivity pattern of *P. mirabilis* to ciprofloxacin, levofloxacin and ceftriaxone was 88.3%, 66.7% and 66.7%, respectively, while Augmentin and ceftazidime were 50% each; resistance to erythromycin was total (100%), while 66.7% resistance was found for both azithromycin and gentamicin.

K. pneumonia isolates showed total (100%) resistance to ceftazidime, azithromycin, erythromycin, Augmentin and gentamicin, but total (100%) susceptibility to ceftriaxone, and modest sensitivity (50%) to levofloxacin and ciprofloxacin.

Multidrug resistance and multiple antibiotic resistance index of the bacterial isolates

The overall prevalence of multidrug resistant (MDR) strains among the isolates was 25.4% (Table 3). Based on their resistance to three or more classes of the antibiotics tested, 13(24.5%) of the total 51 isolates were categorized as MDR strains. In descending order, MDR percentages for the isolates were 100%, 33.3%, 29.4% and 15.3% for *K. pneumonia*e, *P. mirabilis*, *S. aureus*, and *P. aeruginosa*, respectively. However, association between multidrug resistance and the prevalence of infection was not statistically significant ($p>0.05$).

Table 3: Antibiotic susceptibility patterns of isolates to commonly used antibiotics

Pathogens	Status	No. (%) of isolates							
		*CRO	AZI	CAZ	AUG	ERY	LEV	CIP	GEN
P. aeruginosa	Sensitive	20(76.9)	10(38.5)	18(69.2)	24(92.3)	0 -	17(65.4)	17(65.4)	8(30.8)
	Resistant	6 (23.1)	16(61.5)	8(30.8)	2 (7.8)	26(100)	9(34.6)	9(34.6)	18(69.2)
S. aureus	Sensitive	14(82.4)	12(70.6)	11(64.7)	13(76.5)	3(17.6)	10(58.8)	15(88.2)	6(35.3)
	Resistant	3(17.6)	5(29.4)	6(35.3)	4(23.5)	14(82.3)	7(41.2)	2(11.8)	11(64.7)
P. mirabilis	Sensitive	4(66.7)	2(33.3)	3(50.0)	3(50.0)	0 -	4(66.7)	5(83.3)	2(33.3)
	Resistant	2(33.3)	4(66.7)	3(50.0)	3(50.0)	6(100)	2(33.3)	1(16.7)	4(66.7)
K. pnuemoniae	Sensitive	2(100)	0 -	0 -	0 -	0 -	1(50)	1(50)	0 -
	Resistant	0 -	2(100)	2(100)	2 (100)	2(100)	1(50.0)	1(50.0)	2 (100)

* CRO- Ceftriaxone, AZI=Azithromycin, CAZ- Ceftazidime, AUG- Amoxicillin-clavulanate, ERY=Erythromycin, LEV=Levofloxacin, CIP=Ciprofloxacin and GEN=Gentamicin

Table 4: Prevalence of multidrug resistant (MDR) strains among the bacterial isolates

Bacterial pathogens	No. of isolates	Resistance to ≥ 3 classes of antibiotics	MDR (%)	P-value
P. aeruginosa	26	4	4 (15.3)	0.523
Staph. aureus	17	5	5 (29.4)	
P. mirabilis	6	2	2 (33.3)	
K. pneumoniae	2	2	2 (100)	
Total	51	13	13 (25.4)	

Table 5: Multiple antibiotic resistant indices of the ear isolates

Bacterial isolates	No. of antibiotics tested	No. of antibiotics resistant to	No. (%) of isolates	MAR index
P. aeruginosa	8	3	4(15.3)	0.37
(n=26)		4	6 (22.5)	0.50
		5	8 (30.7)	0.62
		6	8 (30.7)	0.75
Staph. aureus	8	4	3 (17.6)	0.50
(n=17)		5	3 (17.6)	0.62
		6	7 (41.1)	0.75
		7	4 (23.5)	0.87
P. mirabilis	8	4	1 (16.6)	0.50
(n=6)		5	3 (50.0)	0.62
		6	2 (33.3)	0.75
K. pneumonia	8	5	1 (50.0)	0.62
(n=2)		7	1 (50.0)	0.87

Multiple antibiotics resistance (MAR) indexing of all the isolates revealed values greater than 0.2; no isolate, however, had an index equal to 1 (Table 4). MAR indices found for different bacterial isolates varied as follows: *P. aeruginosa* (0.42 - 0.85), *K. pneumonia* isolates (0.62 - 0.87), *S. aureus* (0.50 – 0.87), and *P. mirabilis* (0.50 – 0.75).

DISCUSSION

Otitis media is a common disease of childhood, with about 80-90% of children likely to experience one or more episodes by the age of 10 years[18]. This study found the highest prevalence of the disease in subjects aged 1-10 years, while those aged 31-40 years had the least among other age-groups. Although children are generally known to contract infectious diseases more frequently because of indiscriminate playing and poor hygienic habits, vulnerability to OM has been largely associated with underdeveloped immunity and Eustachian tube dysfunction[2,3]. In consistence with some study reports that have identified the male-sex as a host-related risk factor of OM[19,20], the frequency of infection in this study was found to be slightly higher in male subjects than the females. In contrast, however, a number of studies carried out in Nigeria have reported higher prevalence of OM in females than males[21-23]. Variations in gender-based prevalence of OM in diverse epidemiological settings may be attributed to peculiar population dynamics that put one gender at greater risk of exposures to environmental pollution. .

In relation to microbial etiology of OM, this study confirmed the predominance of bacterial pathogens with a prevalence of 72.9% in the study population. *P. aeruginosa* was the most frequently isolated pathogen, among others including *S. aureus, P. mirabilis, and K. pneumoniae*. This finding is consistent with reports of similar studies which found *S. aureus, P. aeruginosa, Proteus species, and K. pneumoniae* among the most common agents of OM in some parts of Nigeria and elsewhere in sub-Saharan Africa[24-26]. .

In spite of a widely endorsed strategy for conservative management of AOM without antibiotics in patients less likely to have any therapeutic benefits[27], treatment with antibiotics has remained a primary tool for clinical intervention of OME[13]. This study found ear pathogens with remarkable variation in their susceptibility to commonly used antibiotic drugs in this setting. For instance, while isolates of *S. aureus* were more susceptible to ciprofloxacin, *K. pneumoniae* strains showed high susceptibility to ceftriaxone. Remarkably, *P. aeruginosa* isolates were found to be highly sensitive to amoxicillin-clavulanate, in contrast to the findings of similar studies that reported high resistance of the pathogen to the drug in other epidemiological settings of sub-Saharan Africa[28]. Compared with other commonly used antibiotics, the high susceptibility of *P. aeruginosa* isolates to amoxicillin-clavulanate in this study may be a consequence of discrete and appropriate usage of the drug in the population. Indiscriminate use of antibiotics has been known to play an important role in development of drug-resistant bacterial strains[29].

Overall, the present study found a high proportion of drug-resistant strains with a MDR prevalence of 25.4% among the isolates. This finding is consistent with the fact that the common isolates of this study, including *P. aeruginosa, S*. *aureus* and *K. pneumonia,* are members of the so-called 'ESKAPE' group of pathogens[30], widely known for their increasing resistance to commonly used antibiotics[28]. Evaluation of MAR index for all the isolates in this study revealed high values ranging from 0.4 to 0.8. MAR indices greater than 0.2 are known to be effective indicators of bacterial pathogens acquired from highly contaminated environments associated with indiscriminate use of antibiotics, whereas indices less than or equal to 0.2 would indicate bacteria from sources with less antibiotic usage.[31] The high MAR indices of ear pathogens in this study may portend dire implications for empiric antimicrobial therapy of OM, with a high likelihood of treatment failures and disease complications in the population. Treatment failures with first episodes of OM has been known to result in recurrent or chronic disease usually associated with hearing loss, decreased learning ability, and poor educational output in children[32,33].

Limitations: This study utilized only ear-swabs/discharge specimens for isolation of bacterial pathogens; absence of tympanocentesis could possibly have compromised the capacity for detection of early onset infections, particularly in children. Similarly, due to inadequate laboratory capacity, bacterial etiology was determined strictly by isolation of pathogens on traditional bacteriological media. Reliance on solid culture isolates, in absence of more sensitive molecular methods, could potentially limit the yield of fastidious ear pathogens, including anaerobes, and possibly eliminate their etiologic contributions. Discrepancies in isolation rates and etiological data of some studies, particularly in sub-Saharan Africa, have been attributed to inadequate laboratory capacity and the quality of microbiological procedures used[28]. Further studies on microbiological etiology of otitis media in this setting should consider utilization of more sensitive methods to maximize the bacterial yield and overall etiological data.

CONCLUSION

This study found a high prevalence of bacteria-associated OM, with *P. aeruginosa* and *S. aureus* as the predominant etiologic agents of the disease in the population. A high susceptibility of the predominant isolates to amoxicillin-clavulanate and ciprofloxacin may underscore the potentials of these drugs for prompt treatment of OM. However, given the overall high resistance indices of the study isolates, and dire implications for empiric antibacterial therapy, choice of antibiotics for clinical intervention of otitis media in this population should be driven mainly by valid results of microbiological susceptibility tests.

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