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Original Article

Identification of Common Bacterial Etiologic Agents, Antimicrobial Susceptibility Pattern & Associated Risk Factors of Otitis Media Among Pediatric Patients of ENT Center of Orotta National Referral Hospital

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Abstract

Background: *Otitis media is a pervasive childhood problem posing great healthcare challenges in developing countries. The magnitude of the problem and the lack of appropriate data about the prevalence, common etiologic agents, and the risk factors responsible for otitis media among children in Eritrea have encouraged us to undertake the present study.*

Methods: *A total of 366 ear discharge samples were collected by an Otorhinolaryngologist. All collected samples were screened by using appropriate media and conditions that favor the growth of bacteria. Antibiotic susceptibility testing was done to record the sensitivity and resistance of bacteria by the disc diffusion method. Socio-demographic and clinical data were collected using standard and structured questionnaires and the data was analyzed using SPSS version 20 software.*

Results: *Of 400, 366 (91.5%) samples were with Otitis media and 305 showed the presence of bacteria (83.33%). The most common pathogenic bacteria was Staphylococcus aureus followed by Hemophilus influenza and Pseudomonas aeruginosa. The results showed that the infection is more common in lower-aged children, daycare attendees, and big families. All are statistically significant (P-value 0.003), (P-value 0.036) (p<0.000), respectively. H.influenza, P.aeruginosa, and S.aureus showed good sensitivity towards ciprofloxacin, which was 85.5%, 100%, and 68.1% respectively.*

Conclusion: *Of 400, 366 (91.5%) samples were with Otitis media and 305 showed the presence of bacteria (83.33%). The most common pathogenic bacteria was Staphylococcus aureus followed by Hemophilus influenza and Pseudomonas aeruginosa. The results showed that the infection is more common in lower-aged children, daycare attendees, and big families. All are statistically significant (P-value 0.003), (P-value 0.036) (p<0.000), respectively. H.influenza, P.aeruginosa, and S.aureus showed good sensitivity towards ciprofloxacin, which was 85.5%, 100%, and 68.1% respectively.*

Keywords: *Otitis Media, Asmara, Eritrea, Antimicrobial Susceptibility Test, Pediatric patient*
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Introduction

Otitis media (OM) is a common problem worldwide. Globally, about 65–330 million people suffer from ear infections and 60 % of them have significant hearing impairment (1). The burden and population demographics of otitis media differ greatly between developed and developing regions and are presumed to be high in low and middle-income countries (2), especially in sub-Saharan Africa (SSA), India, and other developing nations. The effect of low socio-economic status, overcrowding, poor hygiene, and recurrent upper respiratory tract infections on the population is

estimated to cause as high as 11% prevalence of the disease (3) and is accountable for most deaths arising from its complications (4). The incidence of otitis media in sub-Saharan Africa (SSA), South Asia, and Oceania are two-to eight-fold higher than in the rest of the world (5), with the aboriginal population demonstrating the highest incidence (6) and SSA has the second highest incidence of otitis media (5). According to World Health Organization (WHO) surveys, countries can be clustered into those having low ear infections when the prevalence rate of ear infection among children is between 1-2% and high when it is 3-6% (4). Several studies in Ethiopia provide information that there is a high prevalence of OM. Even though the prevalence of ear infection is different in different geographical areas (7,8), despite the expected slight difference, it can be taken as a representative assumption for the prevalence of OM in Horn African nations especially in Eritrea as there is no study existing concerning the prevalence of OM (9 -16).

A periodic review of the etiologic agents of otitis media and their antimicrobial susceptibility profiles is warranted, especially in nations with low socioeconomic status. Due to the limited laboratory diagnosis in developing countries, physicians have often forced the prescription of broad-spectrum antibiotics for most infections, which led to the emergence of drug-resistant bacterial strains (17). Therefore, current information on microbial resistance and the prevalence of pathogenic bacteria needs to be available at national and local levels to guide the rational use of the existing antimicrobials.

Since the disease is insidious and usually seen in small children. It is essential to establish the possible risk factors for OM and identification of children at risk of OM, to prevent any further increase in its occurrence and to determine the proper treatment plan for affected children which are important from medical as well as community perspectives. Nevertheless, in Eritrea and particularly in the study area, there is a scarcity of recent data that indicate the magnitude of the problem. Thus, this study aimed to identify bacterial isolates, determine their drug susceptibility patterns from patients who had ear infections, and identify the main risk factors.

Methods

Study Design

A Cross-sectional Prospective study was conducted from March to March 2022-23 at Orotta ENT Center of Asmara, Eritrea. Socio-demographic and clinical data were collected using a standard and structured questionnaire.

Study size and population

The total number of children who participated in the study was 400 from March to March 2022-23. Out of this, 366 had otitis media and the remaining 34 were without otitis media for the analysis of risk factors.

Inclusion criteria

Pediatric patients below 12 years old were included.

Exclusion criteria

Children whose parents were not willing to fill out the questionnaire were not eligible for participation. Furthermore, patients with antimicrobial treatment 10 days before specimen collection and patients above 12 years old were excluded.

Specimen collection and processing

One sterile swab with transport media was used to aseptically collect ear discharges from each pediatric patient, who was identified to have otitis media. All specimens were transported from the ENT center to the NHL (National Health Laboratory) microbiology lab and cultured within 1 hour of collection. The swabs were inoculated onto Chocolate, Mannitol salt agar, and MacConkey agar plates (18). Bacterial species were identified as per the microbiological methods mentioned in earlier studies done in Asmara, Eritrea (19). All reagents for Gram staining, Biochemical tests, and antibiotic discs were purchased from an Indian company (Hi-Media, Mumbai, India).

Antimicrobial susceptibility testing

The disk diffusion method using Mueller-Hinton agar was used for antimicrobial susceptibility tests. The drug susceptibility pattern was interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2020) (20). The selection of antibiotics was based on the frequency used in the country for the treatment of otitis media. An isolate was defined as being multidrug-resistant if it is resistant to three or more of the antimicrobial agents tested (21).

Questionnaire Survey

The questionnaire forms included personal and familial items. Personal items were: presence of upper respiratory tract infection (URTI) and otitis media (OM) episodes during the previous year; known disease or allergies of the child, attendance at daycare, kindergarten, and elementary school, duration of breastfeeding (months).

Familial questions were: smoking of mother and father; smoking at home; the number of family members; guardian's educational level.

Data Analysis

Data entry was done by using SPSS version 20 software. Chi-square analyses were used to compare group P values with <0 .05 were considered statistically significant. Logistic regression models calculated the independent contribution of risk factors.

Results

Age and Sex Distribution of Patients with Otitis Media

A total of 166 (45.3 %) males and 200 (54.6 %) females were analyzed at the National Health Laboratory Microbiology unit during the study period. The mean age of the study participants was 3.91 (3.5 ±2.96 [SD]) years with the minimum and maximum ages of 1 month and 12 years old, respectively. (Table 1)

Prevalence of Isolated Bacteria from Ear Discharge samples

In this study, bacteria were isolated from 305 (83.33 %) of the patients. Out of the total bacterial isolates, 251 (82.2%) were pathogenic and the remaining 54 (17.7%) were non-pathogenic. (Figure 1) Among the pathogenic bacterial species, the predominant isolates were Staphylococcus aureus 100 (39.8%) followed by Haemophilus influenzae 70 (27.8%) and Pseudomonas aeruginosa 81 (32.2%). Gram-negative pathogenic bacteria 151 (60.1%) were more dominant than Gram-positive pathogenic bacteria 100 (39.8%).

Patients with positive culture results, single and mixed infections were seen in 228 (62.2%) and 77 (21.0%), respectively. 61 (16.6 %) samples showed negative culture results for bacteria. (Figure 2)

The highest number of bacteria 230 (75.4%) were isolated in the age group of less than 5 years. S. aureus 110 (47.8%), P.aeruginosa 65 (28.2%), and H. influenza 55 (23.9%) were the dominant bacterial isolates in this age group. In another age group of 6-10, the number of children affected was 50 (16.3%) and the distribution of S.aureus, P.aeruginosa, and H.influenza was (50%), (30%) and (20%) respectively.

Table1. Socio-demographic of patients with ear discharge at ENT center of Orotta referral hospital, Eritrea.

Variable		Frequency (%) of Otitis Media (n=366)	Positive for Bacterial infection (n=305)
Age	<5	278 (75.9)	230 (75.4%)
	6–10	62 (16.9)	50 (16.3%)
	>10	26 (7.10)	25 (8.19%)
Sex	Female	200 (54.6)	150 (49.1%)
	Male	166 (45.3)	155 (50.8%)
Residence	Rural	94 (25.6)	250 (82%)
	Urban	272 (74.3)	55 (18%)

Overall antibiotic susceptibility pattern of isolated bacteria from ear discharge

During the study period, a total of 20 antimicrobial agents, 10 each for pathogenic Gram-positive and negative bacteria, were used to test their antibiotic susceptibility patterns.

Discussion

This study revealed that S. aureus, P. aeruginosa, and H.influenzae were the most prevalent multi-antibiotic -resistant pathogenic bacteria isolated from suspected patient ear discharges with otitis media admitted to the ENT center of Orotta Hospital.

In this study, the prevalence of bacteria among OM patients was 91.5 % (22-27). This was in tandem with reports from Ethiopia 91.7 %,89.4 % (28), 89.5 % (24), 100 % (29), and Nigeria, 81.9 % (30). Gram-negative bacteria (60.1 %) were the dominant isolates of the discharging ears compared to Gram-positive bacteria (39.8%). Similar reports were seen in Gondar (56.4 %) (24), Dessie (74.2 %) (31), Addis Ababa (60.5 %) (32), and Nigeria (75 %) (33) although the proportion varies. Isolation of Gram-negative bacteria indicates individuals are at risk of infection due to poor hygiene conditions. The predominant bacterial etiology of OM in this study was S. aureus which was (39.8%) and this observation was in line with studies from Addis Ababa (32).

P. aeruginosa and H. influenza were the second and third dominant bacteria in this study which were (32.2%) and (27.8%), respectively, this was in line with reports from Israel (34). Other researchers have also reported P. aeruginosa as the most dominant cause of OM (33).

In this study, a single infection was seen in (62.2%) of the patients. This observation was supported by other researchers elsewhere in the world (30). While

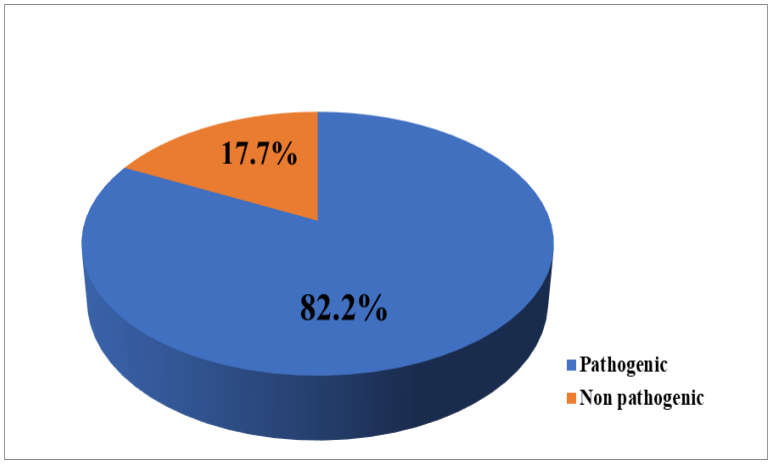

Figure 1. Pathogenic and non-pathogenic bacteria isolated among ear discharge patients attending ENT Orotta Hospital, Eritrea.

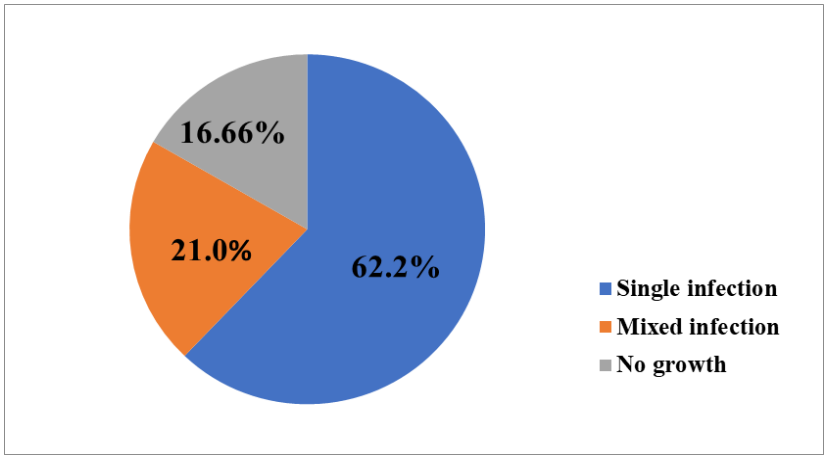

Figure 2. Single and mixed infection among ear discharge patients attending ENT Orotta Hospital, Eritrea.

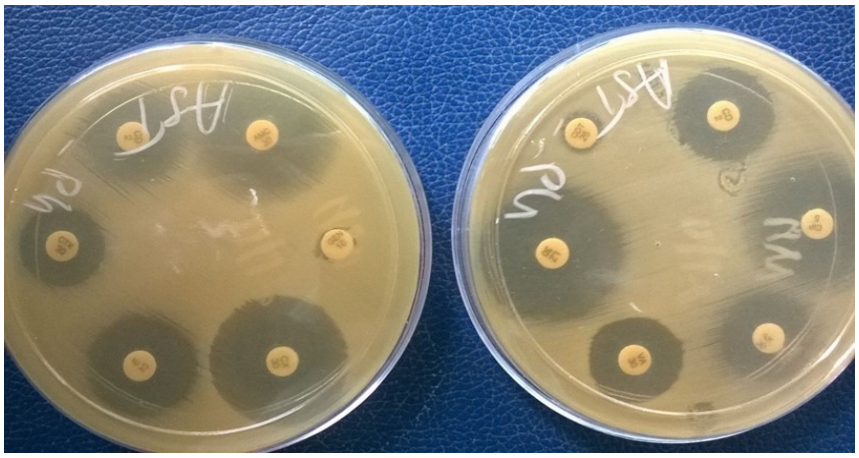

Figure 3: Mueller Hinton agar supplemented with 2-4% NaCl showing Antibiotic susceptibility testing and showing sensitivity towards Oxacillin streaked with S.aureus.

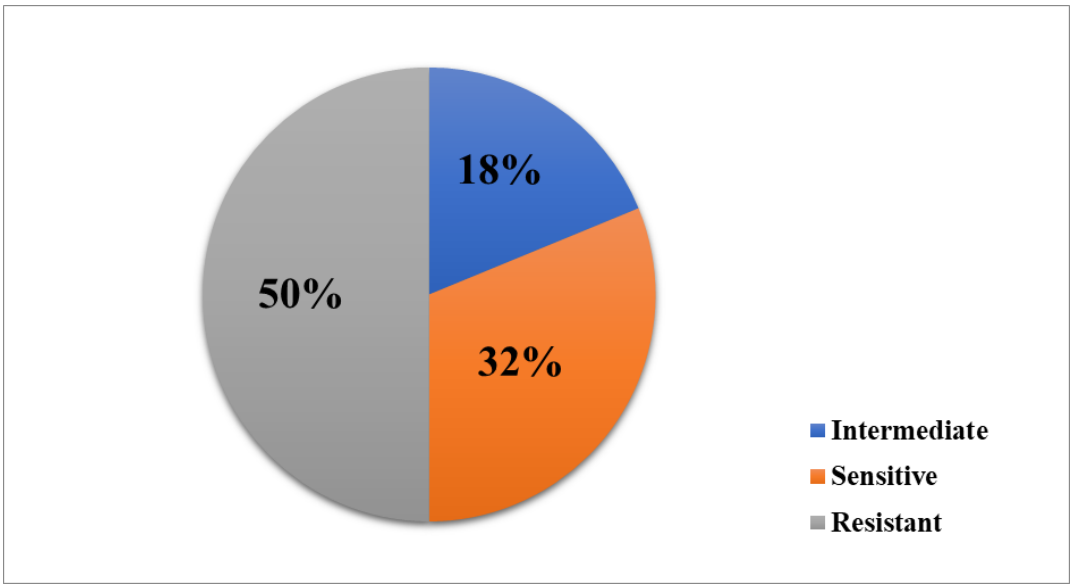

Figure 4. Overall antibiotic susceptibility of Gram-positive bacterial isolates from ear discharge patients attending ENT Orotta Hospital, Eritrea.

Table 2. Antimicrobial resistance pattern of dominant bacterial isolates from ear discharge samples of study participants at ENT, Orotta referral hospital, Eritrea

Antibiotics	**S. aureus (n=100)**		**P.aeruginosa (n = 70)**		**H. influenzae (n = 81)**	
	S%	R%	S%	R%	S%	R%
Ampicillin	NA	NA	0	85. 71	100	0
Ceftazidime	NA	NA	0	100	42.8	57.1
Ceftriazone	NA	NA	0	100	100	0
Chloramphenicol	80.9	14.2	28.5	71.42	71.4	14.2
Ciprofloxacin	63	26.3	100	0	85.7	14.2
Cotrimoxazole	57.8	26.3	0	100	0	100
Gentamycin	36.8	21	14.2	42.85	42.8	42.8
Nalidixic acid	NA	NA	0	100	100	0
Nitrofurantoin	31.5	26.3	0	100	28.5	42.8
Tetracycline	NA	NA	0	100	71.4	28.5
Clindamycin	66.7	9.5	NA	NA	NA	NA
Erythromycin	57.1	28.5	NA	NA	NA	NA
Oxacillin	28.5	61.9	NA	NA	NA	NA
Penicillin	23.8	76.1	NA	NA	NA	NA
Vancomycin	52.3	28.5	NA	NA	NA	NA

Table 4A Questionnaire data results (socio demographic)

Questionnaire Data Results				
Variables	Frequency	Case (366)	Control (34)	p-value
Age	<5	277 (75.6%)	14(41.1%)	0.003
	5-10	25 (6.8%)	18 (52.9%)	
	>10	64 (17.4%)	2(5.8%)	
Sex	Male	167(45.6%)	12(35.2%)	0.002
	Female	199(54.3%)	22(64.7 %)	
Parents Educational level	Elementary	83(22.6%)	10(29.4 %)	0.077
	Junior	110 (30%)	10(29.4 %)	
	High school	173 (47.2 %)	14 (41.1 %)	
Recurrent ear infections in family members	Yes	122(33.3%)	12(35.2%)	0.940
	No	244(66.6%)	22(64.7%)	
Frequent hygiene practice	Yes	132(36.0 %)	22(64.7%)	0.024
	No	234 (63.9%)	12(35.2%)	
Number of children in a family of more than four	Yes	241(65.8%)	32 (94.1%)	0.000
	No	125(34.1%)	2 (5.8%)	
Recurrent URTI	Yes	233 (63.6%)	4 (11.7%)	0.000
	No	133(36.3%)	30(88.2%)	
Birth weight	Normal	283 (77.3%)	32(94.1%)	0.044
	Abnormal	83 (22.6%)	2(5.8%)	
Day care attendance	Yes	249 (68%)	10 (29.4%)	0.036
	No	117 (31.9%)	24 (70.5%)	
Parental smoking and exposure to wood smoke	Yes	139 (37.9%)	12(35.2%)	0.258
	No	227(62%)	22(64.7%)	

Table 4B Questionnaire data results (associated factors)

Material used to clean	**Weak lean**	**81 (22.1%)**	**8 (23.5%)**	
	Cloth	147 (40.1%)	13 (38.2%)	**0.515**
	Cotton	138 (37.7%)	13 (38.2%)	
Duration of disease	Acute	260 (71%)	-----	
	Recurrent	61 (16.6%)	-------	0.258
	Chronic	45 (12.2%)	------	
Otalagia	Yes	272 (74.3%)	16 (47%)	
	No	94 (25.6%)	18 (52.9%)	
Hearing loss	Yes	106 (28.9%)	15 (44.1%)	0.836
	No	260 (71%)	19 (55.8%)	
Duration of breastfeeding	<10 months	158 (43.1%)	8 (23.5%)	
	>10 months	208 (56.8%)	26(76.4%)	0.311
Recent case of measles & pneumonia	Yes	167 (45.6%)	15 (44.1%)	
	No	199 (54.3%)	19 (55.8%)	
Residence	Urban	294 (80.3%)	21(61.7%)	0.224
	Rural	72 (19.6%)	13 (38.2%)	

Table 5. Multivariate analyses of potential risk factors of otitis media

	B	S.E.	Wald	Sig.	Exp(B)	95% C.I.for EXP (B)	
						Lower	Upper
Day care	1.141	.543	4.419	.036	3.131	1.080	9.075
Recurrent URTI	2.256	.588	14.735	.000	9.547	3.017	30.210
Age patient	-.022	.008	8.682	.003	.978	.964	.993
Birth weight	-1.771	.877	4.073	.044	.170	.030	.950
No of children	2.185	.571	14.627	.000	8.889	2.901	27.235
Constant	.686	1.909	.129	.719	1.987		

the mixed infection in our study was only (21.0%), other researchers, however, found mixed infection was more prominent in OM (35).

In vitro, antimicrobial susceptibility patterns revealed that the isolates were highly resistant to most of the antibiotics used. None of the bacterial isolates were sensitive to all antibiotics used. S. aureus showed the highest resistance rate to Penicillin (76.1%). This result was in line with the study done in Ethiopia where a 93 % resistance rate to penicillin was reported (32). H.influenzae and P.aeruginosa were the second dominant bacteria and showed a 100% resistance to some drugs. H.influenzae showed a 100% resistance to Cotrimoxazole, whereas P.aeruginosa exhibited 100% resistance to each of the following antibiotics: Ceftazidime, Ceftriaxone, Nalidixic acid, Tetracycline, Nitrofurantoin, and Cotrimoxazole. In contrast, H. influenza was 100% sensitive to Ampicillin, Ceftriaxone, and Nalidixic acid, whereas P.aeruginosa was 100% sensitive to ciprofloxacin.
P.aeruginosa was the most resistant isolate to many antibiotics in this study, which is in agreement with another researcher (33). Resistance to tetracycline and nitrofurantoin was 100 %; a similar result was reported in Nigeria (30).

This high drug resistance might reflect the degree of misuse of antibiotics, which is a global problem mainly through their purchase without prescription in local pharmacies and drug stores and through inappropriate prescribing habits and an over-zealous desire to treat every infection (28).

Since Otitis media is insidious and usually seen in small children, so early diagnosis is not always possible. Therefore, it is essential to establish the risk factors for OM, both to prevent its occurrence and to determine the proper treatment plan for affected children.

There was a slight statistically significant difference in the prevalence of ear infections in gender which is a similar finding to the report by Hassan (36), and Amusa (37). Females were more affected by ear infections than Males (54.6% vs. 45.4%) (P = 0.017). This may be due to the difference between the ear-cleaning habits of males and females. In some traditions, females use cotton swabs to clean their ears and this may contribute to the introduction of microorganisms from the external surface to the middle ear. However, according to the logistic regression analysis, the sex of subjects was not a potential risk factor.

Episodes of acute otitis media and URTI were found to be statistically significant (<0.001), which was similar to the literature recommending that a child with previous acute otitis media and URTI episodes have regular otorhinolaryngology examinations (39). It is also evident that in developing countries like Eritrea, there can be a greater incidence of chronic or relapsing infections of the upper respiratory tract; some of these infections will become complicated by middle ear infections because these people seldom go to physicians for light diseases. As viral and bacterial otitis has a high degree of spontaneous healing, many of those episodes may never be diagnosed. The higher social classes, with a greater concern for prompt medical care, will go to the physician to resolve every episode of infection and will reduce their risk of suffering OM complications.

In the current study, otitis media were found to be very prevalent in young children of less than 5 years which corroborates results from Ethiopia (32), and Nigeria (33). This indicates that less than five years children were more affected by ear infections. This may be due to different factors such as the anatomy of Eustachian tubes, the nutritional status of the chil-

dren, and other health problems like upper respiratory tract infections which are common in children (39). Children with OM are found to have a larger number of family members in the household than normal children (p= <0.0001). From the multivariate regression model, it is found that having more than 4 family members is one of the potential risk factors of OM (OR= 2.185, 95% CI: 2.901-27.235). This association can only be explained as it is as a result of overcrowding in the family which can lead to recurrent URTI epidemics among the household children that play a great role in the development of otitis media (31) and overcrowding in one room and poorly ventilated houses which consist of housing with a central passageway in which they share same toilet, bathroom and kitchen. This type of housing, which is usually overcrowded, may contribute to the higher proportion of children with OM. Amusa in his/her work had reported a significant association between overcrowding and otitis media (37).

A significant increase in OM in children attending daycare centers has been indicated in this current study. This is linked to the increased prevalence of otitis media in developing countries (11, 40). This is because children who attend daycare centers experience more upper respiratory infections when compared with children cared in a family home (41-43). Parental smoking and exposure to wood smoke were not statistically significant in the current study.

Birth weight results in a statistically significant association (P-value 0.04), and this can be explained as unmeasured socioeconomic factors could be responsible, genetics could also play a role in making these infants more susceptible, also it could result in low immunity that makes them prone to recurrent OM (44) and there were no overweight cases in this research.

Conclusion

The predominant isolates were S.aureus, H. influenzae, and Pseudomonas aeruginosa. The majority of the bacterial isolates had multiple antibiotic-resistant patterns. Gram-positive isolated bacteria were particularly found to be highly resistant to Penicillin and Oxacillin treatments. However, Chloramphenicol, Clindamycin, and ciprofloxacin were effective against most of the Gram-positive bacterial isolates. However, for Gram-negative bacterial isolates Ciprofloxacin, Nalidixic acid, and Ceftriaxone were highly sensitive. Less than 5 years of age, family size of more than 4 members, Recurrent URTI, daycare, and birth weight were found to be risk factors of OM in this study.

Ethical considerations

The study got ethical clearance from the research ethical committee of Asmara College of Health Sciences (ACHS) and the Ministry of Health, Asmara, Eritrea (MOH). Written informed consent was obtained from the parents of each child before inclusion.

Limitations

The study did not isolate fungi and viruses which are also the causative agents for OM. Time constraints to get a sufficient sample size.

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