Original Article
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Correlation of Bacterial Isolates from Middle Ear and Nasopharynx in Patients with Chronic Suppurative Otitis Media in Ilorin, Nigeria

Olushola Abdulrahman Afolabi[1], Foluwasayo Emmanuel Ologe[1], Charles Nwabuisi[2], Adekunle Ganiyu Salaudeen[3], Olalekan Tajudeen Ajiboye[4], Clement Chukwuemeka Nwawolo[5]

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[1] *Department of Ear, Nose and Throat Surgery, University of Ilorin/UITH, PMB 1515, Ilorin, Nigeria*

[2] *Department of Microbiology, University of Ilorin/UITH, PMB 1515 Ilorin, Nigeria*

[3] *Department of Epidemiology and Community Health, University of Ilorin/UITH, PMB 1515, Ilorin, Nigeria*

[4] *Family Medicine Department;UITH, PMB 1459, Ilorin, Nigeria*

[5] *Ear, Nose and Throat Surgery Department, University of Lagos/LUTH, PMB, 56, Akoka, Yaba, Lagos, Nigeria*

Abstract

***Purpose*: To determine the association between isolates in the middle ear (ME) and nasopharynx of patients with chronic otitis media in Ilorin, north-central Nigeria.**

***Methods:* An ethically approved case control study was carried out in the Ear, Nose, and Throat clinic amongst consenting cases using normal subjects as controls. A microbiology investigation form giving the results for otoscopy, aspirate and swabs was filled out for both the ME and nasopharynx. The experimental procedure was carried out and bacteria were identified according to colony characteristics, morphological appearance, Gram-staining, and standard biochemical testing. Data obtained were analysed with SPSS version 16.0 and Epi Info 3.5.1 using the mean, standard deviation and chi-square results.**

***Results:* A total of 140 cases and 70 controls, were recruited. The Gram stain reaction of the ME aspirates were positive in 28.6% and negative in 71.4% of cases. Nasopharyngeal swabs revealed 64.3% Gram positive and 35.7% negative organisms. Overall, there was no relationship between the ME and nasopharyngeal isolates amongst cases, with a P value of 0.000. However, there was a relationship amongst the isolate from the nasopharynx of cases and controls, with the exception of *Klebsiella pneumoniae*, at $P < 0.009$.**

***Conclusion:* There was no relationship amongst the bacterial isolate from the ME and nasopharyngeal specimen of patients with otitis media.**

Keywords: *chronic otitis media, nasopharyngeal swab, middle ear, aspirate, microbiology, correlation, relationship*

Introduction

Chronic otitis media (COM) has been found to be one of the most common chronic infectious diseases of the ear in childhood worldwide, with wide coverage irrespective of race or cultural groups, in both developing and industrialised countries. Chronic suppurative otitis media (CSOM) will be described as chronic otitis media, and it is defined as chronic inflammation of the middle ear (ME) and mastoid mucosa with persistent tympanic membrane perforation and discharge (otorrhoea)(1–3). However, there is no conclusive agreement concerning the duration of symptoms. Some researchers from the World Health Organization (WHO)(4) defined suppurative otitis media as 'otorrhea through a perforated tympanic membrane present for at

least two weeks', while others define the term 'chronic' as symptoms persisting for more than six weeks (1,5–8).

The clinical diagnosis of COM points toward a permanent abnormality of the pars tensa or flaccida, probably as a result of a previous acute otitis media which has been poorly managed; negative ME pressure from the spread of inflammation in the nasopharyngeal area, affecting the opening of the Eustachian tube (ET); or a ME effusion(9). One of the most significant pathogenic factors in the development of otitis media is ET dysfunction, most likely resulting from inflammatory response. From a pathogenic point of view, the nasopharyngeal transmission of pathogenic bacteria is a risk factor for otitis media (10,11), as similar bacteria have been identified in the ME and nasopharynx (12). Children have shorter, straighter, and more compliant ETs than adults (13). This permits a reflux from the nasopharynx to the ME, with the consequence of bacterial contamination (14). Risk factors worldwide include young age, overcrowding, poor housing, poor hygiene, poor nutrition, ET dysfunction and inadequate or unavailable health care (15,16), in addition to poverty in developing countries (17).

To the best of the author's' knowledge, there has been limited study on the association between the isolates from the ME and nasopharynx in Nigeria, as most studies have focussed on either the ME alone or the nasopharynx alone. Thus, the aim of this study is to determine the association between isolates from ME aspirates and nasopharyngeal swabs amongst patients with chronic otitis media (case) in Ilorin, north-central Nigeria.

Materials and Methods

Background study area

This study was carried out at the Ear, Nose and Throat (ENT) Department of the University of Ilorin Teaching Hospital (UITH), Ilorin.

Study population, sampling technique and sample size

This was a case control study of all consenting patients with CSOM (cases) aged 5–65 years attending the ENT outpatient clinic of the UITH with a control group consisting of normal subjects with no ear, nose, and throat complaint within the specified age group seen at the General Outpatient Department (GOPD) of the hospital. Patients with COM who met the inclusion criteria were selected for the study and interviewed using the convenience sampling technique, with written informed consent obtained from each of the patients. All patients were diagnosed clinically as having COM using a 0° 6 cm x 4 mm Karl-Storz rigid telescope attached to a mobile light handle complemented with temporal–mastoid bone computed tomography. The controls were individuals without a history of COM either in the present or in the past and no active upper respiratory tract infection. The sample size was calculated using Fisher's formula for a cross-sectional study as follows (13):

$$N = \frac{Z^2 P Q}{d^2}$$

N = desired sample
Z = the normal standard deviation (SD), which is 1.96 (at a 95% confidence interval)
P = prevalence of the problem (the prevalence of otitis media in north-central Nigeria was 7.3%[18])
Q = 1-P Q =1-0.073 d = 0.05

$$N = \frac{(1.96)^2 \times 0.073 \times 0.923}{(0.05)^2}$$

103.5372 was the calculated minimum sample size. To compensate for the non-response of the subjects, assuming that a 75% response rate would be achieved, the sample size was determined as follows[14]:

Ns = N/0.75 = 104/0.75 = 139
Sample size = 140

A sample size of 140 was chosen and an age- and sex-matched control group of 70 normal subjects was included in the study.

Data collection

A structured study questionnaire was used to obtain the relevant information from the patients, parents or guardians and control subjects. The inclusion and exclusion criteria for the study are given in table 1.

Sample collection

With the subject sitting comfortably opposite the investigator, a head mirror and dry cell battery-powered auroscope with good magnification were used to view the external auditory canal

Table 1: The inclusion and exclusion criteria for patient selection for the study

Inclusion criteria	Exclusion criteria
Patients who fell within the specified age group for the study	Patients < 5 years, as they will not cooperate to allow collection of a nasopharyngeal specimen, and those > 65 years due to possible co-morbidities
Patients with a clinical diagnosis of COM attending the ENT clinic for the first time	Patients taking either topical or systemic antibiotics within the previous two weeks
Patients who consented to take part in the study	Patients who had had surgery for ear discharge
No treatment with antimicrobials for at least two weeks prior to presentation	Patients with distorted normal anatomy of ME structures
	Patients with cleft palate, because of the possibility of persistent contamination of the nasopharynx with oral pathogens due to a direct connection
	Patients who did not consent to the study.

and the tympanic membrane. The external ear was cleaned with 60% alcohol, and 0.1–0.5 ml of ME aspirates were taken through the perforated tympanic membrane using a volume automated micropipette.

With the patient in a comfortable sitting position and mouth widely opened, a swab was taken from the nasopharynx using a 1200 angulated Evepon™ sterile swab stick as the subject was asked to say 'Ah' to elevate the uvula and the soft palate. The tongue was depressed using a lax tongue depressor. Both aspirate and swab were preserved in a separate 'bijou' bottle containing sterile Stuart transport medium microscopy and culture of the various organisms. The controls were normal subjects without ear discharge who also had their nasopharyngeal specimen taken.

Using a sterilised forceps, the swab stick was removed from the Stuart transport medium and inoculated onto blood, chocolate and MacConkey agar plates. The smear was streaked to give discrete colonies using a sterilised inoculating wire loop. Blood agar and MacConkey plates were incubated under an aerobic conditions, while the chocolate agar plates were incubated in a candle extinction jar at 37.0°C for about 18–24 hours. Following this, the discrete colonies on the plates were read and identified based on the standard methods (19).

Statistical analysis

The data collected were entered into the computer using SPSS version 16. The mean and the SD were used in the analysis of the age of the subjects (cases and control). To determine the association between the pattern of Gram staining and types of bacterial isolates in the ME and nasopharynx of cases (patients with CSOM) and controls (persons without CSOM), chi-square was used as a test statistic. The test of significance was set at 0.05 at a 95% confidence interval.

The study was approved by the ethical review committee of the UITH and consent was taken from both the patients and their caregivers and the controls. As part of the ethical consideration, the respondents were also informed of their right to refuse to continue with the study at any time. The confidentiality and anonymity of the data collected were also maintained at all times. The protocol was carried out according to the guidelines set out by the Helsinki Declaration (20). All data generated from the study were checked manually for errors in completion of the responses. Frequency data were generated from the data collected to show the distribution of the study characteristics and the relationship between the various variables was determined using the chi-square test. Statistical significance was taken as a *P* value less than or equal to 0.05. Anaerobic studies were not undertaken at the time of the study because of a lack of facilities.

Results

A total of 140 cases consented to participate in the study. There were 95 males and 45 females, representing a male-to-female ratio of 2:1. Seventy individuals who were suitably matched for age and gender (without COM) were enrolled as controls for the study. There was an equal number of males and females in the control group (35 each). About 33% were in the age range of 5–10 years (Table 2);

the mean age for the patients was 15.0 (SD 14.1 ± 1.30) years and the mean age for control was 14 (SD 13.9 ± 1.8)

Table 2 reveals that the Gram staining in the ME yielded more Gram-negative (71.4%) compared to Gram-positive cultures. Conversely, about two-thirds (64.3%) of the nasopharyngeal isolates yielded Gram-positive compared with Gram-negative cultures (35.7%). The observed difference in the Gram staining pattern of the ME and nasopharynx was statistically significant (χ^2 = 35.9, P = 0.000).

From Table 3, it can be observed that about two-thirds of isolates in the nasopharynx of cases showed that (90, 64.3%) were Gram positive, while among the controls, there were almost equal proportions of isolates with Gram-positive (51.4%) and Gram-negative cultures (48.6%). There was no statistically significance difference in the Gram stain reaction in the nasopharynx of cases and control (χ^2 = 3.21, P =0.073).

In Table 4, it can be seen that of the 140 isolates from the ME and nasopharynx; *Pseudomonas aeruginosa* was isolated in 45 cases (32.1%), while 13 cases (9.3%) revealed the presence of P. aeruginosa in the nasopharynx. There was a statistically significant difference in the presence of P. aeruginosa in the ME and nasopharynx (P = 0.001). Meanwhile, *Klebsiella pneumoniae* was isolated from 22.9% of the MEs of cases and 8.6% of the nasopharynxes of cases. The observed difference in the isolates in these two regions in cases was statistically significant (P = 0.009. Similarly, *Enterococcus faecalis* was isolated in 15 (10.7%) of the MEs while 68 (48.6%) were found in the nasopharynxes of cases. There was a statistically significance difference in the E. faecalis isolates from both the MEs and nasopharynxes of cases (P = 0.001).

Staphylococcus aureus was found in 15.7% of the ME isolates and 10.7% of the nasopharyngeal isolates; there was no statistically significant difference (P = 0.215). Further, *Proteus mirabilis* and Escherichia coli revealed no significant difference in the ME and the nasopharynx (P >0.05).

In the comparison of the pattern of bacterial isolates in the nasopharynx of the cases and the controls showed no statistically significant difference amongst the bacterial isolates in the nasopharynxes of both the cases and control (P > 0.05; Table 5).

Discussion

Upper and lower respiratory bacterial infections, which include pneumonia, acute bronchitis, acute sinusitis, and suppurative or non-suppurative otitis media amongst children and adults represent a major clinical concern (21,22) and this was one of the rationale for our present study. Some of the common bacterial organisms which cause upper respiratory tract infections are *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis* (22). The human nasopharyngeal space is wide, making it an ecological reservoir for a variety of commensal bacterial pathogens which colonise this space, as

Table 2: Distribution for subject Age group n=140

Age (years)	Frequency (%)	
	Case	Control
5-10 years	46 (33)	17 (24.3)
11-20 years	26 (18.6)	13 (18.6)
21-30 years	18 (12.9)	8 (11.4)
31-40 years	20 (14.3)	9 (12.9)
41-50 years	6 (4.3)	7 (10.0)
51-60 years	10 (7.1)	9 (12.9)
61-70 years	14 (10)	7 (10.0)
Total	140 (100)	70 (100)

The mean age for the patients was 15.0±1.30 (14.1SD) years
*n is the number of patients

Table 3: The gram stain reaction of bacterial isolates from the middle ear and nasopharynx in patients of cases and controls

Gram Stain Reactions	Sites Frequency (%)	
	Middle ear	Nasopharynx
Cases		
Gram Positives	40(28.6)	90(64.3)
Gram Negatives	100(71.4)	50(35.7)
Control		
Gram Positives	0	36(51.4)
Gram Negatives	0	34(48.6)

Table 4: Comparison of pattern of bacterial isolates from the middle ear and nasopharynx of cases, n = 140

Types of bacterial isolates	Frequency of isolates with CSOM (%)		χ^2	*P* value
	Middle ear	Nasopharynx		
Pseudomonas aeruginosa	45 (32.1)	13 (9.3)	19.24	0.001*
Klebsiella pneumoniae	32 (22.9)	12 (8.6)	10.88	0.009*
Staphylococcus aureus	22 (15.7)	15 (10.7)	1.54	0.215
Proteus mirabilis	20 (14.3)	28 (20.0)	0.94	0.331
Streptococcus faecalis	15 (10.7)	68 (48.6)	43.10	0.001*
Escherichia coli	06 (4.3)	04 (2.9)	0.42	0.519
Total	140 (100)	140 (100)		

*Statistically significant

Table 5: Pattern of bacterial isolate in the nasopharynx of patient with and without chronic suppurative otitis media.

Bacterial isolates	Frequency of Nasopharyngeal isolate (%)		df, χ^2	*P* value
	With CSOM Patients n = 140	Control n=70		
Streptococcus faecalis	67 (47.8)	30 (42.9)	0.88	0.347
Proteus mirabilis	29 (20.7)	13 (18.6)	0.28	0.597
Staphylococcus aureus	15 (10.7)	04 (5.7)	0.40	0.527
Pseudomonas aeruginosa	13 (9.3)	02 (2.9)	0.91	0.340
Klebsiella pneumoniae	12 (8.6)	20 (28.6)	12.88	0.003
Escherichia coli	04 (2.9)	01 (1.4)	0.02	0.886

n for patient = 140; n for control = 70

evidenced by our findings (23,24). Colonisation of the nasopharyngeal space is an essential step in the development of respiratory bacterial infections (23).

Our study evaluated the relationship between the nasopharynx and chronic ME infection, as both are connected by an ET. Previous studies have shown that otitis media is a major health challenge among children and adolescents, occurring with a high incidence and prevalence in both developed and developing countries (15,25,26). About one-third of the cases in our study were within the age range of 5–10 years, which represents the peak period of occurrence of CSOM in this study. A lower age group has been found in previous studies at our centre (16); however, the age of five years was considered as our lowest limit to ensure cooperation for nasopharyngeal specimen collection. The duration of presentation of the symptoms may be earlier than presented thus it may be more apparent than real, since the age recorded in this study was the age at presentation and not the age at onset of the disease. This supports the view that the high prevalence of COM is evident in the younger age group. Moreover, this is similar to findings by other researchers (25, 27).

The present study showed a higher occurrence of COM among males than females; this may be the result of the higher value placed on males than females in this part of the world, which would be similar to findings by Bakari and Rahman et al. (25, 26, 28) but at variance with a recent study by Chang et al. in Seoul (29). In contrast, a national study on hearing in the United Kingdom found no gender difference (30).

COM is characterised by persistent or recurrent ME discharge through an existing tympanic membrane perforation and can worsen with recurrent respiratory infections (31). Bacteria

can get into the ME via the external auditory canal from a perforated tympanic membrane or following a persistent upper respiratory infection with insufflations of microbial agent via the pharyngeal part of the ET (30). This study found no strong statistical difference in the observed Gram stain reactions using the Epi Info statistical software between the organisms in the ME and nasopharyngeal specimens amongst the cases, which is similar to findings by previous researchers (32–35). Thus, overall, there was no association between the observed Gram reaction in the ME and nasopharyngeal specimens amongst the cases.

The comparison of bacterial isolates revealed a statistical association, as the P value was < 0.05 in specific organisms like *P. aeruginosa, K. pneumoniae* and *E. faecalis* in the ME and nasopharyngeal specimen (Table 3). There was no association between *S. aureus, P. mirabilis* and *E. coli* isolated from the ME and nasopharyngeal specimen of cases ($P > 0.05$).

The most prevalent organism in the nasopharynx was E. faecalis (31), which is a common microbial agent in acute otitis media, as reported by Bakari (25). In comparison, P. aeruginosa which was the most prevalent in the ME of the patients with COM, similar to previous reports from our centre (26, 31, 37). Statistically, there was a significant difference between the prevalence results of P. aeruginosa found in these two regions ($P < 0.001$).

Overall, there was no association between the Gram stain pattern in the ME and nasopharyngeal specimens amongst the cases. The comparison of patterns of bacterial isolates revealed that there was an association in specific organisms like P. aeruginosa, K. pneumoniae and E. faecalis in the ME and nasopharyngeal specimens (Table 3). There was no association between S. aureus, P. mirabilis and E. coli isolated from the ME and nasopharyngeal specimens of cases (patients with CSOM; $P > 0.05$). This is similar to findings by other researchers (31,38–40). The pattern of bacterial isolates in the nasopharyngeal specimens of cases and controls indicated a strong correlation, which revealed no association with a ($\chi^2=3.21$, $P = 0.073$). This indicated that, overall, there was no association amongst the organisms.

E. faecalis was found to more common in the nasopharynxes of cases than controls; however, it rated as the most prevalent organism, with a P value of 0.347, which indicated that there was no association. This showed that irrespective of whether individuals have COM or not, this organism is present in the nasopharynx, as reported by other researchers (21–23,31). Moreover, there was no association of *P. mirabilis*, which is a Gram-negative organism, amongst the subjects and the controls. *K. pneumoniae* showed a significant difference with a chi-square of 12.88 ($P = 0.003$), in the nasopharynx of both the cases and controls. This indicates that there was association in the nasopharyngeal specimens of both the cases and the controls with respect to this organism, which is similar to findings by Konno et al. and Gurnnasson et al. (39,40). In addition, the Gram stain pattern of these regions in the respondents showed no significance, with a chi-square of 3.21 (P = 0.073). Thus, there was no association.

Conclusion

In conclusion, COM was prevalent amongst children under 10 years; Gram-negative and Gram-positive organisms were most prevalent in the ME and nasopharyngeal specimens, respectively. *P. aeruginosa* and *E. faecalis* were the most prevalent organisms in the ME and nasopharyngeal specimens, respectively, of patients with COM. *E. faecalis* was found to be more common in the nasopharyngeal specimens of both subjects with COM and controls, and there was no association between the major organisms in the ME and nasopharyngeal specimen of patients with COM.

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Conflict of Interest

None

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Authors' Contributions

Conception and design, drafting of the article: OAA
Analysis and interpretation of the data: OAA, OGS, REO

Critical revision of the article for the important intellectual content: OTA, CN, REO, CCN
Final approval of the article: CN, REO, CCN
Provision of study materials or patient: OTA
Statistical expertise: OGS, CN
Administrative, technical or logistic support: OTA, CN

Correspondence

Dr Afolabi Olushola Abdulrahman,
MBBS (ABU, Zaria), FWACS (Otorhinolaryngology West Africa), FMCORL (Nigeria)
Department of ENT
University of Ilorin/University of Ilorin Teaching Hospital, PMB 1459
Ilorin, Nigeria
Tel: +234 80357 27069
Fax: +234 80357 27069
Email: droaafolabi@yahoo.com

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