Bima Journal of Science and Technology, Vol. 9(1B) Apr, 2025 ISSN: 2536-6041

DOI: 10.56892/bima.v9i1B.1277

Molecular Detection and Prevalence of Bacterial Pathogens Associated with Ear-Nasal-Throat (ENT) in Patients Attending Aminu Kano Teaching Hospital, Kano, Nigeria

Mustapha Abba[1], Akewula Munirudeen Ajeniyi[1], Abdullahi Mohammed[1], Abdullahi Auwal[1], Adamu Yakubu[1], Aliyu Adamu[1], Bashir Mohammed Abubakar[2], Ibrahim Mustapha[3] Bashir Muhammad[4] and Ibrahim Musa Moi[1]*

[1]Department of Microbiology, Sa'adu Zungur University, Bauchi State Nigeria
[2]Department of Biological Sciences, Sa'adu Zungur University, Bauchi State Nigeria
[3]Department of Medical Laboratory Science College of Health Sciences and Technology Nguru Yobe State, Nigeria
[4]Department of Human Anatomy, Sa'adu Zungur University, Bauchi State Nigeria

Corresponding Author: ibrahimamoi@basug.edu.ng

ABSTRACT

Ear, nose, and throat infections represent a significant public health issue in developing countries including Nigeria, resulting in a considerable burden of illness and economic strain on patients, families, and the healthcare system. Studies on the molecular information of bacterial pathogens associated with Ear-Nasal-Throat (ENT) in Northwest particularly in Kano is limited. This study aimed to determine the Molecular detection and prevalence of bacterial pathogens associated with Ear-Nasal-Throat (ENT) in patient attending Aminu Kano Teaching Hospital Kano (AKTH). A total of 384 samples from patients with ENT infections were collected at AKTH using sterilized swab sticks and cultured on Chocolate and MacConkey agar. Pathogens were identified through Gram staining, biochemical tests, and molecular methods. A questionnaire gathered demographic data on age and gender, and a chi-square test assessed the significance of associations between the variables. The overall prevalence of ENT in this study was 17.0%. The most common pathogen identified was *Staphylococcus aureus* (30.8%), followed by *Providencia stuartii* (23.1%), *Klebsiella pneumoniae* (20.0%), *Escherichia coli* (18.5%), *Serratia marcescens* (6.2%), and *Haemophilus influenzae* (1.5%). Females had a higher prevalence of ENT infections (19.0%) compared to males (14.4%), though not statistically significant ($\chi 2 = 1.482$, $p = 0.223$). The age group 21-30 had the highest prevalence at 27.3%, while those aged 31-50 had the lowest at 11.3%. A significant association was found between infections and age group ($p < 0.05$; $\chi 2 = 10.244$, $p = 0.037$). The current study indicates that the prevalence of bacterial pathogens associated with ear, nasal, and throat infections is moderate in the study area. This finding shows that infections of the ears, nose, and throat are still common, highlighting the urgent need for effective measures to eradicate these pathogens and improve public health.

Keywords: Molecular, Detection, Prevalence, Bacterial pathogens, Ear-nasal and throat, Patients, Infection.

INTRODUCTION

Ear, nose, and throat (ENT) infections are among the most common illnesses prompting medical attention (Witsell *et al.,* 2001). These infections affect individuals of all ages and are a leading cause of morbidity and mortality, particularly in critically ill patients (Witsell *et al.,* 2001). Due to their exposure to the environment and airborne microorganisms, the ear, nose, and throat are frequent infection sites.ENT disorders can significantly disrupt

DOI: 10.56892/bima.v9i1B.1277

daily life for both adults and children (Witsell *et al.,* 2001). As the global population grows, upper respiratory infections may continue to cause hearing loss and learning disabilities in children (Obiajurn and Chukuezi, 2012). Ear infections, like chronic otitis, can lead to delayed language development and academic challenges in developing countries (Newton *et al*., 2001).Symptoms of ENT infections vary in severity and may include pain, fever, headaches (Azeez, 2000), runny or congested noses, a sensation of fullness in the ears (Elaine *et al.,* 1987), and swallowing difficulties. More serious complications can involve meningitis and hearing loss. Factors such as poor hygiene, forceful nose blowing, and excessive sniffing can contribute to these infections (Schnert, 1996). Azeez (2000) highlighted that otitis is more prevalent in children, likely due to their narrower Eustachian tubes, which hinder proper drainage.

Bacterial species such as *Staphylococcus aureus, Streptococcus* spp., *Proteus* spp., *Haemophilus,* and *Escherichia coli* are found to be responsible for most cases of ear, nose, and throat (ENT) infections. Additionally, *Actinomyces israelii, Mycoplasma pneumoniae, Mycobacterium tuberculosis,* and *Corynebacterium diphtheriae* have also been implicated as causes of varying prevalence of ENT infections and diseases (Ikhe *et al.,* 1993; Bailey and Scott, 1992). Furthermore, research indicates that *Pseudomonas, Staphylococcus aureus, Proteus*, and *Klebsiella* are common bacteria causing ENT infections in Japura, India (Kumar *et al.,* 2013). Otitis media is currently recognized as the most common childhood infection, resulting in the deaths of over 50,000 children under the age of 5 each year (Witsel *et al.,* 2001). This condition is a significant health issue, with a high incidence and prevalence in both developed and developing countries. Otitis media can be classified as acute, chronic, or recurrent, and may be present as either suppurative or non-suppurative. Acute suppurative otitis media (ASOM) is a frequent illness in children and can progress to chronic suppurative otitis media (CSOM) (Afolabi *et al.,* 2012).

Ear-nasal-throat infection is a major public health concern in developing countries associated with a high burden of disease and economic impact on patients, families and the health care system. It is one of the most frequently encountered illnesses in children and adults leading to repeated outpatient department (OPD) visits in both developed and developing countries (Mairangthem and Angom, 2012). Ear infection have 2–8-fold higher incidence in Sub-Saharan Africa and South Asia than developed world and the associated complications lead to the death of around 20,000 people annually, the highest mortality rate being in < 5 children ((Acuin *et al.,* 2015). Hearing loss reduced learning ability and low scholastic achievements have been indicated due to the chronic and recurrent form of the disease (Acuin *et al., 2015*). The prevalence of ear, nose, and throat (ENT) disorders in Nigeria is notably high, with numerous studies highlighting significant health concerns (Ohuche *et al.,* 2023). Traditionally, pathogens associated with ENT conditions have been isolated using cultural and common biochemical methods; however, these approaches often yield unreliable and ambiguous results. Therefore, the present study aims to isolate and identify pathogenic bacteria linked to ENT disorders using molecular methods, as well as to determine their prevalence in the study area. This research may contribute to the development of policies for the rational use of antimicrobials and help mitigate the risk of antibiotic resistance.

DOI: 10.56892/bima.v9i1B.1277

MATERIALS AND METHODS

Study Area

The study was conducted at the Ear, Nose, and Throat (ENT) Department of Aminu Kano Teaching Hospital (AKTH) in Kano State, Nigeria. Established in 1988, the hospital is dedicated to providing excellent services through a committed workforce that utilizes resources judiciously, effectively, and efficiently. Kano State, located in the northern part of Nigeria, has coordinates of 11.7471° N latitude and 8.5247° E longitude. According to the 2006 national census, Kano is the most populous state in Nigeria. Subsequent estimates from the National Bureau of Statistics in 2016 confirmed that Kano State continues to be the largest state by population in the country. AKTH serves not only the North-West states but also several other states across Nigeria.

Study Design

The study employed a laboratory-based methodology accompanied by a straightforward questionnaire that collected information on age and gender. A cross-sectional investigation was conducted to identify patients exhibiting symptoms associated with ear, nasal, and throat infections. Informed consent was obtained from all participants, seeking their voluntary involvement in the study. Patients were selected using a simple random sampling technique and subsequently diagnosed in the laboratory to identify the pathogens responsible for their infections.

Study Participants

Participants were selected based on specific inclusion and exclusion criteria. The selection criteria included patients attending AKTH who exhibited various signs and symptoms of ear, nose, and throat (ENT) infections. Eligible participants were both male and female, aged between 1 and 70 years, and had provided their consent to participate. Patients without ENT-related symptoms or those who declined to participate were excluded from the study.

Sample Size Determination

The sample size for prospective cross-sectional study was obtained using Fishers and Fishers, (2009) formula below:

$n = \frac{z^2 pq}{r^2}$, since the entire patients (with ENT infection) population is far above 10,000 in Kano state.

Where n = desired sample size (of the population greater than 10,000)

z = standard normal deviation (usually set at 1.96 or more sample at 2.0) which correspond to 95% confidence.

P = proportion of target population (used as 0.50 if no reasonable population estimate)

q = 1.0 – p

r = degree of accuracy desired (usually 0.05 or occasionally 0.02).

From the above formula; $n = \frac{z^2 pq}{r^2}$

Then, $n = \frac{(1.96)^2 (0.5)(1.0-0.5)}{(0.05)^2}$

Therefore n = 384 samples were used in this study.

Sample/Data Collection

A total of 384 samples were collected from the patients attending Aminu Kano Teaching Hospital Kano (AKTH) with the complaint of Ear-nasal-throat infection. Sterilized swab sticks were used for sample collection. The samples were immediately transported to the microbiology laboratory at AKTH for further investigation. The following data such as; age and gender were recorded using a simple questionnaire.

DOI: 10.56892/bima.v9i1B.1277

Sample Processing

Samples were collected and inoculated onto both chocolate and MacConkey agar plates (OXOID, UK). The MacConkey agar plates were incubated aerobically, while the chocolate agar plates were incubated anaerobically in an anaerobic jar. All inoculated plates were incubated at 37°C. After 24 hours, the organisms that grew were identified using standard methods, which included observing the growth of pathogens, the sizes and shapes of colonies, their elevation, odour, colour, hemolysis patterns, and swarming movements, as well as performing Gram staining. Biochemical screening tests—such as catalase, coagulase, indole, citrate utilization, urease, and oxidase tests—were conducted to identify the organisms (Hailegiyorgis *et al.,* 2018). Additionally, all organisms identified by standard methods were later confirmed using molecular techniques.

DNA Extraction

Genomic DNA was extracted from the overnight cultures of each of the samples (Ear-nasal-throat) using the Wizard Genomic DNA Purification Kit (Promega USA) following the manufacturer's instructions. The concentrations and purities of the genomic DNA were determined using NanoDrop Lite Spectrophotometer (model: Thermo Fisher Scientific, USA) and the DNA was kept at -80°C until further analysis by PCR

PCR Amplification and Sequencing of 16S rRNA

The amplification of the 16S rRNA gene region was conducted using universal primers: the forward primer E16S-F (5′-CCCCCTGGACGAAGACTGAC-3′) and the reverse primer E16S-R (5′ ACCGCTGGCAACAAAGGATA-3′) (Wang *et al.,* 2002). For the PCR setup, the following components were added to the PCR tube: a total reaction volume of 50 µL, which included nuclease-free water, 1 µL of DNA template (300 ng), 25 µL of EconoTaq® PLUS 2X Master Mix (Lucigen, USA), and 1 µL of each primer (forward and reverse, at a concentration of 100 µM). The amplification process was carried out in a thermal cycler for 40 cycles under the following conditions: DNA denaturation at 94ºC for 1 minute, primer annealing at 56ºC for 30 seconds, and primer extension at 72ºC for 1 minute. Following amplification, the PCR product was combined with 5 µL of gel loading buffer. A 1.5% agarose gel was prepared, and the samples were loaded alongside 5 µL of a 1 kb DNA ladder (Thermo Fisher Scientific, USA) as a molecular marker. The gel was then run and visualized under a UV transilluminator (Syngeneic, USA) to observe the bands. The PCR products were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, USA), by the manufacturer's instructions, and were subsequently sequenced using an ABI Prism 3700 DNA Analyzer (Acme Progen Biotech (India) Pvt. Ltd., Salem, Tamil Nadu, India).

Sequence Analysis of 16S rRNA Gene and Construction of Phylogenetic Tree

The validation of the 16S rRNA sequencing data was carried out using BLAST software in the NCBI Gene Bank database, which is accessible at http://www.ncbi.nlm.nih.gov/. Both forward and reverse sequences were analyzed using Sequence Scanner Software v1.0 (Applied Biosystems, Thermo Fisher Scientific). For multiple sequence alignment, the Clustal Omega server provided by the European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI) was utilized. Finally, a phylogenetic tree was constructed from the sequence data employing maximum likelihood methods in MEGA 11.0 software.

DOI: 10.56892/bima.v9i1B.1277

Statistical Analysis

Data analysis was conducted using SPSS version 27.0. A chi-square test was performed to assess whether there is a significant association between the independent variables and the outcome variable. P-values less than 0.05 were considered statistically significant.

Ethical Approval

Ethical approval was secured from review boards of Sa'adu Zungur University Bauchi and Bayero University Teaching Hospital Kano State Nigeria. Informed written consent was obtained from each participant**.**

RESULTS

This research investigated the pathogens associated with ear, nasal, and throat infections. The pathogens were isolated and identified based on various characteristics, including their growth patterns, colony sizes, shapes, elevations, odours, colours, hemolysis properties, swarming movements, Gram staining, and biochemical analyses. The biochemical tests conducted included catalase, coagulase, indole, citrate utilization, urease, and oxidase tests. The overall positive rate of the isolated pathogens from ear, nasal, and throat samples among patients attending Kano Teaching Hospital was approximately 65 out of 384, representing 17.0%.

Among the bacterial pathogens isolated from ear, nasal, and throat samples, a significant majority were Gram-negative organisms, which included *Providencia stuartii, Klebsiella pneumoniae, Escherichia coli, Haemophilus influenzae*, and *Serratia marcescens*. The only Gram-positive bacterium identified was *Staphylococcus aureus*, which emerged as the most prevalent pathogen, comprising 20 out of 65 isolates (30.8%). Following *Staphylococcus aureus*, the Gram-negative bacteria were represented as follows: *Providencia stuartii* with 15 isolates (23.1%), *Klebsiella pneumoniae* with 13 isolates (20.0%), *Escherichia coli* with 12 isolates (18.5%), and *Serratia marcescens* with 4 isolates (6.2%). *Haemophilus influenzae* was the least prevalent, with only 1 isolate (1.5%) (Table 1).

Table 1: Distribution of the Bacterial pathogens isolated from Ear-Nasal-Throat.

Gram-reaction	Isolated species	No. of Positive	Percentage (%)
Gram- negative bacteria	*P. stuartii*	15	23.1
	K. Pneumonia	13	20.0
	E. coli	12	18.5
	H. Influenzae	1	1.5
	S. marcescens	4	6.2
Gram-positive bacteria	*S. aureus*	20	30.8

The bacterial isolates from the Ear-Nasal-Throat samples were further characterized through a molecular approach based on the homology of their 16S rRNA gene sequences, which revealed a single band, as depicted in Figure 1. To accurately identify and confirm the isolated bacterial pathogens, we employed BLAST to compare the 16S rRNA gene sequences with reference sequences available in the NCBI database. A phylogenetic tree was constructed for *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae*, and *Providencia stuartii* using the acquired gene sequences. The isolated pathogens demonstrated 99% similarity with *other S. aureus, E. coli, K. pneumoniae*, and *P. stuartii* sequences found in the NCBI database, as illustrated in Figure 2.

DOI: 10.56892/bima.v9i1B.1277

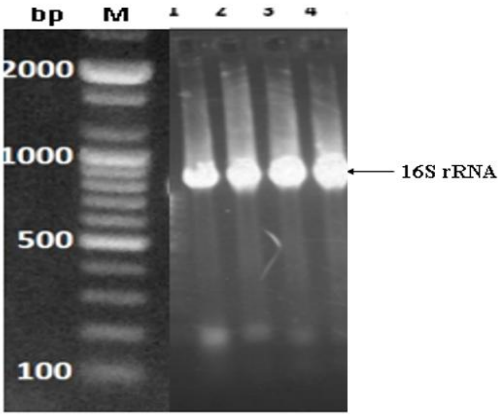
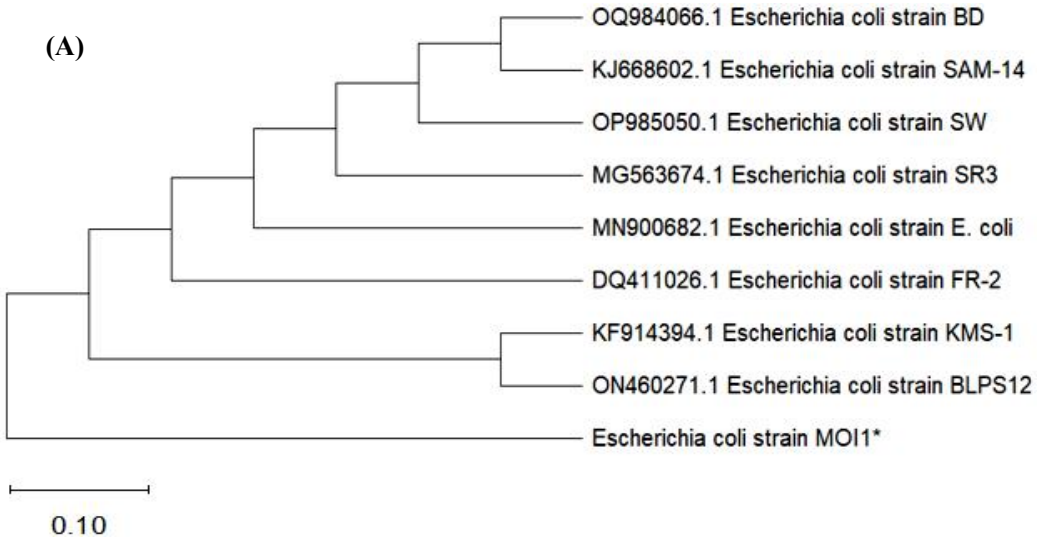
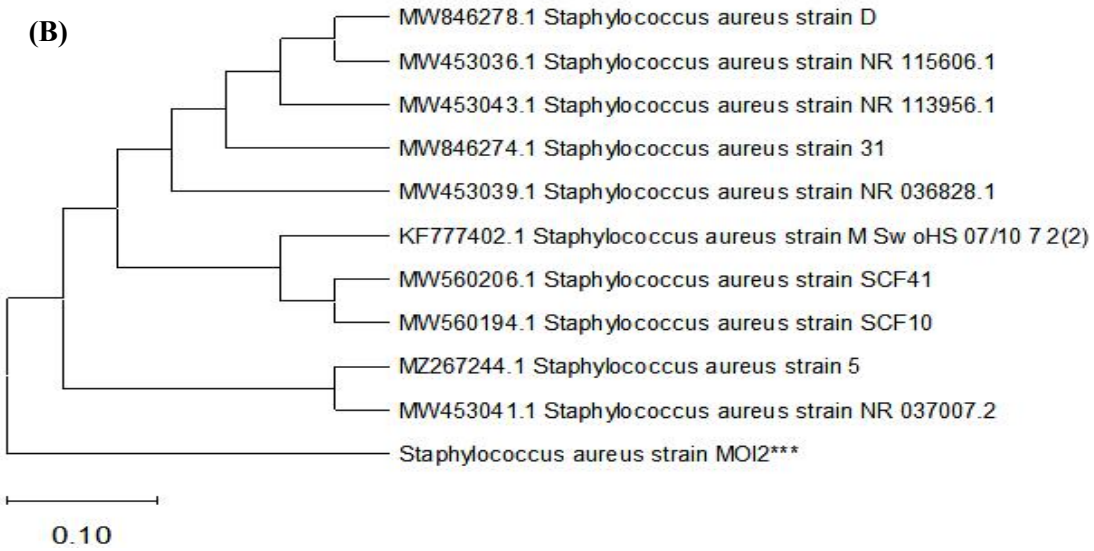


Figure 1: Agarose gel electrophoresis of amplified PCR products using 16S rRNA primers. Lane: M-represents marker, lane 1 represent the isolate of *Escherichia coli* (865 bps), lane 2 represent the isolate of *Staphylococcus aureus* (820 bps), lane 3 represent the isolate of *Klebsiella pneumonia* (866 bps), lane 4 represent the isolate of *Providencia stuarti* (869 bps)i.





DOI: 10.56892/bima.v9i1B.1277

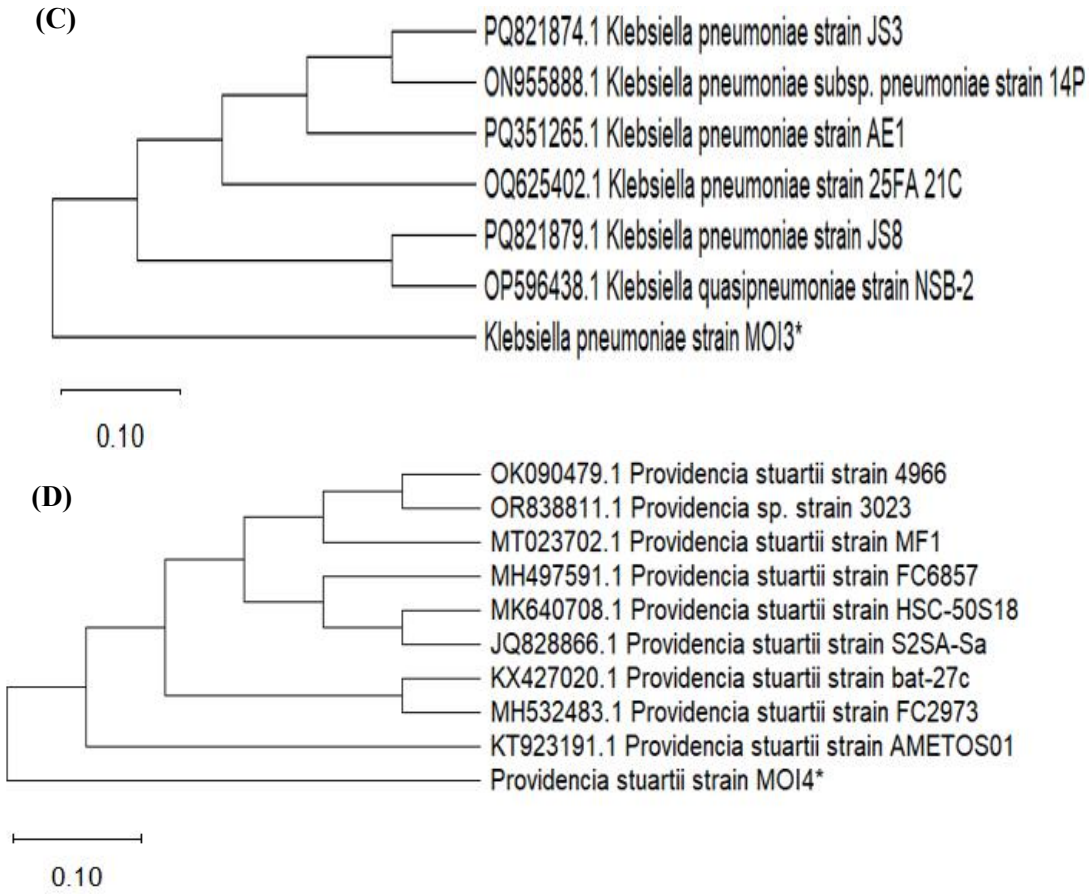


Figure 2 (A-D) Phylogenetic tree analysis constructed using maximum likelihood showing *Escherichia coli* strain MOI1*, *Staphylococcus aureus* strain MOI2*, *Klebsiella pneumonia* strain MOI3*, and *Providencia stuartii* MOI4* with other *Escherichia coli, Staphylococcus aureus*, *Klebsiella pneumonia* , and *Providencia stuartii* found in NCBI.

Prevalence of Bacterial Pathogen Associated with ENT across Sex and Age

In this current study, out of 384 study participants, 174 (45.3%) and 210 (54.7%) were males and females, respectively. Of all the total study participants enrolled in the present study, 124 (32.3%) were between the ages of 1-20 years, followed by 106 (27.6%) between the ages of 31-50 years, 102 (26.6%) were between the age of 51-70 years, 30 (7.8) was above 70 years while the least was 22 (6.3%) between the ages of 21-30 years.

The general prevalence of ENT infections in the current study was found to be 17.0%. However, the results showed that females had a higher prevalence of ENT infection (19.0%) than males (14.4%), but the result was not statistically significant ($\chi 2 = 1.482$: df = 1.00, p = 0.223). Study participants aged 21-30 had the highest prevalence (27.3%), while other participants aged 31-50 had the lowest prevalence (11.3%). The statistical analysis revealed an association between infection occurrence and age group as the p-value is <0.05 ($\chi 2 = 10.244$: df = 4, p=0.037).

DOI: 10.56892/bima.v9i1B.1277

Table 2: Prevalence of Bacterial Pathogen Associated with ENT across Sex and Age.

Variable	Frequency N=384 (%)	Positive (%)	Negative (%)	χ^2	p-value
Age					
1-20	124 (32.3)	30 (24.2)	94 (75.8)	10.244	0.037
21-30	22 (5.7)	6 (27.3)	16 (72.7)		
31-50	106 (27.6)	12 (11.3)	94 (88.7)		
51-70	102 (26.6)	13 (12.7)	89 (87.3)		
>70	30 (7.8)	4 (13.3)	26 (86.7)		
Gender					
Male	174 (45.3)	25 (14.4)	149 (85.6)	1.482	0.223
Female	210 (54.7)	40 (19.0)	170 (81.0)		

DISCUSSION

Our study found that the predominant bacterial pathogens isolated from these regions particularly the ear, nasal passages, and throat were Gram-negative, with notable strains including *Klebsiella pneumoniae, Escherichia coli, Providencia stuartii, Haemophilus influenzae*, and *Serratia marcescens*, all of which are commonly associated with ENT infections. This finding aligns with a study conducted in Addis Ababa, Ethiopia, which reported twelve species of Gram-negative bacteria from ENT samples, highlighting the prevalence of *Klebsiella pneumoniae* and *Escherichia coli* in these infections (Estifanos *et al*., 2018). Another study highlighted *Pseudomonas aeruginosa* and *Proteus spp***.** as common isolates, indicating a diverse range of Gram-negative organisms in these infections (Najeeb *et al.,* 2024). Interestingly, the study also found that the only Gram-positive bacterium detected in ear, nasal, and throat samples was *Staphylococcus aureus*, which emerged as the most prevalent pathogen, accounting for 20 out of 65 isolates (30.8%). This observation corresponds with research from a tertiary care hospital in Islamabad, which emphasizes the predominance of Gram-positive organisms in ENT infections, particularly noting that *Staphylococcus aureus* comprised 67.92% of the isolates (Saqulain *et al.,* 2024).

Moreover, other similar studies around the world including Romania and Iraq highlight the predominance of Gram-positive bacteria in ENT infections, with *Staphylococcus aureus* being the most prevalent isolates (28.72%, and 66.6%) (Togănel *et al.,* 2022; Salahdin *et al.,* 2024). Another study in Bangladesh reported that *S. aureus* accounted for 37% of isolates in chronic suppurative otitis media, showcasing its role as a common pathogen in ear infections (Khatum *et al*., 2021).The prevalence of *S. aureus* is influenced by factors such as poor sanitary conditions, inadequate storage, and handling of food, which contribute to its spread in animal-derived foods (Odetokun *et al.,* 2023). While *Staphylococcus aureus* remains a significant pathogen in Nigeria and other African countries, the prevalence vary widely. This variability is influenced by regional factors, healthcare practices, and environmental conditions

The prevalence of bacterial pathogens isolated from samples taken from the ear, nose, and throat (ENT) in various studies worldwide indicates a significant burden of infections. For example, our study conducted at Aminu Kano Teaching Hospital reported a positive rate of ENT infections at 17.0% from 384 samples. In contrast, a previous study at the same hospital revealed a higher positive rate of approximately 98.89% from just 100

DOI: 10.56892/bima.v9i1B.1277

samples (Ahmad *et al.,* 2016). This suggests a notable prevalence of bacterial pathogens in ENT infections among patients compared to our findings. It is important to note that our study's sample size is larger compared to the earlier study. Moreover, others studies conducted around the worldwide were in contrast with our study. For instance in northern Tanzania, the prevalence of ENT manifestations among HIV-infected patients was 34%, while other studies that reported varying rates, such as 61.8% in Iran and 79% in India, highlighting significant differences in findings across different populations (Shija *et al.,* 2020).

The prevalence of ear, nose, and throat (ENT) infections often varies by gender, with some studies indicating a higher incidence in females, while others show no significant differences. This situation may relate to several factors. One reason could be that female often take on caregiving roles, which can lead to more exposure to childhood illnesses. Additionally, female may report health issues and seek medical care more often. Our results reported females had a higher prevalence of ENT infection (19.0%) than males (14.4%), Our result was similar with the study conducted at a tertiary hospital in Dhaka, where ENT infections were more prevalent in females (37.2%) compared to males (22.6%) (Dey *et al.,* 2018). In another study on children in Telangana found that 61.9% of patients with ENT disorders were female, highlighting a potential gender disparity in prevalence (Surapaneni and Sisodia, 2016). In contrast, another study reported no significant differences in the overall prevalence of ENT diseases between genders, with a nearly equal ratio of male to female patients (0.9:1) (Yasmeen *et al.,* 2023).

The prevalence of ear, nose, and throat (ENT) infections varies significantly across different age groups, with younger adults often experiencing higher rates. Our study indicated that participants aged 21-30 had the highest prevalence of ENT infections at 27.3%, while those aged 31-50 had the lowest at 11.3%, with a statistically significant association ($p < 0.05$). Our results is similar with a study that found 28.6% of patients in the 21-30 age group presented with ENT issues, indicating a similar trend in prevalence(Alam *et al.,* 2022). Another similar study conducted at a Tertiary Hospital in Morang District Nepal indicated that the majority of patients presenting with ENT issues are young adults, particularly those in the 21-30 age (23%) (Pandit *et al.,* 2024). In contrast, older age groups (51-87 years) showed a higher likelihood of misdiagnosis in ENT referrals, suggesting that age impacts both prevalence and diagnostic accuracy (Lukama *et al.,* 2023). While younger adults show a higher prevalence of ENT infections, it is crucial to consider that older populations may experience different health challenges, including misdiagnosis and diverse health outcomes, necessitating tailored healthcare approaches.

CONCLUSION

The most common pathogen identified from ear, nasal, and throat samples among patients at Aminu Kano Teaching Hospital was *Staphylococcus aureus*, followed by *Providencia stuartii, Klebsiella pneumoniae, Escherichia coli,* and *Serratia marcescens. Haemophilus influenzae* was found to be the least prevalent. The incidence of ENT infections was notably higher in females compared to males, although this difference was not statistically significant. Young adults showed the highest prevalence of these infections, while middle-aged individuals had the lowest rates. A significant association was observed between the occurrence of infections and age group. Molecular methods have become essential for the identification of pathogens in various clinical samples, and this

DOI: 10.56892/bima.v9i1B.1277

study strongly advocates for the adoption of molecular techniques to detect a broader range of microbial pathogens.

Acknowledgements

We wish to extend our heartfelt appreciation to the microbiology laboratory staff at Aminu Kano Teaching Hospital for their invaluable support in the collection and processing of clinical samples. We are also grateful to the management of Aminu Kano Teaching Hospital and Sa'adu Zungur University, Bauchi, for their contributions to the success of this study**.**

Funding: This study was funded by research grant TEFT/DR&D/CE/UNI/GADAU/IBR/2023/VOL.I under the Tertiary Educational Trust Fund from the Ministry of Education Federal republic of Nigeria.

REFERENCES

Acuin, J. M., Chiong, C., & Yang, N. (2015). Surgery for chronically discharging ears with underlying eardrum perforations. Cochrane Database of Systematic Reviews, 2015(1).

Afolabi, O. A., Salaudeen, A. G., Ologe, F. E., Nwabuisi, C., & Nwawolo, C. C. (2012). Pattern of bacterial isolates in the middle ear discharge of patients with chronic suppurative otitis media in a tertiary hospital in North central Nigeria. African health sciences, 12(3), 362-367.

Ahmad, M. M., Kurawa, Z. M., Shu'aibu, I., & Yahaya, G. (2016). Microbiological Assessment of Bacterial Isolates from Ear, Nose And Throat (ENT) Among Patients Attending Aminu Kano Teaching Hospital, Kano, Nigeria. *Nigerian Journal of Basic and Applied Sciences*, *24*(1), 15–18.

Alam, A. U., Ullah, M. K., Shakil, M., Rahman, S. T., Ahamed, M. K., & Sarower, M. G. (2022). Incidence of Otolaryngological Diseases in General Population: A Study in Eastern Medical College Hospital. *Eastern Medical College Journal*, *7*(1), 6-10.

Azeez, M.M. (2000). Bacteriology of Otitis Media in Oyo Nigeria. *Journal of Medical Laboratory Science*,11(1): 34-39.

Bailey, W.R. and Scott, E.G. (1992). *Diagnostic Microbiology* 7th edition CV Mosby co. St. Louis pp 88 – 175.

Dey, B. K., Datta, A., Rhaman, M. M., & Sayeed, M. A. (2018). Pattern of ear nose and throat diaseses in a tertiary hospital of Dhaka city. *IMC Journal of Medical Science*, *12*(1), 22-26

Elaine, P.P., Michael, A.N. and Anne, L.B. (1987).Predictors of Phonologic Inadequacy in Young Children Prone to Otitis Media. *The Journal of Speech and Hearing Disorders,* 52: 232-242.

Estifanos, T., Mesele, A., & Meseret, C. (2018). Microbiological assessment of gram-negative bacterial isolates from ear, eye, nose and throat among patients attending aresho advanced medical laboratory, Addis Ababa, Ethiopia. *J Appl Microb Res*, 1(2), 27-32.

Hailegiyorgis, T. T., Sarhie, W. D., & Workie, H. M. (2018). Isolation and antimicrobial drug susceptibility pattern of bacterial pathogens from pediatric patients with otitis media in selected health institutions, Addis Ababa, Ethiopia: a prospective cross-sectional study. BMC Ear, Nose and Throat Disorders, 18, 1-7.

Ikeh E.A., Adebayo, E.O., Okuonghae, H.O. and Ighogboja, L.S. (1993). Bacteriology of chronic discharging ears in children in Jos, Nigeria,*Journal of Medical Laboratory Science*; 3: 27 – 30.

Kumar, R.P., Sriyasteva, M. Sharma, S. Rishi, P.S. and Nwawan, P. (2013). Isolation and Antimicrobial Sensitivity profile of Bacterial Agents in Chronic Supportive Otitis media in patients at NIMS Hospital,

DOI: 10.56892/bima.v9i1B.1277

Jaipur. *Journal of Research in Nursing and Midwifery*, 2(5): 265 – 269.

Khatun, M. R., Alam, K. M. F., Naznin, M., & Salam, M. A. (2021). Microbiology of chronic suppurative otitis media: an update from a tertiary care hospital in Bangladesh. Pakistan journal of medical sciences, 37(3), 821.

Lukama, L., Aldous, C., Michelo, C., & Kalinda, C. (2023). Ear, Nose and Throat (ENT) disease diagnostic error in low-resource health care: Observations from a hospital-based cross-sectional study. *Plos one*, 18(2), e0281686.

Mairangthem, A. and Angom, L.L., (2012). Spectrum of beta haemolytic infections in ENT patients. *Journal of Medical Science and Technology,* 1(3): 16 – 23.

Najeeb, B., Shakeela, Q., Qureshi, S. A., Ahmed, S., Uzma, B., Nazir, A., & Ali, F. (2024). Bacteriological evaluation, antibiogram, and phenotypic detection of MBL-producing gram-negative bacteria isolated from outdoor patients with Otorrhea attending Ayub Medical Complex. *Kuwait Journal of Science*, 51(3), 100233.

Newton, V. E., Macharia, I., Mugwe, P., Ototo, B., & Kan, S. W. (2001). Evaluation of the use of a questionnaire to detect hearing loss in Kenyan pre-school children. International journal of pediatric otorhinolaryngology, 57(3), 229-234.

Obiajuru, I. O., & Chukuezi, A. B. (2012). Microbiological Assessment of ENT infections and diseases: Clinical case study at Orlu Imo State, Nigeria. *Journal of Research in Nursing and Midwifery*, 2(5) pp. 72-76.

Ohuche, I. O., Iloanusi, N. I., Dike, C. M., & Chime, E. N. (2023). Clinical presentation, radiographic findings, and treatment outcomes in children with adenoid hypertrophy in a paediatric outpatient clinic in Enugu, Nigeria. Ghana Medical Journal, 57(3), 204-209.

Pandit, S. B., Shah, R., & Gyawali, P. R. (2024). Clinical and Epidemiological Profile of Otorhinolaryngology Diseases at a Tertiary Hospital in Morang District: A Descriptive Cross-Sectional Study. *Birat Journal of Health Sciences*, 9(1), 23–28

Saha, T. K., Gazi, E., Samanta, A. K., & Bhandari, K. (2023). Cross-sectional Study on Pattern of Ear, Nose and Throat Disorders among School Children in a Rural Area of West Bengal. 14(4), 352.

Salahdin, O. D., Saleh, R. O., Jasim, S. A., & Al-Janabi, S. S. Detection of Virulence Genes Hla (α-hemolysis), CrtN (staphyloxanthin) for Staphylococcus aureus Isolated from Patients with Otitis and Rhinitis. *Acta Microbiolologica Bulgarica,* 3(40). 331-335.

Saqulain, G., Naqvi, S. A. R., & Imtiaz, N. (2024). Antimicrobial Susceptibility Pattern of Bacterial Pathogens in Our Setup: Ear, Nose and Throat Perspective: Antimicrobial Susceptibility of Bacterial Pathogens. Pakistan BioMedical Journal, 26-31.

Schnert, K.W. (1996). *How to be your own doctor sometimes*. Grosset and Fulap Publishers, New York; pp 20 – 21.

Shija, P. S., Karaba, J. A., Philemon, R., Minja, B. L., Mtenga, P. P., & Katundu, D. (2020). Prevalence of Ear Nose and Throat (ENT) Manifestations Among HIV Seropositive Patients at a Tertiary Hospital in Northern Tanzania: A Descriptive Cross-Sectional Study. HIV/AIDS : Research and Palliative Care, 12, 425–429.

Surapaneni, H., & Sisodia, S. S. (2016). Incidence of ear, nose and throat disorders in children: a study in a teaching hospital in Telangana. *International Journal of*

DOI: 10.56892/bima.v9i1B.1277

Otorhinolaryngology and Head and Neck Surgery, *2*(1), 26-9.

Togănel, R. O., Coșeriu, R. L., Vintilă, C., & Simion, A. (2022). An overview of the microflora of the patients admitted in an Ear Nose and Throat tertiary hospital from Romania. Acta Marisiensis-Seria Medica, 68(3).

Wang G, Clark CG, Rodgers FG. Detection in Escherichia coli of the genes encoding the major virulence factors, the genes defining the O157: H7 serotype, and components of the type 2 Shiga toxin family by multiplex PCR. Journal of clinical microbiology. 2002;40(10):3613-9.

Witsell, D. L., Dolor, R. J., Bolte, J. M., & Stinnett, S. S. (2001). Exploring health-related quality of life in patients with diseases of the ear, nose, and throat: a multicenter observational study. Otolaryngology—Head and Neck Surgery, 125(4), 288-298.

World Health Organization. (2004). Chronic suppurative otitis media: burden of illness and management options. In Chronic suppurative otitis media: burden of illness and management options.

Yasmeen, N., Shumon, A. M., Atikuzzaman, K., Hoque, F., Malakar, M., & Salam, J. (2022). Prevalence of ENT Diseases in Medical College for Women and Hospital, Dhaka-Six Months Retrospective Study. *Bangladesh Journal of Otorhinolaryngology*, *28*(2), 186-192.