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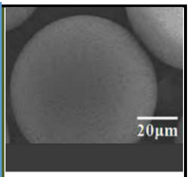

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Antimicrobial Resistance Profiles of Bacteria Isolated from Ear Swabs Specimens in a Tertiary Health Facility, North-eastern Nigeria

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ABSTRACT

An ear infection is among the common diseases in children. There is an increased rate of antimicrobial resistance to pathogens that cause ear infections worldwide. We analyzed two years of data on bacterial isolates associated with suspected cases of ear infection and their antibiotic-resistant patterns. All records on ear swabs (n= 134) sent for culture and sensitivity, at the medical microbiology laboratory of a tertiary Health Facility in Northeastern Nigeria, from 2017 to 2018 were reviewed. The patients' socio-demographic information, bacteria isolated, antibiotics tested, and their sensitivity patterns were extracted. Kirby-Bauer method of antibiotic sensitivity testing and CLSI guidelines were adopted. Descriptive analyses of the data were conducted. Of a total of 134 patients' records extracted 50.7% were female, median age 12 (IQR 9-15 years). Of the 134 samples cultured, 124 (92.5%) yield bacterial growth. Eight (8) different isolates obtained were *Staphylococcus aureus* (38.7%), *Pseudomonas aeruginosa* (17.7%), *Proteus mirabilis* (16.1%) *Proteus vulgaris* (9.7%), *Klebsiella oxytoca* (9.0%), *Klebsiella pneumoniae* (5.6%) *Escherichia coli* (2.4%) and *Enterococcus* spp. (0.8%), *Staphylococcus aureus* was the predominant isolate, while *Enterococcus* spp. *was* the least. A high resistant rate was recorded against many of the antibiotics tested. All isolate demonstrated resistance to amoxicillin and cotrimoxazole, and high sensitivity to ciprofloxacin. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the most common pathogens isolated. Most isolates showed high sensitivity to ciprofloxacin. The high resistance rate recorded on most of the antibiotics tested highlight the need for appropriate prescription of antimicrobials based on local susceptibility profile in the treatments of ear infection.

INTRODUCTION

Ear infections include acute otitis media (AOM), otitis media with effusion, chronic suppurative otitis media (CSOM), and otitis externa. Ear infections are among the most frequent disorders in children worldwide. [1] Acute otitis media is still a major public health issue around the world. Around 65-330 million individuals worldwide suffer from ear infections, with 60% having substantial hearing damage [2]. Recurrent otitis media (OM) has been linked to a decrease in quality of life[3]. Furthermore, OM causes more than 25,000 deaths worldwide each year, particularly in low-income countries, due to intracranial problems caused by OM expansion in the lack of adequate treatment [4]. Although OM is the most common ear infection, they can be treated effectively and disappear without complications. However, it has been linked to hearing loss and long-term consequences [5],[6]. It is highly frequent in children and is usually accompanied with a viral upper respiratory infection (URI), with a peak prevalence between the ages of 4 and 7 years old [7]. The frequency of ear infection varies globally based on health and economic level. For example, in the United States and Europe, the frequency is falling due to improved hygiene and knowledge, whereas in underdeveloped nations, the incidence is increasing due to inadequate hygiene practices and a lack of health education [8]. In these countries, nearly half of all children will have three or more ear infections within their first three years of life. Although OM is typically a condition in newborns and young children, it can also affect adults [9]. Socioeconomic level, cultural, seasonal, and age characteristics, as well as a family history of middle ear infection, are all major risk factors in OM. According to some studies, males are more likely to be affected than females [9]. Varied geographical areas have different etiologies and incidences of ear infections [2]. Bacteria, however, continue to be the most prominent aetiological agent in suppurative or discharging otitis media. While non-typable *Haemophilus influenzae, Streptococcus pneumonia,* and *Moraxella catarrhalis* are frequently reported as etiological agents in acutely discharging ears in developed countries,[10] local studies in Nigeria suggest that *Moraxella catarrhalis* is not a predominant organism in acutely discharging otitis media [11].

Similarly, several investigations in Sub-Saharan Africa and other underdeveloped countries have revealed that the most prevalent organisms isolated from cases of ear infections, particularly in CSOM, are *Pseudomonas* sp., *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Proteus* sp. [11-17]. CSOM can have serious side effects such as intra- and extracranial problems that can be fatal. Because of the invention of antibiotics, the complications of CSOM have been decreased to a greater extent [18]. However, irrational antibiotic use has resulted in the creation of drug-resistant microbes. Antibiotic resistance occurs when bacteria evolve in a way that diminishes or eliminates the effectiveness of medications, chemicals, or other agents designed to cure or prevent infection.

As a result, the germs survive and multiply, causing greater harm. [19] The growing antibiotic resistance of microbial pathogens responsible for numerous infectious illnesses constitutes a significant threat to infectious disease management globally [19]. The antibiotic resistance profile of bacteria varies by the community due to differences in geography, local antimicrobial prescribing patterns, and the prevalence of resistant bacterial strains in a given area [2]. Furthermore, the World Health Organization lists antibiotic resistance as one of the biggest dangers to human health [19]. The microbiological characteristics of ear infections are widely described in the developed world. However, few investigations have been undertaken in most developing countries[20-22], despite the fact that medical microbiology laboratories throughout the region house a wealth of data on the microbiological profile and sensitivity pattern of the etiological agents. As a result, it is critical to investigate the microbiological profiles of ear infections as well as the amount of antibiotic resistance in these developing nations in order to properly manage patients with ear infections.

Although research has been undertaken on the antibiotic susceptibility and resistance pattern of bacterial pathogens causing otitis media in various parts of Nigeria [5,11,13,14,23]. However, there is a scarcity of published data from the country's northeastern region. In this study, we showed a two-year retrospective analysis of culture and antibiotic susceptibility test results from patients suspected of having an ear infection who were referred to a medical microbiology laboratory of a tertiary hospital in northeastern Nigeria. This analysis of laboratory data was carried out to know the bacterial aetiology of ear infections encountered and their antibiotic susceptibility pattern throughout the study period in the study area which served This understanding is critical for doctors to manage cases appropriately, preventing or minimizing complications, unnecessary economic loss, patient discomfort, and, most importantly, antibiotic resistance[5]. As a result, there should be up-to-date information on microbial resistance patterns at the national, regional, and local levels to guide the sensible use of existing antimicrobial medications, especially in this era of rising microbial resistance.

MATERIALS AND METHODS

Study design and data source

This is a retrospective assessment of ear swab samples sent for culture and sensitivity testing at the medical microbiology laboratory of Abubakar Tafawa Balewa University Teaching Hospital (ATBUTH) Bauchi, a tertiary health facility in Nigeria's northeastern region, from January 2017 to December 2018. The retrospective chart review data collecting approach was used to acquire patient data. As a consequence, from January 2017 to December 2018, all patients' information reported in microbiology laboratory unit patient registration books was collected, including patient sociodemographic data, isolated bacteria from ear discharge, and antibiotic susceptibility results. This two-year data would provide insight into the many etiological agents of ear infection and their resistance profile.

Study setting

The research was carried out at the Abubakar Tafawa Balewa University Teaching Hospital (ATBUTH). It is a 650-bed capacity tertiary hospital that provides multi-specialized services and is a key referral center in Nigeria's North-Eastern geographical zone. It is one of the two tertiary health institutions owned by the federal government in the state. The hospital is a university teaching hospital located in Bauchi, the state capital of Bauchi. It sees around 300,000 patients per year. The hospital's medical microbiology laboratory provides a variety of diagnostic services, the most important of which is culture and sensitivity; around 7,000 samples are cultivated in the laboratory each year.

To assure the accuracy and reliability of the antimicrobial susceptibility test, the reference strains *S. aureus* (ATCC25923), *Escherichia coli* (ATCC25922), and *P. aeruginosa* (ATCC 27853) were used as internal quality controls in that laboratory. Study population include Any patient who underwent an ear swab collection at the Ear Nose and Throat (ENT) clinic, Pediatric outpatient (POD) and General outpatients' departments (GOPD) sent for culture and sensitivity, at the medical microbiology laboratory of a tertiary Health Facility in North Eastern Nigeria, while data with incomplete records or illegible handwriting were excluded from this study.

Data collection

The data for the patients was gathered utilizing the retrospective chart review data gathering approach. As a result, from January 2017 to December 2018, all patients' information registered in microbiology laboratory unit patient registration books were collected, as well as socio-demographic information of patients with ear infections from pediatric out-patient, ENT clinics, and GOPD across all age groups. A two-year laboratory record also provided us with information on the growth of swab cultures, the type of bacteria isolated, and the antibiogram/sensitivity patterns of the bacterial isolates.

Laboratory Diagnostic Methods

The laboratory scientist gave the following specific information on the laboratory methods used: Some swabs were taken in the laboratory, while others were gathered in clinics by laboratory scientists and doctors, respectively. Each patient had one swab collected aseptically using sterilized cotton swabs, and each swab was infected into blood, chocolate, and MacConkey agar plates.

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The duplicate blood and chocolate plates were inoculated. Each pair and MacConkey agar plate were incubated aerobically, while the other pair was incubated overnight at 37 degrees Celsius in an elevated CO2 environment.

Bacterial isolation and identification were performed in accordance with standard microbiological methods as outlined in Monica Cheesbrough's important work [24]. And the antimicrobial sensitivity pattern was assessed using the Kirby-Bauer method of antibiotic sensitivity testing and the CLSI guidelines for evaluating antibiotic susceptibility.The drugs tested for both Gram negative and Gram positive bacteria were amoxicilin (10 µg), ciprofloxacin (5 µg), ofloxacin (10 µg), Perfloxacin (10 µg) cefuroxime (30 µg), gentamicin (10 µg), sparfloxacin (30 µg), chloramphenicol (30 µg), ampiclox (20 µg), Augmentin (15 µg), ceftriaxone (30 µg), erythromycin (15 µg) and Cotrimoxazole (30 µg). To assure the accuracy and reliability of the antimicrobial susceptibility test, the reference strains *S. aureus* (ATCC25923), *Escherichia coli* (ATCC25922), and *P. aeruginosa* (ATCC 27853) were used as internal quality controls.

Data management and analysis

Using Microsoft Excel® 2016, the data was altered, cleaned, inputted, and evaluated. A descriptive study of socio-demographic data, isolate type, antibiotics utilized, and isolate sensitivity pattern was performed, and the results were given in frequencies, proportions, and tables.

Ethical Considerations

The ATB University Teaching Hospital's Ethical Review Committee granted ethical approval. This acted as an official supporting document given to the hospital's head of the medical microbiology department in exchange for cooperation and authorization to obtain the two-year data record. Deidentification of records safeguarded patient privacy. Patients' names were substituted with initials. All data collected throughout the trial was kept anonymous and utilized only for this study.

RESULT

A total of 134 patients' records were extracted, with 68 (50.7 percent) being female and the median age being 12 years (IQR 9-15 years) (**Table 1**). 124 (92.5 percent) of the 134 samples cultivated yielded bacterial growth, all of which were clinically significant. There were no fungi recorded during the study period, although 75 (60.5 percent) of the total isolates were Gram negative and 49 (39.5 percent) were Gram positive. The highest bacterial growth (100 percent) was obtained in the age group of one to four years, followed by 95.7 percent in the age group of five to nine years, and the lowest bacterial growth was obtained in the age group of twenty years.

Staphylococcus aureus (38.7 percent), *Pseudomonas aeruginosa* (17.7 percent), *Proteus mirabilis* (16.1 percent), *Proteus vulgaris* (9.7 percent), *Klebsiella oxytoca* (9.0 percent), *Klebsiella pneumoniae* (5.6 percent), *Escherichia coli* (2.4 percent), and *Enterococcus* spp. (0.8 percent) were the most common isolates (**Table 2**). Gram-negative bacteria account for a higher percentage in all age groups. A significant percentage of Gram-positive (46.7) was found in the age group 10-14 years, however, all isolates recovered within the age group less than a year were Gram-negative.

Table 1. Demographic characteristics of the patients seen at a tertiary hospital in Northeastern Nigeria.

Variable	Frequency	Percentage (%)
Age group (years)		
<1	4	2.9
1-4	21	15.7
5-9	23	17.2
10-14	49	36.5
15-19	22	16.4
≥20	15	11.2
Sex		
Male	66	49.3
Female	68	50.7

Table 2. Frequency of bacterial isolates from ear swab cultures at a tertiary Hospital in Northeastern Nigeria.

Isolate	*Frequency*	*Percentage (%)*
S. aureus	48	38.7
P. aeruginosa	22	17.7
P. mirabilis	20	16.1
P. vulgaris	12	9.7
K. oxytoca	11	9.0
K. pneumoniae	07	5.6
E. coli	03	2.4
Enterococcus spp.	01	0.8
Total	124	100

Many of the antibiotics examined to have a high resistance rate. Amoxicillin and cotrimoxazole resistance were found in all isolates (**Table 3**). *S. aureus* was shown to be highly resistant to amoxicillin (72.9%) but extremely susceptible to ciprofloxacin (97.9 percent). *Proteus* spp. was also resistant to amoxicillin (78.2%), but highly sensitive to ciprofloxacin. 87.0 percent. P. aeruginosa was discovered to be completely resistant to cotrimoxazole, but very sensitive to pefloxacin and streptomycin (86.6 percent) *Klebsiella* spp. is highly resistant to Augmentin and chloramphenicol (77.8%) but is extremely sensitive to ciprofloxacin (94.5 percent). All *E. coli* isolates are resistant to cotrimoxazole, although they are sensitive to gentamycin, streptomycin, amoxicillin, ciprofloxacin, sparfloxacin, chloramphenicol, and amoxiclav (66.7 percent).

Table 3. Antibiotic resistance patterns of the bacterial isolates from ear swab cultures at a tertiary hospital Northeastern Nigeria.

		Resistance Pattern of the Bacterial isolate					
S/ No.	Antib-iotics	*S. aureus,* n=48 (%)	*Proteus* spp.,n=32 (%)	*P. aeru-ginosa,* n=22 (%)	*Klebsiella* spp., n=18 (%)	*E. coli* *n*=03	*Entero-coccus* spp., n=01 (%)
1	SXT	29 (60.4)	17 (73.9)	22 (100)	10 (55.6)	3 (100)	1 (100)
2	S	7 (14.6)	7 (30.4)	3 (13.6)	3 (37.5)	NT	1 (100)
3	CN	15 (31.2)	6 (26.1)	7 (31.8)	6 (33.3)	1 (33.3)	1 (100)
4	AMX	35 (72.9)	18 (78.2)	16 (72.7)	12 (66.6)	1 (33.3)	1 (100)
5	CPX	1 (2.0)	3 (13.0)	7 (31.8)	1 (5.5)	1 (33.3)	NT
6	PEP	7 (14.5)	8 (34.7)	3 (13.6)	3 (16.7)	NT	1 (100)
7	SPF	NT	8 (34.7)	8 (36.3)	11 (61.1)	1 (33.3)	1 (100)
8	OFX	NT	7 (30.4)	4 (18.1)	10 (55.6)	NT	1 (100)
9	CH	NT	14 (60.8)	17 (77.2)	14 (77.8)	1 (33.3)	1 (100)
10	AU	NT	16 (69.5)	7 (31.8)	14 (77.8)	1 (33.3)	1 (100)
11	APX	29 (60.4)	NT	NT	NT	NT	NT

Key: SXT=Cotrimoxazole, S=Streptomycin, CN=Gentamycin, AMX= Amoxicillin, CPX= Ciprofloxacin, PEF= Pefloxacin, SPF= Sparfloxacin, OFX= Ofloxacin, CH= Chloramphenicol, AU= Amoxiclav, APX= Ampiclox, CTR= Ceftriaxone, CXM= Cefuroxime, E= Erythromycin. NT= Not Tested.

DISCUSSION

Ear infection is a frequent clinical concern worldwide and the leading cause of preventable hearing loss in underdeveloped nations. [25] Microbial agents can infect both the middle and outer ear. In this investigation, the burden of bacteria as aetiological agents linked with otitis media was shown to be extremely high. The majority of the patients were between the ages of 10 and 14, which is consistent with previous research [26],[6]. In contrast, Loy et al found an increased prevalence of ear infection in people aged 30 to 40 in their study [27]. Bacterial isolates were found in 92.5 percent of the ear discharge samples. This is consistent with previous findings from different parts of Nigeria, for example, from the North West region. Bilkisu et al.,[13] reported 84.9 percent, which is close to Afolabi's claim from the North Central region,[28] and Omosigho et al.,[29] recently recorded 89.8 percent in the same central region. Philomena [14] from the South-South recorded 84.6 percent, whereas Elechuku from the South East reported 91.0 percent. However, Fayemiwo et al. [12] from the country's Southwest recorded the highest frequency of 95.6. Kasahun et al.,[30] recorded 92.5 percent in Ethiopia, and Kiran et al.,[15] reported 84.6 percent in India. However, Dhakar [31] and Saudi Arabia [8] research indicated 74% and 75%, respectively.

Gram-negative bacteria were found to be the most prevalent bacteria identified in this investigation, accounting for 60.5 percent of the total compared to gram-positive bacteria. Similar reports were seen from North Central Nigeria, which recorded 71.6 percent, [28], and South-South Nigeria, which recorded 70.0 percent, [14]. South to South, 57.4 percent are Gram-negative. However, this differs from the study from the North-Western region, where Bilkis et al.,[13] found Gram-positive bacteria to be the most common isolate. Similarly, research from Ethiopia[2,16] and Yemen [32] found Gram-negative bacteria to be the most prevalent bacterium isolated.

This study found that the most common bacterial species isolated during the period were *Staphylococcus aureus* (38.7 percent), followed by proteus spp. (25.8 percent), *Pseudomonas aeruginosa* (17.7 percent), and least was *Enterococcus* spp. (0.8 percent), which is consistent with findings from Zamfara state North-West, Benin South-South[14], and Bidda North-Central[29]. However, in the Ibadan South-West region, Staphylococcus was not among the most common organisms isolated, but *Pseudomonas aeruginosa* and proteus spp. were [5] similar in North-Western Ethiopia. [33]

With the exception of *Pseudomonas, Staphylococcus* and *Proteus* were found as the most commonly isolated organisms in another investigation in Chennai [18]. *Proteus* spp. and pseudomonas are among the most commonly isolated organisms in Angola, with the exception of *Staphylococcus* [17]. This could be linked to climate and weather variations. While non-typable *Haemophilus influenzae, Streptococcus pneumonia,* and *Moraxella catarrhalis* are the most commonly reported aetiological agents in acutely discharging ears in developed countries [10], this could be attributed to geographical areas and climate conditions, as well as levels of hygiene, education, and economic development in developed and developing countries.

The antibiotic susceptibility profile of the identified organisms in this review caused considerable worry, as the majority of the isolates were discovered to be resistant to routinely used and available antibiotics. Many of the drugs examined showed a significant rate of resistance. For example, *Staphylococcus aureus* (the most commonly reported organism) resistance to antibiotics ranged from 72.9 percent 8 percent for amoxicillin to 2.0 percent for ciprofloxacin, which is consistent with prior findings that most organisms show the least resistance to ciprofloxacin [5,28,29]. All pathogens showed substantial resistance to cotrimoxazole, a routinely used antibiotic in the area of this analysis. Similar resistance patterns have been documented in other research [18,34]. The major concern as revealed by this review there is an increased in antimicrobial resistance which is one of the greatest global public health challenges.,

CONCLUSION

In this investigation, the majority of the isolates were *Staphylococcus aureus*, *Proteus* spp., and *Pseudomonas aeruginosa*. These are the same organisms that have been described as the aetiology of ear infections by many studies in Sub-Saharan Africa and other underdeveloped nations. On the majority of isolates, most fluoroquinolones were shown to be active antibiotics. A variety of reasons could contribute to high levels of resistance to amoxicillin, cotrimoxazole, and chloramphenicol. Inappropriate usage and the development of enzymatic resistance are two examples. Empiric antibiotic therapy may be ineffective at times and, in the long run, may contribute to the development of antimicrobial resistance. It is consequently advised that a nationwide antimicrobial surveillance system and strict adherence to antibiotic prescription policies be supported, as well as the improvement of appropriate clinical and laboratory standards.

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COMPETING INTERESTS

The authors declare no competing interest.

AUTHORS' CONTRIBUTIONS

Hafiz Halilu: analyzed the laboratory data and wrote the first draft of the manuscript. Abdulmumin Ibrahim Sulaiman, Adamu Garba Yusuf, Muhammad Abdullahi and Muhammad M. Barma: extracts the data from laboratory records and wrote the introduction section of the manuscript. Ibrahim Abdurrasul reviewed the manuscript.

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