
African Journal of Pharmaceutical Research and Development

Available online at <https://ajopred.com>

Vol. 18 No.1; pp. 599-607 (2026)



Original Research Article

SUSCEPTIBILITY PROFILES OF MULTIPLE ANTIBIOTIC-RESISTANT BACTERIAL ISOLATES FROM HEALTHY EARS OF STUDENTS TO SELECTED BRANDS OF GENTAMICIN AND CHLORAMPHENICOL EAR DROPS MARKETED IN NIGERIA

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ABSTRACT

The human ear has a diverse microbial community, some of which may turn into opportunistic pathogens under favourable conditions. Antimicrobial resistance among ear flora is a rising public health concern in healthy individuals. In our study, we investigated the susceptibility profiles of bacteria isolated from healthy ears of students to systemic antibiotics and selected commercial eardrop formulations. Ear swab samples from healthy students were cultured, and isolates were identified through morphological, Gram stain, and biochemical tests. The susceptibility of the identified organism to commonly used antibiotics was evaluated using the disc diffusion method and interpreted in accordance to CLSI guidelines. The organisms recognized to be multidrug resistant were further tested against five brands of gentamicin ear drops and one brand of chloramphenicol ear drops using the agar well diffusion method, with constant volumes, controls, and formulation details recorded. A total of 100 isolates were recovered, comprising *Staphylococcus*, *Micrococcus*, and *Bacillus* species. There was high susceptibility to levofloxacin, ciprofloxacin, chloramphenicol, and gentamicin, with resistance mainly to azithromycin and Meropenem among the isolates. Majority of the isolates were resistant to multiple antibiotics. However, all identified multiple antibiotic-resistant isolates were susceptible to the tested eardrop formulations. The findings highlight the persistence of multidrug resistant bacteria in the normal ear flora. It also affirms the retained efficacy of quality topical preparations, thus providing a basis for their value in localized infections.

ARTICLE INFO

Received 19 November, 2025

Accepted 26 April, 2026

Published 30 April, 2026

KEYWORDS

Healthy ear, antimicrobial resistance, gentamicin, chloramphenicol, ear drops, multi-drug resistance.

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INTRODUCTION

The human ear functions as an organ responsible for hearing and balance. It receives sound waves from the surroundings and converts them into signals that the brain can understand. This process is called transduction [1]. The human ear has a natural flora that comprises a vast number of microorganisms, most of which are significantly essential for the functionality and maintenance of this body organ [2]. These microorganisms include bacteria, fungi, and sometimes viruses [2, 3]. Most of these organisms exist in a symbiotic

relationship with the host, except for the pathogenic ones. They help boost human natural immunity as they compete and inhibit other dangerous pathogens from colonizing and causing illnesses [3]. However, due to some disturbances and disruptions that might occur due to changes in the environment, improper usage of antibiotics, and a poor or weak human immunity system, these organisms can become opportunistic pathogens that can lead to ear infections [4]. Ear infections, also known as otitis, can affect the

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<https://doi.org/10.59493/ajopred/2026.1.20>

ISSN: 0794-800X (print); 1596-2431 (online)

outer, middle, or inner part of the ear. These are referred to as otitis externa, media, or interna, respectively [5]. Common organisms that cause these infections include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* [6, 7]. In Nigeria and similar resource-limited settings, empirical antibiotic treatment remains common due to limited access to culture-based diagnostic services [8]. The rise in antimicrobial resistance among bacteria that cause diseases and harmless ones represents a major public health concern. This is particular to low- and middle-income countries where antibiotics are often accessible without prescription [9, 10]. Consequently, it is important to conduct surveillance of both harmful and commensal ear organisms in order to track emerging resistance patterns that may complicate empirical therapy.

Topical antimicrobial agents remain the most commonly used medication for localized ear infections [11]. Gentamicin and chloramphenicol ear drops are among the most commonly used. Gentamicin is effective against Gram-negative organisms, such as *P. aeruginosa* [12]. On the other hand, chloramphenicol is commonly used for infections that affect the middle and external ear [13, 6]. However, there is a growing concern. The increased circulation of substandard and falsified medicines in the Nigerian pharmaceutical market raises concerns about the actual efficacy of some products that are marketed locally [14, 15]. In another context, commensal bacteria in healthy ears may harbour resistant genes that are transferable. Such genes may reduce the effectiveness of standard medications [9]. Hence, they can directly affect the outcomes of patients.

Only a few studies in Nigeria have examined how much bacterial isolates from healthy human ears are susceptible to commonly used antibiotics, despite their potential role as carriers of resistance genes [9]. It is important to monitor these organisms. It not only gives insight into resistance patterns that occur outside obvious clinical infections, but also into the performance of commonly used medications. Together, these will provide useful insight into the effectiveness of available formulations of antibiotics, particularly in cases of resistance.

Therefore, this study investigates the bacterial flora of healthy ears of university students. It examines how susceptible the isolates recovered are to commonly used systemic antibiotics. It also evaluates the efficacy of selected locally marketed brands of

gentamicin and chloramphenicol ear drops against multidrug-resistant isolates.

MATERIALS AND METHODS

Study Area and Ethical Approval

The study was conducted in the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Obafemi Awolowo University (OAU), Ile-Ife, Osun State, Nigeria. Ethical clearance for the study was obtained from the Health Research Ethics Committee of the Institute of Public Health, OAU (Ethical Approval Number: IPH/OAU/12/2793). Also, all procedures involving human participants were conducted with strict adherence to ethical considerations as highlighted in the protocol for the study. The consent forms were signed by all participants before sample collection. Confidentiality and anonymity were maintained throughout the research process. This was done by assigning codes to participant data and by ensuring secure storage of collected information.

Sample Collection

Earwax samples were collected aseptically from both ears of fifty (50) volunteers who are apparently healthy. The volunteers were students aged 16 years and above without any ear infection at the time of sampling. Individuals with ear infections, those who had taken antibiotics within 3 weeks, or those with a history of ear surgery/trauma were excluded from this study. The process of collection of earwax from the ears of students was done by using aseptic method. It was collected from the external auditory canal (EAC) using cotton swabs that are sterile. The collected swabs were immediately inoculated onto freshly prepared nutrient agar plates. This was done in order to ensure that the organism remains viable and to reduce accidental contamination.

Isolation and Characterization of Organisms

The collected samples were inoculated directly on freshly prepared agar plates. The streak plate method for the isolation of organism was used to ensure that each colony is isolated. The inoculated plates were incubated at 37 °C for 24 h. After incubation, distinct colonies were examined for their morphological properties for mixed growth/colonies. Mixed growth is defined by distinct colonies with different morphological properties, that is, size, colour, shape, elevation, and margin. Where mixed growth was observed, the different colonies were picked

separately with a thin loop and inoculated onto freshly prepared agar plates. The streaking method is used again to isolate colonies. This process continued until pure isolated colonies were obtained.

The pure isolates were then identified by assessing the colony morphology. Gram staining and standard biochemical tests were also performed. The biochemical test carried out included catalase, oxidase, indole, citrate utilization, urease, methyl red, coagulase, hemolysis, and carbohydrate fermentation tests [16]. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* NCTC 6571, and *Pseudomonas aeruginosa* ATCC 10145 were used as control organisms. They were used to validate how good the culture media is and the biochemical test methods used in the identification process. The known positive and negative reactions of these strains served as references for the interpretation of results obtained from the tested isolates.

Susceptibility of Isolates to Selected Antibiotics

The antimicrobial susceptibility of isolates was determined using the Kirby-Bauer disk diffusion method [17]. Distinct colonies of each organism were dissolved in sterile distilled water to get an organism suspension. The organism suspension was standardized to 0.5 McFarland turbidity standard ($\approx 10^8$ CFU/mL) using sterile distilled water. Sterile swab stick was used to spread the bacterial suspension on the surface of a well-dried Mueller-Hinton Agar. This ensured that each organism suspension had approximately similar density. A pair of sterile forceps was used to place antibiotic discs on the inoculated agar surface. The following antibiotics were tested using Biomark® Gram-positive discs: azithromycin 15 µg, Meropenem 10 µg, ceftriaxone 30 µg, erythromycin 5 µg, co-trimoxazole 25 µg, levofloxacin 5 µg, gentamicin 10 µg, ciprofloxacin 5 µg, Augmentin 30 µg, vancomycin 30 µg, chloramphenicol 10 µg, and cephalixin 30 µg.

The inoculated plates with antibiotic discs were incubated at 37 °C for 18 – 24 h. After incubation, the diameter of the zones of inhibition was measured and recorded in millimetres. Results were interpreted using the Clinical and Laboratory Standards Institute (CLSI) guidelines [18] as Resistant, Susceptible and Intermediate. Isolates resistant to three or more antibiotic classes were noted. They were defined as the multidrug-resistant (MDR) bacterial isolates.

Susceptibility of Multidrug Resistant Isolates to Marketed Ear Drops

The MDR bacterial isolates identified from the first phase of antimicrobial susceptibility testing were further evaluated for their susceptibility to five commercially available brands of gentamicin ear drops and one brand of chloramphenicol ear drops marketed in Ile-Ife, Nigeria. These brands were selected based on their popular use in the treatment of ear infections. Also, their availability in local pharmaceutical outlets as at the time of this study informed their usage. Table 1 shows the packaging and product information for the selected brands. For the susceptibility testing in this phase, the agar-well diffusion method was employed. In this method, seeded Mueller-Hinton agar plates were prepared with standardized bacterial suspensions (0.5 McFarland Turbidity). Wells (about 8mm in diameter) were made in agar plates with sterile cork-borer. The wells were filled with equal volumes of each ear-drop formulation. Sterile distilled water served as the negative control and streptomycin (1 mg/mL) as the positive control. Following incubation at 37 °C for 18–24 h, inhibition zones were measured.

Table 1: Packaging and Product Information for Gentamicin and Chloramphenicol Ear Drops Used in the Study

Brand Name	Active Ingredient	Manufacturer	Batch No	Mfg Date	Exp Date	Strength	Packaging size	Country of Manufacture
Aventra	Gentamicin	Fidson	F18224006	09/24	08/26	0.3%	10ml	Nigeria
Drugent	Gentamicin	Drugfield	47GO128	07/23	06/28	0.3%	10ml	Nigeria
Chumath	Gentamicin	Chumath	230984	09/23	09/26	0.3%	10ml	China
Gentalab	Gentamicin	Embassy	23GD06	01/23	12/25	0.3%	10ml	Nigeria
Venita	Gentamicin	Alpha Lab	VE3038	02/23	01/26	0.3%	10ml	Nigeria
MCA	Chloramphenicol	M.C.A	22CT16	09/22	08/25	5%	10ml	India

Statistical Analysis

The data obtained from this study were entered and analyzed using Microsoft Excel (Microsoft Corporation, USA). Descriptive statistics such as frequencies, percentages and mean deviation were used to summarize the data. No inferential statistical analysis was performed as the study was primarily descriptive.

RESULTS

Isolation and Characterization of Organisms

In this study, a total of 100 isolates were obtained. Table 2 presents the distribution of bacterial species isolated from earwax samples. The largest proportion of isolates belonged to *Staphylococcus spp.* This was followed closely by *Micrococcus spp.*, while *Bacillus spp.* formed a smaller share of the total.

Table 2: Distribution of the organisms isolated from the earwax

Organism	Number of Isolates	Percentage Distribution
<i>Bacillus spp</i>	18	18.00%
<i>Micrococcus spp</i>	39	39.00%
<i>Staphylococcus spp</i>	43	43.00%
Grand Total	100	100.00%

Susceptibility of Isolates to Selected Antibiotics

Table 3 presents the collective susceptibility of all the isolated organisms to commonly used antibacterial agents. Susceptibility was highest for levofloxacin (93%), chloramphenicol (92%), gentamicin (92%), ciprofloxacin (91%), ceftriaxone (90%), and co-trimoxazole (84%). vancomycin (87%), and Augmentin (76%) also demonstrated strong activity. Erythromycin showed moderate susceptibility at 62%, while azithromycin and cephalexin were lower at 59% and 52%, respectively. Meropenem had the least activity, with only 23% of the isolates susceptible. The table further presents the susceptibility patterns of isolates in groups to all twelve antibiotics. Overall, the *Micrococcus spp.* exhibited complete susceptibility to most of the antibiotics tested. On the other hand, *Bacillus spp.* and *Staphylococcus spp.* showed high sensitivity to vancomycin, levofloxacin, chloramphenicol, gentamicin, and ceftriaxone, but

reduced susceptibility to azithromycin and meropenem was observed in several of the species.

Susceptibility of Multidrug Resistant Isolates to Marketed Ear Drops

Table 4 summarizes the susceptibility patterns of multi-resistant isolates to selected brands of gentamicin ear drops and a chloramphenicol ear drop brand marketed in Ile-Ife, Nigeria. The multidrug-resistant isolates demonstrated pronounced susceptibility to the tested formulations. Sterile distilled water and 1 mg/mL streptomycin were used as negative and positive controls, respectively. All the organisms were susceptible to streptomycin, while no zone of inhibition was observed with sterile distilled water.

Table 3: The susceptibility patterns of organisms isolated to the Gram-positive antibiotic disc

Organism	VAN	COT	LBC	CHL	GEN	ERY	AZN	AUG	CTR	CP	MEM	CIP
<i>Bacillus spp</i>	83%	89%	89%	94%	100%	56%	44%	72%	100%	44%	28%	94%
<i>Micrococcus spp</i>	92%	90%	95%	95%	92%	79%	79%	85%	92%	72%	26%	97%
<i>Staphylococcus spp</i>	84%	77%	93%	88%	88%	49%	47%	70%	84%	37%	19%	84%
Percentage of isolate susceptible	87%	84%	93%	92%	92%	62%	59%	76%	90%	52%	23%	91%

KEY: VAN: Vancomycin, LBC: Levofloxacin, CIP: Ciprofloxacin, CHL: Chloramphenicol, GEN: Gentamicin, CTR: Ceftriaxone, COT: Co-trimoxazole, CP: Cephalexin, AUG: Augmentin, ERY: Erythromycin, AZN: Azithromycin, MEM: Meropenem.

Table 4. Mean inhibition zones (mm) of MDR isolates to marketed ear-drop brands

Brand	Active ingredient	Mean zone (mm)	Susceptibility
Aventra®	Gentamicin	36.4 ± 8.0	Susceptible
Drugent®	Gentamicin	33.8 ± 7.5	Susceptible
Chumath®	Gentamicin	35.1 ± 8.3	Susceptible
Gentalab®	Gentamicin	35.0 ± 9.2	Susceptible
Venita®	Gentamicin	35.1 ± 8.0	Susceptible
MCA®	Chloramphenicol	39.1 ± 6.4	Susceptible
Positive Control	Streptomycin (1 mg/mL)	29.0 ± 6.8	Susceptible
Negative Control	Sterile distilled water	0	Resistant

DISCUSSION

This study identified 100 bacterial isolates from healthy students' cerumen, with *Staphylococcus spp.* (43%) being the most common, followed by *Micrococcus spp.* (39%) and *Bacillus spp.* (18%). This distribution represents the normal flora of the EAC for this study. The dominance of *Micrococcus spp.* and coagulase-negative staphylococci (CoNS) can be linked with the findings of previous studies that have documented these organisms as part of the ear's normal flora [19, 20]. These bacteria are generally non-pathogenic under normal conditions, but they play an important role in colonization resistance, whereby they occupy ecological niches and limit pathogenic colonization [3].

The detection of the potentially pathogenic members of *Staphylococcus spp.* and *Bacillus spp.*, although in smaller proportions, is noteworthy. These members are recognized opportunists. Their presence in a healthy ear suggests that the EAC can act as a silent reservoir for bacterial organisms that are potentially

pathogenic. This becomes relevant in clinical cases when the ear's natural defences are disrupted by trauma, moisture, or immunosuppression [4]. Also, the absence of Gram-negative organisms, such as *Proteus mirabilis*, *Pseudomonas aeruginosa*, among others, indicates that the sampled population was healthy and free of active infection at the time of sampling.

The antibiotic susceptibility testing revealed several important details. Despite the fact that most of the organisms recovered were commensal organisms, resistance still existed. The existence of resistance to several antibiotics by the organisms could be attributed to environmental acquisition of resistance genes. It could also be by horizontal transfer from pathogenic bacteria, among other factors [21]. Also, the relatively high susceptibility of isolates observed with fluoroquinolones, chloramphenicol, and gentamicin indicates that these antibiotics remain effective against commensal ear flora. The same way, the moderate to low resistance observed for macrolides and β -lactams such as azithromycin and

Augmentin means that the previously existing concerns over the rising level of macrolide resistance among *Staphylococcus spp* and *Micrococcus spp.* is valid [22]. Interestingly, Meropenem (a broad-spectrum carbapenem) exhibited poor efficacy against the isolates. This is consistent with the fact that Gram-positive organisms in normal flora may possess natural resistance to antibiotics because of their structure [23]. It may also mean that carbapenem diffusion and binding are limited in such species that are resistant to it [23].

The analysis at the genus level showed that isolates within the *Micrococcus spp* and *Staphylococcus spp.* exhibited complete susceptibility to virtually all antibiotics tested. However, some of them exhibited multidrug resistance. In particular, certain isolates in the *Staphylococcus spp.* showed particularly low susceptibility to azithromycin, cephalexin, and Meropenem. These findings are similar to reports of methicillin-resistant *S. aureus* (MRSA) and multi-resistant CoNS as major contributors to healthcare-associated and community-acquired infections. There has been a report of multidrug resistance (MDR) in CoNS in about 23% of isolates in Europe [24]. A similar result was found in Africa. 5% of individuals who have no active infections or symptoms carry MRSA [25]. These findings give perspective to the silently increasing challenge of resistance globally.

Furthermore, the pattern of resistance in *Bacillus spp.* varied for different species. Few of the isolates in this genus demonstrated resistance to multiple agents, hence defined as multi-resistant. However, a good number maintained the generally high susceptibility to fluoroquinolones, chloramphenicol, and gentamicin observed in other organisms. These results again show that healthy carriers can act as reservoirs of resistant bacteria. This could become a problem in cases of opportunistic infections or in cases where there is transfer of resistance determinants to more virulent species [21].

Organisms showing the MDR properties during the susceptibility testing were mainly seen in *Staphylococcus spp.* and *Micrococcus spp.* In fact, there was an isolate within the *Staphylococcus spp.* that was resistant to all 12 antibiotics tested. This was a disturbing observation because it was isolated from the ear of a healthy individual. This suggests that resistance is not solely acquired during infection treatment. It may arise and persist in the normal flora of the body. It may be as a result of environmental antibiotic exposure, substandard pharmaceutical

products, or community-level misuse. Remarkably, almost all isolates were completely susceptible to all eardrop formulations tested. This includes those that are resistant to virtually all 12 antibiotics in the standard disc panel. This suggests that, at least *in vitro*, the tested products retain their antimicrobial activity despite the presence of multidrug resistance to systemic antibiotics. No correlation was found between systemic resistance and topical susceptibility. This likely reflects the pharmacodynamic advantage of topical agents, which achieve high local concentrations of active agents which is capable of overcoming resistance mechanisms.

In this study, all tested brands – Aventra®, Drugent®, Chumath®, Gentlab®, Venita® (gentamicin-based), and MCA® (chloramphenicol-based) – demonstrated potent and comparable *in-vitro* antimicrobial activity against multidrug-resistant isolates. The mean inhibition zones observed for all the brands are within acceptable potency limits. This indicates that the brands were adequately formulated. It also confirms that the products are microbiologically effective under laboratory conditions, indicating brand quality. Nevertheless, variability in inhibition diameters across brands shows the need for ongoing quality control and post-marketing surveillance to ensure therapeutic consistency.

This study has implications for the health of the public, and they are broad. Asymptomatic carriers of multidrug-resistant organisms (MDROs) may unknowingly spread them to others. This is risky for infants, the elderly, or hospital patients. These categories have weak immune systems. Once resistant infections occur, they become prolonged and complicated. Sometimes, it may lead to death. Global studies also show that resistance can be transferred. For instance, a study in Europe reports that 1 in every 4 individuals has CoNS that are resistant to multiple antibiotics living freely on their skin [24]. The same study further reveals that 2-6% of healthcare workers are carriers of MRSA [24]. South America has more difficult results. 1 in every 3 healthy children of the school age have multidrug resistant on their skin [25]. These findings prove that resistant organisms are capable of spreading widely within and among communities. This can happen in any population regardless of colour, race, or geography.

There are public health implications of this study in Nigeria, also. First, the presence of resistance in normal bacterial organisms in healthy humans shows that more surveillance data is needed. This will help Nigeria to comprehend the magnitude of the problem. Also, this surveillance must extend beyond the four walls of the hospital. When such data is routinely collected, it would help track early-warning signs for resistance trends. Secondly, post-marketing quality monitoring of drugs and antibiotics in the market is important, although the result obtained from this study shows the preserved efficacy of the tested brands of eardrops. Continuous drug monitoring and surveillance will help regulatory bodies to discover counterfeit products circulating the market. Thirdly, this study also provides evidence for drug choice. We found that fluoroquinolones and common agents like chloramphenicol and gentamicin are excellent drugs of choice for ear infections. This generalization is based on the *in vitro* results obtained. The same way, this study reveals that macrolide therapy is less suitable for ear infections. In all, these generalizations require additional studies for confirmation. Lastly, the detection of severe MDR with *Staphylococcus spp* from healthy ears is worthy of attention. It emphasizes the need for a more stringent control of antibiotics. This should be done by educating the public on the rational and good use of antibiotics and limiting access.

All these observations put further perspective on the “One Health” approach to antimicrobial resistance. One Health means that humans, animals, and the environment are all connected together [21]. Changes in one automatically affects the other. So, humans, animals and the environment are all implicated in the transfer of resistance [21]. This study also shows this reality. The discovery of multi-drug resistant (MDR) bacteria in students' healthy ears is not just a coincidence or a serendipitous discovery. It tells a deeper story about the status of the environment's health. As healthy humans are becoming more and more resistant, the animals and the environment must also be harbouring or have harboured a lot of resistance. This raises the possibility of quiet transmission between participating factors in One Health.

While this study offers valuable data and insights, certain limitations should be considered. Firstly, the participants were restricted to students from a single institution. This may limit how much results can be

generalized to represent the wider Nigerian population. Secondly, molecular studies on the mechanism of resistance of organisms were not carried out. This limits how much is known about how resistance genes are transferred or how resistance occurs in individual organisms. Lastly, the strong *in vitro* activity of the ear drops does not necessarily mean that the same result will be achieved in clinical conditions. *In vivo* performance of topical preparations can be influenced by cerumen composition, patient adherence, and biofilm formation in organisms. Therefore, the study leaves a gap for a multicentered studies incorporating molecular analyses and clinical outcome studies. This will provide a more comprehensive understanding.

CONCLUSION

The study indicates the presence of multidrug-resistant organisms in healthy ears of humans. It also highlights the continuous effectiveness of locally marketed gentamicin and chloramphenicol ear drops, particularly the tested brands. Based on these findings of the study, it is important to emphasize the importance of stewarding antimicrobial use, and to continually carry out surveillance of resistance patterns beyond clinical premises. This will help to provide public data on resistance profiles. There is also a need for sustained quality standards of pharmaceutical products in the Nigerian market. Public health strategies should focus on resistance surveillance in communities, education on rational antibiotic use, and regulation of antimicrobial sales. All these will assist in curbing the spread of resistance from harmless carriers to pathogenic contexts.

ACKNOWLEDGMENT

The authors wish to acknowledge Mrs. Roseline Aderonke Adewusi, the laboratory technologist, for her technical support throughout the duration of the study.

AUTHORS' CONTRIBUTION

MOO conceptualized the idea and designed the work with FOA. HAB carried out the collection of ear samples, isolation of organisms, and the characterization of isolates under the supervision of MOO, FOA and DIO. HAB wrote the first draft of the manuscript. MOO, FOA, and DIO revised the manuscript. All authors approved the manuscript for submission.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

This research work was funded by the authors with no external funding.

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