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Emmanuel SUNDAY

sundayemmanuel1638@gmail.com

Anchor University

Michael B. OSHO

McPherson University

Kayode O. AFOLABI

Anchor University Lagos

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Hidden Reservoirs, Emerging Threats: Herbal Medicines Fuel the Spread of Antimicrobial Resistance

Emmanuel SUNDAY^{1*}; Michael B. OSHO²; Kayode O. AFOLABI^{1,3,4};

¹Molecular Epidemiology and Public Health Research Group (MEPHREG), Department of Biological Sciences, Anchor University, Lagos, Lagos state, Nigeria

²Department of Microbiology & Biotechnology, McPherson University, Seriki Sotayo, Ogun State, Nigeria

³Anchor University Centre for Global Health, Anchor University, Lagos, Lagos state, Nigeria

⁴Department of Microbiology, University of Ibadan, Ibadan, Oyo state, Nigeria

Corresponding Author: Emmanuel Sunday, sundayemmanuel638@gmail.com, 09034846493

Abstract

Contaminated herbal medicinal products are increasingly recognized as a potential reservoir for the dissemination of antimicrobial-resistant bacteria within communities. This study provides a systematic characterization of the resistance phenotypes and genotypes of bacteria contaminating herbal medicinal products sold across six Local Council Development Areas of Alimosho Local Government Area, Lagos State, Nigeria. A cross sectional study of 150 herbal products in six Local Council Development Areas of Alimosho Local Government Area, Lagos State, Nigeria was conducted. The bacteria were isolated by using the standard method and susceptibility was determined by Kirby–Bauer disc diffusion following CLSI recommendations. The MAR indices and MDR, MDR and PDR phenotypes were calculated. The resistant isolates were subjected to PCR for tetA, sul1, blaCTX-M, ant(3'')-Ia, qnrA, qnrB, qnrC and qnrS to identify the genotype–phenotype associations and statistical analysis was performed. The total number of bacteria isolated was 520, which included *Staphylococcus*, *Escherichia*, *Bacillus*, *Enterobacter*, *Klebsiella*, *Citrobacter*, *Proteus*, *Pseudomonas*, *Salmonella* and *Shigella*. A high level of resistance was seen in sulfamethoxazole-trimethoprim (67.7%), imipenem (63.1%), augmentin (60.2%), erythromycin (50.4%), and tetracycline (49.2%) and a high level of sensitivity was shown in ciprofloxacin (96.3%) and gentamicin (88.5%) in the antimicrobial susceptibility test with MDR phenotypes being common and MAR indices > 0.20 in most strains. Molecular analysis revealed the presence of several resistance genes, including sul1 (60.6%), tetA (49.7%), QnrC (24.2%), ant(3'')-Ia (21.8%), QnrA (20.6%), blaCTX-M (18.8%), QnrB (7.3%), and QnrS (3.6%). The distribution of genes was significantly different among LCDAs and sample types ($p < 0.05$); some genes had significant genotype–phenotype associations prior to multiple testing correction. Herbal products sold within Alimosho LGA constitute a significant source of resistant bacteria and transferable resistance genes, potentially contributing to the spread of antimicrobial resistance within the community. Strengthened microbiological and molecular surveillance, alongside more rigorous regulatory control, would meaningfully improve the safety of these products and reduce the risk they pose to public health.

Introduction

Herbal medicine is an integral part of primary health care in many developing countries, with an estimated 80% of the population relying on herbal remedies for their primary health needs (World Health Organization (WHO) 2024). This is especially apparent in Nigeria. The powders, infusions and decoctions can be used to control a wide variety of ailments, such as malaria, typhoid fever, gastrointestinal disorders, respiratory ailments, hypertension, diabetes and infertility. The commercial production of these preparations has grown, and so has their distribution, making them increasingly available to the growing population of consumers (Omoruyi *et al.*, 2024). However, there is a lesser known worry from this general acceptance, and that is the significant microbiological variability between such preparations (Ejimofo *et al.*, 2023). Herbal medicines begin their life as raw plant material gathered from farms, bush or roadside vegetation and each stage of the drying, grinding, packaging, storage and display process to the end product for sale is a potential source of contamination, particularly when hygienic practice is lax and there is little or no quality control (Alharbi *et al.*, 2024). Most are used without further processing and pathogenic organisms present in these products are transferred directly to the user, which can be more of a concern for immunocompromised individuals, pregnant women, and the elderly (Ahiabor *et al.*, 2024). A theoretical worry is not a real worry. The presence of bacteria in herbal preparations has been detected many times in Nigeria. Fawehinmi *et al.* (2023) isolated a wide spectrum of pathogenic bacteria from herbal preparations sold in Lagos, Lagos State, Nigeria and Oladosun *et al.* (2020) recorded similar findings in the South-West of Nigeria. Further north, in Kaduna metropolis, Abba *et al.* (2009) similarly reported that over half of the herbal remedies sampled were contaminated with organisms such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Shigella* species. These organisms recur throughout the literature, alongside *Klebsiella pneumoniae*, *Proteus* species, *Pseudomonas aeruginosa*, and various *Bacillus* and *Enterobacter* species, underscoring that contamination of herbal products is a widespread problem with direct implications for shelf life and consumer safety (Hassan *et al.*, 2024).

On top of that is another major issue that could have even more impact, namely antimicrobial resistance, which the WHO has identified as one of the top 10 threats to global health (Nkosi *et al.*, 2024), and has stated that the constant reduction in antibiotic effectiveness is putting 30 years of improvements in treating common infections at risk (WHO, 2021). Resistance occurs as a consequence of the combined pressure of the inappropriate use of antibiotics in human medicine, veterinary medicine and agriculture (Ahiaboe *et al.*, 2024; Alharbi *et al.*, 2024), and is transferred effectively between bacteria via horizontal gene transfer (Ovuru *et al.*, 2023). This mobility is mainly attributed to mobile elements such as mobile genetic elements, plasmids, integrons, transposons and gene cassettes carried by these mobile elements, which can spread resistance characteristics among distantly related bacterial species, and across different environments with ease (Partridge *et al.*, 2018). Herbal products are not protected from the effects of bacteria contaminating them during manufacture or handling; they can develop resistance to the antibiotic residues, disinfectants and heavy metals that they continually encounter, just like any other organism in their environment (Kumar *et al.*, 2023; Li *et al.*, 2023). When a consumer ingests a contaminated preparation, he or she is exposed to two concurrent risks: one, the pathogenicity of the organism ingested; and two, the possibility that the organism contains resistance genes that can establish themselves in the consumer's gut microbiota and spread further through community and environmental routes, exactly what the One Health framework was intended to address. This picture can only be captured by susceptibility testing, not by the underlying genetics; understanding how resistance is encoded and transmitted (Opuni *et al.*, 2023). Genes that confer resistance to tetracyclines, sulfonamides, β -lactams, quinolones, and aminoglycosides are often transferable on mobile plasmids, meaning that the presence of these resistance genes in a cluster of one bacterial population may indicate a threat of continued spread, not just a single incident (Ahiabor *et al.*, 2024; Mekonnen *et al.*, 2023). Much of the published work on the microbiological quality of herbal medicinal products in Nigeria has concentrated on determining microbial load and identifying contaminating organisms, with far fewer studies extending to antimicrobial susceptibility testing. Adeleye *et al.* (2005), Esimone

et al. (2007), Abba *et al.* (2009), and Fawehinmi *et al.* (2023), for instance, all reported on contamination levels, the range of microbial species recovered, and, in some cases, susceptibility patterns in herbal preparations, yet none of these studies ventured into the molecular mechanisms underlying antimicrobial resistance. While some Nigerian studies have included antimicrobial susceptibility testing, molecular characterization of resistance determinants is still limited and the presence of resistance genes and the epidemiology of resistance still need to be detected. This observation is in line with the last systematic review of microbial contamination of herbal medicines in Africa where it was reported that only about 4% of the published studies used molecular techniques while the remaining 96% used only conventional culture and biochemical identification. This information will be required to fill existing gaps in knowledge about the presence, prevalence and genetic origin of antimicrobial resistance in bacteria related to herbal medicines in Lagos. This study, therefore, investigated the bacterial contaminants present in the commercially available herbal preparations in the six LCDAs of Alimosho, their genotype–phenotype relationships with geographical variation in the study area and the detection of antimicrobial resistance genes by polymerase chain reaction (PCR). The results provide evidence of the role of contaminated herbal preparations in the burden of antimicrobial resistance in Alimosho, and indicate the type of surveillance and regulatory attention that is required to minimize the threat these products pose to public health.

Materials and methods

Study Site

The study was carried out in Alimosho Local Government Area (LGA) of Lagos State, Nigeria. Alimosho Local Government Area (LGA) is divided into six Local Community Development Areas (LCDAs), namely: Agbado/Oke-Odo LCDA; Ayobo/Ipaja LCDA, Alimosho LCDA (main LCDA, Egbe/Idimu LCDA, Igando/Ikotun LCDA and Mosan Okunola LCDA.

Study Design

In the present study, a cross sectional design was adopted and samples of bacteria isolate recovered April– August, 2025 from herbal drugs in six LCDAs of Alimosho LGA were collected (Usanga *et al.*, 2023).

Sample Size

All the 520 isolates recovered from 150 liquid and powdered herbal preparations where collected for this study. The sample size was initially calculated with the formula for an unknown population ($N = Z^2_{1-\alpha}[P(1 - P)] \div D^2$) for a 95% confidence interval. The standard error of the mean ($Z_{1-\alpha}$) was fixed at 1.96, the estimated prevalence of bacterial contamination that exceeded the permissible levels by the WHO of herbal preparations at 90% and the tolerable sampling error (D) at 0.05. Substituting these values gave $N = 1.96^2[0.9(1 - 0.9)] \div 0.05^2 = 138.3$, rounded up to a minimum of 138 samples (Omoruyi *et al.*, 2024; Ovuru *et al.*, 2023).

Sampling Techniques

Convenience sampling technique were applied. All the 520 previously identified bacteria isolates belonging to the genus; *Staphylococcus* spp, *Escherichia coli*, *Klebsiella* spp, *Enterobacter* spp, *Citrobacter* spp, *Salmonella* spp, *Shigella* spp, *Pseudomonas* spp and *Proteus* spp were used for this study.

Sample Collection

A total of 520 identified bacteria isolate from herbal preparations (liquid and powder) were obtained between the months of April – August, 2025 from the Microbiology Laboratory, Anchor University were provided for Phenotypic Antimicrobial Susceptibility Testing and molecular detection of resistance genes.

Resuscitation of Resistant Isolates

Each isolate was resuscitated by inoculating into Luria-Bertani (LB) broth and incubated at 37 °C for 18 - 24 hours to allow for active growth and recovery. The revived cultures were subsequently maintained under appropriate storage conditions for downstream analyses.

Phenotypic Antimicrobial Susceptibility Testing

Susceptibility profiles were obtained using Kirby-Bauer disk diffusion procedure according to CLSI methodology (CLSI, 2024). The antibiotic panel was designed to cover the major classes used in human medicine: fluoroquinolone antibiotics (ciprofloxacin 5 µg and nalidixic acid 30 µg), aminoglycoside antibiotics (gentamicin 5 µg and streptomycin 25 µg), cephalosporin antibiotic (ceftriaxone 30 µg), macrolide antibiotic (erythromycin 10 µg), phenicol antibiotic (chloramphenicol 30 µg), tetracycline antibiotic (tetracycline 10 µg), β-lactam antibiotic (ampicillin 10 µg), folate pathway inhibitor (trimethoprim-sulfamethoxazole 25 µg) and a carbapenem antibiotic (imipenem 30 µg). Each bacterial suspension was matched to 0.5 McFarland turbidity (standard inoculum) in sterile normal saline, and then even swabbed across Mueller-Hinton agar (Neogen Media, United Kingdom) to create a confluent lawn of bacteria. The inoculated plates were covered with aseptically placed discs and incubated at 35 °C for 18–24 h, aerobically. All zone diameters were measured to the nearest mm using a ruler, and susceptibility, intermediate or resistance was determined by the CLSI breakpoints. Multiple Antimicrobial Resistance (MAR) index was calculated for each isolate as the ratio of number of antibiotics which the isolate is resistant to, and the total number of antibiotics tested (CLSI, 2022).

Molecular Detection of Antibiotics Resistance Genes

Primers and PCR conditions listed in Supplementary Table 1 were used to detect antimicrobial resistance genes that correspond to key classes of antibiotics. The target genes were tetA (tetracycline resistance), ant(3'')-Ia (aminoglycoside resistance), sul1 (sulfonamide resistance), blaCTX-M (β-lactam resistance) and the qnr genes (quinolone resistance). PCR was carried out in 10 µL volumes according to the standard protocols, using the optimized annealing temperatures for each of the gene targets (Ahiabor *et al.*, 2024). Each reaction was 25 µL consisting of 0.5 µL forward primer, 0.5 µL reverse primer, 4 µL 5x FIREPol master mix (Solis BioDyne) (0.4 M Tris-HCl, pH 8.5, (NH₄)₂SO₄, 7.5 mM MgCl₂, 0.1% w/v Tween 20, 1 mM dNTPs, FIREPol DNA polymerase, blue and yellow dye), 3 µL template DNA, and 17 µL molecular-grade water, with a negative control of nuclease-free water. Amplicons were generated by denaturation at 95 °C for 5 min followed by 34 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min and then extended at 72 °C for 5 min and resolved on 1.5% agarose gels (70 V, 60 min) on a VWR Imager2 system (Germany) with amplicon sizes reminiscent of the expected target sizes (Ode *et al.*, 2026).

Supplementary Table 1: Oligonucleotides used in this study

Target gene	Primer direction and sequence	Amplicon size (bp)	Annealing temperature	References
SulA	F: 5'-AC TGC CAC AAG CCG TAA-3' R: 5'-GTC CGC CTC AGC AAT ATC-3'	350	60 °C	Zulkikam <i>et al.</i> , 2024
tetA	F: 5'-GCTACATCCTGCTTGCCTTC-3' R: 5'-CATAGATCGCCGTGAAGAGG-3'	210	55 °C	Nelly <i>et al.</i> , 2024
bla-CTX-M	F: 5'-ATGTGCAGYACCAGTAARGTKATGGC-3' R: 5'-TGGGTRAARTARGTSACCAGAAYCAGCGG-3'	593	60 °C	Ahiabor <i>et al.</i> , 2024
ant(3)-La	F: 5'-TTATCCAGCTAAGCGCGAA-3' R: 5'-ATGAGGGAAGCGGTGATCG-3'	526	60 °C	Omoruyi <i>et al.</i> , 2024
QnrA	F: 5'-CCGCTTTTATCAGTGTGACT-3' R: 5'-ACTCTATGCCAAAGCAGTTG-3'	188	55 °C	Fasema <i>et al.</i> (2024)
QnrB	F: 5'-GATCGTGAAAGCCAGAAAGG-3' R: 5'-ACGATGCCTGGTAGTTGTCC-3'	469	55 °C	
QnrC	F: 5'-CTGAAACCGTGCCAACTATG-3' R: 5'-TTTGCCTGCGTTTGGTATTC-3'	308	55 °C	
QnrS	F: 5'-ATGGAAACCTACAATCATAC-3' R: 5'-AAAAACACCTCGACTTAAGT-3'	417	55 °C	

Statistical analysis

Data were analyzed using descriptive and inferential statistical methods. Summary statistics included frequency distributions, means, and measures of dispersion. Inferential analyses included ANOVA, Cramer's V test, and Pearson's correlation coefficient, performed using GraphPad Prism1 and R with significance set at $p < 0.05$.

Results

Antimicrobial Susceptibility Profile of Bacterial isolates

A significant level of resistance was found for 11 of the antibiotics tested. In general, the most resistance was noted on tetracycline, amoxicillin-clavulanic acid (Augmentin), sulfamethoxazole-trimethoprim, erythromycin, streptomycin and imipenem, while the resistance was still low to ciprofloxacin, gentamicin, chloramphenicol, ceftriaxone and nalidixacid. Antimicrobial resistance pattern showed geographical heterogeneities with differences in resistance profile being significantly different across the six LCDAs ($p < 0.05$) (table 1). The resistance frequencies of the liquid herbal preparations derived isolates were consistently higher than for the powdered ones for most antibiotics, including sulfamethoxazole-trimethoprim, augmentin, erythromycin, tetracycline and streptomycin whereas lower resistance proportions were observed for isolates picked up from powdered formulations (Figure 1). There were also significant differences in resistance to the five antibiotics showing the highest resistance rates when compared using a geographical approach. Generally, the highest resistance frequencies were recorded in Ayobo/Ipaja, Agbado/Oke-Odo and Idimu/Egbe while the lowest resistance frequencies were recorded in isolates from Igando/Ikotun (Figure 2).

Table 1: Antibiotic Susceptibility Profile of Bacterial Isolates

Antibiotic Resistance and Susceptibility Patterns Across the Six LCDAs in Alimosho LGA													
Antibiotic	ALIMOSHO (n=117)		AGBADO (n=116)		MOSAN (n=118)		IDUMU (n=84)		AYOBO (n=52)		IGANDO (n=29)		
	%R	%S	%R	%S	%R	%S	%R	%S	%R	%S	%R	%S	P Value
Streptomycin	30.8	69.2	54.3	45.7	28.0	72.0	51.2	48.8	48.1	51.9	48.3	51.7	0.000
Tetracycline	29.9	70.1	55.2	44.8	56.8	43.2	44.0	56.0	75.0	25.0	44.8	55.2	0.000

Augmentin (Amox-Clav)	60.7	39.3	58.6	41.4	51.7	48.3	76.2	23.8	73.1	26.9	34.5	65.5	0.012
Nalidixic Acid	7.7	92.3	17.2	82.8	7.6	92.4	25.0	75.0	13.5	86.5	20.7	79.3	0.000
Ciprofloxacin	4.3	95.7	4.3	95.7	1.7	98.3	0.0	100.0	0.0	100.0	0.0	100.0	0.009
Sulfamethoxazole-Trimethoprim	67.5	32.5	79.3	20.7	66.1	33.9	66.7	33.3	67.3	32.7	34.5	65.5	0.000
Erythromycin	41.0	59.0	53.4	46.6	55.9	44.1	51.2	48.8	65.4	34.6	31.0	69.0	0.001
Gentamicin	5.1	94.9	12.1	87.9	5.1	94.9	11.9	88.1	13.5	86.5	10.3	89.7	0.000
Chloramphenicol	13.7	86.3	13.8	86.2	15.3	84.7	41.7	58.3	9.6	90.4	3.4	96.6	0.000
Cefuroxime	10.3	89.7	21.6	78.4	23.9	76.1	26.2	73.8	28.8	71.2	6.9	93.1	0.036
Imipenem	52.1	47.9	69.8	30.2	71.8	28.2	57.1	42.9	73.1	26.9	37.9	62.1	0.000

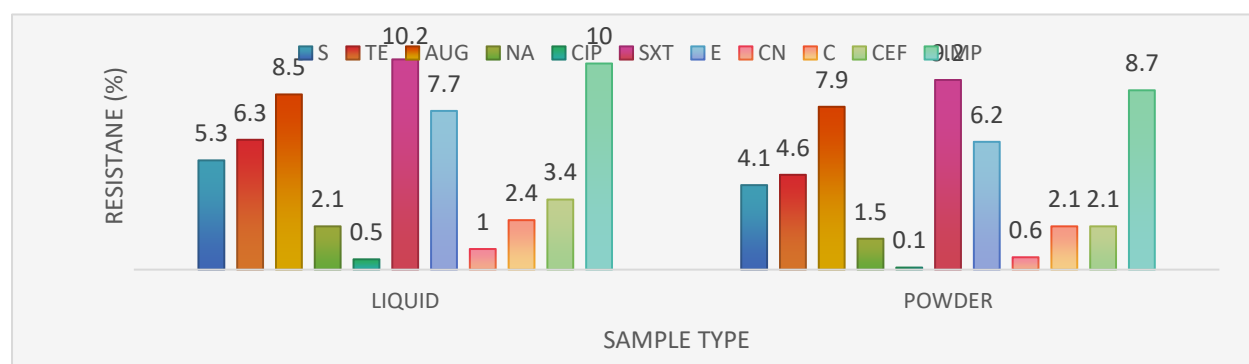


Figure 1: Resistance across sample types in all the six LCDAS in Alimosho LGA, Lagos State. S: Streptomycin, TE: Tetracycline, AUG: Augmentin, NA: Nalidixic Acid, CIP: Ciprofloxacin, SXT: Sulfamethoxazole-trimethoprim, E: Erythromycin, CN: Gentamicin, C: Chloramphenicol, CEF: Ceftriaxone, IMP: Imipenem.

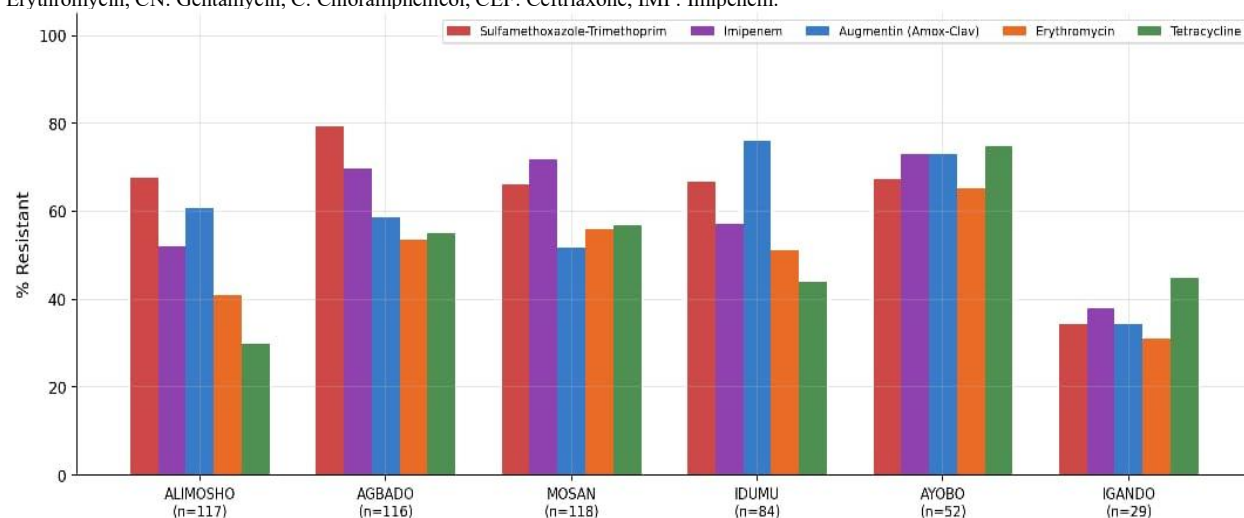


Figure 2: Resistance Rate for Top 5 Antibiotics Across LCDAs in Alimosho LGA

Distribution of Multidrug Resistance Phenotypes

Multidrug resistance was widespread and was confirmed by classification of isolates by internationally accepted definitions. Multidrug resistant (MDR) bacterial isolates were more than half while the proportions which were classified as extensively drug-resistant (XDR) and pandrug-resistant (PDR) was small (figure 3). There was considerable geographical variation in the distribution of resistance phenotypes (Figure 4). The proportion of MDR isolates was greatest at Ayobo/Ipaja, while the proportion of highly resistant isolates was highest at Idimu/Egbe (11% XDR and 14% PDR) indicating the highest burden of advanced resistance phenotypes at all study sites. A comparison of the antimicrobial resistance burden, on the other hand, showed a higher percentage of non-MDR isolates in Igando/Ikotun, as none of the isolates was found to be XDR. Agbado/Oke-Odo and Mosan/Okunola exhibited intermediate resistance profiles which had high MDR rates in addition to moderate rates of

XDR and PDR isolates (figure 4).

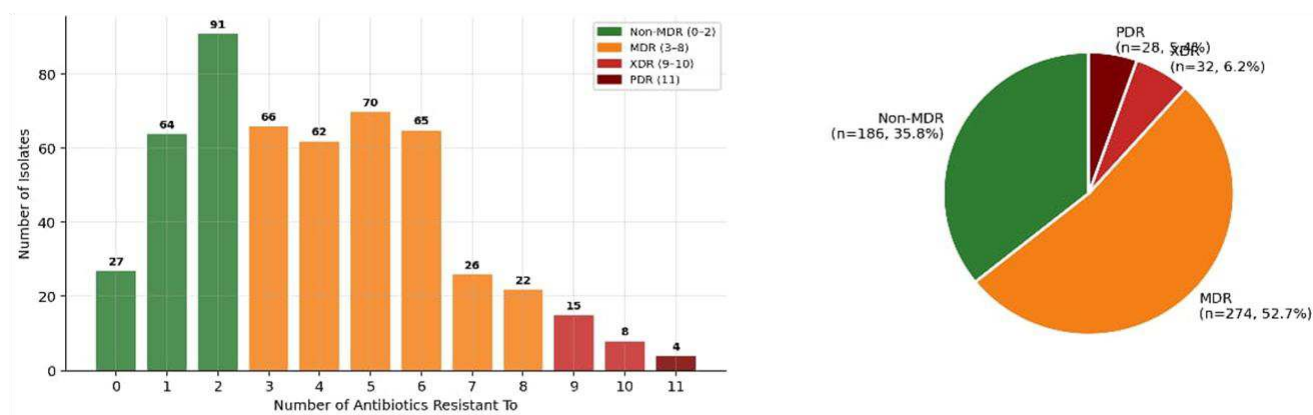


Figure 3: Distribution of Antibiotics resistance number per isolate and MDR Category Distribution (n=520)

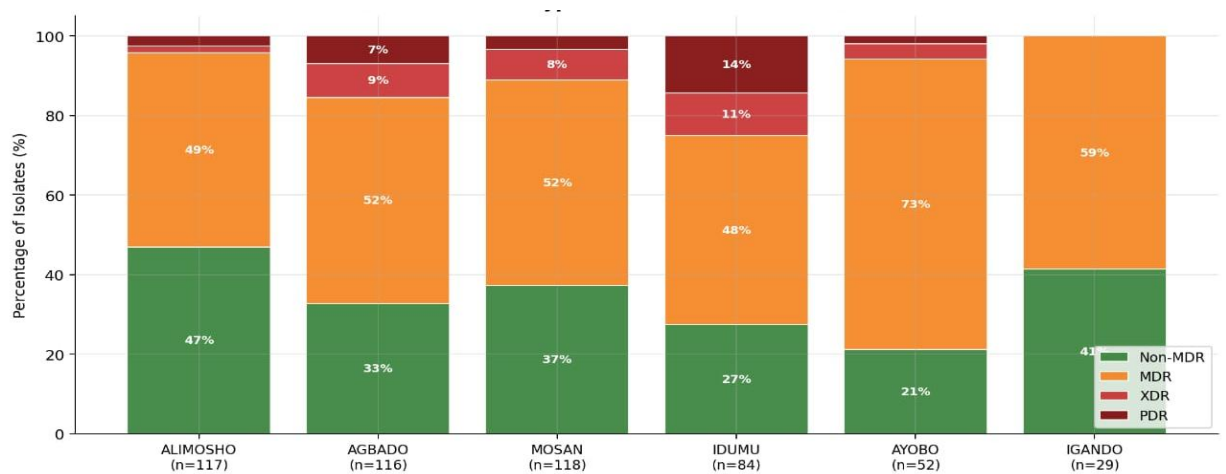


Figure 4: Geographic Variation in MDR Phenotype Across LCDAs

Geographic Correlation of Antimicrobial Resistance

Strong similarities in the resistance patterns were found between the majority of the LCDAs through Spearman correlation analysis (Figure 5). Very high correlation was found between Alimosho and Idimu/Egbe ($\rho = 0.99$, $p < 0.001$) and Alimosho and Agbado/Oke-Odo ($\rho = 0.96$, $p < 0.001$), which are closely related antimicrobial resistance profiles. In contrast, Igando/Ikotun had relatively low correlations with the other LCDAs (0.67-0.81) suggesting a unique regional resistance pattern.



Figure 5: LCDAs x LCDAs Spearman Correlation (based on 11 antibiotic resistance rate vectors)

Antibiotic Co-resistance Patterns

Non-random associations between resistance to some of the antibiotics were shown by the pairwise correlation analysis (Figure 6). There were found to be moderate positive correlation between erythromycin resistance with tetracycline resistance ($\rho = 0.43$),

streptomycin resistance with tetracycline resistance ($\rho = 0.40$), and sulfamethoxazole-trimethoprim resistance with augmentin resistance ($\rho = 0.38$). In comparison, there were comparatively low correlations between ciprofloxacin, gentamicin, ceftriaxone, and chloramphenicol with resistance to other.

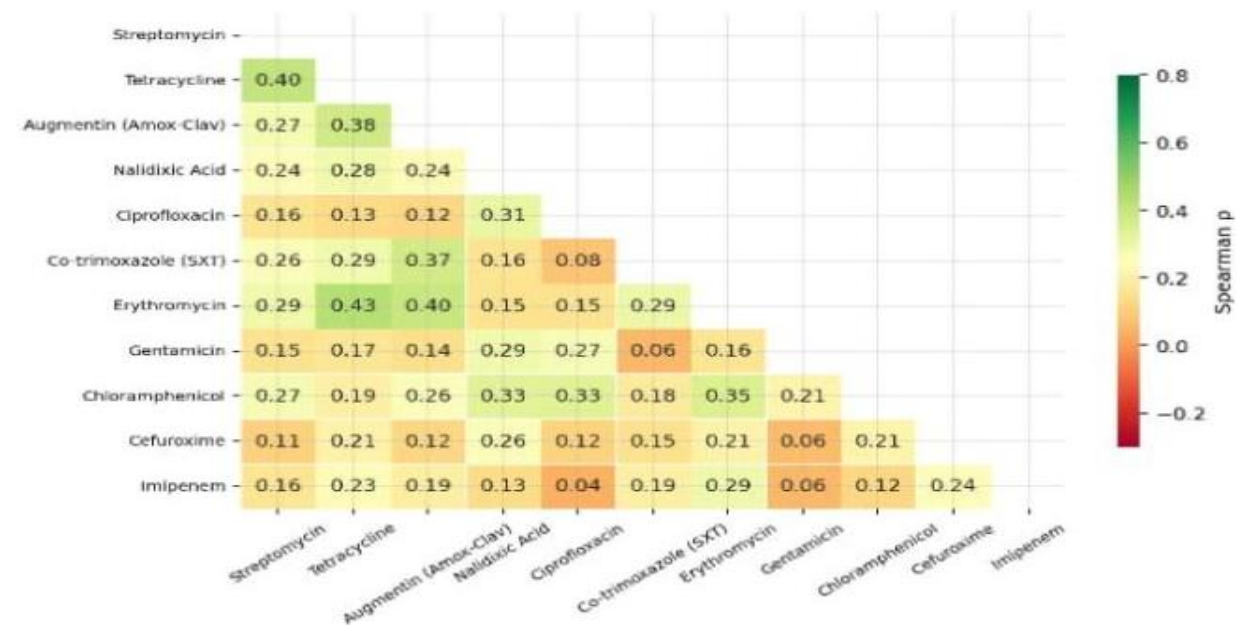


Figure 6: Antibiotic Co-resistance Correlation (Spearman p, all 520 isolates, lower triangle) antimicrobial agents ($p < 0.20$), suggesting different resistance mechanisms.

Hierarchical Clustering of Resistance Profiles

Antibiotics were clustered in two major resistance groups by hierarchical clustering (Figure 7). The lowest resistance frequency group was Ciprofloxacin, Nalidixic acid, Gentamicin, Chloramphenicol and Ceftriaxone, while Augmentin, Sulfamethoxazole-Trimethoprim, Imipenem, Streptomycin, Tetracycline & Erythromycin were grouped together due to their high resistance frequency. The location of Igando/Ikotun was separated from the other five LCDAs, indicating its antimicrobial resistance pattern was different from the other study areas.

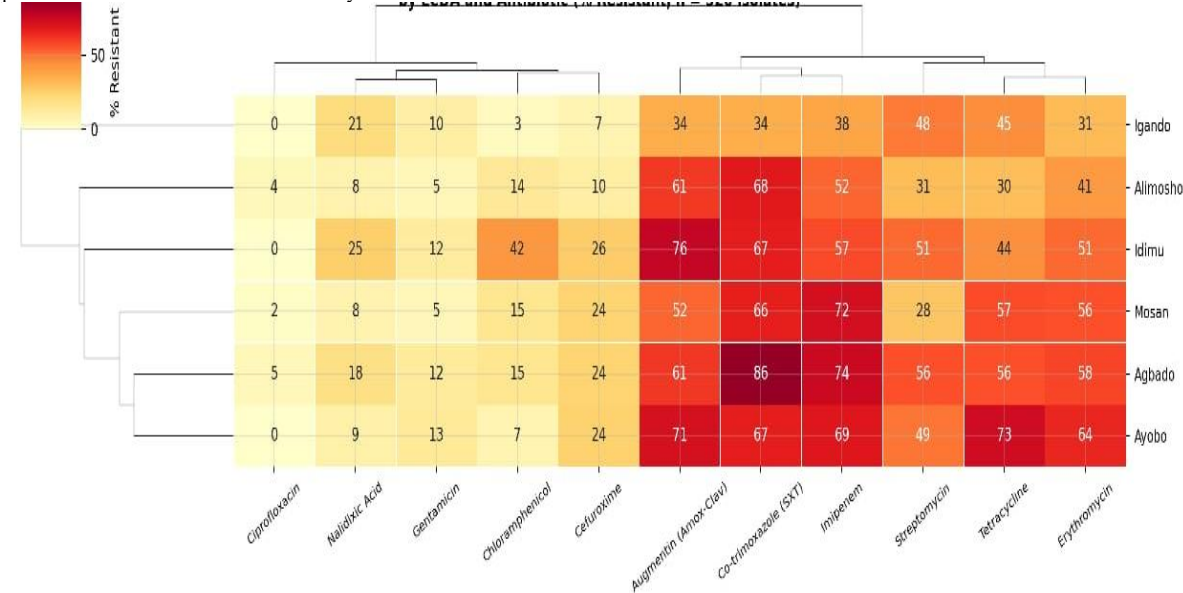


Figure 7: Hierarchically Clustered Resistance Rate Heat-map by LCDA and Antibiotics (% Resistant, $n = 520$ isolates)

Detection of Antimicrobial Resistance Genes

All eight targeted antimicrobial resistance genes were detected in 165 representative bacterial isolates using PCR (Figure 8). The *sul1* gene was the most common resistance determinant, detected in 60.7% of the gene-positive isolates, and *tetA* was the second most common (49.7%). Intermediate prevalence was recorded for *qnrC* (24.2%), *ant(3'')-Ia* (21.8%), *qnrA* (20.6%), and *blaCTX-M* (18.8%), whereas *qnrB* (7.3%) and *qnrS* (3.6%) were the least frequently detected genes (See also the gel images in figure S1, 2, 3, and 4).

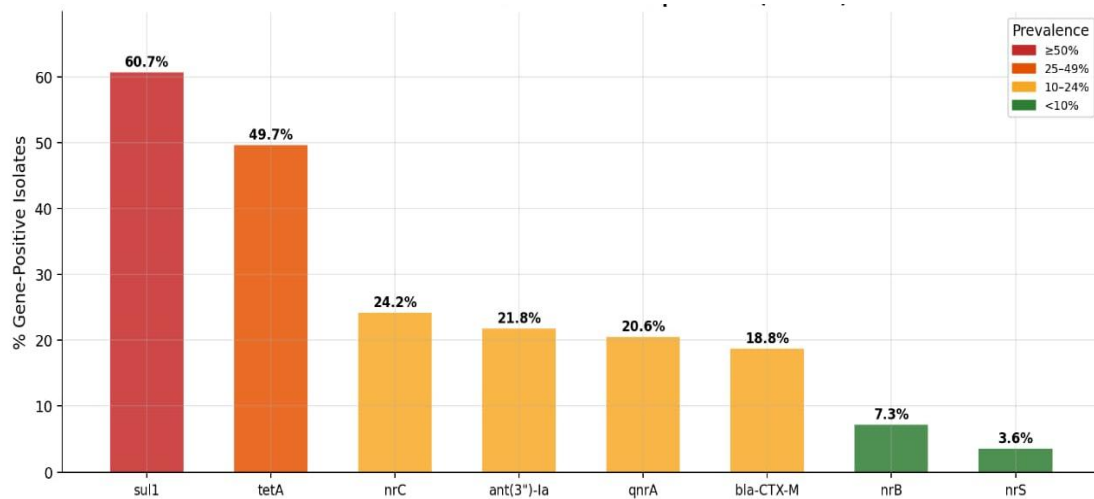


Figure 8: Prevalence of Antibiotic Resistance Genes in Bacterial Isolates from Herbal Preparations (n = 165)

Distribution of Resistance Genes According to Sample Type

Distribution of resistance genes based on sample type is described below. Bacterial isolates from liquid herbal formulations showed higher level of resistance to these antibiotics than those from powdered herbal formulations (Figure 9). There were significant differences for tetA ($p < 0.001$) and sul1 ($p = 0.005$) with a significant increase in both cases in the liquid formulations. However, no statistically significant differences were seen among the different formulations for blaCTX-M, qnrA, qnrB, qnrC, qnrS or ant(3'')-Ia ($p > 0.05$).

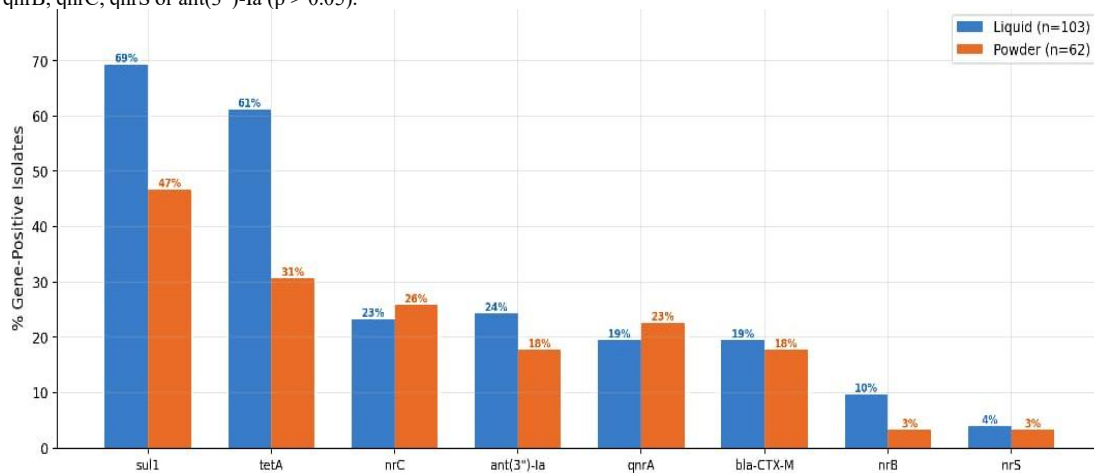


Figure 9: Distribution of Resistance Genes by Sample Type (Liquid vs Powdered Herbal Preparations)

Geographic Distribution of Resistance Genes

There was a large geographical variation in the distribution of several antimicrobial resistance genes (Table 2). Chi-square analysis demonstrated significant differences among the six LCDAs for sul1 ($\chi^2 = 19.40$, $p = 0.0016$), blaCTX-M ($\chi^2 = 24.21$, $p = 0.0002$), ant(3'')-Ia ($\chi^2 = 16.46$, $p = 0.0056$), and qnrA ($\chi^2 = 12.15$, $p = 0.0327$). The results for tetA, qnrB, and qnrS did not show any significant geographical variability; although qnrC approached statistical significance ($p = 0.0529$). Further, post hoc analyses showed that sul1 and ant(3'')-Ia differed significantly between Agbado/Oke-Odo and Idimu/Egbe ($p = 0.012$, $p = 0.044$, respectively) and blaCTX-M was significantly different between Agbado/Oke-Odo and Mosan/Okunola ($p = 0.002$). Interestingly, blaCTX-M was isolated at high frequencies in Igando/Ikotun (60%) and Mosan/Okunola (38.1%).

Table 2: Distribution of Antibiotic Resistance Genes Across the Six LCDAs

Resistant Gene	LOCATION						p-Value
	Agbado/Oke N(%)	Alimosho N(%)	Ayobo/Ipaja N(%)	Idimu/Egbe N(%)	Igando/Ikotun N(%)	Mosan/Okunola N(%)	
tetA	24(29.3)	12(14.6)	5(6.1)	16(19.5)	2(2.4)	23(28.0)	0.230
bla1CTX1M	3(9.7)	1(3.2)	3(9.7)	5(16.1)	3(9.7)	16(51.6)	0.000*
qnrA	19(55.9)	3(8.8)	3(8.8)	3(8.8)	1(2.9)	5(14.7)	0.033*

nrB	6(30.1)	0(0.0)	2(16.7)	0(0.0)	0(0.0)	4(0.0)	0.297
nrC	18(45.0)	2(5.0)	2(5.0)	6(15.0)	3(7.5)	9(22.5)	0.053
nrS	4(66.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(33.3)	0.384
ant³La	8(22.2)	2(5.6)	5(13.9)	13(36.1)	2(5.6)	6(16.7)	0.006*
sul1	19(19.0)	14(14.0)	11(11.0)	21(21.0)	4(4.0)	31(31.0)	0.001*

Association Between Resistance Genes and Phenotypic Resistance

Eighteen gene–antibiotic combinations had a p value < 0.15, seven of which were statistically significant (Figure 10). The best correlation was found between qnrS and resistance to ciprofloxacin (OR = 21.15, 95% CI: 3.16–141.51, p = 0.004). There were also significant positive associations between the presence of tetA and cefuroxime resistance (OR = 2.82, p = 0.004), qnrC and ciprofloxacin resistance (OR = 5.13, p = 0.007), qnrB and cefuroxime resistance (OR = 4.22, p = 0.025), sul1 and augmentin resistance (OR = 2.67, p = 0.042), and qnrA and chloramphenicol resistance (OR = 2.24, p = 0.049). A strong inverse correlation was found between qnrC and tetracycline resistance (OR = 0.38, p = 0.032). All of the targeted resistance genes were successfully amplified by PCR yielding the expected size of the amplicon, demonstrating the validity of the molecular detection assays (figure 11). Weaker to moderate genotype–phenotype relationships were present overall, with the highest correlation found between the genotype of qnrS and the resistance to ciprofloxacin ($\phi = 0.34$, p < 0.05). None of the associations observed were statistically significant, however, after Benjamini–Hochberg correction, which shows that the genes that were not tested in this study also contribute to the phenomenon of antimicrobial resistance among the bacterial isolates.

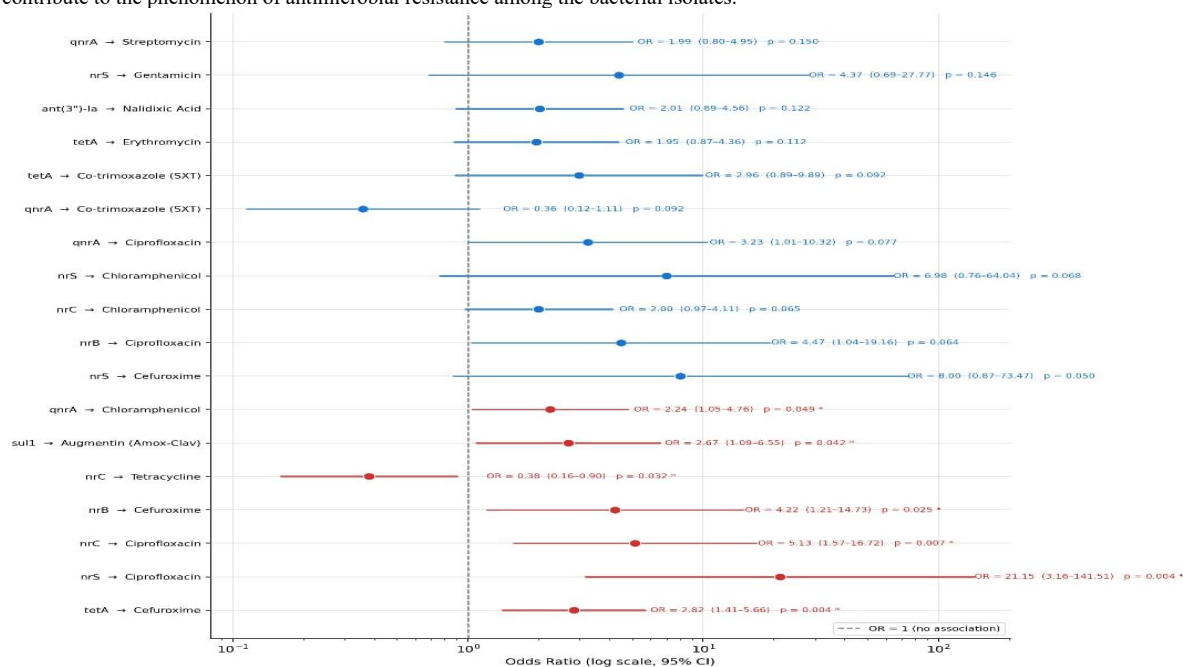


Figure 10: Forest Plot: Gene–Antibiotic Resistance Associations (Pairs with p<0.15 shown; red = p<0.05; ♦= Survives BH PDR)

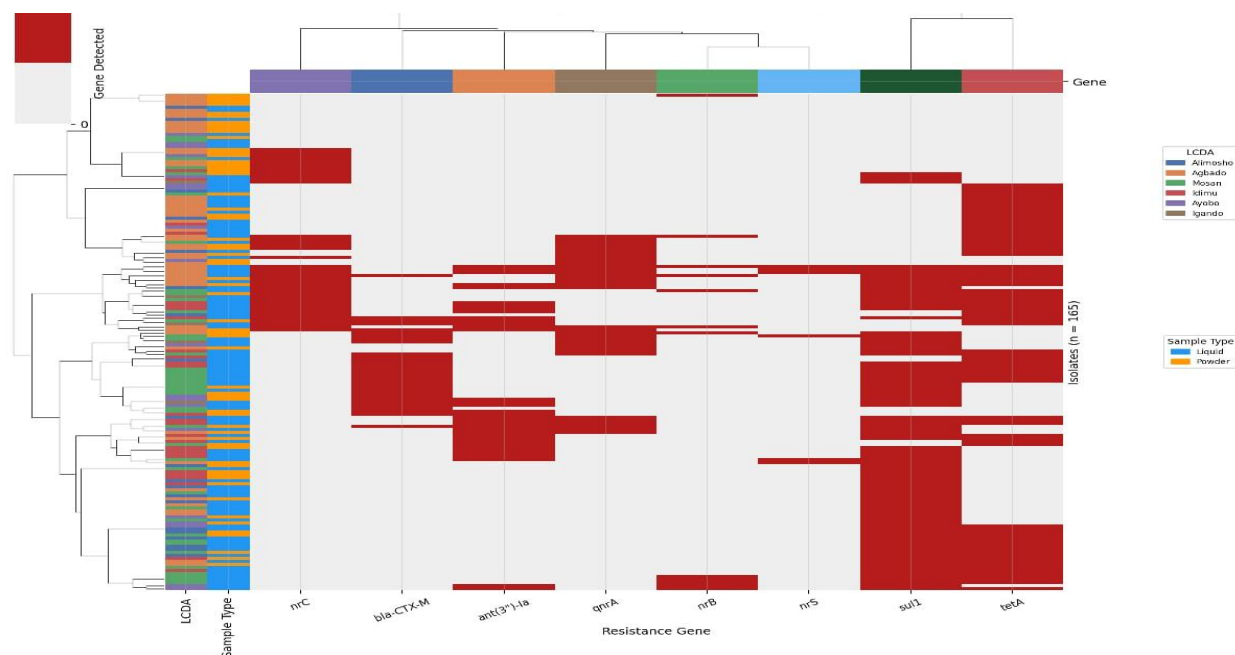


Figure 11: Hierarchically Clustered Heatmap of the Resistance Gene Distribution Among Isolates (n = 165, 8 genes). Red= Gene present, Grey = Gene absent, Left bar:LCDAs, Right bar: Sample Type

4.0 Discussion

Extensive resistance was seen with tetracycline, sulfamethoxazole-trimethoprim, augmentin (amoxicillin-clavulanic acid), erythromycin, streptomycin and imipenem while comparatively more resistance was seen with ciprofloxacin, gentamicin, chloramphenicol, ceftriaxone and nalidixic acid. Extended and sometimes indiscriminate use of commonly used antibiotics in sub-Saharan Africa has resulted in continued antimicrobial selective pressure and similar resistance patterns for bacterial contaminants found in environmental, ready to eat and herbal medicinal products across the region (Walusansa *et al.*, 2022; Opuni *et al.*, 2023; Omoruyi *et al.*, 2024; WHO, 2024). Similar observations have been made in Nigeria, showing that environmental and foodborne bacterial isolates are highly resistant to β -lactam, sulfonamides, and tetracyclines (Adesokan *et al.*, 2020; Chinakwe *et al.*, 2023). This lower resistance observed for the drugs ciprofloxacin and gentamicin may be because they are relatively restricted in their use, more expensive and not as readily available as other Oral Antimicrobials used routinely (Murray *et al.*, 2022; Odo *et al.*, 2023; WHO, 2024). In particular, high levels of resistance were found to imipenem, a carbapenem drug considered to be a last-line treatment for serious infections caused by bacteria. In the present study, the phenotypic resistance was high, but carbapenemase genes were not studied; therefore, the existence of carbapenem-resistant bacterial populations should be considered in the community-associated environments. This trend of carbapenem resistance being detected outside hospitals has also been observed in carbapenem-resistant bacteria found in the environment and in food products (Murray *et al.*, 2022; WHO, 2024; Omoruyi *et al.*, 2024). These results highlight the need to ensure ongoing molecular monitoring of carbapenem resistance mechanisms in herbal medicinal products, due to the importance of carbapenems in clinical use. The level of multidrug resistant bacteria detected in this work is high and indicates the establishment of antimicrobial resistance among commercial HMMP-associated bacterial contaminants. Based on the standardized definitions proposed by Magiorakos *et al.* (2012), a high proportion (> 50%) of isolates demonstrated multidrug resistant phenotypes and smaller proportion exhibited pandrug resistant and the extensively drug resistant phenotype. The level of MDR in Uganda was reported as 9.4% (Walusansa *et al.*, 2022), 10.4% in Ghana (Darkwah *et al.*, 2022) and 15.0% in Nigeria (Esimone *et al.*, 2007; Omoruyi *et al.*, 2024) with contaminated herbal products identified as possible reservoirs of MDR bacteria. These results have been alarming for public health with consumers potentially exposed to multiple-resistant bacterial pathogens at the same time, which decreases therapeutic choices in the event of infection. There were significant geographical differences in antimicrobial resistance in the six LCDAs. The highest proportions of MDR isolates were seen in Ayoba/Ipaja, while the IDimu/Egbe had the highest proportion of XDR and PDR isolates. In contrast, the proportions of non-MDR, and resistance level in Igando/Ikotun remained comparatively low. These geographical differences likely represent community differences in sanitation, production methods, water quality, environmental contamination and/or antibiotic exposure. Other African countries have reported similar spatial heterogeneous antimicrobial resistance, with local, ecological, and socioeconomic factors playing a major role in the distribution of antimicrobial resistance in Nigeria (Opuni *et al.*, 2023; Kalumbi *et al.*, 2020; Aharbi *et al.*, 2024). Furthermore, the strong correlation coefficients (0.96–0.99) and p values < 0.001 obtained between the resistance profile of Alimosho and Idimu/Egbe, and Alimosho and Agbado/Oke-Odo mean there is similarity in contamination sources or production/distribution practices, while the resistance profile of Igando/Ikotun was comparatively different and could be investigated for possible epidemiological differences.

Co-resistance patterns were analyzed and showed that resistance to several antibiotics were not independent or random. There is moderate positive correlation among erythromycin and tetracycline (0.43), streptomycin and tetracycline (0.40) and sulfamethoxazole-trimethoprim and augmentin (0.38) suggesting that isolates that are resistant to one antimicrobial were often resistant to the others. Co-resistance is also observed between environmental and food-borne bacteria, and is generally attributed to the accumulation of multiple resistance mechanisms in bacteria that are exposed to a number of antimicrobial selection pressures (Blair *et al.*, 2015; Van Hoek *et al.*, 2011; Walusansa *et al.*, 2022). This is in contrast to the weaker associations found with ciprofloxacin, gentamicin, chloramphenicol and ceftriaxone, which indicate that these antibiotics may involve different genetic or physiological mechanisms in their resistance. These findings were confirmed by hierarchical clustering analysis which divided the antibiotics into groups of high resistance and low resistance. The consistently high frequencies of resistance were the reason that tetracycline, erythromycin, augmentin, sulfamethoxazole-trimethoprim, streptomycin, and imipenem were grouped together, while ciprofloxacin, nalidixic acid, gentamicin, chloramphenicol, and ceftriaxone were in a comparatively low

frequency cluster. Clustering has also been found in antimicrobial surveillance studies, in which antibiotic groups that have similar resistance profiles tend to have similar patterns of epidemiological behaviour (Walusansa *et al.*, 2022; WHO, 2024). The results of the independent clustering of Igando/Ikotun further corroborates the distinct resistance profile revealed by the geographical analyses.

The molecular characterization of the bacterial isolates recovered from the herbal medicinal products was found to be the presence of clinically important antimicrobial resistance (AMR) genes in wide-spread distribution. Of the genes found, *sul1* and *tetA* were most common, and both are known to confer resistance to sulfonamides and tetracyclines. This is in line with earlier studies that showed these resistance genes are ubiquitous in environmental, foodborne and community-associated bacterial flora (Gillings, 2018; Zheng *et al.*, 2021; Iweriebor *et al.*, 2022). Likewise, the relatively high occurrence of *bla*_{CTX-M} highlights the increasing spread of extended-spectrum β -lactamase (ESBL)-producing bacteria beyond clinical settings. This has also been documented in Nigeria and other African countries, where the resistance is now firmly established in the environmental reservoirs and other non-clinical reservoirs (Ogbolu *et al.*, 2021; Chinakwe *et al.*, 2023). The plasmid-mediated quinolone resistance genes (*qnrA*, *qnrB*, *qnrC* and *qnrS*) were present at lower levels, but their presence suggests the existence of transferable quinolone resistance determinants in bacterial contaminants of herbal medicinal products and this could contribute to transmission of the resistance via the food chain and the environment (Zheng *et al.*, 2021; Ruiz, 2019; Jacoby *et al.*, 2014). Resistance genes did not evenly distribute themselves in the six Local Council Development Areas (LCDAs). Comparing the geographical distribution of these genes, there were significant differences found for *sul1* ($p = 0.0016$), *bla*_{CTX-M} ($p = 0.0002$), *ant*(3'')-Ia ($p = 0.0056$) and *qnrA* ($p = 0.0327$) while *tetA*, *qnrB* and *qnrS* exhibited relatively similar geographical distribution. The results indicate that local environmental conditions and human activities may have an impact on the presence and distribution of certain resistance genes. There are other reports of geographical variation in antimicrobial resistance in other countries such as Nigeria, Ghana, Malawi and Saudi Arabia, which have correlated the geographical variations in antimicrobial resistance with sanitation, waste disposal, antibiotic use, and environmental contamination (Kalumbi *et al.*, 2020; Darkwah *et al.*, 2022; Alharbi *et al.*, 2024; Omoruyi *et al.*, 2024). The higher prevalence of *bla*_{CTX-M} in Igando/Ikotun and Mosan/Okunola further shows that there is not a uniform distribution of resistance throughout the study area, but rather ecological and anthropogenic factors play a role. The molecular results were compared to antimicrobial susceptibility data and some statistically significant associations between resistance phenotype and the corresponding resistance genes were found. The best correlation was found with *qnrS* and ciprofloxacin resistance (OR = 21.15, $p = 0.004$). Other significant associations were: *tetA* resistance to cefuroxime, *qnrC* resistance to ciprofloxacin, *qnrB* resistance to cefuroxime, *sul1* resistance to augmentin, and *qnrA* resistance to chloramphenicol. None of these relationships, however, held up with multiple comparisons correction (Benjamini–Hochberg). This result suggests there is more to the resistance phenotypes detected than just the resistance genes studied in this paper. These differences between genotype and phenotype have been noted in environmental populations of bacteria, where antimicrobial resistance can be mediated by several mechanisms such as chromosomal mutation, decreased membrane permeability, up-regulation of efflux pumps, biofilm formation, and presence of other antimicrobial resistance mechanisms that may not have been identified during molecular screening (Blair *et al.*, 2015; Hendriksen *et al.*, 2019; de Sousa Lima *et al.*, 2020; WHO, 2024). In summary, these results add to the growing body of evidence that HMPs are significant environmental sources of antimicrobial resistant bacteria and clinically significant antibiotic resistance genes. The presence of a number of transferable resistance determinants in bacteria found in these products indicate that they may spread AR into the environment. This highlights the importance of better quality control in manufacturing and distribution of herbal medicinal products, regular microbiological surveillance and better regulatory control to minimise public health risks due to spread of antimicrobial resistance.

The presence of a widespread contamination with bacteria, high levels of multidrug resistance, considerable geographical variability and different sets of antimicrobial resistance determinants underline the need for regular microbiological quality control of HMPs. Improving good manufacturing practices, applying regulatory requirements for the production of HMPs, conducting routine antimicrobial resistance surveillance and increasing awareness amongst the population about the microbiological safety of HMPs are important measures to reduce the spread of ARB through these popular products (WHO, 2023; WHO, 2024; CLSI, 2024). Conclusion This study revealed that high levels of antimicrobial resistance can be obtained from the microbial population of the commercial herbal medicinal products sold in Alimosho LGA with a wide variety of bacterial species. The high rate of MDR and presence of clinically relevant resistance genes such as *sul1*, *tetA*, *bla*_{CTX-M}, *qnrA*, *qnrB*, *qnrC*, *qnrS* and *ant*(3'')-Ia suggest that these products may act as reservoirs for antimicrobial resistance. Resistance has also been geographically variable, with different profiles and different genes being present in different areas, which further highlights the need to have local surveillance. The results indicate that there is a need for more robust microbiological quality control, good manufacturing practices and regular monitoring for antimicrobial resistance, to ensure the safety of HMPs and minimize public health risks. Study

Limitations

This study only focused on selected antimicrobial resistance genes and did not use whole genome sequencing, so other resistance genes could not be identified. Moreover, the samples of one LG were taken, which could not make the results easy to generalize across the LGAs of the community.

Recommendations

1. Increase microbiological quality control and regulatory surveillance of HMPs. Implement Good Manufacturing Practices (GMP) in manufacturing and distribution.
2. Integrate regular antimicrobial resistance monitoring in quality assessment of herbal medicines.
3. Carry out future studies through whole genome sequencing to fully understand the mechanisms of resistance.

Author contributions

SE: Data curation, Formal analysis, Methodology, Software, Investigation, Writing – review & editing, Writing – original draft. MO: Writing – original draft, Supervision, Conceptualization, Project administration, Writing – review & editing. KA: Supervision, Methodology, Investigation, Writing – review & editing, Conceptualization, Writing – original draft, Project administration, Funding acquisition.

Declarations

Ethical Approval: Not applicable in this research

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Availability of data and materials

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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