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# Phenotypic Resistance Pattern of Bacterial Isolates from Gold Mine Tailings in Ile Ife, Osun State, Nigeria

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author EF designed the study, managed the analyses of the study and wrote the initial draft of the manuscript, Author KA collected the data and Authors AO and CF managed the literature search. Author AO managed the graphics and literature search. All authors read and approved the final manuscript.*

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## ABSTRACT

Artisanal gold mining and the resultant environmental degradation and health hazards have been a cause for public health concern. This, specifically as heavy metal contaminated environments have been pointed out as major players in the dissemination of resistant pathogens of public health concern. This study aimed to determine the phenotypic antibiotic resistance profiles of bacteria

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isolated from gold mine tailings in a community in Osun State, Nigeria. Bacteria were isolated from excavation soil samples using standard microbiological methods, with general-purpose and selective media. Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method. Isolates recovered were identified and their zone of growth inhibition was determined. Of the 32 isolates identified, 100% were resistant to ceftriazone, and also 100% resistance respectively to oxacillin and amoxicillin-clavulanic acid by all the isolates tested, while 96.67% were resistant to ceftazidime. The isolates exhibited 12 phenotypic resistance pattern of which 100% were resistant to at least three types of antibiotics and over 45% MDR-multidrug resistance. These findings indicate a high frequency occurrence of antibiotic resistance in the goldmine tailings including multidrug resistant bacteria in the environment. Hence, this calls for the concerned stakeholders to take necessary measures in safeguarding the catchment community against this public health menace.

*Keywords: Gold mining; antibiotic; MDR-multidrug resistance; environment.*

## 1. INTRODUCTION

Antibiotics resistance has continued to be a global public health concern that could lead to a post-antibiotic era, in which even minor infections would be life-threatening, as declared by World Health Organization [1]. Despite measures to counter this phenomenon, antibiotic resistance in pathogens is still prevalent [2]. This implies the presence of extraneous factors, other than the antibiotics, that may be responsible for the evolution, selection and dissemination of antibiotic-resistant strains. In that regard, antimicrobial resistance (AMR) in environmental micro-organisms has been pointed out as a major culprit [3, 4].

Heavy metals can co-select for antimicrobial resistance by fostering environments where resistance genes, whether within closely related taxa or across genera, undergo horizontal transfer [5, 6]. Heavy metal (HM) contaminants have also been implicated in selection of microbial virulence factors and with the potential to alter the antibiotic resistome of contaminated water bodies [7,8]. Exposure to HM predisposes bacteria to antibiotic resistance, as both phenomena create selection pressure on the microbes [6,9]. It has been established that in heavy metal contaminant ecosystem, bacteria can exhibit selection for heavy metal resistance and antibiotic resistance simultaneously [5]. This phenomenon could be by either co-selection when there are two or more genetically related resistance genes or cross-selection due to presence of single genetic element which provides tolerance to more than one antimicrobial agents [6, 10].

Extrachromosomal resistance is commonly implicated in AMR in environmental microbes. These resistance determinants are transferrable to clinical pathogenic bacteria, as have been previously documented [2]. Therefore, researchers are focusing on the effects of mining on the environment, among which is the impact and relationship between HM contamination and antibiotics resistance [2,6,11].

Although gold mining provides huge socio-economic benefits to nations, the long-term deleterious impact on the environment and the public health cannot be overlooked [12, 13]. Large quantities of waste are produced during gold mining activities, during which over 99% of the extracted ore is released as waste to the environment [14]. The type of mining process applied in gold extraction and the quantity of waste generated is determined by the location of the gold deposit [15]. Mine tailings accumulate either in the form of large hills or water ponds, which could constitute severe environmental problems [16].

In Nigeria, gold mining is predominantly artisanal, carried out through open-pit methods that generate substantial environmental waste and heavy metal contamination, with adverse health implications [17-19]. Previous studies have examined the environmental impacts of these activities, including contamination of water, soil and vegetation [20, 21], yet data on the phenotypic antibiotic resistance profiles of soil bacteria in mining areas remain scarce. This study investigates the phenotypic antibiotic resistance profiles of bacteria isolated from gold mine tailings in Iloro Araromi village, Ile-Ife, Osun State, Nigeria, to provide insights into the potential public health risks posed by these contaminated environments.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The study area is Iloro Araromi village originally known as Araromi Okeodo village situated in Kere area of Ife South (Fig. 1), Osun State, Nigeria and its geographical coordinates are 7° 9' 9" North, 4° 27' 14" East. The climate is tropical, farming combines coco and banana plantation by residents of the community.

### 2.2 Sample Collection

Soil samples were aseptically collected from mine tailings with a sterile spatula into sterile screw capped bottles. Composite samples were obtained by collecting at different excavations sampling points from top to a depth of 0-10cm below the top soil surface from two separate points respectively [15]. The soil samples were transported immediately to the laboratory for microbiological analysis.

### 2.3 Preparation of Media

Eosin methylene blue agar (EMBA), *Salmonella Shigella* agar (SSA) and Nutrient agar (NA) were prepared according to the manufacturers' instruction and were sterilized by autoclaving at 121°C for 15mins. SSA was sterilized by bringing to boil on a laboratory hot plate. All sterile media were poured into petri dishes at about 45°C and allowed to solidify. EMBA plates were used to isolate *E. coli* and other Gram-negative enteric bacteria, while SSA plates were solely for isolation of *Shigella* and *Salmonella* species. All media except otherwise stated were purchased from Hi-Media laboratories (Mumbai, India).

### 2.4 Bacterial Isolation

Serial dilution of each soil sample was carried out using normal saline as diluent in test tubes containing 9ml of sterile water each. A 1 g of the soil sample was dispensed in a diluent test tube and homogenized. Thereafter, 1ml suspension of the homogenate was transferred using a sterile pipette into another diluent test tube and swirled gently to make $10^{-1}$ dilution. This procedure was repeated for the successive dilutions up to $10^{-6}$ dilution. One milliliter from dilutions $10^{-4}$ to $10^{-6}$ was inoculated in various culture media by spread plate method. However, for the isolation of *Salmonella* and *Shigella* 1ml $10^{-1}$ and $10^{-2}$ dilutions were inoculated into petri dishes then the prepared SSA was introduced and the plates were gently swirled to ensure even distribution of the inoculum. All inoculation of dilutions was in duplicate plates. The inoculated and blank control plates were incubated in an inverted position for 24hrs at 37°C in an incubator. The colonies that emerged were counted and colony forming units per ml was calculated. Sub culturing of colonies was done and pure cultures obtained were stored at 4°C in NA slants for further study.

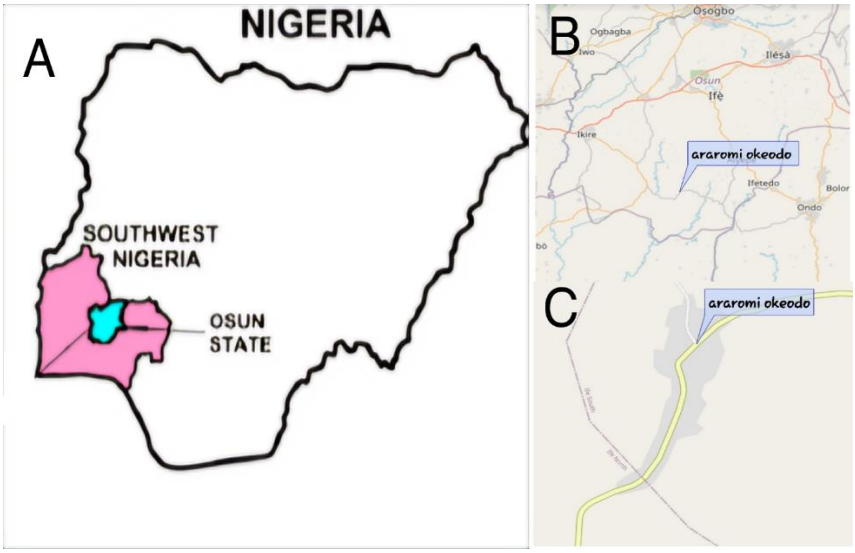


**Fig. 1. Maps highlighting the study area. (A) Map of Nigeria displaying the sample location base on region (B) Map of Osun State showing the sample location (C) Outlined map of the study area**

### 2.5 Bacterial Identification and Characterization

Gram staining and biochemical tests were performed to differentiate organisms based on the structure of their cell wall and metabolic characteristics respectively. The biochemical tests comprised catalase, coagulase, IMViC (Indole, Methyl red, Voges proskauer) tests.

### 2.6 Antibiotics Sensitivity Testing

The isolates from gold mine soil were tested for antimicrobial susceptibility by disc diffusion using the Kirby Bauer disk diffusion method on Muller Hilton agar (BIOTEC) was performed with slight modification as described by [22], and Clinical and Laboratory Standards Institute [23] guideline was used to interpret the result. The following antibiotics with their different concentrations were used for testing: Ceftazidime (30µg), Gentamicin (10µg), Oxacillin (1µg), Ciprofloxacin (5µg), Ceftriazone (30µg) and Amoxicilin-clavulanic acid (30µg). *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 served as the control strains. The definition of multidrug resistance (MDR) was in accordance with Centre for Disease Control (CDC) for resistance of bacterial isolates to at least one antibiotic in three or more drug classes [24].

### 2.7 Statistical Analysis

Data generated from bacterial isolation and antibiotic sensitivity tests were analyzed by entering into Microsoft Excel to determine the frequency of occurrence and resistance profile of the bacterial isolates using descriptive statistics.

## 3. RESULTS AND DISCUSSION

### 3.1 Total Heterotrophic and Coliform Bacterial Count

The total bacterial population of the gold mine excavation soil recorded during the study is presented in Table 1. Heterotrophic plate count from the soil samples showed a highest value of 1.2 x $10^6$ CFU/g observed at site C2, followed by 9.6 x $10^5$ in site C1, while the lowest was 3.3 x $10^5$ recorded at site CP1. Furthermore, the highest coliform count of 8.4 x $10^2$ CFU/g was also documented at site C2 while the least count of 1.1 x $10^2$ was observed at site C1. Comparatively, a previous study on a mining site in north-west Nigeria recorded highest and lowest bacterial counts of 4.5 × $10^6$ cfu/g and 3.3 × $10^4$ CFU/g respectively [25]. A low microbial density is usually associated with presence of heavy metal [26] which could explain the low HTB count recorded in this study. This is because, although needed in trace amounts, at higher concentrations heavy metals become toxic to microbes [27]. Nevertheless, the presence of coliform in the mining sites is a cause for environmental health concern as vast amounts of the contaminated soil can be spread to the community via aeolian and water erosion [28].

### 3.2 Bacterial Identification and Frequency Rate

Thirty-two bacteria were isolated from the goldmine soil of which 26 (81%) were Gram negative and 6 (19%) were Gram positive. A total of 10 genera were identified, and the highest frequency rate of 16% recorded was for *Klebsiella*, *Proteus* and *Citrobacter* species respectively. This is followed by *Providencia* and *Staphylococcus* species with 13 and 12% frequency rate respectively, while the least was *Edwardsiella* sp. with 3% frequency rate. The result is depicted in Fig. 2. This result implies that gold mine excavations in the studied area habour a number of enteric bacterial genera constituting public health threat as opportunistic pathogens. This is probably due to ‘bush method’ of defecation and human waste management system, common in many rural and peri-urban communities [29]. Specifically, *Citrobacter* spp. occur in animal and human guts and in some environment including soil and sewage sludge water, but have also been implicated in 3-6% of all *Enterobacteriaceae* isolates responsible for hospital acquired infections [30]. In addition, *Proteus*, *Klebsiella* and *Providencia* species although occurring in natural environment, are capable of colonizing skin and mucosal tissues. These have been implicated as agents of healthcare-associated infections (HAIs) such as urinary tract infection and burn wounds as well as gastroenteritis, including travelers' diarrhea [31]. A report on AMR status in WHO African regions stated that *Klebsiella* spp. were the most common bacterial infectious agents in Africa [32]. Noteworthy also is the incidence (6% respectively) of *E. coli* and *Salmonella* spp. recorded in this study, which are common agents of enteric fever and gastrointestinal disorders. For instance, illegal miners were reported to be the predominant group in an enteric fever outbreak in South Africa probably due to consumption of contaminated ground water while working in a gold mine shaft [33].

**Table 1. Bacterial load of gold mine excavation soil**

| S/N | Site Code | Total heterotrophic count CFU/g | Total coliform count CFU/g |
|---|---|---|---|
| 1 | C1 | $9.6 \times 10^5$ | $1.1 \times 10^2$ |
| 2 | C2 | $1.2 \times 10^6$ | $8.4 \times 10^2$ |
| 3 | CP1 | $3.3 \times 10^5$ | $7.1 \times 10^2$ |
| 4 | CP2 | $5.6 \times 10^5$ | $6.2 \times 10^2$ |

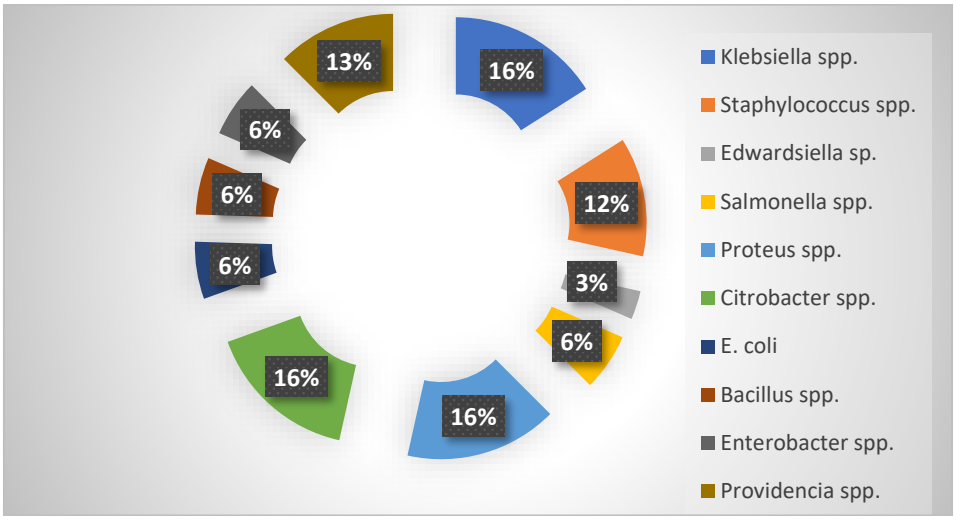


**Fig. 2. Frequency rate of bacterial genera isolated from gold mine excavation soil**

Previous studies have reported several genera and bacterial phyla in mining sites; Proteobacteria, Actinobacteria, and Firmicutes were reported as the predominant phyla in abandoned gold mine tailings in Krugersdorp, South Africa [15]. Furthermore, other studies on HM contaminated environments in Nigeria and some other countries have reported different bacterial genera including those carrying HM-resistant genes such as *Bacillus* and *Gemmata* [34], *Pseudomonas* and *Bacillus* [35], *Priestia aryabhattai* and *Enterobacter cloacae* [36].

However, it should be noted that frequency and diversity of microbial population in HM contaminated sites are a function of the HM concentration and the microbes that have been able to adapt to the prevailing conditions in such environment [5, 27, 37].

### 3.3 Resistance Profile of Bacterial Isolates

All bacterial isolates in this study demonstrated complete resistance (100%) to ceftriaxone, oxacillin, and amoxicillin-clavulanic acid, with 96.67% also resistant to ceftazidime (Fig. 3). On the other hand, gentamicin and ciprofloxacin retained susceptibility in 84.38% and 65.63% of the isolates, respectively. The high resistance to first-line antibiotics such as ceftriaxone and amoxicillin-clavulanic acid, both widely used in primary and emergency care globally, is particularly concerning, indicating limited treatment options for infections by these isolates [38]. Oxacillin, is a β-lactam antibiotic member also referred to as penicillinase resistant penicillin being resistant to β-lactamase hydrolytic enzyme. The antibiotic has a limited spectrum of activity mostly on Gram-positive bacteria with resistance being common particularly among *Enterobacteriaceae* [39]. Conversely, ceftazidime and ceftriazone are third-generation cephalosporin antibiotics used in the management of most Gram-negative bacterial infections, especially those resistant to first- and second-generation cephalosporin or other β-lactam antimicrobials [40]. Moreover, amoxicillin-clavulanic acid (β-lactam/β-lactamase inhibitor combinations) is the most frequently used antimicrobial combination by primary care givers and in emergency conditions globally [38]. Amoxicillin is a broad spectrum β-lactam penicillin derivative with similar activity against Gram- positive and negative bacteria by inhibiting cell wall biosynthesis. The clavulanic acid is a β-lactamase inhibitor that prevents microbial degradation of the β-lactam, hence the

combination offers extended antibiotic coverage and reduce resistance [38]. Nevertheless, the high resistance observed in this study suggests that reliance on these antibiotics for managing infections linked to this environment may be ineffective, underlining the need for continuous antimicrobial resistance surveillance.

Major mechanisms of β-lactam resistance include resistance by β-lactamase enzyme hydrolysis of the antibiotic, resistance by active efflux of the antibiotic and resistance by penicillin-binding protein modification and increased peptidoglycan synthesis [39].

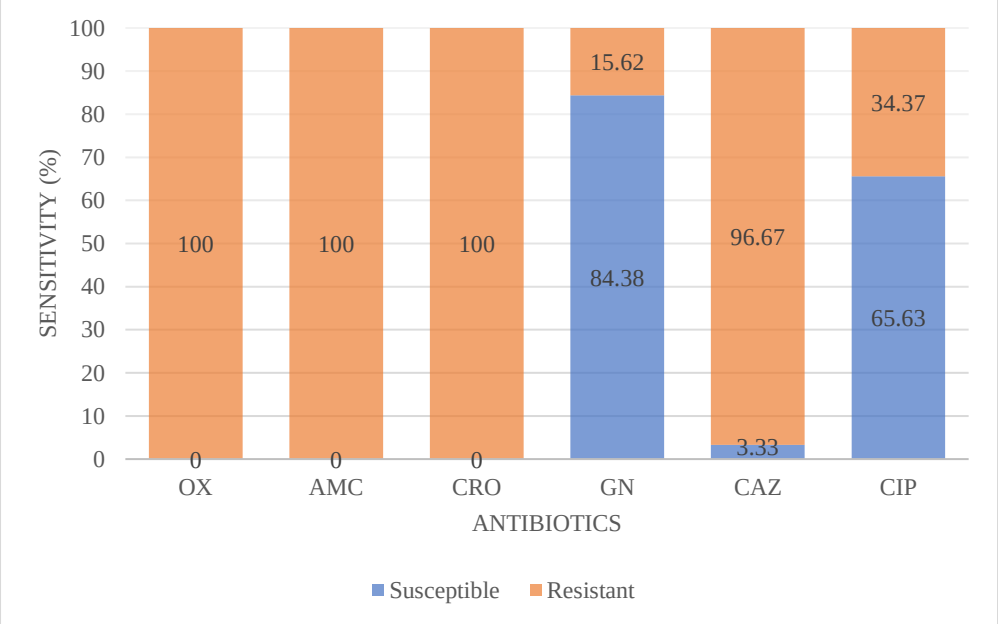


**Fig. 3. Antibiotics sensitivity profile of bacterial isolates from gold mine excavation soil**

**Table 2. Phenotypic resistance pattern of bacteria isolated from gold mine excavation soil**

| No. of Antibiotics | Resistance Phenotype | No. of isolates (%) with resistance | Bacteria |
|---|---|---|---|
| 3 | OX, CRO, CAZ | 2(6.3%) | Kleb(BB1) and Staph(BB2) |
| | AMC, CRO, CAZ | 2(6.3%) | Sal(BB3) and Prov(B2b) |
| | OX, AMC, CRO | 2(6.3%) | Prov(B8) and Prot(B10b) |
| 4 | OX, AMC, CRO, CAZ | 1(3.1%) | Staph(BP5) |
| | AMC, CRO, CAZ, CIP | 2(6.3%) | Prot(BB4) and Prov(A1) |
| | OX, CRO, GN, CAZ | 1(3.1%) | Edws(BP3) |
| | OX, AMC, CRO, CIP | 1(3.1%) | Prot (A3) |
| | OX, CRO, CAZ, CIP | 2(6.3%) | Sal(BB5) and Staph(AP3) |
| | OX, AMC, CRO, CAZ | 10(31.3%) | Kleb(AB4), Entero(B7), *E. coli*(AP4) and (BP2), Prot(B6), Entero(A6), Prov(A7), Kleb(A9), Citro(B1a) and (B1b) |
| 5 | OX, AMC, CRO, GN, CAZ | 3(9.4%) | Bac(A10), Kleb(AB5) and Citro(A5) |
| | OX, AMC, CRO, CAZ, CIP | 5(15.6%) | Staph(BP4), Citro(AB3), Prot(A2), Kleb(A8) and Bac(B5) |
| | AMC, CRO, GN, CAZ, CIP | 1(3.1%) | Citro(AP5) |

*Key: Kleb-Klebsiella; Staph-Staphylococcus; Sal-Salmonella; Prov-Providencia; Prot-Proteus; Edws-Edwardsiella; Entero-Enterobacter; Citro-Citrobacter; Bac-Bacillus*

**Table 3. Multidrug resistant bacterial isolates from goldmine tailings**

| No. of Antibiotics | Resistance Phenotype | No. of isolates (%) with resistance | Bacteria |
|---|---|---|---|
| MDR | AMC, CRO, CAZ, CIP | 2 | Prot(BB4) and Prov(A1) |
| | OX, CRO, GN, CAZ | 1 | Edws(BP3) |
| | OX, AMC, CRO, CIP | 1 | Prot (A3) |
| | OX, CRO, CAZ, CIP | 2 | Sal(BB5) and Staph(AP3) |
| | OX, AMC, CRO, GN, CAZ | 3 | Bac(A10), Kleb(AB5) and Citro(A5) |
| | OX, AMC, CRO, CAZ, CIP | 5 | Staph(BP4), Citro(AB3), Prot(A2), Kleb(A8) and Bac(B5) |
| | AMC, CRO, GN, CAZ, CIP. | 1 | Citro(AP5). |
| **Total number of isolates(%)** | | **15(46.88%)** | |

*Key: Kleb-Klebsiella; Staph-Staphylococcus; Sal-Salmonella; Prov-Providencia; Prot-Proteus; Edws-Edwardsiella; Citro-Citrobacter; Bac-Bacillus; MDR-Multidrug resistance*

## 3.4 Multidrug Resistance (MDR)

The isolates recovered from the gold mine excavation soils exhibited twelve (12) phenotypic resistance pattern (Table 2) of which 100% were resistant to at least three types of antibiotics while 31.3% were resistant to four (4) types of antibiotics comprising oxacillin, amoxicillin-clavulanic acid, ceftriazone and ceftazidime. A 46.88% multidrug resistance (MDR) comprising eight bacterial genera (Table 3) was also recorded. Furthermore, resistance to the highest number of antibiotics tested in this study was typical for *Klebsiella* spp. followed by *Citrobacter* spp. Likewise, the two *E*. *coli* isolates in this study were resistant to all the β-lactam class and the third-generation cephalosporins (Table 3), corroborating an earlier report by WHO which documented significant *E*. *coli* resistance to third generation cephalosporins and fluoroquinolones in communities and hospitals in African region [1]. Previous studies on bacterial isolates from heavy-metal contaminated freshwater farms, seawater farms, and their markets, and dumpsite respectively reported 40 and 50% MDR [41, 42]. This is somewhat similar to that of [8] in which bacteria phyla Proteobacteria, Firmicutes and Bacteriodetes were 87 and 50% resistant to at least one antibiotic and more than three antibiotic classes respectively. On the other hand, *Staphylococcus* spp. was reported as having high tolerance to several antibiotics tested for heterotrophic bacteria isolated from mine tailings in Poland [43].

Empirical administration of antibiotics is a common practice in most African countries, in which antibiotics are prescribed for patients without antibiotic sensitivity testing and in non-bacterial infections including cases of COVID-19 [44]. In Nigeria as in many other developing countries, antibiotics are unregulated, cheap and easily accessible over the counter without prescription [44]. This is despite the WHO established guidance on the appropriate use of antibiotics for common infections [45]. It is noteworthy that the result of our study indicates high resistance to the Access and Watch antibiotic groups comprising those recommended by WHO for the empiric treatment of most common infections, are less costly and have lower potential for the selection of antimicrobial resistance, and the ones that the use should be carefully monitored to avoid overuse due to a higher potential for the selection of antimicrobial resistance respectively [45]. Therefore, the resistance pattern in this study implies the types of antibiotics usage in the community, since non-metabolized antibiotics can be discharged into the environment [27]. The multidrug resistant bacterial genera recovered from the mining sites could serve as potential transmission channel to the nearby community.

## 4. CONCLUSION

This study highlights the significant presence of antibiotic-resistant bacteria including multidrug resistant bacteria in gold mining soils from Iloro Araromi village, Ile-Ife, Osun State, Nigeria. The

findings revealed a high prevalence of resistance to commonly used antibiotics suggesting that environmental exposure in heavy metal-contaminated soils may contribute to the selection and spread of resistant bacteria. These findings call for urgent intervention to the environmental and public health impacts of artisanal mining practices, as well as the need for targeted surveillance and mitigation strategies to control the spread of antibiotic resistance in such environments. Broader regional sampling and molecular techniques are recommended to better understand the links between heavy metal contamination and antimicrobial resistance development in mining areas.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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