

# Prevalence of Multidrug-Resistant Oral Bacteria and Demographics of Dental Outpatients in Awka, Anambra State, Nigeria

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## Abstract

Oral diseases and the rise of multidrug-resistant (MDR) bacteria in dental care pose significant public health concerns. This study investigated the antibiogram of Multidrug-resistant (MDR) bacterial isolates from dental patients in Awka. It analyzed associated demographic factors for a possible link to the presence of the identified pathogens. Oral samples were collected and analyzed using standard microbiological methods and 16S rDNA sequencing. Antimicrobial susceptibility testing was determined using the Kirby-Bauer disc diffusion method. Identified MDR isolates included *Mammalicoccus sciuri*, *Enterococcus casseliflavus*, *Enterococcus gallinarum*, and *Glutamicibacter creatinolyticus*. Among these, *E. gallinarum* showed the highest resistance but was the least prevalent (8%), while *M. sciuri* was the most prevalent (46%). Demographic and lifestyle variables—age, sex, education, marital and employment status, oral hygiene practices, and confectionery consumption—significantly influenced clinic visits. Enterococcal species were sensitive to Gentamicin, Ceftriaxone, Levofloxacin, Ofloxacin, Cefotaxime, Erythromycin, Ciprofloxacin, and Azithromycin, but resistant to Cefuroxime, Augmentin, Cefixime, and Imipenem. Notably, *E. gallinarum* was resistant to Erythromycin and Azithromycin, unlike *E. casseliflavus*. *M. sciuri* was sensitive to Gentamicin, Levofloxacin, Ciprofloxacin, and Ofloxacin, but resistant to Cefixime, Imipenem, and Augmentin. *G. creatinolyticus* was generally sensitive to several antibiotics, including Cefuroxime and Levofloxacin, but resistant to Cefixime, Imipenem, and Augmentin. Although these bacteria share morphological and biochemical traits, molecular analysis revealed significant genetic differences. These MDR strains are believed to be transient oral pathogens resident across all age groups.

**Keywords:** Prevalence; Multidrug-Resistant; Oral Bacteria; Demographics; Oral Disease

## 1. Introduction

Oral hygiene involves maintaining cleanliness of the mouth through brushing, flossing, and other practices to prevent dental diseases such as tooth decay and gum disease Agbulu *et al.*, [1]. In Nigeria and other developing countries, a large proportion of oral and facial diseases stem from microbial infections Olufemi, [2]; Ndukwe *et al.*, [3]. The frequent and sometimes inappropriate use of antibiotics in dental care in these regions contributes significantly to the development of multidrug-resistant (MDR) bacteria Akande-Sholabi and Oyesiji, [4].

To combat rising antibiotic resistance, alternative antimicrobial approaches are needed, especially in dental care Adeoti *et al.*, [5]. Dental caries, a common oral condition, is caused by biofilm (plaque) accumulation and the metabolic activities of microbial communities, exacerbated by poor oral hygiene Prabha and Anoop, [6]; Spatafora *et al.*, [7]. Pathogens involved include *Streptococci*, *Spirochetes*, *Bacteroides*, *Escherichia coli*, and *Candida albicans*.

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In Nigeria, poor oral hygiene and limited access to dental care contribute to high rates of dental plaque, caries, halitosis, and periodontal diseases. These conditions are further complicated by the resistance of oral pathogens to commonly used antibiotics and the high cost of dental treatment. Globally, oral diseases are among the most prevalent chronic conditions and are the fourth most expensive to treat, accounting for around 10% of global health expenditure Bilge and Meltap, [8].

Periodontal diseases—classified as gingivitis and periodontitis—affect the tooth-supporting structures, including gums and alveolar bone. Gingivitis involves inflammation of the gingiva, while periodontitis leads to the destruction of periodontal tissues and can result in tooth loss Idu *et al.*, [9]. These diseases are primarily caused by bacterial plaque and affect individuals regardless of age, gender, or race.

The use of natural oral cleaning tools such as chewing sticks has shown promising results in some populations, offering both mechanical and chemical cleaning benefits Malik *et al.*, [10].

Antibiotics, whether naturally derived or synthetically produced, are essential in managing infections. Their modes of action include bactericidal (killing bacteria) and bacteriostatic (inhibiting growth) mechanisms. Common classes include Aminoglycosides, Cephalosporins, Quinolones, Macrolides, and Penicillins, among others Pancu *et al.*, [11]; Wright, [12].

However, the growing threat of antimicrobial resistance (AMR)—defined as the failure of antibiotics to inhibit or kill targeted microorganisms—is alarming. MDR arises when bacteria develop resistance to multiple antibiotic classes. Contributing factors include antibiotic misuse, genetic mutations, enzyme production (e.g., beta-lactamases), and environmental pressures Cassir *et al.*, [13]; James *et al.*, [14].

To mitigate MDR spread, rational antibiotic use under medical supervision is essential. Reducing inappropriate prescriptions and public awareness of antibiotic stewardship are key Mousumi and Agniswar, [15]; Hendricks and Nemeth, [16].

## 2. Materials and Methods

### 2.1. Instruments and Materials Used for the Study

The instruments and equipment used in course of this research study, include: Incubator Model gp/50/CLAD/250/HYD, China, Micro pipettes (Thermo Scientific, USA), Syringes (BD Bioscience), Refrigerator (Nexus, China), Brookfield Viscosity, USA, Electronic weighing balance (Ohaus Corp., USA) Equitron Partially Automatic Autoclave (Medica Instrument Manufacturing CO., India), Light Microscope (Nikon Esol, Japan), pH meter (PHS-25 Helmreasin), China.

### 2.2. Culture Media and Reagents

All culture media used for the work were prepared in accordance to the instructions given by the manufacturers. Culture media used were Nutrient broth (Oxoid, Uk), Nutrient Agar (Oxoid, Uk), Blood Agar (Oxoid, Uk), Mueller Hinton broth (Oxoid, Uk), Mueller Hinton Agar (Oxoid, Uk), Dimethyl Sulfoxide (Sigma – Aldrich Co., Germany), Ethanol (BDH Chemicals Ltd, England), Sheep blood (Hidden impact diagnostics, Abuja), hydrogen peroxide (BDH Chemicals Ltd, England).

### 2.3. Antibiotics Used

In vitro diagnostics (IVD), gram positive antibiotic discs (Celtech Diagnostics, Belgium Code BD R002), were used. This includes Imipenem Cilastatin (IMP, 10µg), Cefuroxime (CXM 30µg), Erythromycin (ERY, 15µg), Gentamycin (GN, 10µg), Azithromycin (AZN, 15µg), Amoxacillin Clavulanate (AUG, 30µg), Cefotaxime (CTX, 25µg), Ceftriaxone Subactam (CRO, 45µg), Cefexime (ZEM, 5µg), Levofloxacin (LBC, 5µg) and Ciprofloxacin (CIP, 5µg).

### 2.4. Ethical Consideration

Ethical approval for this study was obtained from Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH), Amaku, Awka, Anambra State, Nigeria. Participants were given information sheets, consent forms, and questionnaires assuring confidentiality. Only those who gave informed consent participated.

## 2.5. Sample Size Determination

An average of 210 patients visited the clinics monthly. A total of 230 patients were recruited, including an extra 10% to account for possible attrition.

## 2.6. Inclusion and Exclusion Criteria

Included were adults (18+) attending dental clinics in Awka who voluntarily consented and completed the questionnaire. Excluded were children, patients on antibiotics, those who came for only routine checks or cleanings, and anyone who refused to give consent or complete the forms.

## 2.7. Source of Bacterial Isolates

Oral bacteria were isolated over 12–15 months from patients who completed a questionnaire covering antibiotic use, diet, and hygiene habits. Participation was voluntary and fully explained.

## 2.8. Isolation, Cultivation, and Preservation

Oral swabs were collected using sterile swab sticks and cultured in nutrient broth. Subcultures were made on sterile blood agar and incubated at 37 °C for 24 hours. Pure cultures were maintained on agar slants and stored at 4 °C.

## 2.9. Standardization of Cultures

Pure cultures were inoculated into 0.1% peptone water and adjusted to 0.5 McFarland standard ( $1 \times 10^8$  cfu/ml).

## 2.10. Identification of Isolates

Isolates were identified using standard microbiological methods including Gram staining and biochemical tests at COOUTH lab. Molecular identification, including 16S rDNA sequencing and PCR, was conducted at Iqaba Biotech West Africa Ltd, Ibadan.

## 2.11. Gram Staining

Gram staining followed standard procedures. Gram-positive bacteria appeared purple, while Gram-negative appeared red or pink under oil immersion microscopy.

## 2.12. Antibiotic Sensitivity Tests

After incubation, 0.1 ml of standardized pure bacterial cultures was inoculated into 20 ml of sterile, cooled molten Mueller-Hinton agar in McCartney bottles. The mixture was poured into sterile petri dishes and allowed to solidify. Using sterile forceps, standard Gram-positive antibiotic discs were placed evenly on the agar surface and gently pressed for adherence. Discs used included: Imipenem (10 µg), Cefuroxime (30 µg), Erythromycin (15 µg), Gentamicin (10 µg), Azithromycin (15 µg), Augmentin (30 µg), Cefotaxime (25 µg), Ceftriaxone (45 µg), Cefixime (5 µg), Levofloxacin (5 µg), and Ciprofloxacin (5 µg). The test followed the modified Kirby-Bauer disc diffusion method per CLSI guidelines and was conducted in duplicates. Plates were incubated at 37°C for 24 hours. Inhibition zone diameters (IZD) were measured in millimeters, and results interpreted as “Resistant,” “Intermediate,” or “Susceptible” using Cheesbrough’s [17] chart. Only isolates classified as resistant were selected for further analysis.

## 2.13. Molecular Analysis

Phenotypic characteristics alone are often insufficient for bacterial identification; therefore, 16S rDNA sequencing was used. Universal primers 27F and 1492R were employed to amplify the 16S rRNA gene region Lane *et al.*, [18].

Genomic DNA was extracted from MDR bacterial isolates using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005). PCR amplification was carried out with One-Taq Quick-Load 2X Master Mix (NEB, M0486). Amplified products were confirmed by gel electrophoresis and cleaned using the EXOSAP method.

Sequencing was performed bidirectionally with the Nimagen BrilliantDye Terminator Cycle Sequencing Kit v3.1 and purified using the ZR-96 DNA Sequencing Clean-Up Kit (Zymo Research, D4050). Samples were analyzed on the ABI 3500xl Genetic Analyzer (Thermo Fisher Scientific).

Resulting sequence files (.ab1) were processed using BioEdit v7.2.5 and matched to known sequences through BLAST search (NCBI), following Altschul *et al.*, [19].

**Table 1** 16S Primer sequence

| Name of Primer | Target           | Sequece 5'3'         |
|----------------|------------------|----------------------|
| 16S27F         | 16SrDNA sequence | AGACTTTGATCMTGGCTCAG |
| 16S1492R       | 18SrDNAsequence  | CGGTTACCTTGTTACGACTT |

### 3. Results and Discussion

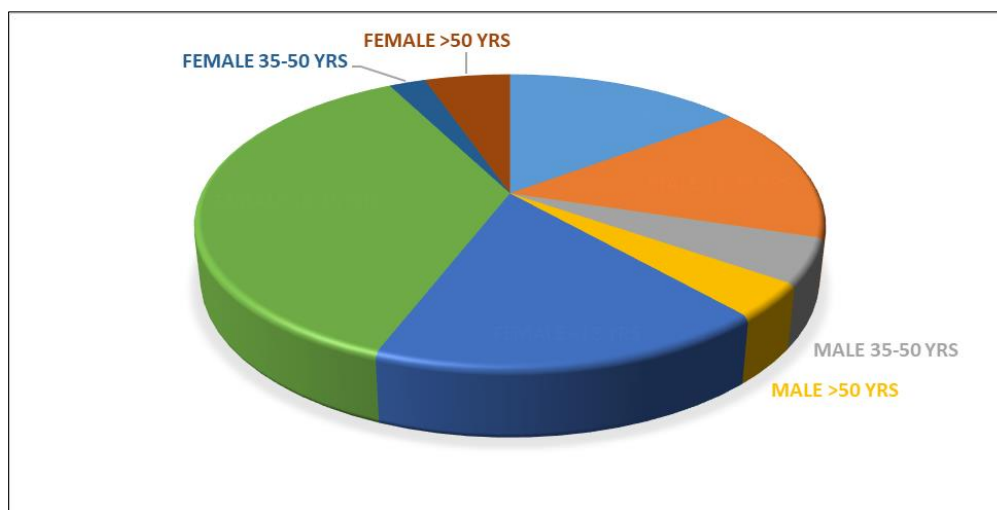
#### 3.1. Demographic Details of the Patients

The demographic data of patients attending dental clinics in Awka revealed that the highest number were females aged 18–35 years, followed by females below 18. Males below 18 and males aged 18–35 followed closely. Fewer participants were in the 35–50 and above 50 age brackets, with the least being males aged above 50 and females aged 35–50 (Fig. 1.0).

In terms of marital status, single females (18–35 years) were the most represented, followed by single males under 18, then married females (19–35), and married males over 50. Divorced females under 18 were among the least represented (Fig. 1.1).

Regarding educational background, most patients had tertiary education, followed by those with primary and secondary education.

The least were those holding second degrees (Fig. 1.2). Employment status showed that unemployed individuals aged 18–35 years made up the largest group, followed by employed individuals under 18.

**Figure 1** Demographic Details of the Patients

The retired population (50+ years) had the lowest attendance (Fig. 1.0). Most patients visited clinics labeled as “unspecified” (names withheld), followed by those attending COOUTH Amaku, with fewer visiting unlicensed clinics (Table 1.1). On oral hygiene practices, most patients used only toothbrush and paste, followed by those using both toothbrush and chewing sticks. Few used chewing sticks alone.

Most brushed once daily, fewer brushed twice, and only a small number brushed thrice daily (Table 1.2). Confectionery consumption showed that candies were most consumed, followed by confectionaries, ice cream, and cakes (Table 1.3). Awareness of the need for dental check-ups twice yearly was low—fewer than 50% were aware, regardless of age or education level (Table 1.2).

Tooth extraction data showed that 68% had experienced it once, while fewer than 1% had extracted more than one tooth (Table 1.3). Regarding mouthwash use, 58% reported using it, while 42% did not (Table 1.3).

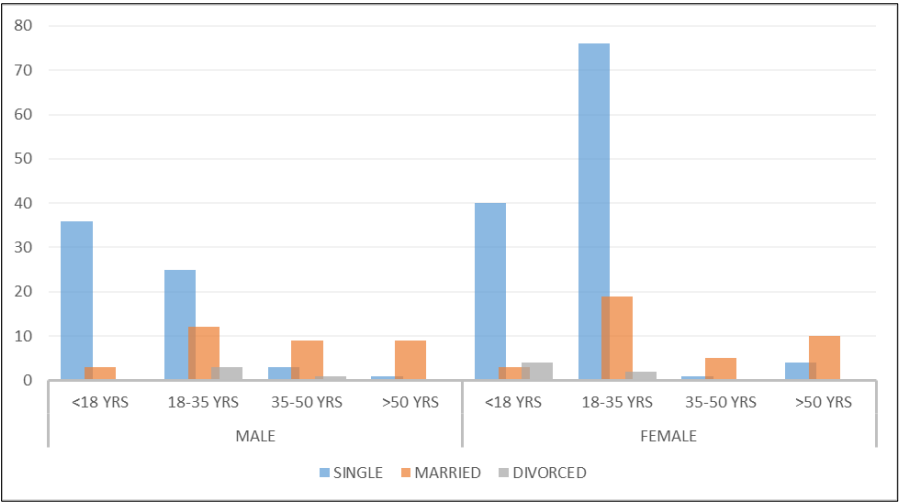


Figure 2 Marital Status of the Dental Patients

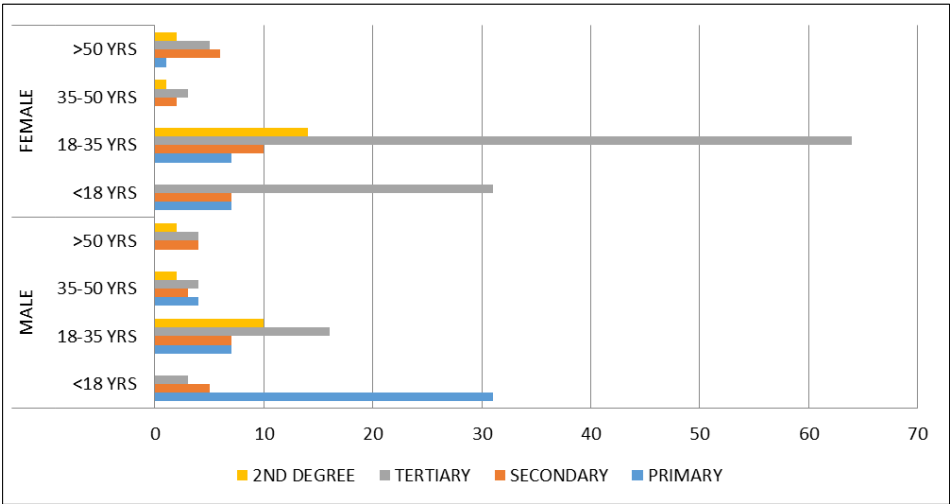


Figure 3 Educational status of the Patients

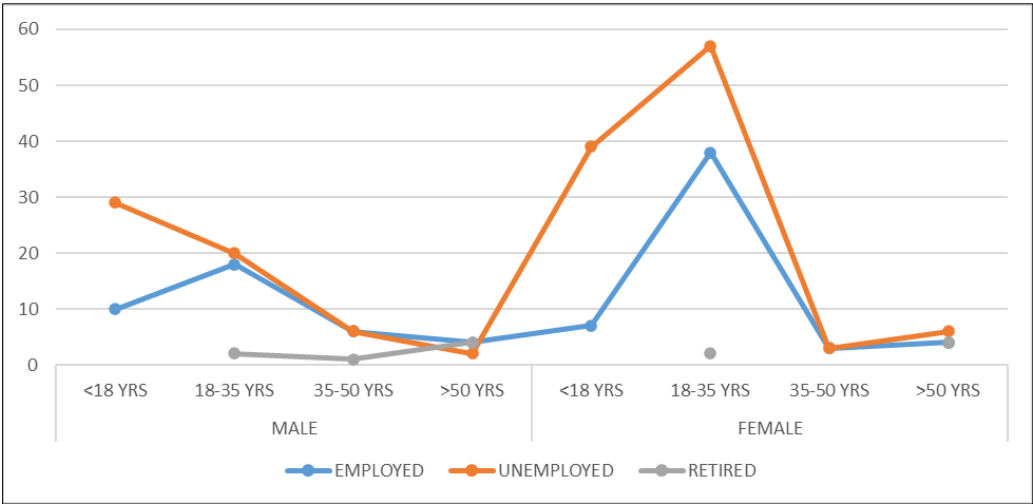


Figure 4 Employment Status of Patients attending Dental Clinics in Awka

**Table 2** Dental Hygiene Practices among the Patients

| ITEM                         | MALE        |                          |             |            | FEMALE       |                           |            |             |
|------------------------------|-------------|--------------------------|-------------|------------|--------------|---------------------------|------------|-------------|
| AGE                          | <18 YRS     | 18-35 YRS                | 35-50 YRS   | >50 YRS    | <18 YRS      | 18-35 YRS                 | 35-50 YRS  | >50 YRS     |
| NO.                          | 39          | 40                       | 13          | 10         | 46           | 97                        | 6          | 14          |
| HEALTHCARE FACILITY ATTENDED |             |                          |             |            |              |                           |            |             |
| AMAKU HOSP.                  | 2           | 6                        | 1           | 4          | 4            | 27                        | 2          | 7           |
| OTHERS                       | -           | -                        | -           | -          | 6            | 11                        | -          | -           |
| UNSPECIFIED                  | 37          | 34                       | 12          | 6          | 36           | 59                        | 4          | 7           |
| DENTAL CARE                  |             |                          |             |            |              |                           |            |             |
| DENTIST VISIT                | Y N<br>38 1 | Y N<br>33 2<br>UNSP. = 5 | Y N<br>11 2 | Y N<br>7 3 | Y N<br>34 12 | Y N<br>63 23<br>UNSP. =11 | Y N<br>5 1 | Y N<br>5 9  |
| TOOTHBRUSH                   | 30          | 21                       | 9           | 7, UNSP=1  | 28           | 64                        | 2          | 7           |
| CHEWING STICK                | 6           | 8                        | 2           | 1          | 15           | 8                         | -          | 2           |
| BOTH                         | 3           | 11                       | 2           | 1          | 2            | 22                        | 4          | 5           |
| OTHERS                       | -           | -                        | -           | -          | UNSP=1       | 2, UNSP=1                 | -          | -           |
| MOUTHWASH USE                | Y N<br>33 6 | Y N<br>19 21             | Y N<br>3 10 | Y N<br>4 6 | Y N<br>26 20 | Y N<br>57 40              | Y N<br>2 4 | Y N<br>10 4 |
| FREQUENCY OF USE             |             |                          |             |            |              |                           |            |             |
| ONCE A DAY                   | 32          | 10                       | 1           | 2          | 21           | 23                        | -          | 8           |
| TWICE                        | 1           | 9                        | 2           | 2          | 5            | 32                        | 1          | 1           |
| THRICE                       | -           | -                        | -           | -          | -            | -                         | 1          | -           |
| OTHERS                       | -           | -                        | -           | -          | -            | 2                         | -          | 1           |

**Table 3** Oral Health Challenge of the Patients

| ITEM                           | MALE        |             |            |            | FEMALE       |              |            |             |
|--------------------------------|-------------|-------------|------------|------------|--------------|--------------|------------|-------------|
| AGE                            | <18 YRS     | 18-35 YRS   | 35-50 YRS  | >50 YRS    | <18 YRS      | 18-35 YRS    | 35-50 YRS  | >50 YRS     |
| NO.                            | 39          | 40          | 13         | 10         | 46           | 97           | 6          | 14          |
| EDUCATIONAL LEVEL              |             |             |            |            |              |              |            |             |
| PRIMARY                        | 31          | 7           | 4          | -          | 7            | 7            | -          | 1           |
| SECONDARY                      | 5           | 7           | 3          | 4          | 7            | 10           | 2          | 6           |
| TERTIARY                       | 3           | 16          | 4          | 4          | 31           | 64           | 3          | 5           |
| 2 <sup>ND</sup> DEGREE         | -           | 10          | 2          | 2          | -            | 14           | 1          | 2           |
| ORAL HEALTH CHALLENGE SUFFERED |             |             |            |            |              |              |            |             |
| ORAL HEALTH CHALLENGE          | Y N<br>36 3 | Y N<br>32 8 | Y N<br>9 4 | Y N<br>7 3 | Y N<br>36 10 | Y N<br>55 42 | Y N<br>5 1 | Y N<br>13 1 |

|                  |             |              |            |            |              |                        |            |             |
|------------------|-------------|--------------|------------|------------|--------------|------------------------|------------|-------------|
| TOOTH EXTRACTION | Y N<br>30 9 | Y N<br>32 8  | Y N<br>7 6 | Y N<br>5 5 | Y N<br>22 14 | Y N<br>36 19           | Y N<br>3 2 | Y N<br>11 3 |
| >1 EXTRACTION    | 1           | -            | -          | 1          | -            | -                      | -          | 6           |
| ON ANTIBIOTICS   | Y N<br>31 8 | Y N<br>21 11 | Y N<br>8 5 | Y N<br>2 8 | Y N<br>14 32 | Y N<br>15 79<br>UNSP=3 | Y N<br>3 3 | Y N<br>4 10 |

**Table 4** Dental Caries Prevention Practices of the Patients

| ITEM                          | MALE                  |              |            |            | FEMALE       |              |            |            |
|-------------------------------|-----------------------|--------------|------------|------------|--------------|--------------|------------|------------|
| AGE                           | <18 YRS               | 18-35 YRS    | 35-50 YRS  | >50 YRS    | <18 YRS      | 18-35 YRS    | 35-50 YRS  | >50 YRS    |
| NO.                           | 39                    | 40           | 13         | 10         | 46           | 97           | 6          | 14         |
| EDUCATIONAL LEVEL             |                       |              |            |            |              |              |            |            |
| PRIMARY                       | 31                    | 7            | 4          | -          | 7            | 7            | -          | 1          |
| SECONDARY                     | 5                     | 7            | 3          | 4          | 7            | 10           | 2          | 6          |
| TERTIARY                      | 3                     | 16           | 4          | 4          | 31           | 64           | 3          | 5          |
| 2 <sup>ND</sup> DEGREE        | -                     | 10           | 2          | 2          | -            | 14           | 1          | 2          |
| KNOWLEDGE OF VISIT TO DENTIST |                       |              |            |            |              |              |            |            |
| KNOWLEDGE OF VISIT TO DENTIST | Y N<br>9 29<br>UNSP=1 | Y N<br>18 22 | Y N<br>4 9 | Y N<br>5 5 | Y N<br>24 22 | Y N<br>58 39 | Y N<br>5 1 | Y N<br>6 8 |
| ONCE A YEAR                   | -                     | -            | -          | -          | -            | -            | -          | -          |
| >ONCE A DAY                   | 2                     | 1            | -          | -          | -            | -            | -          | -          |
| CONFECTIONARIES CONSUMPTION   |                       |              |            |            |              |              |            |            |
| CONFECTIONARIES CONSUMPTION   | Y<br>39               | Y N<br>30 10 | Y N<br>8 5 | Y N<br>5 5 | Y N<br>45 1  | Y N<br>71 16 | Y N<br>4 2 | Y N<br>9 5 |

#### 4. Antibiotic sensitivity profile result

The antibiotic sensitivity profile result, revealed that *Mammalicoccus sciuri*, was 100% sensitive to Gentamicin, Levofloxacin, Ciprofloxacin and Ofloxacin, 100% resistant to Cefixime and 90% resistant to Imepenem. *E. caseliflavus* was 100% sensitive to Gentamicin, Ceftriaxone, Levofloxacin and Ofloxacin, 90% sensitive to Cefotaxime, Erythromycin, and Azithromycin. They were 100% resistant to Cefuroxime and Augmentine but 90% resistant to Cefixime. *Enterococcus galinarum* was 100% sensitive to Gentamicin, Cefotaxime, Ceftriaxone, Levofloxacin, Ciprofloxacin and Ofloxacin. They were 100% resistant to Cefuroxime, Cefixime, Imepenem, Erythromycin, Azithromycin and Augmentin. *Glutamicibacter creatinolyticus* was 100% sensitive to Cefuroxime, Gentamicin, Ceftriaxone, Levofloxacin and Ofloxacin, 50% sensitive to Ciprofloxacin, Erythromycin and Azithromycin. They were however, 100% resistant to Cefixime, Imipenem and Augmentin. Generally, all the MDS used for the study, were found to be resistant to at least four (4) different antibiotics and also to three different classes of antibiotics. as shown in table 4.7, Percentage occurrence of MDRs revealed *M.sciuri*, was highest with about 46, next was *E. caseliflavus*, about 31, then *G. creatinolytticus*, about 15, and *E. galinarum* was the least occurring with about 8.

**Table 5** Table showing oral bacterial resistant to at least four different classes of antibiotics (Multy Drug Resistant Bacteria)

| ISOLATE                                | CXN | GN | CTX | CRO | ZEM | LBC | CIP | IMP | OFX | ERY | AZN | AUG |
|----------------------------------------|-----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| <i>Mammalicoccus Sciuri</i>            | S   | S  | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| <i>Enterococcus caseliflavus</i>       | R   | S  | S   | S   | S   | S   | R   | S   | S   | S   | S   | R   |
| <i>Enterococcus caseliflavus</i>       | R   | S  | S   | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| <i>Enterococcus caseliflavus</i>       | R   | S  | S   | S   | S   | S   | S   | R   | S   | S   | S   | S   |
| <i>Mammalicoccus Sciuri</i>            | S   | S  | R   | S   | R   | S   | R   | R   | S   | S   | R   | R   |
| <i>Enterococcus caseliflavus</i>       | R   | S  | R   | S   | R   | S   | S   | R   | S   | R   | R   | R   |
| <i>Mammalicoccus Sciuri</i>            | S   | S  | S   | S   | R   | S   | S   | R   | S   | S   | R   | S   |
| <i>Mammalicoccus Sciuri</i>            | R   | S  | R   | S   | R   | S   | S   | R   | S   | R   | S   | S   |
| <i>Enterococcus galinarum</i>          | R   | S  | S   | S   | R   | S   | S   | R   | S   | R   | R   | R   |
| <i>Mammalicoccus Sciuri</i>            | R   | S  | R   | S   | R   | S   | S   | S   | S   | S   | R   | R   |
| <i>Mammalicoccus Sciuri</i>            | S   | S  | R   | S   | R   | S   | S   | R   | S   | R   | S   | R   |
| <i>Glutamicibacter creatinolyticus</i> | S   | S  | R   | S   | R   | S   | S   | R   | S   | R   | S   | R   |
| <i>Glutamicibacter creatinolyticus</i> | S   | S  | R   | S   | R   | S   | R   | R   | S   | S   | S   | R   |

Key: CXN: Cefuroxime; GN: Gentamicin; CTX: Cefotaxime; CRO: Ceftriaxone; ZEM: Cefixime; LBC: Levofloxacin; CIP: Ciprofloxacin; IMP: Imipenem; OFX: Ofloxacin; ERY: Erythromycin; AZN: Azithromycin; AUG: Augmentin; R: Resistant; S: sensitive

**Table 6** Percentage Occurrence of isolated Multidrug Resistant Strains (MDS)

| Name of organism                       | Percentage occurrence (%) |
|----------------------------------------|---------------------------|
| <i>Enterococcus caseliflavus</i>       | 30.8                      |
| <i>Enterococcus galinarum</i>          | 7.7                       |
| <i>Glutamicibacter creatinolyticus</i> | 15.4                      |
| <i>Mammalicoccus sciuri</i>            | 46.2                      |

Statistical data showed that females aged 18–35 years were the highest number attending dental clinics in Awka, followed by females under 18, then males under 18 and 18–35 years. The least were males and females aged 35–50 and above. This agrees with Oluwarotimi et al. [20], who reported more dental patients among middle-aged groups with a higher percentage of females. Similarly, Mofoluwaso et al. [21], found that people in their third and fourth decades of life represent the highest proportion seeking dental care, with more females than males attending clinics. Younger females and males are more prone to dental issues, likely due to higher consumption of sugary foods and more physical activities that could cause dental injuries from play or fights, which occur less frequently in older adults. Regarding marital status, unmarried younger adults visited dental clinics more often, possibly due to parental support. Patients with tertiary education attended clinics more frequently than those with lower qualifications, likely due to better awareness and access. This aligns with Oluwarotimi et al. [20], who noted that lower socio-economic groups in Nigeria have less access to dental care. Unemployed individuals under 35 were more common clinic attendees than employed or retired ones, possibly due to parental sponsorship. Older, retired patients may lack financial resources, reducing their clinic attendance, supporting findings by Oluwarotimi et al. [20] on the influence of socio-economic status on dental care seeking. Most patients attended unspecified dental clinics rather than the Amaku Teaching Hospital, differing from Oluwarotimi et al. [20], who suggested cultural beliefs influence dental care behavior. This may reflect easier or quicker access, lower costs, or willingness to participate in the study at unspecified clinics. Regarding oral hygiene, more patients used only toothbrushes than both toothbrushes and chewing sticks, with chewing stick users being the fewest. This aligns with Mafoluwaso et al. [21], who linked socio-economic and cultural factors to oral hygiene practices. Urban residents often view toothbrushes as modern and chewing sticks as outdated, reflecting personal or medical

preferences. Most patients washed their mouths once daily, with fewer washing twice or thrice, consistent with Mofoluwaso et al. [21]. This may relate to lifestyle, personal conviction, or medical advice. Younger females consumed more ice cream and confectionaries than older patients, possibly due to age-related preferences or advice from health providers. Awareness of the need for biannual dental visits was low; less than 50% knew they should visit the dentist regularly regardless of age, gender, or education. This reflects findings by Mofoluwaso et al. [21] and Oluwarotimi et al. [20], indicating poor public sensitization by health agencies. Tooth extraction data showed that 68% of patients had at least one tooth extracted, unlike Oluwarotimi et al. [20], who reported patients sought care mainly for systemic conditions like pain or diabetes. This discrepancy could result from high carbohydrate consumption, poor oral hygiene, and poverty.

Cultural, morphological, biochemical, and molecular analyses identified thirteen multidrug-resistant oral bacterial species from patients: six *Mammalicoccus sciuri*, four *Enterococcus casseliflavus*, one *Enterococcus gallinarum*, and two *Glutamicibacter creatinolyticus*. These bacteria share similar cultural and biochemical traits but differ molecularly, supporting Komiyama et al. [22], who described Enterococci as transient oral bacteria found across age groups. Antibiotic sensitivity showed *Enterococcus* species were sensitive to Gentamicin, Ceftriaxone, Levofloxacin, Ofloxacin, Cefotaxime, Erythromycin, Ciprofloxacin, and Azithromycin but resistant to Cefuroxime and Imipenem, corroborating Haslem and Geme [23]. However, *E. gallinarum* was resistant to Erythromycin and Azithromycin, unlike *E. casseliflavus*. *M. sciuri* was sensitive to Gentamicin, Levofloxacin, Ciprofloxacin, and Ofloxacin but resistant to Cefixime and Imipenem, consistent with Haslem and Geme [23] except for Ciprofloxacin sensitivity. *G. creatinolyticus* was sensitive to Cefuroxime, Gentamicin, Ceftriaxone, Levofloxacin, and Ofloxacin, partially sensitive to Ciprofloxacin, Erythromycin, and Azithromycin, but resistant to Cefixime, Imipenem, and Augmentin.

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## 5. Conclusion

This study has yielded several significant conclusions regarding the factors influencing dental clinic attendance, the characterization of oral bacterial isolates, and the antibacterial properties of selected plant extracts.

Demographic and lifestyle factors such as age, sex, academic qualification, marital status, employment status, oral hygiene practices, types of oral hygiene kits used, and consumption of confectionaries significantly impact the number of patients visiting dental clinics in Awka.

Thirteen bacterial strains were identified: six *Mammalicoccus sciuri* (Accession No. CP041879.1), four *Enterococcus casseliflavus* (Accession No. CP046123.1), one *Enterococcus gallinarum* (Accession No. MT5977046.1), and two *Glycolyticus creatinolyticus* (Accession Nos. KY814694.1 and CP034412.1).

While these bacteria share cultural, morphological, and biochemical traits, they differ in molecular characteristics and gene sequences. These bacteria are transient residents in the oral cavity of individuals across various age groups.

*Enterococcal* species exhibited sensitivity to Gentamicin, Ceftriaxone, Levofloxacin, Ofloxacin, Cefotaxime, Erythromycin, Ciprofloxacin, and Azithromycin. However, they demonstrated resistance to Cefuroxime, Augmentin, Cefixime, and Imipenem. Notably, *E. gallinarum* was resistant to Erythromycin and Azithromycin, unlike *E. casseliflavus*.

*Mammalicoccus sciuri* was sensitive to Gentamicin, Levofloxacin, Ciprofloxacin, and Ofloxacin; slightly sensitive to Ciprofloxacin, Erythromycin, and Azithromycin; and resistant to Cefixime, Imipenem, and Augmentin.

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## Compliance with ethical standards

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### Disclosure of conflict of interest

The authors declare no conflict-of-interest

### Statement of informed consent

Informed consent was obtained from all study respondents after description of the study objectives individual participants included in the study.

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