**ANTIMICROBIAL ACTIVITY OF *VERNONIA AMYGDALINA* LEAVES AQEUOUS EXTRACTS AGAINST MULTIDRU-RESISTANT BACTERIAL ISOLATES FROM POULTRY CHICKENS IN BAUCHI METROPOLIS**

**ABSTRACT**

**Background and Aim:** The rise of Multidrug-resistant (MDR) bacteria in poultry due to excessive antibiotic use presents a serious public health concern, especially in regions like Bauchi Metropolis where poultry farming is widespread. There is increasing interest in exploring natural plant-based alternatives to antibiotics. *Vernonia amygdalina* (bitter leaf) is a medicinal plant traditionally used for its antimicrobial properties, but its effectiveness against MDR bacteria from poultry sources remains under-researched. The study aims to evaluate the antimicrobial activity of aqueous extracts of *Vernonia amygdalina* leaves against Multidrug-resistant bacterial isolates obtained from poultry chickens in Bauchi Metropolis.

**Place and Duration of Study:** This study was carried out in the Abubakar Tafawa Balewa University (ATBU), Bauchi, Nigeria, in a period extended from August 2024 to March 2025.

**Methodology:** Cloacal rectal swab samples of poultry birds were collected using a swab stick. The samples were immediately transported and processed in the Microbiology laboratory at Yelwa Campus. Bacterial identification was performed using standard microbiological techniques, including Gram staining, selective media culture, and microscopy. Antimicrobial susceptibility was evaluated using the Kirby-Buer disc diffusion method, while the effects of *V. amygdalina* leaf extracts were tested using aqueous extractions.

**Results:** Eight bacterial species were identified, with *Escherichia coli* being the most prevalent (26.6%), followed by *Staphylococcus aureus* (14.4%) and *Streptococcus pyogenes* (14.4%). Antibiotic susceptibility testing revealed high resistance rates to commonly used antibiotics, with 111 isolates classified as MDR. *E. coli* exhibited the highest MDR prevalence (31.5%), showing significant resistance to azithromycin, amoxicillin, ceftriaxone, and ciprofloxacin. The antimicrobial susceptibility testing of *V. amygdalina* extracts demonstrated inhibitory effects on most MDR isolates, with minimum inhibitory concentrations (MIC) of 50.0 mg/ml for the aqueous extract. However, 28 isolates (25.2%) remained resistant to the extracts

**Conclusion:** The study reveals broader concern of antibiotic resistance among poultry-associated bacteria. It also shows Vernonia amygdalina possesses antimicrobial potential and could serve as an alternative to synthetic antibiotics for managing MDR bacterial infections in poultry. Further research is required to standardize its application and explore additional treatment options, as some isolates exhibited resistance to Vernonia amygdalina extracts.

*Keywords: poultry birds; Vernonia amygdalina; Aqueous extract; Antimicrobial Resistances; Multidrug Resistance.*

**1. INTRODUCTION**

The potential of the poultry industry to contribute to the gross domestic product (GDP) of any nation cannot be underestimated. Poultry meat and eggs have significantly improved living standards, particularly in developing countries. The use of antibiotics in poultry began with the discoveries of penicillin, streptomycin, and chlortetracycline in the 1940s. These drugs were instrumental in outbreak control, helping maintain the health of birds' digestive tracts and allowing chickens to gain weight efficiently with less feed [1]. Additionally, antibiotics have been shown to promote a beneficial microbiome, support improved immune function, and enhance gut health, thereby leading to better feed efficiency [2]. However, it has been observed that the advantages associated with antibiotics, especially when used uncontrollably, have led to several serious consequences, such as antibiotic resistance in birds, other animals, and humans [3].

Multidrug resistance (MDR) is a significant public health issue that occurs when bacteria become resistant to two or more antimicrobial drugs, rendering previously effective treatments ineffective [4]. Antibiotic-resistant (AMR) bacteria are naturally present in the environment because many antibiotics are produced by other organisms, such as fungi (e.g., penicillin) and soil bacteria (e.g., streptomycin, chloramphenicol, and tetracycline [5]. Acquired bacterial resistance can occur through several general mechanisms, including inactivation, target alteration, decreased permeability, and increased efflux [4]. This research focuses on isolating common bacterial pathogens associated with poultry birds. Bacteria frequently reported in connection with poultry include *Enterobacter aerogenes, Salmonella spp., Listeria monocytogenes, Staphylococcus aureus, Proteus spp., Escherichia coli, Riemerella anatipestifer, Pseudomonas spp., Klebsiella pneumoniae, Acinetobacter spp., Enterococcus spp.,* and *Streptococcus spp.* [6,7,8]. Some of these pathogens are capable of spreading virulence and resistance genes throughout the microbial population, making infections more challenging to treat and potentially increasing mortality rates in birds, other animals, and humans [9].

Recognizing the growing threat of antimicrobial-resistant microorganisms due to indiscriminate antibiotic use, many poultry farmers by practice are exploring alternatives. One such alternative is the use of organic extracts instead of synthetic antibiotics. For instance, *Vernonia amygdalina*, or bitter leaf, aqueous extract possesses bioactive phytochemicals that have demonstrated significant antibacterial activity [10].

1. **MATERIALS AND METHODS**

**2.1 Study Area**

Bauchi state is located between latitude 903 and 1203 north of the equator. Longitudinally, it lies between 805 and 110 east of Greenwich meridian. This work was carried out in the microbiology Laboratory of Abubakar Tafawa Balewa University ATBU. Bauchi is dominated by tropical Sudan Savannah, characterized by agricultural activities both on a large and small scale, which include poultry production and cattle rearing.

**2.2 Sample Collection**

A total of ninety-nine (99) samples of cloacal swabs were collected for this study within the time frame of three weeks and were transported to the Microbiology Laboratory of Abubakar Tafawa Balewa University (ATBU) for Processing according to standard laboratory techniques as described by [11,12].

**2.3 Sample Processing**

99 cloacal swab samples were collected over three weeks and transported to the Microbiology Laboratory at Abubakar Tafawa Balewa University (ATBU), adhering to the standard laboratory protocols. Gram-negative bacteria are inoculated on MacConkey agar and incubated at 37°C for 18-24 hours. MacConkey agar is selective for gram-negative bacteria and differentiates lactose fermenters based on colony morphology [11]. For gram-positive Bacteria, Mannitol salt agar (MSA) was used for the isolation of gram-positive types, following the same incubation conditions. Following growth, subculturing was carry out on Blood agar [11]. Isolates were evaluated morphologically, confirmed via Gram staining and microscopy. Biochemical tests included catalase, citrate, indole, coagulase, and urease tests [11].

Antimicrobial Susceptibility was conducted using the disc diffusion method on Mueller-Hinton agar (Himedia, India) with bacterial concentrations standardized to 0.5 McFarland units. Antibiotics tested included aminoglycosides, beta-lactams, macrolide etc. [13]. Following CLSI guidelines (2018) s. Extraction of Vernonia amygdalina aqueous extractions of *Vernonia amygdalina* leaves were performed. Leaves were dried, crushed, mixed with distilled water, shaken for three days on electronic shaker, centrifuged, and filtered, followed by mild heating for drying. extract was assessed for antibacterial efficacy against Multidrug-resistant isolates on Mueller-Hinton agar [13]. The Shapiro-Wilk test assessed normality in the distribution of bacterial and multidrug-resistant isolates, using a significance threshold of p < 0.05 for non-normal distribution.

**3. RESULTS AND DISCUSSION**

The morphological and biochemical characteristics of the bacteria isolated are shown in the table below, which shows the species of bacteria based on phenotypic characteristics.

# **Table 1: Morphological and Biochemical Characteristics of Bacterial Isolates From this Study**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Morphological Characteristics** | | **Biochemical Characteristics** | | | | | **Probable Bacterial species** |
| **Colonies** | **Gram Reactions** | **Catalase** | **Citrate** | **Indole** | **Coagulase** | **Urea** |  |
| Pink to red | **-** | **-** | **-** | **+** | **-** | **-** | *Escherichia coli* |
| Pink to red | **-** | **-** | **-** | **-** | **-** | **+** | *Enterobacter aerogenes* |
| Golden Yellow | **+** | **+** | **-** | **-** | **+** | **+** | *Staphylococcus aureus* |
| White to grayish | **+** | **-** | **-** | **-** | **-** | **-** | *Streptococcus pyogenes* |
| Colorless or pale | **-** | **-** | **+** | **-** | **-** | **-** | *Salmonella typhi* |
| Mucoid Pink to red | **-** | **-** |  | **-** | **-** | **+** | *Klebsiella pneumoniae* |
| Colorless or pale | **-** | **-** | **-** | **-** | **-** | **-** | *Shigella sp* |
| Pink to red | **-** | **+** | **+** | **-** | **-** | **+** | *Proteus mirabilis* |

Isolates were identified morphologically (Gram staining, color, size of colonies), while microscopic observations included shape and size of bacteria cells. For biochemical characteristics, bacteria isolates were tested for the presence of catalase enzymes, citrate test was used to determined their ability to utilize citrate as a sole source of carbon and energy, indole test was to check for bacteria that can convert tryptophan to indole, Coagulase test was used to identify *Staphylococcus* species which produces coagulase enzymes and finally urea test was used to identify bacteria that can utilize urea to form ammonia [14].

The table below shows some common bacteria that are found in poultry birds and their distribution based on the frequency of each isolate in the cloacal swabs’ samples. Most of these bacteria are associated with poultry bacterial infection.

# **Table 2: Distribution of Bacteria Species From Cloacal Swabs According to Frequency of Isolates**

| **Bacterial Species** | **No. of Isolates (n=139)** | **Percentage (%)** | **Normality (p < 0.05)** |
| --- | --- | --- | --- |
| *Enterobacter aerogenes* | 17.00 | 12.20 |  |
| *Escherichia coli* | 37.00 | 26.60 |  |
| *Proteus mirabilis* | 14.00 | 10.10 |  |
| *Salmonella typhi* | 17.00 | 12.20 | 0.7 |
| *Staphylococcus aureus* | 20.00 | 14.40 |  |
| *Streptococcus pyogenes* | 20.00 | 14.40 |  |
| *Shigella* spp. | 4.00 | 2.90 |  |
| *Klebsiella pneumoniae* | 10.00 | 7.20 |  |

Poultry cloacal swabs can harbor a diverse range of bacteria. Based on this study, eight different species of bacteria have been isolated, as shown in the table above. *Escherichia coli* has the highest percentage of 26.6%, followed by *Staphylococcus aureus* and *Streptococcus pyogenes,* which are 14.4% each, while *Shigella species* has the lowest percentage of only 2.90%. Statistically, the isolates are not normally distributed in the samples using the Shapiro-Wilk Test of normal distribution. The overall diversity of bacterial species associated with poultry birds in this study is consistent with other studies conducted by Kamel, Oueslati, and Telli. Kamel reported that *Escherichia coli* was the most predominant bacterial species with 80.5%, and he also reported 75% of the bacterial isolates that were reported in this study [3,15,16]. *E. coli* is a well-known global problem, causing significant economic losses in the poultry sector and representing one of the main reasons for seizures during slaughterhouse inspections [16]. Commonly, beta-lactams and fluoroquinolones are used to treat these infections; however, infections caused by strains that are resistant to these antibiotics or by multidrug-resistant strains limit the number of available treatment options and increase the severity of infections [16,17].

Bacteria colonizing healthy poultry are widely found in the natural environment and the digestive systems of birds, mammals, and humans. Several species are major pathogenic bacteria in human clinical settings, as well as in food-producing animals [16]. However, some of these bacteria can be the normal host flora of the poultry, which may serve a beneficial function to the birds [18]. Poultry is the second most consumed meat in the world [19]. In Africa, chicken production and processing are practiced both formally and informally, with smallholders constituting the majority in this sector. Informal practices are vulnerable to the production and processing of chicken, which is easily contaminated by pathogens such as *Escherichia coli, Staphylococcus aureus, Salmonella*, and *Campylobacter* [19]. Hence, interactions with the fecal products of these birds should be in a hygienic manner to prevent the spread of these potential pathogenic microorganisms to the human host and the environment.

# **Table 3: Distribution of Bacterial Isolates According to Multidrug Resistance Pattern in this Study**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | **No. (%) of bacterial isolates (n=139) and multidrug resistant pattern** | | | | | | |
| **Antibiotics(µg)** | ***E. aerogenes (*n=17)** | ***E. coli* (n=37)** | | ***P*. *mirabilis* (n=14)** | ***S. Typhi***  **(n=17)** | ***S. aureus***  **(n=20)** | ***S. pyogenes* (n=20)** | ***Shigella sp.* (n=4)** | ***K. pneumoniae* (n=10)** |
| Azithromycin (10) | 10(64.7) | 37(100) | | 12(85.7) | 15(88.2) | 16(80.0) | 14(70.0) | 3(75.0) | 7(70.0) |
| Amoxicillin (20) | 14(82.3) | 36(97.2) | | 13(92.8) | 14(82.3) | 16(80.0) | 14(70.0) | 2(50.0) | 9(90.0) |
| Erythromycin (30) | 15(88.2) | 35(94.6) | | 9(64.2) | 13(76.4) | 19(95.0) | 12(60.0) | 4(100) | 10(100) |
| Ofloxacin (10) | 15(88.2) | 37(100) | | 12(85.7) | 14(82.3) | 15(75.0) | 16(80.0) | 3(75.0) | 9(90.0) |
| Augmentin (30) | 16(94.1) | 37(100) | | 13(92.8) | 15(88.2) | 18(90.0) | 19(95.0) | 3(75.0) | 10(100) |
| Levofloxacin (20) | 14(82.3) | 36(97.2) | | 11(78.5) | 15(88.2) | 7(35.0) | 5(25.0) | 2(50.0) | 4(40.0) |
| Ceftazidime (30) | 16(94.1) | 37(100) | | 12(85.7) | 15(88.2) | 18(90.0) | 19(95.0) | 2(50.0) | 7(70.0) |
| Gentamycin (10) | 16(94.1) | 37(100) | | 9(64.2) | 14(82.3) | 16(80.0) | 13(65.0) | 2(50.0) | 7(70.0) |
| Ciprofloxacin (10) | 7(41.1) | 28(75.6) | | 5(35.7) | 7(41.1) | 8(40.0) | 8(40.0) | 2(50.0) | 4(40.0) |
| Ceftriaxone (30) | 16(94.1) | 37(100) | | 12(85.7) | 15(88.2) | 15(75.0) | 14(70.0) | 4(100) | 8(80.0) |
| Streptomycin (30) | 16(94.1) | 34(91.8) | | 12(85.7) | 14(82.3) | 17(85.0) | 16(80.0) | 3(75.0) | 9(90.0) |
| Cefalexin (10) | 16(94.1) | 37(100) | | 12(85.7) | 15(88.2) | 19(95.0) | 18(90.0) | 3(75.0) | 9(90.0) |

*Zone of inhibition interpreted according to CLSI (2022) guidelines*

# **Antimicrobial Resistance Pattern of Bacterial Isolates from Poultry Cloacal Swabs in this Study**

In this Study, Table 3 shows antimicrobial resistance was found in all isolates, with *E. coli* being the most resistant isolates with 100% resistant to 7 of the 12 antibiotics that were tested include, Azithromycin, Ofloxacin, Augmentin, Ceftazidime, Gentamycin, Ceftriaxone and Cefalexin. This is followed by *K. pneumoniae* that is 100% resistant to Erythromycin and Augmentin. The least resistant bacteria among the isolates are *S. pyogenes,* which shows only 25% resistance to Levofloxacin, followed by *P. mirabilis,* which is 35.7% resistant to Ciprofloxacin.

The results indicate a high prevalence of antimicrobial-resistant bacteria, including MDR in poultry birds. This higher prevalence of resistant microbes could be due to poultry farming practices that rely heavily on the use of antibiotics [5]. Other studies, including modeling studies, have shown similar conclusions, including a gradual increase in antibiotic consumption and associated AMR in the animal health sector by 2030 [2]. Although antibiotics have rarely been used in backyard free-range chicken, resistance has been reported in India and Pakistan [20,21,22]. This is possibly due to either sharing a common environment with broilers (which are treated with antibiotics) or environmental transmission through the liquid manure of livestock and human excreta [23]. In addition, some species of bacteria, such as *E. coli*, are intrinsically resistant to different first-line antimicrobial agents (e.g., low-level resistance to β-lactams and aminoglycosides) and have the capacity to acquire resistance to several other antimicrobial agents, including last-resort antibiotics, such as glycopeptides and Vancomycin [24].

*Vernonia amygdalina* **(**VA) has been used traditionally for a variety of medical purposes, including antibacterial, anti-diabetic, anticancer, and anti-inflammatory effects. The plant has a wide spectrum of uses in traditional African medicine and has been used in the management and treatment of several health conditions [15].

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# **Table 4: Minimum Inhibitory Concentration (MIC) of Extract against Non-Resistant Isolates and the Diameter of Zone of Inhibitions**

| **Solvent** | **MIC (mg/ml)** | **Zone of Inhibition (mm)** |
| --- | --- | --- |
| Aqueous | 50.0 | 27.0 |

Susceptibility criteria =Diameter of ZOI ≥10mm compared with Negative control. (26)

Based on this study, the extracts have significantly shown antibacterial properties with 27mm zoneof inhibition at 50mg/ml MIC value tested against same isolates. *Vernonia amygdalina* contains bioactive phytochemicals that are responsible for its antibacterial properties. More research is needed to allow the purification of unique bio-potential chemicals and their subsequent transformation into chemotherapeutic agents [27,28].

# **Table 5: Antimicrobial Activity of *Vernonia amygdalina* Aqueous extraction against MDR bacterial Isolates in this Study**

|  |  |  |  |
| --- | --- | --- | --- |
| **MDR Bacterial Isolates** | **No. of MDR Bacteria (n=111)** | **No. (%) of MDR Bacteria Resistant to the Extracts(n=28)** | **Normality**  **(p > 0.05)** |
| *Enterobacter aerogenes* | 14 | 04 (14.2) |  |
| *Escherichia coli* | 35 | 09 (32.1) |  |
| *Proteus mirabilis* | 11 | 03 (10.7) |  |
| *Salmonella typhi* | 13 | 04 (14.2) | 0.856 |
| *Staphylococcus aureus* | 16 | 04 (14.2) |  |
| *Streptococcus pyogenes* | 14 | 02 (7.10) |  |
| *Shigella species* | 02 | 00 (0.00) |  |
| *Klebsiella pneumoniae* | 06 | 02 (7.10) |  |

Susceptible criteria =Diameter of ZOI ≥10mm compared with Negative control. (26)

Based on this study, Most of the Multidrug resistant bacterial isolates were susceptible to the aqueous extracts; only 28 out of 111 isolates were found resistant to the extracts. *Escherichia coli* has the highest resistance of 32.1%, followed by followed by *E. aerogenes,* S. *typhi* and *S. aureus* which show 14.2%. *Shigella species* was not found resistant with 0.0%. Statistically, the MDR isolates are normally distributed in resistance to both conventional drugs and extract the using Shapiro-Wilk Test of normal distribution. The wide use of antibiotics (drugs) in the treatment of bacterial infections has led to the emergence and spread of resistant strains. The emergence of multiple drug-resistant bacteria (MDR) has become a major cause of failure of the treatment of infectious diseases [30]. The antibacterial activity of *V. amygdalina* leaf extract on MDR Bacteria shows effectiveness even at low concentration, proving the plant can be an alternative [31]. The result of this study also opens doors to the possibilities of overcoming antimicrobial resistance using *Vernonia amygdalina* and also calls for intensive research to look for alternatives to synthetic drugs.

**4. CONCLUSION**

The study found that 79.8% of 139 bacterial isolates from poultry cloacal swabs exhibited multidrug resistance (MDR) to conventional antibiotics. Notably, 25.2% of the MDR isolates were resistant to aqueous extracts of *Vernonia amygdalina* (bitter leaf), *Escherichia coli* showed the highest number of MDR isolates and significant resistance to the plant extract. Despite this, *Vernonia amygdalina* may offer potential as an alternative treatment for MDR infections in poultry farming and other clinical infections.

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