**Occurrence and Antibiotic-resistant Profile of *Escherichia coli* in Pastoral Fresh Cow Milk Vended around Galadimawa, Abuja, Nigeria.**

**Abstract**

The quality and safety of fresh cow milk are of critical concern due to the potential presence of pathogenic microorganisms. The high nutritional content of milk makes it susceptible to contamination by various pathogenic bacteria and thus serves as a vector for pathogens in humans. This study aimed to assess the incidence, identification, and antibiotic susceptibility (antibiogram) profile of *Escherichia coli* (*E. coli*) in fresh cow milk sold in the Galadimawa area of the Federal Capital Territory (FCT), Nigeria. The *E. coli* characterization in pastoral fresh cow milk samples was carried out using standard microbiological procedures. The antimicrobial susceptibility test was performed on Mueller-Hinton Agar using the disc diffusion method and compared with the Clinical and Laboratory Standards Institute (CLSI) standards. The commercially available antibiotic discs used include Augmentin, Ceftriaxone, Ciprofloxacin and Gentamycin, among others. This study established the presence of *E. coli* in all the purchased milk samples. Furthermore, 38.5% of the total isolated *E. coli* exhibited gamma hemolysis while 61.5% exhibited beta hemolysis in blood. Also, all the *E. coli* isolates were resistant to Augmentin and 92.3% were susceptible to levofloxacin. The findings in this study indicated a significant prevalence of *E. coli* in the milk samples, underscoring the potential risk of milk-borne diseases in the community. Also, the high rate of antibiotic-resistant *E. coli* is a serious threat to public health, as it could result in difficult-to-treat bacterial infections.

**Introduction**

Milk is a good growth medium that favours the growth of several microorganisms due to the presence of some essential nutrients like calcium, magnesium, zinc and potassium (Reta and Addas, 2021). Microorganisms may contaminate milk easily at various stages before and during consumption. For instance, fresh cow milk can be contaminated from within the udder, outside the udder and from the equipment used for milking and during storage (Belbachir *et al*., 2019). The presence of these microorganisms can affect the nutritional quality and also threaten consumers’ well-being by inducing foodborne illness, thus posing a threat to public health if found to be pathogenic (Logan, 2022).

Foodborne diseases are a widespread global problem. Several outbreaks have been reported as a result of consuming milk that may appear normal but is contaminated with a large number of harmful pathogens, including *Escherichia coli*. The presence of which is direct or indirect faecal contamination and a general lack of cleanliness in handling, as well as improper storage. Nowadays, people consume raw cow milk for its affordability without bothering about the dangers that could be incurred with its consumption. According to recent analysis by the Centre for Disease Control and Prevention (CDC, 2024), unpasteurized milk is 150 times more likely to cause foodborne illness and result in 13 times more hospitalizations than illnesses involving pasteurized dairy products. The rising resistance of these organisms to antibiotics also poses a serious health challenge.

It was recently reported by the World Health Organization that roughly 1 in 10 people in the world gets sick from foodborne illness, with 420,000 people dying every year (WHO, 2024). Also, *E. coli* is one of the most common foodborne pathogens that affects millions of people, sometimes leading to hospitalizations and fatal outcomes. The enterohaemorrhagic form of this pathogen is often associated with unpasteurized milk, such as is sold in the Galadimawa area, Abuja. Adequate education and information on the presence of this pathogen in the raw milk and its possibility of causing serious unwanted health issues would not only improve the methods employed in handling and processing the milk for sale but also influence the choice of consuming it, all of which would reduce the occurrence of foodborne illness and enhance wellbeing.

The study is aimed at investigating the occurrence and antibiogram of *E. coli* in pastoral fresh cow milk vended around Galadimawa, Abuja. This would provide information on the general hygiene of the fresh milk consumed in and around Galadimawa, Abuja, Nigeria and the serious risk it might pose to the health and well-being of its consumers.

**Materials and methods**

**Study Area**

The study was focused on pastoral fresh milk sold in and around Galadimawa, Abuja Municipal Area Council (AMAC), Federal Capital Territory, Abuja, Nigeria. It is geographically situated at a Latitude. 9.0062° or 9° 0' 22" North; Longitude. 7.4045° or 7° 24' 16" East.

**Sample Collection**

Raw cow milk samples were purchased from various locations in Galadimawa, FCT, Abuja. The samples were placed inside an airtight container containing ice packs and immediately transported to the Microbiology Laboratory of Baze University, Abuja, for analysis.

**Enumeration of *E. coli* from Raw Cow Milk**

Exactly 5mL of milk sample was dispersed into 45mL of sterile distilled water and mixed thoroughly. This was serially diluted in a 10-fold serial dilution (1mL of milk sample into 9 m of sterile distilled water). The procedure was repeated until a 10-3 dilution was achieved. 0.1mL of each dilution was then plated on a sterile molten Eosin Methylene Blue (EMB) agar, using the spread plate method, the plates were then incubated at 37oC for 24 hours and colonies were purified by sub-culturing distinct colonies on fresh EMB agar plates using the streaking method. Pure colonies with green metallic sheen on EMB agar plates after appropriate incubation were kept in sterile nutrient agar slants and maintained in 4oC for further procedure (Bushra *et al*., 2020).

**Biochemical Characteristics of *E. coli* Isolates**

The following biochemical tests; Indole, Methyl Red, Voges Proskauer and Citrate were performed to confirm the identity of *E. coli* isolates phenotypically in conformity with the IMVIC. Other tests that were performed include catalase, motility, hydrogen sulfide production, carbohydrate utilization and urease using standard microbiological procedures.

**Hemolysis test**

Sterile blood agar base, supplemented with 7% human blood, was poured into sterile petri dishes and allowed to gel. Using a sterile inoculating loop, each of the isolates was streaked onto agar plates. The inoculated plates were then incubated at 37°C for 24 hours, after which the medium was observed for telltale signs of alpha- or beta-hemolysis (Saggar, 2022).

**Antibiotic Sensitivity Test**

Standard bacterial inoculum was spread on the surface of Mueller-Hinton agar (MHA) plate under aseptic conditions. The antibiotic disc was placed on the surface using a sterile forcep. The antibiotics used are Augmentin, Ceftriaxone, Cefixime, Ofloxacin, Nitrofurantoin, Gentamycin, Cotrimoxazole, Levofloxacin, Piperacillin, Tetracycline, Clindamycin, and Ciprofloxacin. The plates were incubated at 37˚C for 24 hours. After incubation, the zones of inhibition were measured in millimetres and the results were interpreted by Clinical and Laboratory Standards Institute (CLSI, 2020).

**Results**

**Enumeration of *E. coli* from milk samples.**

*Escherichia coli* was isolated from all the milk samples that were analyzed. The *E. coli* count ranged between 2.5 ×102 and 3.0 ×102 CFU/mL on EMB agar.

**Biochemical Characteristics of *E. coli* Isolates.**

All the isolates that appeared as green metallic sheen on Eosin Methylene Blue agar were observed to be Gram-negative cocci after Gram staining. The biochemical reactions of isolates were observed to vary as some of the isolates exhibited negative and positive reactions to the respective tests carried out. Given the sugar utilization test, some *E. coli* isolates were able to utilize the sugars as indicated (Ab, Gb), others slightly utilized the sugars (As, Gs), while some did not utilize the sugars (-, -). The result of Gram staining and biochemical reaction, as well as sugar fermentation, is presented in Table 1.

**Production of Hemolysin by *E. coli* isolates**

Table 2 shows the hemolysin production profile of *E. coli* isolates. Some isolates were observed to exhibit gamma hemolysis, which indicates no hemolysis, others exhibited beta hemolysis, which indicates complete lysis of the blood cell, characterized by clear zones around the line of streak on blood agar. However, none of the *E. coli* isolates exhibited alpha hemolysis, which indicates partial lysis of the blood cell.

**Antibiotic Susceptibility Pattern of *E. coli* isolates**

The result of the antibiotic susceptibility pattern of E. coli isolates is presented in Table 3. All the isolates were resistant to Augmentin. While some of the isolates were resistant to a few antibiotics, including Cefixime, Nitrofurantoin, Co-Trimoxazole and Tetracycline, some were observed to be susceptible to Ciprofloxacin, ofloxacin, gentamycin, Levofloxacin and Piperacillin.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ISOLATE CODE** | **GRAM****REACTION** | **CATALASE** | **HYDROGEN****SULFIDE** | **INDOLE** | **MOTILITY** | **MR** | **VP** | **UREASE** | **CITRATE** | **SUGAR UTILIZATION** |
| **GLUC** | **SUC** | **MAL** | **MAN** | **GLA** | **STA** |
| **FA1** | **Negative****Cocci** | **-ve** | **+ve** | **-ve** | **+ve** | **+ve** | **+ve**  | **+ve** | **+ve** | **Ab, Gb** | **Ab, Gb** | **As, Gb** |  **-, Gb** | **Ab, Gb** | **\_** |
| **FA2** | **Negative** **Cocci** | **-ve** | **+ve** | **-ve** | **+ve** | **+ve** | **+ve**  | **+ve** | **+ve** | **Ab, Gb** | **Ab, Gb** | **As, Gb** |  **-, Gb** | **Ab, Gb** | **\_** |
| **FA3** | **Negative Cocci** | **-ve** | **+ve** | **-ve** | **+ve** | **+ve** | **+ve**  | **+ve** | **+ve** | **Ab, Gb** | **Ab, Gb** | **As, Gs** |  **-, Gb** | **Ab, -** | **\_** |
| **FA4** | **Negative****Cocci** | **-ve** | **+ve** | **-ve** | **+ve** | **+ve**  | **+ve**  | **+ve** | **+ve** | **Ab, Gb** | **Ab, Gb** | **As, Gb** |  **\_** | **Ab, Gb** | **\_** |
| **FA5** | **Negative** **Cocci** | **-ve** | **+ve** | **-ve** | **+ve** | **+ve**  | **+ve**  | **+ve** | **+ve** | **Ab, Gb** | **Ab, Gb** | **As, Gb** |  **As, Gb** | **Ab, Gb** | **-, Gs** |
| **FA9** | **Negative** **Cocci** | **-ve** | **+ve** | **-ve** | **+ve** | **+ve**  | **+ve**  | **+ve** | **+ve** | **Ab, Gb** | **Ab, Gb** | **As, Gb** |  **As, Gb** | **Ab, Gb** | **-, Gs** |
| **FA10** | **Negative****Cocci** | **-ve** | **+ve** | **-ve** | **+ve** | **+ve**  | **+ve**  | **+ve** | **+ve** | **Ab, Gb** | **Ab, Gb** | **As, Gb** |  **\_** | **Ab, Gb** | **-, Gs** |
| **FA12** | **Negative****Cocci** | **-ve** | **-ve** | **-ve** | **+ve** | **+ve**  | **+ve**  | **+ve** | **+ve** | **Ab, Gb** | **Ab, Gb** | **As, Gb** |  **As, -** | **Ab, Gb** | **\_** |
| **FA13** | **Negative** **Cocci** | **-ve** | **-ve** | **-ve** | **+ve** | **+ve** | **+ve**  | **+ve** | **+ve** | **Ab, Gb** | **Ab, Gb** | **As, Gb** |  **-, Gb** |  **\_** | **Ab, Gb** |
| **FA14** | **Negative****Cocci** | **-ve** | **-ve** | **-ve** | **+ve** | **+ve** | **+ve**  | **+ve** | **+ve** | **Ab, Gb** | **Ab, Gb** | **As, Gb** |  **As, Gb** | **Ab, Gb** | **-, Gs** |
| **FA15** | **Negative****Cocci** | **-ve** | **-ve** | **-ve** | **+ve** | **+ve**  | **+ve**  | **+ve** | **+ve** | **Ab, Gb** | **Ab, Gb** |  **\_** |  **As, Gb** | **Ab, Gb** | **As, Gs** |
| **FA17** | **Negative** **Cocci** | **-ve** | **-ve** | **-ve** | **+ve** | **+ve**  | **+ve**  | **+ve** | **+ve** | **Ab, Gb** | **Ab, Gb** | **Ab, Gb** |  **As, Gb** | **Ab, Gb** | **\_** |
| **FA18** | **Negative****Cocci** | **-ve** | **-ve** | **-ve** | **+ve** | **+ve**  | **+ve**  | **+ve** | **+ve** | **Ab, Gb** | **Ab, Gb** | **As, Gb** |  **As, Gb** | **Ab, Gb** | **As, Gs** |

Table 1: Gram and Biochemical Reaction of *E. coli* Isolates from Profile

Key: -ve = Negative, +ve = Positive, Ab = acid, full color change, As = small amount of acid, Gb = gas (1/4 full), Gs = slight gas (less than 1/4).

**Table 2: Hemolysin Production Profile of E. coli Isolates**

|  |  |  |  |
| --- | --- | --- | --- |
| **ISOLATE CODE** | **GAMMA HEMOLYSIS**  | **BETA HEMOLYSIS** | **ALPHA HEMOLYSIS** |
| **FA1** | **-** | **+ve** | **-** |
| **FA2** | **-** | **+ve** | **-** |
| **FA3** | **-** | **+ve** | **-** |
| **FA4** | **-** | **+ve** | **-** |
| **FA5** | **-** | **+ve** | **-** |
| **FA9** | **-** | **+ve** | **-** |
| **FA10** | **-** | **+ve** | **-** |
| **FA12** | **+ve** | **-** | **-** |
| **FA13** | **+ve** | **-** | **-** |
| **FA14** | **-** | **+ve** | **-** |
| **FA15** | **+ve** | **-** | **-** |
| **FA17** | **+ve** | **-** | **-** |
| **FA18** | **+ve** | **-** | **-** |

**Key: -ve Negative, +ve=Positive**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Antibiotics**  | **FA1** | **FA2** | **FA3** | **FA4** | **FA5** | **FA9** | **FA10** | **FA12** | **FA13** | **FA14** | **FA15** | **FA17** | **FA18** |
| **Augmentin**  | R | R | R | R | R | R | R | R | R | R | R | R | R |
| **Ceftriaxone** | S | R | R | S | R | S | I | R | R | S | R | I | I |
| **Cefixime** | R | R | R | I | R | I | I | I | I | R | I | R | I |
| **Ofloxacin** | S | S | S | S | I | I | S | S | S | I | I | S | S |
| **Nitrofurantoin** | I | R | R | I | R | I | R | R | R | I | R | R | I |
| **Gentamycin** | R | I | I | S | I | R | S | S | S | S | S | S | I |
| **Co-trimoxazole** | S | R | I | S | R | R | I | S | S | I | R | R | S |
| **Levofloxacin** | I | I | S | S | S | S | S | S | S | S | I | S | I |
| **Piperacillin** | I | I | S | I | I | R | I | I | S | I | R | I | I |
| **Tetracycline**  | I | R | I | I | I | I | I | R | R | I | R | I | I |
| **Clindamycin** | R | R | R | R | R | R | R | R | R | R | R | R | R |
| **Ciprofloxacin** | R | I | S | R | R | R | S | S | R | S | S | I | R |

**Table 3: Antibiotic Susceptibility Profile of E. coli Isolates**

**Key: R= resistant, S= susceptible, I= intermediate**

**Discussion**

Milk is a good growth medium that supports the growth of several microorganisms, especially bacteria due to its high-water content, nearly neutral pH, and a variety of available essential nutrients such as calcium, magnesium, zinc and potassium (Reta and Addas, 2021). The adverse effects caused by pathogenic strains of *E. coli* have been reported in several regions around the globe and have raised great concern among researchers.

The results obtained from this study on the occurrence of *E. coli* show that the bacteriological quality of the pastoral raw milk sold around Galadimawa is substandard and could be a potential cause of bacterial infections. In Galadimawa, where ambient temperatures can be high due to urbanization, proper cooling and refrigeration are often challenging, especially, if vendors lack access to cooling facilities. This creates an ideal environment for pathogens. Higher temperatures accelerate bacterial multiplication, raising the total aerobic bacterial load, consequently compromising milk quality and safety.

The growth of *E. coli* in particular can be exacerbated in uncooled milk. Thus, temperature control especially cooling milk to below 4°C can significantly reduce bacterial proliferation and help maintain the microbiological quality of milk. Without it, there’s an increased risk of bacterial contamination, antibiotic resistance development, and potential health hazards for consumers. The contamination level recorded in this study might be due to poor hygienic practices, as most microorganisms enter the raw milk from contaminants on the outer surface of the udder, milking utensils and milkers (Okeke *et al*., 2020). Also, the quality of water used for washing utensils, the mode of packaging and methods of handling could be part of the reasons for obtaining a poor microbiological quality in these milk samples. The presence of coliform bacteria in the milk samples indicates faecal contamination and poor hygienic practices in the rearing and milking processes, and also shows that cow milk from pastoral herds can serve as a medium for the transmission of bacterial infections, as *E. coli* has been implicated in several human diseases. However, a high percentage of the herders who are the handlers of these animals in the study area are illiterate and might be ignorant of the possibility of contamination of milk through water and utensils used during milk processing and packaging.

*Escherichia coli* is one of the most common pathogens causing urinary and gastrointestinal tract infections (Ahmed *et al*., 2023). In the present study, it was observed that some strains of isolated *E. coli* produced hemolysin and exhibited beta hemolysis after the hemolysis test, indicating their ability to lyse blood cells and cause serious health concerns. This report agrees with Oladipo *et al*. (2022), who reported the presence of pathogenic strains of *E. coli* isolated from raw cow milk from different dairy farms in Ogbomosho, Oyo state, Nigeria.

Antibiotics are medicines that help fight bacterial infections by killing or inhibiting the growth of microorganisms. They are the first line of defense against microorganisms. Organisms are resistant to antibiotics due to mutation or indiscriminate use of certain antibiotics (Ahmed *et al*., 2023). Antibiotic resistance of *E. coli* has been reported worldwide, and the increasing rate of resistance is a growing concern in both developed and developing countries (Prestinaci *et al*., 2022). Here, *E. coli* resistance may be linked to mutation or the use and misuse of antibiotics in most farms to treat bacterial infections. Some isolates were susceptible to Ceftazidime, Cefuroxime, Gentamycin, Ciprofloxacin, Ofloxacin, Augmentin, Nitrofurantoin and Levofloxacin. This observation agrees with Oladipo and Omo-Adua (2023), who reported the susceptibility of *E. coli* to Ceftazidime, Cefuroxime, Gentamycin, Ciprofloxacin, Ofloxacin, Augmentin and Nitrofurantoin in dairy milk isolated from Kenya.

**Conclusion**

This study showed that the bacterial load of fresh pastoral cow milk sold around Galadimawa is high due to poor hygiene and sanitary practices by handlers. The recorded resistance of the isolated *E coli* to antibiotics is a cause for public health concern, as the impact of bacterial resistance is widespread and constitutes a serious threat to humanity.

**Disclaimer**

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

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