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Isolation, Identification and Antimicrobial Susceptibility Profilling of *Escherichia Coli* from Clinically Non-Mastitis Animals in Sokoto Metropolis

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ABSTRACT
Mastitis, which is the inflammation of the mammary gland, is among the most common and very significantly devastating potential dairy disease occurring in all dairy herds worldwide. This study investigated the prevalence of environmental pathogens specifically *Escherichia coli* in clinically non mastitis animals. A total of 200 samples from cows and goats were cultured on nutrient agar. From the result after culturing, 178 samples showed colonial growth which were then sub cultured on selective media (EMB) and characterized by Gram staining in which the prevalence was found to be 18.5% and the result of Gram staining revealed short to medium sized rod organisms staining pinkish. Representatives of positive isolates were then subjected to antimicrobial susceptibility profiling where all were found to be resistant to Amoxicillin/clavulanic acid, Ampicillin, and tetracycline but susceptible to chloramphenicol and gentamycin. The current study has shown a little decrease in the prevalence of *Escherichia coli* causing mastitis which may be because of regular treatment, and improvement of sanitary condition which must be maintained. Also, sensitivity of the test employed and the period during which the work was conducted might be some of the reasons for the low prevalence. Enhanced sanitary measures during milking and education on hygienic practices are recommended to further reduce contamination risks.

Keywords:
Escherichia coli,
Dairy cattle,
Non-mastitis,
Milk hygiene,
Sokoto, zoonosis

INTRODUCTION

Dairy cow has been called the foster mother of the human race with milk being a universal and nearly a very nutritional perfect food. Milk contains all the nutrients required by the neonate (it is rich in carbohydrate, proteins, fats, vitamins, minerals and also a rich source of enzymes and growth factors) and has thus long been recognized as perhaps nature's ultimate food (Polidori et al., 2022; Sanjulián et al., 2025; Tirfie, 2023).

In Nigeria however, milk production often does not satisfy the country's milk requirements due to a multitude of associated factors and constraints such as inadequate management, low genetic potential, nutritional insufficiency, poor reproductive performance, and various diseases in particular mastitis, which is among the most important impediments confronting the economic milk production in dairy cow (Mode et al., 2023; Wang et al., 2021).

Due to its high incidence, mastitis is one of the most common and a very significantly devastating potential dairy disease occurring in all dairy herds worldwide (Ali et al., 2024; Girma & Tamir, 2022; Morales-Ubaldo et al., 2023; Tomanić et al., 2023). The term is derived from Greek word "Mammae" or "Mastos" meaning breast and inflammation, respectively (Ansari et al., 2025). According to the National Mastitis Council's current concepts of bovine mastitis, mastitis is an inflammation of the mammary gland characterized by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissues as response to injury for the purpose of destroying and neutralizing the infectious agents and to prepare the way for healing and return to normal function (Goulart & Mellata, 2022; Sharun et al., 2021; Ansari et al., 2025; Saleem et al., 2024). Inflammation can be caused by many types of injury including infectious agents and their toxins,

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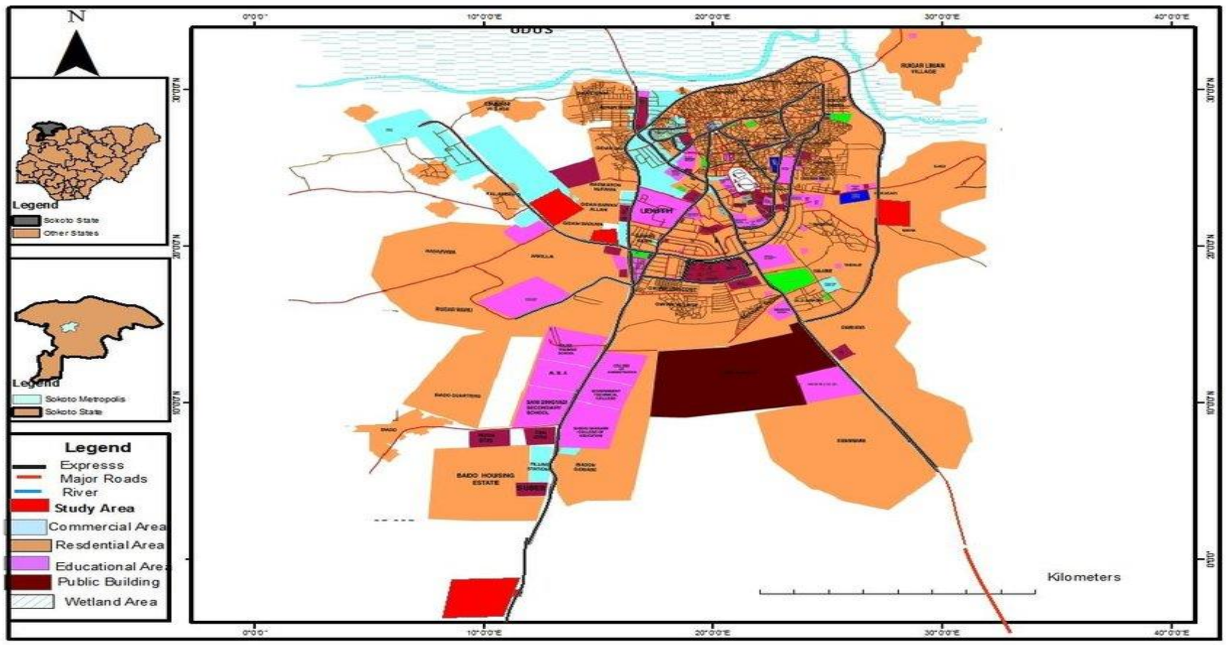
physical trauma or chemical irritants (Tomanić et al., 2023; Urrutia-Angulo et al., 2024; Andreeva et al., 2024). Bovine mastitis is the most economically important disease in dairy milk production worldwide (Duse et al., 2021; Morales-Ubaldo et al., 2023; Rifatbegović et al., 2024). This disease can have an infectious or non-infectious aetiology, and infectious mastitis is most important, frequently due to pathogens such as bacteria, viruses, mycoplasmas, yeasts, fungi and algae (Morales-Ubaldo et al., 2023; Rifatbegović et al., 2024). Fortunately, the vast majority of mastitis is of bacterial origin and just a few species account for most cases, such as *Escherichiacoli*, *Staphylococcusaureus*, *Streptococcus uberis*, *Streptococcus dysgalactiae* and *Streptococcus agalactiae* (Duse et al., 2021; Kabelitz et al., 2021; Morales-Ubaldo et al., 2023; Rifatbegović et al., 2024; Zhao et al., 2025). From previous studies on mastitis aetiology, it has been revealed that Enterobacteriaceae (mainly coliforms such as *E. coli* and *Klebsiella* spp.) are among the commonest causes of clinical mastitis, while *S. aureus*, *S. dysgalactiae* and *S. agalactiae* also account for an important proportion of clinical and subclinical cases, with the exact distribution varying by region (Belay et al., 2022; Dyson et al., 2021; Morales-Ubaldo et al., 2023; Abebe et al., 2023; Zhao et al., 2025). To give an insight into the current profile of mastitis organisms and a true reflection of herd problems and cow status, investigating the prevalence of pathogens most frequently associated with clinical and subclinical infections is therefore critical (Abed et al., 2021; Duse et al., 2021; Belay et al., 2022; Michira et al., 2023; Rifatbegović et al., 2024).

Escherichia coli causes infection and inflammation of the mammary gland in dairy cows mainly around parturition and during early lactation, with striking local and sometimes severe systemic clinical symptoms (Cheng et al., 2022; Iso-Touru et al., 2024; Noleto et al., 2023). This so-called environmental type of mastitis may affect productivity of high-producing cows in dairy herds and can occasionally result in fatalities (Cheng et al., 2022; Goulart & Mellata, 2022; Jung et al., 2024). Escherichia coli invades the udder through the teat canal where it grows and initiates a prompt inflammatory reaction, with rapidly increasing numbers of neutrophils appearing in milk and mammary tissue (Goulart & Mellata, 2022; Noleto et al., 2023; Schneider et al., 2023). This neutrophil infiltration is one of the first steps of the inflammatory reaction because these cells must prevent escape and multiplication of the pathogens (Schneider et al., 2023; Wagner et al., 2023). It is well known that bacterial, environmental or management, and cow-related factors can change susceptibility to mastitis, and these determinants are interdependent, with the relative impact of each depending on the type of pathogen (Abegewi et al., 2022; Bari et al., 2022; Cheng et al., 2022; Duse et al., 2021; Iso-Touru et al., 2024; Makolo & Suleiman, 2025). Mastitis is a common and economically important disease in dairy cattle (Morales-Ubaldo et al., 2023; Pascu et al., 2022). It remains one of the main reasons for extensive antimicrobial use in dairy farms, contributing to the emergence of antimicrobial-resistant mastitis pathogens (Sharun et al., 2021; Majumder et al., 2021; Xu et al., 2022; Camsing et al., 2024). Mastitis is also a food safety problem and among the biggest economic challenges facing dairy farms due to reduced yield, discarded milk and treatment costs (Morales-Ubaldo et al., 2023; Ali et al., 2021; Fesseha et al., 2021). Bacterial contamination of milk from affected cows renders it unfit for human consumption and facilitates the transmission of zoonotic agents through raw or improperly treated milk, underlining its public health importance (Morales-Ubaldo et al., 2023; Abed et al., 2021; Bobade et al., 2021). This study was aimed to identify Escherichia coli from fresh milk from bovine mastitis cases in Sokoto State, Nigeria, by means of morphologic examination, in line with recent work that has characterized E. coli from mastitic milk and highlighted its resistance patterns and public health relevance (Goulart & Mellata, 2022; Liu et al., 2021; Xu et al., 2022; Dahesa et al., 2025).

MATERIALS AND METHODS

Study area

The study was conducted in Sokoto metropolis, the capital of Sokoto State of Nigeria. Geographically the state is situated on latitude 12ºN and 13º58N and is 308m above the sea level (Sokoto, 2001). Sokoto state occupies an area of short grass savannah vegetation in the south and thorn in the north. It shares boundaries with Zamfara State to the east, Niger Republic to the North and Kebbi State to the west and southwest (Sokoto, 2001). The sampling locations were associated farms and selected houses within Sokoto metropolis. Sokoto metropolis is mainly made of Sokoto north and south local government areas, however some part of Dange Shuni, Wamakko Local government and Kware local government constitute part of this metropolis.



Source: Dankani (2018)

Sample Collection

A total of 200 samples were collected from 47 cows and 6 goats. The breeds of cattle comprised of Sokoto Gudali, White Fulani and Friesian, whilst that of goat was only Red Sokoto goat. The animals were physically restrained and prior to sampling, teat ends were scrubbed with 70% alcohol. Milk was expressed using hand milking technique. The initial milk stripped from each udder was discarded and the next 10 ml were collected in a sterile container. The milk samples were collected in plain sample bottles. Separate samples were chilled to 4°C and transported to the Veterinary public health laboratory of Usmanu Danfodiyo University Sokoto for processing and analysis**.**

Bacterial pre-culturing

About 1ml of raw milk was transferred into separate tubes containing 9ml of buffered peptone water and incubated at 37°C for 24 hours (Oyeleke and Manga, 2008).

Isolation and identification of *Escherichia coli*

From each pre-cultured sample, a loop full of bacteria suspension was streaked on nutrient agar plate and then incubated at 37°C for 24 hours under aerobic conditions. Each presumptive bacteria isolate was characterized based on gram staining. The isolates were subcultured on EMB media to confirm *E. coli* as described by Oyeleke and Manga (2008).

Morphological characteristics

Colonial appearance

The colonies on nutrient agar are whitish to greyish. Eosin methylene blue (EMB) agar is occasionally used in diagnostic laboratories and on this medium *E. coli* colony have a unique and characteristic metallic green sheen in accordance with Oyeleke and Manga (2008).

Cellular morphology

Escherichia coli as a Gram-negative, short to medium-sized rods (0.4–0.6 × 2–3 µm) with morphology of peritrichate arrangement of flagella was observed in accordance with Oyeleke and Manga (2008).

Antimicrobial susceptibility profiling

Isolated bacteria were tested for resistance to a panel of antibiotics using a standard Kirby-Bauer disk diffusion procedure. The antibiotics tested include chloramphenicol (30-µg disk), ciprofloxacin (10-µg disk), gentamicin (30-µg disk), tetracycline (30-µg disk), trimethoprim-sulfamethoxazole (1.25/23.75-µg disk), ampicillin (10-µg disk), amoxicillin/clavulanic acid (20/10-µg disk) and ceftriaxone (30-µg disk), according to Clinical and Laboratory Standards Institute guidelines (CLSI 2023). Intermediate isolates were classified as susceptible isolates. Resistance to “any antibiotic” included resistance to any individual antibiotic tested, while multi-drug resistance was defined as resistance to at least one antibiotic in three or more of the classes of antibiotics tested.

RESULTS AND DISCUSSION

Bacterial Culture and Isolation

The culture and isolation of the 200 milk samples across the farms yielded 37 isolates that show characteristics of green metallic sheen which is positive for *Escherichia coli* (Table 1).

Table 1: Showing a total of 200 samples from both Farms and herds which were cultured and 37 samples yielded green metallic sheen.

S/N	Species/breeds	Number of samples	Growth on EMB (*E. coli* positive)	Percentage
1	Red Sokoto goat	12	0	0
2	White Fulani	60	10	5
3	Sokoto Gudali	96	20	10
4	Friesian	32	7	3.5

Prevalence = 18.5%

Out of the 200 samples examined for bacterial isolation and culture, the overall prevalence from this study was found to be 18.5%. Sokoto Gudali has the highest prevalence of 10%, followed by White Fulani with prevalence of 5%, then Friesian with prevalence of 3.5% while the prevalence of Red Sokoto goat was found to be zero.

Gram Staining

The microscopic appearance of the smear was found to be pinkish (gram negative organism) which are short to medium sized rods.

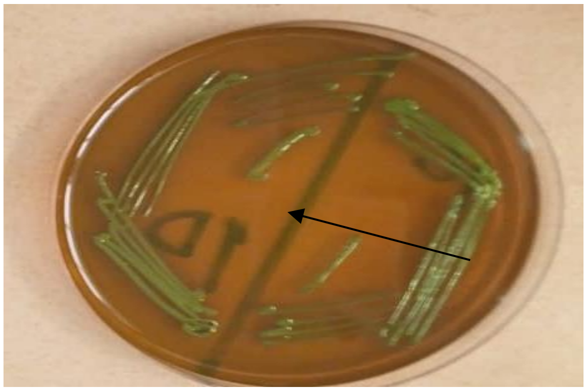


Figure 1: Showing colonies of *E. coli* displaying the characteristic green metallic sheen on EMB agar.

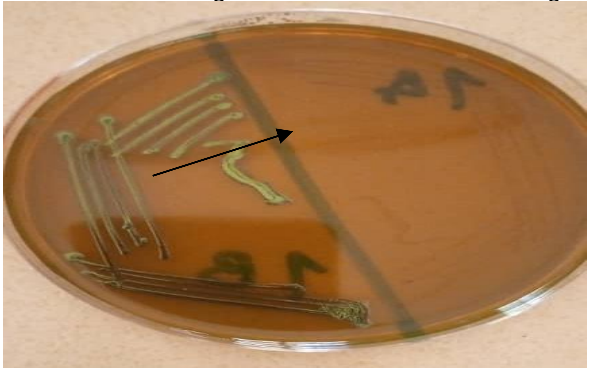


Figure 2: From the left, showing green metallic sheen while the right side is negative

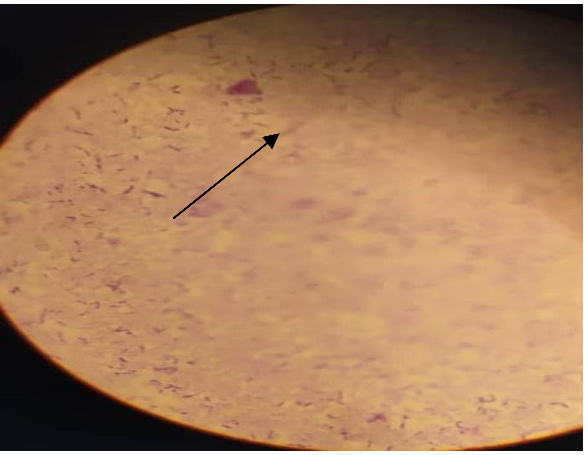


Figure 3: Showing pinkish short rod bacterial cells

Antimicrobial susceptibility profiling

Ten of the positive isolates were randomly selected as representatives and subjected to antimicrobial susceptibility profiling using Kirby-Bauer disk diffusion method. All the samples subjected were found to be resistant to Amoxicillin/clavulanic acid, Ampicillin, and tetracycline but susceptible to chloramphenicol and gentamycin (Table 2).

Table 2: Showing antimicrobials sensitivity test

Antimicrobials	Susceptible	Intermediate	Resistance
Chloramphenicol	10	0	0
Tetracycline	2	1	7
Gentamycin	9	0	1
Ceftriaxone	0	6	4
Sulphamethazone	2	3	5
Amoxicillin/clavulanic acid	2	1	7
Ampicillin	1	2	7
Ciprofloxacin	2	4	4

Figure 4: Showing zones of inhibition during antibiotic susceptibility testing

In this study, the prevalence of mastitis was 18.5%, which is relatively low compared to other similar studies reporting overall mastitis burdens of 39–54% or more and this had agreed with the finding of Abegewi et al. (2022), Kitila et al. (2021) and Mekonnen et al. (2023). Specifically, *E. coli* was responsible for about 40.9% of mastitis cases, a rate lower than what has been reported in various local and international studies, where *E. coli* typically accounts for 7–21% of mastitis isolates (Abebe et al., 2023; Belay et al., 2022; Mekonnen et al., 2024). The need to understand common mastitis and the range of microbes involved as well as the factors that contribute to the disease are essential for improving prevention strategies and guiding treatment decisions as reported by Abegewi et al. (2022), Abebe et al. (2023) and Girma & Tamir (2022). Yakubu et al. (2018) found E. coli prevalence in animal products ranging from 9% to 50% depending on the product type, and meta-analyses from Africa have shown even higher rates of coliform-related mastitis (Khasapane et al., 2022; Tora et al., 2022). These differences likely stem from variations in farm management, environmental conditions, breed susceptibility, and diagnostic methods (Girma & Tamir, 2022; Klaas & Zadoks, 2018; Tora et al., 2022).

The notably low prevalence in this study may be explained by effective mastitis control programs, good environmental conditions, and high hygiene standards in the study areas, as well as the sensitivity of the diagnostic methods used (Abegewi et al., 2022; Belay et al., 2022). The authors recommend advancing to molecular techniques to identify different *E. coli* strains, in line with recent work emphasizing molecular characterization of mastitis and milk-borne *E. coli* (Shoaib et al., 2023; Mangroliya et al., 2025). The study found a low rate of subclinical mastitis caused by environmental pathogens like *E. coli*, with no *E. coli* isolates found in goat milk thereby supporting the idea that cattle are the main carriers of this pathogen in dairy settings (Sarba et al., 2023; Makolo & Suleiman, 2025). *E. coli* often enters the udder through fecal contamination during milking or via dirty bedding and water (Abegewi et al., 2022; Belay et al., 2022; Khasapane et al., 2022). Hand-milking without proper udder hygiene is a major risk factor, highlighting the need for better milking practices (Abebe et al., 2023; Mbindyo et al., 2020; Makolo & Suleiman, 2025).

Breed-specific differences were noted, with a higher prevalence in Sokoto Gudali cattle, possibly due to genetic or management-related factors (Abegewi et al., 2022; Girma & Tamir, 2022; Tora et al., 2022). The overall low prevalence may also reflect increased farmer awareness of mastitis control measures, such as regular cleaning of udders and equipment, and timely veterinary care (Abegewi et al., 2022; Belay et al., 2022; Mbindyo et al., 2020). Importantly, even subclinical *E. coli* carriage poses a public health risk, as it can be transmitted through raw milk or cross-contamination during processing (Sarba et al., 2023; Elbastawesy et al., 2025; Drugea et al., 2025). Recent research points to the rise of antimicrobial-resistant strains among commensal *E. coli* in dairy environments, stressing the need for cautious antibiotic use (Mwasinga et al., 2023; Shoaib et al., 2023; Sobur et al., 2019; Weber et al., 2021).

The detection of drug-resistant *Escherichia coli* in clinically healthy, non-mastitis cows is of considerable concern, as it indicates the presence of covert reservoirs of antimicrobial resistance in apparently normal animals (Liu et al., 2021; Majumder et al., 2021; Mim et al., 2024). Such animals may continuously shed resistant bacteria into milk and the surrounding environment without exhibiting clinical signs, thereby increasing the likelihood of contaminating raw milk commonly consumed in the area and predisposing humans to infections that are difficult to treat (Mwasinga et al., 2023; Nahar et al., 2023; Widodo et al., 2022). This observation suggests that antimicrobial resistance is not confined to overtly diseased animals, complicating both detection and control, and points to the probable misuse or overuse of antibiotics in livestock even in the absence of clinical illness (Hoteit et al., 2022; Mahmoudi et al., 2025; Widianingrum et al., 2024).

These findings raise important public health concerns regarding unrecognized foodborne transmission and underscore the need for routine surveillance that includes healthy animals, rather than focusing solely on clinically affected cases (Drugea et al., 2025; Khasapane et al., 2025; Vijay et al., 2025). From an economic perspective, the circulation of resistant *E. coli* in the dairy value chain may undermine milk safety, erode consumer confidence, and negatively impact the marketability of dairy products (Mwasinga et al., 2023; Widodo et al., 2022). Framed within a One Health paradigm, this situation illustrates the interconnected transmission of resistance among animals, humans, and the environment, and highlights the urgent need to strengthen surveillance systems, promote rational antimicrobial use, and improve dairy hygiene practices in the region (Arbab et al., 2025; Dushayeva, 2025; Zalewska et al., 2024).

CONCLUSION

In conclusion, subclinical mastitis remains a highly prevalent and economically significant problem in dairy production, especially in resource-poor settings such as seen Nigeria. Its largely hidden nature allows infections to persist in herds, leading to production losses, increased treatment costs, and potential public health risks. The detection of drug-resistant *E. coli* in apparently healthy animals during this study has brought to light the imminent dangers such as zoonotic transmission and spread of antimicrobial resistance among unsuspecting consumers of raw or unpasteurized milk. Addressing this disease therefore requires sustained attention at both farm and policy levels, with emphasis on early detection, effective treatment, and long-term prevention strategies supported by research and stakeholder engagement.

This study therefore, recommends routine screening of animals to detect subclinical mastitis early, followed by prompt treatment and regular follow-up of chronic cases. Good flock and pen hygiene, proper milking practices, and the use of milk screening tests before milk is collected from each animal should be standard procedures. Prophylactic therapy, including appropriate mastitis treatments and dry-cow therapy, should be implemented to reduce disease burden.

Farmers need better awareness of the economic and health implications of subclinical mastitis, and poor milking techniques, dirty housing, and inadequate personal hygiene should be avoided to limit cross-contamination and new infections. Regular use of effective disinfectants and available vaccines in endemic areas is also advised.

Further comprehensive research on the prevalence and risk factors of mastitis is required to guide targeted control programmes. Finally, coordinated efforts by international and national public health bodies, universities, and research institutions are essential to minimize the impact of mastitis on animal welfare, human health, and global food safety.

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