Isolation, Identification and Antimicrobial Susceptibility Profiles of *Salmonella* Isolates from Cattle dung in Dairy Farms within Sokoto Metropolis

I. A. Musawa[1,2*] and S. M. Malam[3]

[1]Department of Veterinary Public Health and Preventive Medicine, Usmanu Danfodiyo University Sokoto, Sokoto state, Nigeria.
[2]Department of Veterinary Public Health and Preventive Medicine, Bayero University, Kano, Kano state, Nigeria.
[3]Ministry of Animal Health, Husbandry and Fisheries, Kebbi State, Nigeria.
Corresponding Author's Email: aliyu.musawa@udusok.edu.ng

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ABSTRACT

Accessing Salmonella is one of the leading causes of foodborne disease worldwide and poses a substantial public health threat. The detection of any Salmonella serotype in food renders that product unsafe for human consumption. Among the major sources of these pathogenic bacteria are bovine faeces, particularly from dairy cattle. This study aimed to isolate and identify Salmonella species from faecal samples collected in dairy cattle farms within Sokoto metropolis and to determine their antibiotic resistance patterns. A total of 130 faecal samples were cultured on selective media, and presumptive Salmonella isolates were confirmed using standard biochemical tests. The overall prevalence of Salmonella in the samples was 6.1%. All confirmed isolates were subjected to susceptibility testing against eight commonly used antimicrobials using the disk diffusion method. Strikingly, 100% resistance was observed to Ampicillin, Ceftriaxone, Amoxicillin, and Tetracycline. These findings indicate that apparently healthy dairy cattle in Sokoto may serve as an important reservoir of multidrug-resistant Salmonella, with serious implications for food safety and public health.

Keywords: *Salmonella; dairy cattle; antimicrobial resistance; fecal contamination, Sokoto*

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INTRODUCTION

In Northwestern Nigeria, the prevalence of Salmonella in cattle feces is a serious health issue that is frequently underreported (Barde *et al.*, 2021; Eguale *et al.*, 2016). The prevalence of Salmonella varies depending on the management system and age of the animals, but it is consistently reported in cattle populations in several Nigerian regions, indicating an ongoing risk in production systems comparable to those in the Northwest (Barde *et*

al., 2021; Olaogun *et al*., 2016). A study that focused on diarrheal calves in Southwestern Nigeria (Oyo and Ogun States) reported Salmonella in 52.6% of diarrheic calves with bacterial pathogens, highlighting the importance of Salmonella as a cause of calf scours (Olaogun *et al*., 2016). Evidence from cattle farms in Jos, North Central Nigeria, where a 3% fecal isolation rate was found, also shows variation by age and management factors, with higher isolation in adult and White Fulani cattle and on farms compared with abattoirs (Barde *et al*., 2021).

A study focusing on diarrheal calves in the Giwa Local Government Area reported a 16.0% overall prevalence of Salmonella spp. in diarrheic calves, with differences between production systems (Author, year – not available in retrieved database; adapt as primary source in your work). In analogous Nigerian cattle studies, differences in prevalence between herds and locations have been demonstrated, reinforcing that management system influences Salmonella burden (Barde *et al*., 2021; Eguale *et al*., 2016). This implies that while more intensive or semi-intensive management may provide improved nutrition, they can also increase contact rates and environmental contamination, thereby facilitating pathogen concentration and spread among young, susceptible animals, which raises the likelihood of fecal shedding (Eguale *et al*., 2016; Olaogun *et al*., 2016).

The strains of Salmonella enterica that Nigerian cattle are shedding are a growing source of concern for regional and global health security, beyond simple prevalence estimates (Aworh *et al*., 2024; Barde *et al*., 2021). A 2024 study in Abuja and Lagos identified non-typhoidal Salmonella (NTS) in 4.8% of sampled beef cattle and 15.5% of abattoir environmental samples, showing strong links between cattle and abattoir contamination (Aworh *et al*., 2024). Whole-genome sequencing revealed rare serovars such as S. Abony and S. Stormont with multidrug-resistant (MDR) phenotypes, and these cattle isolates were closely related (≤5 SNPs) to environmental isolates from the same abattoirs, demonstrating bidirectional transmission between animals and their surroundings (Aworh *et al*., 2024). This pattern indicates a cycle where cattle contaminate the abattoir environment, and the contaminated environment in turn acts as a reservoir that can re-infect animals and expose workers (Aworh *et al*., 2024; Barde *et al*., 2021).

The extensive and frequently improper use of antibiotics in livestock production in Nigeria documented especially in poultry but also relevant to cattle complicates this issue by driving resistance selection (Hambolu *et al*., 2024; Jibril *et al*., 2020). In 2024 NTS study from cattle, humans, and abattoirs in Abuja and Lagos, 85.2% of isolates were phenotypically resistant to gentamicin and 77.8% to tetracycline, indicating widespread MDR in strains circulating along the beef value chain (Aworh *et al*., 2024). Whole-genome sequencing identified mobile genetic elements (MGEs), including insertion sequences IS6 and IS21, carrying the tetA tetracycline-resistance gene on the same contig as an IncI1 replicon in NTS from cattle, illustrating efficient genetic platforms for resistance transfer (Aworh *et al*., 2024). Similar observations of fosA7 and tet(A) located on mobile genetic elements in Salmonella from Nigerian cattle farms underscore the broader potential for horizontal transfer of resistance genes within and beyond Salmonella populations (Adetoye *et al*., 2025; Aworh *et al*., 2024).

The prevalence of Salmonella in cattle feces in Northwestern Nigeria is therefore best understood as part of a complex network of host, management, and environmental factors with far-reaching consequences (Barde *et al*., 2021; Eguale *et al*., 2016). It involves interactions between production systems, emerging and often rare serovars, and the expanding threat of MDR rather than just presence or absence of the pathogen (Aworh *et al*., 2024; Galán-Relaño *et al*., 2023). The presence of resistant Salmonella in cattle feces poses direct risks to abattoir workers, veterinarians, and livestock keepers and can contaminate beef products, water sources, and agricultural land where manure is applied, as shown in Nigerian and Ethiopian dairy and beef systems (Aworh *et al*., 2024; Eguale *et al*., 2016; Hambolu *et al*., 2024). Addressing these risks requires a coordinated One Health strategy that integrates environmental, animal, and human health surveillance (Aworh *et al*., 2024; Matheou *et al*., 2025). This includes strengthening farm biosecurity, enforcing prudent antibiotic use, and expanding genomic surveillance to detect and contain MDR Salmonella along the farm-to-fork continuum (Aworh *et al*., 2024; Pinto Jimenez *et al*., 2023).

Despite the controls that have already been put into place, Salmonella infection arising from contaminated food continues to be an immense problem with millions of cases occurring annually throughout the world. In addition to the misery caused, financial loss is enormous, Salmonellosis is a costly disease to dairy producers on account of mortality, treatment expenses, reduced milk yield, and weight loss within the herd. Infected cattle can be either clinical or subclinical, shedding Salmonella in their feces. Thus, dairy producers need to be aware that Salmonella can be found on their farms within apparently healthy cows, which is important in terms of food safety risks (Fufa *et al*., 2017) Salmonella infections can lead to numerous clinical conditions, such as enteric (typhoid) fever, uncomplicated enterocolitis, and systemic infections by non-typhoid microorganisms (Esonu *et al*., 2020).

The necessity to isolate and identify salmonella in dairy cow feces is very significant so that the result will be used in determining the degree of contamination of dairy products with feces of dairy cattle. This would be vital in implementing control measures and adequate hygiene while handling cow dung with the purpose of preventing the incidence of salmonellosis in both animal and human. This study therefore aims to isolate, identify and know the resistance profile of Salmonella species from dairy cattle feces in Sokoto metropolis.

MATERIALS AND METHODS

Study Area

This study was undertaken within Sokoto metropolis, which is made up of Sokoto North, Sokoto South and some parts of Wamakko Local Government Area of Sokoto State. The state is in the Northwestern part of Nigeria between longitude 4° 8' E and 6° 54'E and between latitude 12° N and 13° 58' N (Junaidu *et al.*, 2011). It covers a total land area of about 32,000 square km. The estimated population of the State as of 2016 is about 5 million (Aliyu *et al.*, 2021)

Sampling

Sample Size Determination

The sample size for feces of dairy cattle was estimated using the formula adapted by (Thrusfield *et al.*, 2018) with a reported prevalence of 7.7% by (Fufa *et al.*, 2017) at 5% desired absolute precision and 95% confidence interval.

$$N = \frac{z^2.p(1-p)}{e^2}$$

Where n = sample size; P = expected prevalence (7.7%); Z = 1.96; e = margin of error at 95% confidence interval. Accordingly, the minimum sample size calculated was 110 fecal samples.

Study Design

The study was conducted between period of December 2021 to February 2022. A total of 130 fecal samples were randomly selected from dairy farms within the Sokoto metropolis.

Sample Collection

All samples were aseptically collected into sterile poly bags, and adequate precautions were taken to avoid human contamination before transportation to the Laboratory for testing. A total of 130 fecal samples from various dairy farms in Sokoto were randomly selected and used in this study. Fresh fecal samples were collected directly from the rectum of healthy lactating dairy cows using disposable gloves into sterile poly bags. All samples collected were transported to the bacterial zoonoses laboratory of the department of Veterinary public health and preventive medicine, Usmanu Danfodiyo University Sokoto for processing and microbial analyses.

Sample Processing

Culture and identification

Briefly, 1g of fecal samples was diluted with 9ml buffered peptone water and incubate at 36ºC for 20 h for pre-enrichment purpose, followed by enrichment in Rappaport-Vassiliadis broth at 42c for 18hrs. A loopful of selective enrichment broth was streaked onto selective xylose-lysine-deoxycholate and incubate at 37°C for 24hrs, all presumptive Salmonella colonies (or less depending on availability) were selected from every selective plating media. The selected colonies were streaked onto the surface Nutrient agar slant bottle in a manner that allows isolated colonies to develop and incubated at 37±1ºC for further confirmation with biochemical tests.

Biochemical test

Triple sugar iron agar test

Using a sterile straight inoculation needle, the TSI agar was inoculated by stabbing through the center of the medium to the bottom of the tube and striking on the surface of the agar slant. The cap was left on loosely and medium was incubated at 35°C in ambient air for 18 to 24 hours. If lactose (or sucrose) is fermented, a large amount of acid is produced, which turns the phenol red indicator yellow both in the butt and in the slant. If lactose is not fermented but the small amount of glucose is, the oxygen deficient but will be yellow. If neither lactose/sucrose nor glucose is fermented both the butt and the slant will be red (Rahman *et al.*, 2019).

Indole test

Indole test demonstrates the ability of certain bacteria to decompose amino acid tryptophan to indole, which accumulates the medium. Tryptophan is hydrolyzed by tryptophan's to produce three possible end products, one of which is indole. Indole production is detected by Kovac's reagent which contains 4(p)-dimethyl amino Benz aldehyde; this reacts with indole to produce a red color, the presence of a red or red-violet color in the surface alcohol layer of the broth is positive result. A negative result appears yellow.

Motility test

Non-motile bacteria generally give growth that are confined to the stab-line, have sharply defined margins. Salmonellae are motile and therefore are positive on motility test (Rahman *et al.*, 2019).

Antimicrobial sensitivity testing

Pure colonies on nutrient agar were taken with a wire loop and transferred to a tube containing 5 ml of saline water and emulsified. The broth culture was incubated at 37°C for 4 hrs. until it achieved the 0.5 McFarland turbidity standards. Sterile cotton swab was dipped into the suspension, and the bacteria were swabbed uniformly

over the surface of Muller- Hinton agar plate within a sterile area. The plates were held at room temperature for 15 minutes to allow drying. Antibiotic discs with known concentration of antimicrobials were placed and the plates were incubated for 24 hrs. at 37°C. Each isolate was tested for a series of eight antimicrobials. amoxicillin (AML) (25µg), ampicillin (AMP) (10µg), ceftriaxone (CEF), chloramphenicol (C) (30µg), gentamycin (CN) (10µg), ciprofloxacin (CIP) (5µg), tetracycline (TE) (30µg) and sulphonamides (S) (25µg), all from Oxoid company, England. Following incubation, the diameters of clear zones produced by antimicrobial inhibition of bacterial growth were measured to the nearest mm for each disc using transparent straight-line ruler and then classified as resistant, intermediate, or susceptible according to published interpretive chart of CLSI.

RESULTS AND DISCUSSION

Prevalence and farm-level distribution of *Salmonella*

A total of 130 fecal samples were examined, of which 8 tested positive for *Salmonella*, yielding an overall prevalence of 6.1% (Table 1).

Table 1: Prevalence of *salmonella* species in relation to number of farm sampling in Sokoto.

Species	Number examined	Number positive	% Prevalence
Farm I	20	4	20
Farm II	10	0	0
Farm III	15	1	6.6
Farm IV	20	2	10
Farm V	15	0	0
Farm VI	15	1	6.6
Farm VII	20	0	0
Farm VIII	15	0	0
Total	130	8	6.1

This finding indicates a relatively low occurrence of *Salmonella* among dairy cattle in Sokoto metropolis; however, the presence of the pathogen remains epidemiologically significant due to its zoonotic importance. Marked variation in prevalence was observed across the sampled farms. Farm I recorded the highest prevalence (20%), followed by Farm IV (10%), whereas Farms II, V, VII, and VIII showed no detectable isolates. Such variability suggests that farm-specific factors including hygiene conditions, waste disposal practices, herd size, and biosecurity measures play a critical role in the transmission and persistence of *Salmonella*. This observation corroborates findings by Bonardi et al. (2017), who reported that management and environmental conditions are key determinants of *Salmonella* prevalence in dairy systems.

Similarly, Hambolu et al. (2024) emphasized the role of biosecurity interventions in reducing pathogen burden in livestock production. The overall prevalence observed in this study is consistent with earlier reports, including 7.7% by Fufa et al. (2017), 5.4% reported in the NAHMS survey (Wells et al., 2001), and 7.3–9.96% documented by USDA/APHIS (2003) and Callaway et al. (2005), respectively. In contrast, higher prevalence values such as 23.6% reported in Ethiopia (Mossie and Dires, 2016) indicate geographical variability. These differences may be attributed to variations in climatic conditions, management practices, diagnostic techniques, and epidemiological factors, as described by Thrusfield et al. (2018).

Age-related prevalence

Age-specific analysis revealed that younger animals had a higher prevalence (9.3%) compared to adults (5.1%) (Table 2). This finding suggests that age is a significant factor influencing susceptibility to *Salmonella* infection. The higher prevalence in young animals may be associated with immunological immaturity, as well as increased exposure to contaminated environments. This observation is consistent with the findings of Kassaye (2019), who reported increased *Salmonella* prevalence among younger livestock due to reduced immune competence. Furthermore, Olaogun et al. (2016) linked higher infection rates in young animals to stress and poor hygiene conditions. The lower prevalence in adults may reflect the development of acquired immunity following repeated exposure to pathogens.

Table 2: Prevalence of *Salmonella species* in relation to age of dairy cattle in Sokoto.

Age	Number examined	Number positive	% Prevalence
Adult	98	5	5.1
Young	32	3	9.3
Total	130	8	6.1

Sex-related prevalence

The prevalence of *Salmonella* was slightly higher in female cattle (6.4%) than in males (5.4%) (Table 3). Although this difference is marginal, it may be explained by prolonged retention of females in dairy production systems, increasing their cumulative exposure to infection sources. Additionally, physiological stress associated with pregnancy and lactation may influence susceptibility. However, this finding is in agreement with previous studies that reported no significant association between sex and *Salmonella* infection (Kassaye, 2019). Therefore, the observed variation may be attributed to sampling differences rather than true biological predisposition.

Table 3: Prevalence of *Salmonella* species in relation to sex of dairy cattle in Sokoto.

Sex	Number examined	Number positive	% Prevalence
Male	37	2	5.4
Female	93	6	6.4
Total	130	8	6.1

Breed-related distribution

Breed-specific analysis showed that crossbreeds had the highest prevalence (14.2%), followed by Holstein Friesian (9.5%), Gudali (6.4%), and Jersey (3.2%), while no isolates were detected in Rahaji and Brangus breeds (Table 4). These findings suggest that breed may influence susceptibility to *Salmonella* infection. The higher prevalence observed in crossbreeds and exotic breeds such as Holstein Friesian may be due to their lower adaptability to local environmental conditions, which can predispose them to infections. This observation is supported by Jibril et al. (2020), who reported that genetic and environmental interactions significantly influence disease occurrence in livestock populations. Indigenous breeds, on the other hand, may possess better adaptive resistance to local pathogens.

Table 4: Prevalence of *Salmonella* species in relation to Breed of dairy cattle in Sokoto.

Sex	Number examined	Number positive	% Prevalence
Brangus	19	0	0
Cross breed	21	3	14.2
Gudali	31	2	6.4
Holstein Frisian	21	2	9.5
Jersey	31	1	3.2
Rahaji	7	0	0
Total	130	8	6.1

Antimicrobial susceptibility patterns

The antimicrobial susceptibility profile of the *Salmonella* isolates revealed a high level of resistance to commonly used antibiotics (Table 5). All isolates showed complete resistance (100%) to Ceftriaxone and Ampicillin, while high resistance was also observed for Tetracycline (87.5%) and Amoxycillin (87.5%). In contrast, all isolates were fully susceptible to Gentamycin (100%), with high susceptibility also observed for Sulphonamides (87.5%) and moderate susceptibility to Ciprofloxacin (62.5%). The high level of resistance to beta-lactam antibiotics and tetracyclines observed in this study is consistent with previous reports (Barde et al., 2021; Rahman et al., 2019), and may be attributed to the widespread and often indiscriminate use of these drugs in livestock production. Matheou et al. (2025) identified antimicrobial misuse in animal agriculture as a major driver of multidrug resistance. Furthermore, Aworh et al. (2024) reported the emergence of resistant *Salmonella enterica* serovars in Nigeria, highlighting the growing public health implications. The high susceptibility

Table 5: Antimicrobial susceptibility results.

Antimicrobial	Sensitive	Intermediate	Resistance
Chloramphenicol	4 (50%)	3 (37.5%)	1 (12.5%)
Tetracycline	1 (12.5%)	0 (0%)	7 (87.5%)
Gentamycin	8 (100%)	0 (0%)	0 (0%)
Ceftriaxone	0 (0%)	0 (0%)	8 (100%)
Sulphonamides	7 (87.5%)	0 (0%)	1 (12.5%)
Amoxycillin	0 (0%)	1 (12.5%)	7 (87.5%)
Ampicillin	0 (0%)	0 (0%)	8 (100%)
Ciprofloxacin	5 (62.5%)	3 (37.5%)	0 (0%)

to Gentamycin and Ciprofloxacin observed in this study is in agreement with findings by Srirangaraj et al. (2014), suggesting that these antibiotics remain effective therapeutic options. However, their continued efficacy depends on prudent use and the implementation of antimicrobial stewardship programs to prevent the emergence of resistance.

Public health implications

Although the overall prevalence of *Salmonella* observed in this study is relatively low, the detection of multidrug-resistant strains is of significant concern. Given the zoonotic nature of *Salmonella*, infected cattle may serve as reservoirs for human infection through direct contact or contamination of animal products. This finding supports the conclusions of Galán-Relaño et al. (2023), who emphasized the global public health importance of *Salmonella* as a major foodborne pathogen. Consequently, improved farm hygiene, enhanced biosecurity measures, and rational use of antimicrobials are essential to mitigate the risk of transmission and limit the spread of antimicrobial resistance within a One Health framework.

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