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Molecular Characterization and Antimicrobial Susceptibility Pattern of Sorbitol and Non-Sorbitol Fermenting *Escherichia Coli* Isolated from Unpasteurized Milk in Ohimini LGA, Benue State, Nigeria

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ABSTRACT

Background: The presence of pathogenic *Escherichia coli* in unpasteurized milk increases the risk of infection. This study investigated the prevalence, extended-spectrum beta-lactamase (ESBL) indicators, and antibiotic susceptibility pattern of pathogenic *E. coli* in unpasteurized milk in Ohimini Local Government Area (LGA) of Benue State.

Materials & Methods: In this study, 100 milk samples were collected from Ohimini LGA, and *E. coli* isolates were identified through various methods including morphological, biochemical, serological, and PCR (polymerase chain reaction) tests. ESBL production was screened using the double-disc diffusion method, and β-lactamase genes (bla_{TEM}, bla_{SHV}, bla_{CTX-M}) were detected through simplex PCR. Antibiotic susceptibility was assessed against multiple antibiotics using the Kirby–Bauer disc diffusion method.

Findings: About 13% of the samples tested positive for *E. coli*, with four (30.8%) and nine (69.2%) cases classified as non-sorbitol fermenting (NSF) and sorbitol fermenting (SF) isolates, respectively. All isolates were resistant to gentamicin. SF isolates showed significant resistance to ceftazidime (n=6, 66.7%), aztreonam (n=4, 44.4%), ciprofloxacin (n =4, 44.4) and amoxicillin–clavulanate (n=4, 44.4%) but were less resistant to imipenem (n=1, 11%) and other β-lactams. In contrast, NSF isolates exhibited higher resistance rates across multiple agents, with three (75%) isolates showing resistance to aztreonam, ceftazidime, amoxicillin-clavulanate, cefotaxime, and two (50%) isolates showing resistance to cefepime, ciprofloxacin, and meropenem. All NSF isolates were multidrug-resistant and tested positive for at least one ESBL gene (bla_{TEM}, bla_{SHV}, or bla_{CTX-M}) when analyzed via PCR.

Conclusion: Multidrug-resistant *E. coli* with ESBL genes detected in unpasteurized milk samples in Ohimini LGA emphasizes the necessity of mandatory pasteurization, enhanced dairy hygiene, and stricter veterinary antibiotic regulations to protect public health.

Keywords: *Escherichia coli*, Milk, Antimicrobial susceptibility tests, Drug resistance, Bacterial, Beta-lactamases, Polymerase chain reaction

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Introduction

Escherichia coli is a Gram-negative, rod-shaped, facultative, anaerobic bacterium that is frequently found in the lower intestines of both humans and animals. When this bacterium is found in food, it is a serious public health concern [1, 32, 33].

Sorbitol fermentation alone does not determine the virulence of *E. coli*. Although some non-sorbitol fermenting isolates are non-pathogenic, *E. coli* O157:H7, traditionally known as non-sorbitol fermenter, is pathogenic and significantly impacts public health. On the other hand, several *E. coli* strains, including sorbitol-fermenting strains and diarrheagenic pathotypes such as enterotoxigenic *E. coli* (ETEC) and enteropathogenic *E. coli* (EPEC), which are recognized as sorbitol fermenters, are pathogenic [2, 34]. *E. coli* O157:H7, an enterohaemorrhagic serotype, significantly impacts public health due to its association with severe diarrhoea outbreaks [3, 26, 27, 31].

Dairy milk is a significant dietary product consumed by a large portion of the Nigerian population and the people of Ohimini Local Government Area (LGA) because of its nutritional content. A major portion of the current dairy milk produced in Ohimini LGA is produced by nomadic pastoralists who lack the necessary knowledge on safe food handling practices, sanitation, and hygiene, as well as access to equipment that allows for proper preservation. This milk is infrequently pasteurized by farmers and consumers, increasing the risk of contamination and spoilage [4, 35, 37, 38].

The possibility of pathogenic microorganisms growing in contaminated processed and unprocessed dairy milk has been established, even under the highest levels of hygiene [5, 6, 14]. Minor diarrhoea, hemorrhagic colitis (HC), thrombotic purpura with thrombocytopenics (TTP), hemorrhagic uraemic syndrome (HUS), and death have all been associated with faecal contamination of dairy milk and other food items with pathogenic microbes such as *E. coli* [7, 29]. *E. coli* comprises several diarrhoeagenic pathovars, including enterohemorrhagic *E. coli* (EHEC), diffusively adherent *E. coli* (DAEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), and enteropathogenic *E. coli* (EPEC) [8, 10, 12]. Residents of Ohimini LGA frequently consume both pasteurized and unpasteurized milk, while raw milk is the typical means of EHEC transmission from animals to humans. There is limited molecular-level data on both sorbitol fermenting (SF) and non-sorbitol fermenting (NSF) *E. coli* in dairy milk in Ohimini LGA of Benue State, especially regarding their extended-spectrum beta-lactamase (ESBL) gene profiles.

EHEC and other *Enterobacteriaceae* have been the subject of numerous investigations in various regions of Nigeria [9, 16, 38, 39], while there is no information on the prevalence, antibiotic susceptibility patterns, and molecular characteristics of *E. coli* isolates from unpasteurized milk produced and sold in Ohimini.

Objectives: The current study on the molecular characterisation and antibiotic susceptibility patterns of *E. coli* strains isolated from milk samples in Ohimini LGA is critical, as information on the prevalence of pathogenic *E. coli* will provide baseline data that will also be important for public health planning and regulatory policy decisions.

Materials and Methods

Study area: This study was performed in Ohimini Local Government Area (LGA) of Benue State, Nigeria, located in Benue South Senatorial District (Figure 1). LGA is located at a latitude of 7° 44' 1.5" north of the equator and a longitude of 8° 31' 17.00" east of the Greenwich Meridian. Ohimini borders Otukpo, Okpokwu, and other nearby local government areas. The region comprises a limited land area characterized mainly by rural inhabitants, the majority of whom depend significantly on agriculture for their livelihood and income. Ecologically, Ohimini is located in the Guinea Savannah belt, distinguished by a mosaic of woodland and grassland flora, pronounced wet and dry seasons, and a moderate annual precipitation pattern. The National Population Commission estimates that the LGA has a population of over 100,000 residents, spread among many wards and villages. The rural characteristics of this community, along with the prevalent livestock rearing, especially cattle husbandry, establish an environment conducive to raw milk intake and potential zoonotic disease transmission, warranting its inclusion in this study [40].

Sample collection: Using the Thrusfield formula (2018), a sample size of 100 unpasteurized milk samples was calculated, assuming a 50% expected prevalence, a 95% confidence level, and a 5% margin of error. Considering an estimated population of about 135 milk sources in the study area, the finite population correction led to a minimum required sample size of 100 cases.

Thus,100 unpasteurized raw milk samples were randomly collected from four primary sources: Idekpa market, Onyagede market, nomadic pastoralist cattle camps, and smallholder dairy farms. To prevent contamination, samples were aseptically collected directly from vendor containers or milking buckets and transferred into sterile screw-capped test tubes. To preserve the integrity of the microorganisms, all samples were promptly transported in an insulated, ice-packed container to the Microbiology Laboratory at Joseph Sarwuan Tarka University, Makurdi, Benue State. Upon collection, the samples were subjected to bacteriological analysis within six hours.

Culture and isolation of *E. coli:* Milk samples were mixed by gentle inversion and vortexed for 10 s prior to processing. For direct culture, 100 µL of each raw milk sample was spread-plated (using a sterile pipette) onto MacConkey agar (HiMedia, Mumbai, India) and sorbitol–MacConkey agar (HiMedia, Mumbai, India) plates using a sterile bent glass spreader; the plates were then incubated aerobically at 37 °C for 24–18 hrs. In addition, to increase recovery from samples with low bacterial load, 1.0 mL of each milk sample was added to 9.0 mL of buffered peptone water (BPW) for enrichment and incubated at 37 °C for 8–6 hrs; 100 µL of the enriched culture was subsequently spread-plated onto the same agars and incubated as above. After incubation, presumptive *E. coli* colonies (pink lactose-fermenting colonies on MacConkey medium; colorless colonies on sorbitol-MacConkey medium indicating no sorbitol fermentation) were selected based on colony morphology. Up to three representative colonies per plate (if present) were picked and sub-cultured onto nutrient agar (Merck, Darmstadt, Germany) for purification. Pure isolates were stored on nutrient agar slants at 4 °C for short-term storage and in 20% glycerol at −80 °C for long-term storage. All plating steps were done using sterile technique in a Class II biosafety cabinet. Positive and negative laboratory controls (sterile blank milk and *E. coli* ATCC 25922) were included periodically to monitor culture performance. Gram staining was performed on heat-fixed smears of pure colonies using crystal violet, Gram iodine, ace-

tone/ethanol decolorizer, and safranin (all reagents: Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). Microscopic examination under oil immersion conditions revealed short, rod-shaped, Gram-negative bacteria typical of E. coli. To distinguish sorbitol-fermenting (SF) from non-sorbitol-fermenting (NSF) E. coli, isolates were sub-cultured onto sorbitol-MacConkey agar (HiMedia, Mumbai, India) and incubated at 37 °C for 24 hrs. Pink colonies indicated sorbitol fermentation (SF), whereas colourless colonies represented NSF strains. Pure isolates from each category were further sub-cultured in tryptone soya broth (Merck, Darmstadt, Germany) and incubated for subsequent serotyping, antimicrobial susceptibility testing, and molecular analyses [2, 11, 13].

Serological identification of *E. coli* O157:H7: Serological detection of pathogenic *E. coli* O157:H7 was performed using a latex agglutination test kit (Oxoid, Basingstoke, United Kingdom) following the manufacturer's instructions. Pure NSF colonies were emulsified in sterile saline, mixed with the latex reagent on a reaction card, and observed for agglutination within one minute. Visible clumping indicated a positive reaction for *E. coli* O157:H7 [41].

Biochemical identification of isolates: Suspected colonies were subjected to a series of standard biochemical tests following conventional microbiological protocols to confirm *E. coli* identity. The tests included:

a. Indole test: The test was performed using SIM medium (HiMedia, Mumbai, India) along with Kovács reagent (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). A distinct red ring after reagent addition indicated positive indole production.

b. Methyl red and Voges-Proskauer (MR-VP) tests: Buffered glucose broth (Merck, Darmstadt, Germany) was inoculated and incubated. A red color after adding methyl red indicated a positive MR test; VP was expected to be negative for *E. coli*.

c. Citrate test: Simmons citrate agar slants (HiMedia, Mumbai, India) were inoculated and incubated; a color change from green to blue denoted citrate utilization.

d. Catalase test: This test was performed by adding 3% hydrogen peroxide (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) to bacterial growth; effervescence indicated catalase activity.

e. Oxidase test: The test was conducted using freshly prepared oxidase reagent (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany); the absence of purple color indicated oxidase-negative organisms.

f. Urease test: Urease agar slants (HiMedia, Mumbai, India) were used; a colour change indicating ammonia production denoted urease activity.

Biochemical reactions were read and interpreted according to standard microbiological keys [44]. Presumptive identification of *E. coli* required the following profile: indole (+), MR (+), VP (−), citrate (−), catalase (+), oxidase (−), and urease (−). Where results were ambiguous, isolates were confirmed using the API 20E system (bioMérieux, Marcy-l'Étoile, France) following the manufacturer's interpretation chart.

Antimicrobial susceptibility testing: The Kirby–Bauer disc diffusion method on Mueller–Hinton agar (Oxoid, Basingstoke, UK) was used for antimicrobial susceptibility testing (AST) according to CLSI M100 (2025) guidelines. The antibiotics and disc potencies (discs from Oxoid) tested were aztreonam (30 µg), imipenem (10 µg), meropenem (10 µg), ceftazidime (30 µg), cefotaxime (30 µg), cefepime (30 µg), amoxicillin-clavulanate 20/10(µg, labelled as 30 µg on the box), ciprofloxacin (5 µg), and gentamicin (10 µg). Inoculum was prepared at a 0.5 McFarland concentration, and plates were incubated at 37 °C for 18–24 hrs; inhibition zone diameters were read and inter-

preted based on CLSI M100 (2025) breakpoints. E. coli ATCC 25922 (Federal Institute of Industrial Research, Oshodi, Lagos) was used as the quality control strain. Multidrug resistance (MDR) was defined as resistance to three or more antimicrobial classes [42].

Detection of extended-spectrum beta-lactamase production: Presumptive extended-spectrum beta-lactamase (ESBL) production was screened using cefotaxime, cefepime, and ceftazidime discs. Confirmatory testing was performed using the double-disc synergy test (DDST) and the combination-disc method. For DDST, an amoxicillin-clavulanate disc was placed in the center, and cefotaxime, cefepime, and ceftazidime discs were placed 15 mm (edge-to-edge) away; enhancement of the inhibition zone toward the clavulanate disc was recorded as an indication of ESBL production.

For the combination-disc test, a ≥5 mm increase in the inhibition zone diameter for cephalosporin plus clavulanate compared with cephalosporin alone was considered as confirmation of ESBL production [11]. Positive and negative control strains were included in all tests.

Molecular detection of extended-spectrum beta-lactamase gene production: Isolates confirmed to express extended-spectrum beta-lactamases (ESBLs) were subjected to polymerase chain reaction (PCR) analysis to identify prevalent ESBL genes. The genes of interest included bla_{TEM}, bla_{SHV}, and bla_{CTX-M}. A total of 20 representative isolates, representing both SF and NSF isolates, were selected for molecular examination. Genomic DNA was extracted using boiling lysis. PCR amplifications were conducted in 20 µL reaction volumes comprising Qiagen HotStarTaq Master Mix (Qiagen, Hilden, Germany), primers (Integrated DNA Technologies, Coralville, IA, USA), template DNA, and nuclease-free water.

The cycling parameters were as follows: initial denaturation at 95 °C for 3 min; 35 cycles consisting of denaturation at 95 °C for 30 s, annealing at 56 °C for 40 s, and extension at 72 °C for 50 s; with a final extension at 72 °C for 3 min. Amplicons were separated on %1.5 agarose gels (Agarose, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) utilizing tris-borate-EDTA (TBE) buffer and visualized with ethidium bromide (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). Electrophoresis was conducted at around 120 V for about 20 min, and the gels were visualized with a gel documentation system (Bio-Rad Laboratories, Hercules, CA, USA).

To determine the sizes of the DNA fragments, a 1-kb DNA ladder (Thermo Fisher Scientific, Waltham, Massachusetts) was used. The primer sequences used are presented in Table 1 [15].

Table 1) Primers used to detect beta-lactamases and antibiotic resistance genes in E. coli isolates from unpasteurized dairy milk samples in Ohimini LGA

Target	Sequence (5' → 3')	Amplicon Size (bp)	References
bla_{TEM}	F: ATGAGTATTCAACATTTCCGTG R: TTACCAATGCTTAATCAGTGAG	~850 bp	15
bla_{CTX-M}	F:ATGTGCAGYACCAGTAARGT R: TGGGTRAARTAR G T SACCAGA	~620	41
bla_{SHV}	F:ATGCGTTATATTGGCCTGTCTC R: TTAGCGTTGCCAGTGCTCG	~760	15

Statistical analysis

Data from culture, biochemical, serological, antimicrobial susceptibility, and molecular assays were compiled in Microsoft Excel and analyzed using Origin 2025b.Ink. Descriptive statistics (frequencies, proportions, and means) were calculated for relevant variables. The prevalence of *E. coli* at each sampling location was reported as a proportion with 95% confidence intervals computed by Wilson method. Because several contingency-table cells had small counts, associations were evaluated using exact tests: Fisher's exact test for 2×2 comparisons and the Freeman–Halton extension of Fisher's exact test for larger RxC tables. Where appropriate, sampling sites were aggregated into "markets" versus "farms" to permit 2×2 comparisons. Effect sizes were presented as odds ratios with 95% confidence intervals. Firth's penalized logistic regression was employed to model predictors of sample positivity while minimizing small-sample bias.



Figure 1) Map of Ohimini LGA showing study sites

Findings

Of the 100 unpasteurized milk samples examined, 13 (13.0%; 95% CI: 7.8–21.0) were found to be contaminated with *E. coli.* The prevalence of *E. coli* in market samples was greater (16.0%; 95% CI: 8.3–28.5) compared to farm samples (10.0%; 95% CI: 4.4–21.4); however, this disparity was not statistically significant (Fisher's exact test, p= .554; OR = 1.71, 95% CI: 0.52–5.66). Among *E. coli* isolates, SF *E. coli* isolates were predominant (69.2%), especially in agricultural samples (80.0%). Firth's penalized logistic regression, utilized to mitigate small-sample bias, revealed no significant correlation between *E. coli* positivity and sampling site (market versus farm), milk origin, or sorbitol fermentation phenotype (p> 0.05 for all variables). The findings revealed extensive yet minimal contamination across sampling sites, with no significant predictors of *E. coli* positivity

Table 2) Prevalence and distribution of sorbitol- and non-sorbitol-fermenting E. coli isolates from unpasteurized milk samples from markets and farms in Ohimini LGA

Location	Samples (n)	*E. coli* Positives N (%)	*E. coli* Prevalence % (95% CI, Wilson)	NSF *E. coli* N (%)	SF *E. coli* N (%)
Onyagede market	25	3 (12.0)	12.0 (4.2–30.0)	1 (33.3)	2 (66.7)
Idekpa market	25	5 (20.0)	20.0 (8.9–39.1)	2 (40.0)	3 (60.0)
Total markets	50	8 (16.0)	16.0 (8.3–28.5)	3 (37.5)	5 (62.5)
Onyagede cattle farm	25	2 (8.0)	8.0 (2.2–25.0)	0 (0.0)	2 (100.0)
Idekpa cattle farm	25	3 (12.0)	12.0 (4.2–30.0)	1 (33.3)	2 (66.7)
Total farms	50	5 (10.0)	10.0 (4.4–21.4)	1 (20.0)	4 (80.0)
Overall	100	13 (13.0)	13.0 (7.8–21.0)	4 (30.8)	9 (69.2)

SF: sorbitol-fermenting, NSF: non-sorbitol-fermenting

in this dataset (Table 2).

Figures 2-4 present the antibiotic susceptibility profiles of SF and NSF *E. coli* isolates from unpasteurized milk samples in Ohimini LGA. Both groups were generally resistant to gentamicin (100%). SF isolates showed substantial resistance to ceftazidime (n=6, 66.7%), aztreonam (n=4, 44.4%), ciprofloxacin (n=4, 44.4%), and amoxicillin–clavulanate (Augmentin) (n=4, 44.4%) but remained less resistant to imipenem (n=1, 11%), cefepime (n=2, 22.2%), cefotaxime (n=2, 22.2%), and meropenem (n=3, 33.3%). In contrast, NSF isolates exhibited higher resistance across multiple antimicrobial agents, including aztreonam (n=3, 75%), ceftazidime (n=3, 75%), Augmentin (n=3, 75%), cefotaxime (n=3, 75%), ciprofloxacin (n=2, 50%), meropenem (n=2, 50%), and cefepime (n=2, 50%), but showed lower resistance only to imipenem (n=1, 25%).

Resistance profile of ESBL-producing NSF *E. coli* isolates to third-generation cephalosporins was determined by disk-diffusion susceptibility testing and interpreted using CLSI criteria: three (75%) ESBL-positive NSF isolates were resistant to cefotaxime, one (25%) to ceftazidime, and 0% to cefepime (Figure 5).

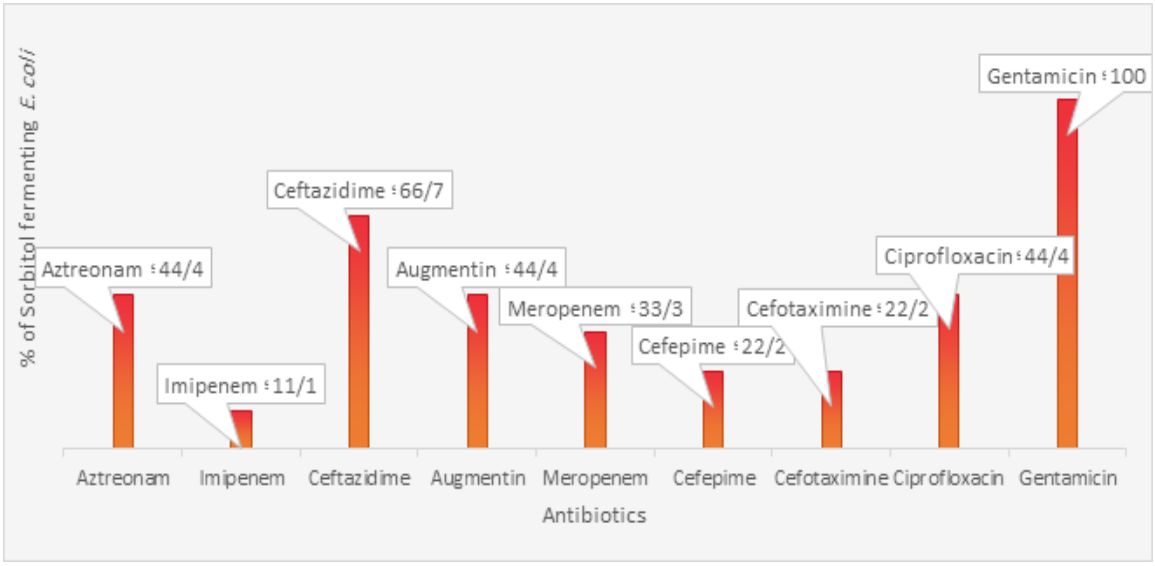


Figure 2) Antibiotic susceptibility pattern of sorbitol fermenting E. coli isolates from unpasteurized Milk samples in Ohimini LGA

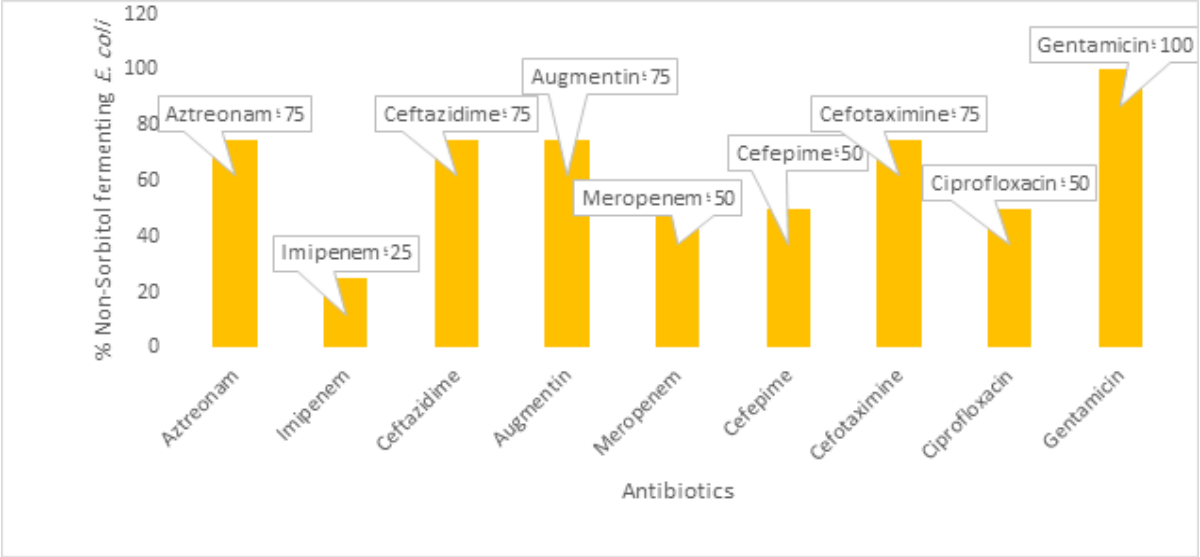


Figure 3) Antibiotic susceptibility pattern of non-sorbitol fermenting E. coli isolates from unpasteurized milk samples in Ohimini LGA

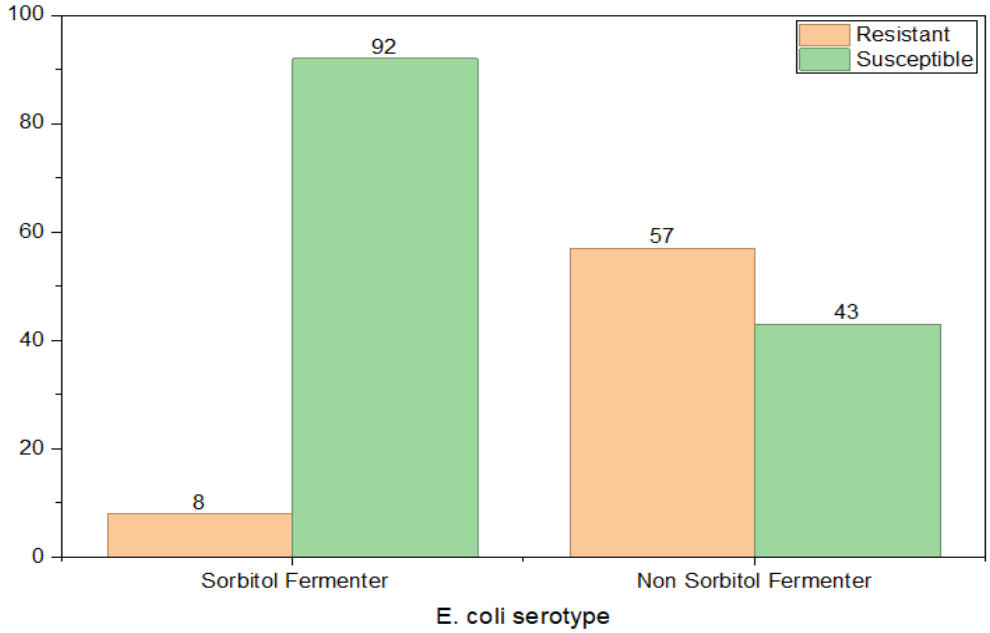


Figure 4) Multidrug resistance profile of sorbitol fermenting and non-sorbitol fermenting E. coli isolates from unpasteurized milk samples in Ohimini LGA

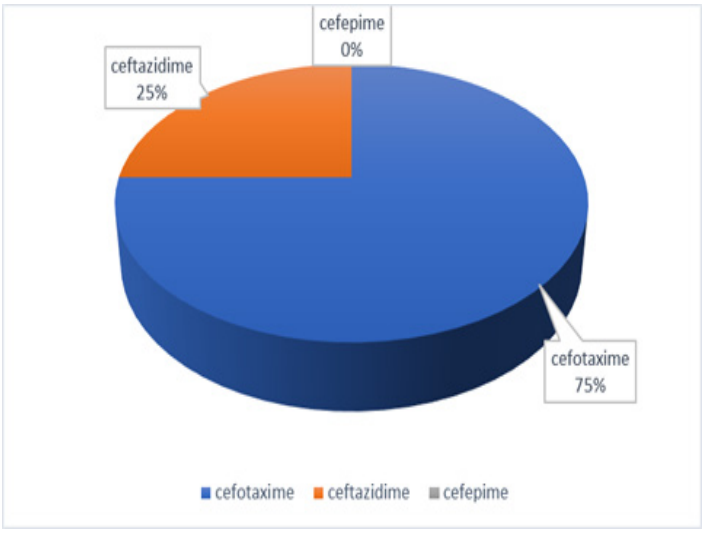


Figure 5) Distribution of ESBL-producing non-sorbitol fermenting E. coli isolates based on resistance to third-generation cephalosporins

No ESBL production was observed among the sorbitol-fermenting *E. coli* serotypes. The phenotypic and genotypic characteristics of SF and NSF *E. coli* isolates from unpasteurized milk samples in Ohimini LGA are presented in Table 3. The NSF isolates showed a consistently stronger resistance profile, displaying ESBL activity in all cases and exhibiting higher resistance to β-lactam antibiotics and fluoroquinolones. In contrast, the SF isolates demonstrated comparatively lower resistance levels. Multidrug resistance (MDR) was defined as resistance to three or more antimicrobial classes. Molecular screening for bla_{TEM}, bla_{CTX-M}, and bla_{SHV} revealed that these ESBL-associated genes were more prevalent in NSF isolates, aligning with the pronounced ESBL phenotype observed in this group. The bla_{TEM} target yielded a distinct 850 bp amplicon. Lanes 2–6 and 8–12 showed this 850 bp band, indicating positivity for the bla_{TEM} gene in

Table 3) Phenotypic and genotypic characteristics of sorbitol-fermenting (SF) and non-sorbitol-fermenting (NSF) *E. coli* isolates from unpasteurized milk samples (Ohimini LGA)

Characteristic	SF (n = 9)	NSF (n = 4)
No. of isolates	9	4
ESBL phenotype	0 (0.0%)	4 (100.0%)
Gentamicin resistance	9 (100.0%)	4 (100.0%)
Ceftazidime resistance	6 (66.7%)	3 (75.0%)
Aztreonam resistance	4 (44.4%)	3 (75.0%)
Amoxicillin–clavulanate resistance	4 (44.4%)	3 (75.0%)
Cefotaxime resistance	2 (22.2%)	3 (75.0%)
Cefepime resistance	2 (22.2%)	2 (50.0%)
Meropenem resistance	3 (33.3%)	2 (50.0%)
Ciprofloxacin resistance	4 (44.4%)	2 (50.0%)
Multidrug resistance (≥3 classes)	5 (55.6%)	4 (100.0%)
Bla-TEM (PCR)	4 (44.4%)	3 (75.0%)
Bla-CTX-M (PCR)	2 (22.2%)	3 (75.0%)
Bla-SHV (PCR)	3 (33.3%)	3 (75.0%)

these isolates. Amplification of the $bla_{CTX\text{-}M}$ gene resulted in a 602 bp product. Lanes 3-4, 7, and 9–10 presented a sharp 602 bp band, verifying the presence of the $bla_{CTX\text{-}M}$ gene in these samples. PCR amplification of the bla_{SHV} gene produced an approximately 747 bp fragment on 1.5% agarose gel. Lanes 3-6, and 9-10 each displayed a clear 747 bp band, confirming that these *E. coli* isolates carried the bla_{SHV} gene**.**

Discussion

The general prevalence of *E. coli* (13.0%) identified in this study aligns with findings from similar environments where unsanitary milking practices, inadequate refrigeration, and improper handling lead to bacterial contamination of raw milk [1, 4, 5, 9, 23]. Although the market samples displayed a higher prevalence than farm samples, this variance was not statistically significant, as demonstrated by the overlap of the 95% confidence intervals and Fisher's exact test. Likewise, Firth's penalized logistic regression did not recognize sampling sites or milk origins as significant predictors of *E. coli* positivity, indicating generally comparable levels of contamination throughout the milk supply chain in the study area.

A significant observation in this study was the predominance of sorbitol-fermenting (SF) *E. coli* isolates (69.2%), while non-sorbitol-fermenting (NSF) isolates, which are epidemiologically vital due to their association with potentially more virulent serotypes, accounted for 30.8% of the recovered isolates [2, 36]. The presence of NSF *E. coli* in milk intended for direct human consumption is significant and concerning, as NSF phenotypes often correspond to pathogenic

variants, including shiga toxin-producing *E. coli* and other diarrheagenic lineages [20, 30]. The antimicrobial susceptibility findings revealed a marked disparity between SF and NSF *E. coli* isolates. While all isolates were resistant to gentamicin, resistance to most β-lactams and fluoroquinolones was substantially higher among NSF isolates. NSF isolates demonstrated extreme resistance rates of 75-100% to ceftazidime, aztreonam, amoxicillin-clavulanate, cefotaxime, cefepime, ciprofloxacin, and meropenem. In contrast, SF isolates remained mostly susceptible to 3rd- and 4th-generation cephalosporins and carbapenems, with resistance generally ≤ 22% to cefotaxime, cefepime, meropenem, and ciprofloxacin. This noticeable difference suggests that NSF isolates circulating in the milk chain may be under greater selective pressure, possibly due to prior antibiotic exposure in cattle or environmental dissemination of resistant strains [9, 23, 25]. Multidrug resistance patterns support this disparity: all NSF isolates met MDR criteria (resistance to ≥3 antibiotic classes), whereas just over half of SF isolates were MDR. These findings mirror reports from other African livestock systems, where the unregulated use of antimicrobials in animal husbandry often drives the selection and persistence of highly resistant strains [17, 19, 24]. ESBL production was detected exclusively among NSF isolates, with 100% phenotypic positivity. This finding aligns with their elevated resistance to third-generation cephalosporins. Genotypic screening further supported this pattern: NSF isolates carried ESBL-associated genes (bla_{TEM}, bla_{CTX-M}, and bla_{SHV}) at substantially higher frequencies. The high co-occurrence of multiple ESBL genes in NSF isolates (75% positivity for each gene) shows the circulation of multidrug-resistant *E. coli* harboring diverse β-lactamase determinants. In contrast, SF isolates showed much lower gene carriage rates (22–44%), consistent with their largely susceptible phenotypic profiles.

Detection of bla_{CTX-M} is of particular importance, as CTX-M-type ESBLs continue to dominate global ESBL epidemiology and have been increasingly reported in Nigerian livestock and food products. The co-presence of bla_{TEM} and bla_{SHV} further indicates genetic diversity and potential plasmid-mediated transfer of resistance genes among milk-associated *E. coli* populations [15, 18, 21, 22, 28, 41].

The presence of ESBL-producing NSF *E. coli* strains in unpasteurized milk poses a significant public health concern. In many rural and peri-urban Nigerian communities, people still consume unpasteurized milk, which puts them at risk of zoonotic strains that could cause serious illnesses or act as reservoirs of transferable resistance genes [15, 19, 43]. The complete absence of ESBL production among SF isolates suggests that pathogenic and non-fermenting phenotypes may represent more clinically relevant reservoirs of resistance within the milk chain.

Conclusion

This study finding highlight the coexistence of both sorbitol-fermenting and highly resistant NSF *E. coli* in raw milk produced and sold in Ohimini LGA. The detection of multiple ESBL genes in NSF isolates highlights the urgent need for improved hygiene practices during milking, routine surveillance of antimicrobial resistance in food-producing animals, and strict enforcement of regulations regarding antibiotic use in livestock. Interventions targeting both farm-level management and market handling practices are essential to reduce the risk of transmission of multidrug-resistant *E. coli* to consumers.

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Ethical permissions: Ethical approval was not required for this study because it involved the microbiological analysis of cow milk samples and did not involve human participants or experimental procedures on live animals. Permission to collect the samples was obtained from the relevant milk vendors and/or farm owners, as applicable.
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