

# Isolation and Identification of Enteric Bacteria from Raw Cow Milk from Carysburg Slaughter House in New Island Community, Carysburg, Montserrado County Liberia

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## ABSTRACT

Raw cow milk from informal dairy systems in developing countries, including Liberia, is frequently contaminated with enteric bacteria, posing significant public health risks due to widespread consumption without pasteurization. This study investigated microbial safety of raw cow milk produced at Carysburg Slaughter House, Montserrado County, Liberia. Hence, isolating, identifying, and characterizing enteric bacteria from raw cow milk and determine their antibiotic susceptibility profiles. Four raw milk samples were collected aseptically from individual cows at Carysburg Slaughter House in 2025. Samples were cultured on MacConkey agar, HiCrome agar, and nutrient agar. Presumptive enteric isolates were identified using Gram staining, standard biochemical tests (Kligler Iron Agar, citrate, motility indole-ornithine, urea), and colony characteristics. Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method against gentamicin (10 µg), ciprofloxacin (5 µg), amoxicillin (30 µg), ceftriaxone (30 µg), and chloramphenicol (30 µg), with results interpreted according to CLSI guidelines. Results: Two of the four milk samples (50%) yielded enteric bacteria. The isolates were identified as *Citrobacter freundii* and *Salmonella enterica* serovar Typhi. Both isolates were resistant to amoxicillin (100%) but fully susceptible to gentamicin, ciprofloxacin, ceftriaxone, and chloramphenicol. No multidrug resistance was observed. Raw milk from Carysburg Slaughter House is contaminated with potentially pathogenic enteric bacteria, including the alarming presence of *Salmonella* Typhi, indicating severe hygienic deficiencies and human fecal contamination. Immediate interventions—improved milking hygiene, routine milk testing, and promotion of pasteurization—are urgently needed to prevent foodborne outbreaks in this community.

**Keywords:** Antibiotic Susceptibility, Enteric Bacteria, *Salmonella typhi*

## INTRODUCTION

Enteric bacteria are a large family of Gram-negative bacteria. It includes over 30 genera and more than 100 species (Don J.et. al, 2005). Enteric bacteria include, along with many harmless symbionts, many of the more familiar pathogens, such as *Salmonella*, *Escherichia coli*, *Klebsiella*, and *Shigella*. Other disease-causing bacteria in this family include *Enterobacter* and *Citrobacter* (Corkery and Liz, 2020). Milk is a nutrient-dense food source, providing essential proteins, vitamins, and minerals that contribute significantly to human nutrition, particularly in developing countries where it serves as a staple for children and vulnerable populations (FAO, 2020). However, raw cow milk, which is unpasteurized and unprocessed, poses substantial public health risks due to potential contamination with pathogenic microorganisms. Enteric bacteria, such as *Escherichia coli*, *Salmonella* spp., *Shigella* spp., and *Campylobacter jejuni*, are among the primary contaminants in raw milk, originating from fecal matter, udder infections, or environmental sources during milking (Oliver et al., 2009).

The goal of this study is to isolate, identify enteric bacteria from cow's milk and determine if the enteric bacteria isolated is pathogenic from Carey Burg Slaughter House in New Island Community, Montserrado County, Liberia.

## MATERIALS AND METHODS

### Research Setting

This research was conducted at the National Public Health Institute of Liberia (NPHIL), specific the National Reference Laboratory. This Laboratory is the only biggest government lab in the country and the reference lab for the Liberia.

### Research Design

This study used experimental design. This is a structured process for planning and conducting experiments to test hypotheses and determine causes and effects relationships by carefully controlling and arranging variables.

### Sampling Techniques and Sample Size

The sampling techniques Random sampling. The total sample size was 4 and from four different cows. cow 1 milk, cow 2 milk, cow 3 milk, and Cow 4 milk

### Sample collection and techniques

Four different cow’s milk were collected using sterile containers for each cow’s milk. Samples were transported to the Lab on ice and stored at 4c till experiment time.

### Isolation and Biochemical characterization of bacteria

MacConkey, Nutrient and Hicrome agar (MAC) were used for culturing of the samples while Gram stain was performed to countercheck the bacteria appearance and morphology (Red or Pink).

Further identification of the bacterial isolates was carried out using various biochemical tests including Catalase, Coagulase, Oxidase, Indole, Urea hydrolysis, and sugar fermentation test following standard microbiological protocols as described by Dawodu and Akanbi (2021)

### Antibiotic Susceptibility Test (AST)

To assess the antibiotic susceptibility of the isolates, the Kirby-Bauer disk diffusion method, as outlined by Hudzicki, (2009) was employed. An inoculum was prepared and administered onto Mueller-Hinton susceptibility agar plates utilizing a sterile swab stick. Following this, the plates were allowed to air-dry for a period of 5-10 minutes. The antibiotic susceptibility test was carried out using various such as Gentamicin, Amoxicillin, Ciprofloxacin, Ceftriaxone, Chloramphenicol. Using sterile forceps, the antibiotic discs were positioned onto the inoculated plates, and subsequently, the plates were incubated at a temperature of  $37 \pm 2$  °C for a duration of 24 hours. Following incubation, the plates were carefully examined to identify zones of inhibition, indicative of the susceptibility of each isolate to the antibiotics utilized.

## RESULT

Table 3.1: Culturing And Identification of Enteric Bacteria Using MacConkey and Hicrome Agar

Source	Sample	# Collected	Media used	Colony	Enteric Bacteria
	Type			Color	Positive
Cow1	Milk	1	MAC, NAM& Hicrome	No growth	
Cow2	Milk	1	MAC, NAM& Hicrome	No growth	
Cow3	Milk	1	MAC, NAM& Hicrome	Pink	<i>Citrobacter freundii</i>

Cow4	Milk	1	MAC, NAM&	Light pink	<i>Salmonella typhi</i>
			<b>Hicrome</b>		
TOTAL		4			2

**Key:** MAC represents (MacConkey agar); NAM represents (Nutrient agar media); **Hicrome** represents (Hicrome agar)

According to Table 3.1, Cow1 and 2 milk cultured on both MacConkey, Nutrient and Hicrome agar exhibited no growth. Cow 3 milk cultured on MacConkey, Nutrient and Hicrome agar, only MacConkey agar has better growth by displaying colony morphology of pink which was further confirmed by biochemical and gram stain tests to be *Citrobacter freundii*. Cow 4 was also cultured the same set of media mentioned above, but only Hicrome agar has better growth by displaying colony morphology of light pink which was further confirmed by biochemical and gram stain tests to be *Salmonella typhi*.

**Table 3.2: Biochemical and Gram stain tests for Enteric Bacteria identification**

Key: + represents (positive); - represents (negative)

As pointed out clearly in table 2, Cow3 milk containing the isolate of *Citrobacter freundii* and Cow 4 milk containing the isolate of *Salmonella typhi* were confirmed by both biochemical and gram stain tests as positive.

Isolate	Sample	Biochemical tests	Test Result	Gram stain	Test
<i>Citrobacter freundii</i>	Cow 3 milk	Kligler_Iron_Agar	(y/y,G+,H2S +)	Gram negative Pink rod shape Bacillus	+
		Citrate	(+)		
		Motility_Indole_Ornith	M(+),I(-),O(-)		
		Urea	(+)		
<i>Salmonella typhi</i>	Cow 4 milk	Kligler_Iron_Agar	(r/y,G(-),H2S (-)	Gram negative Pink rod shape Bacillus	+
		Citrate	(+)		
		Motility_Indole_Ornith	M(+),I(-),O(+)		
		Urea	(-)		

Table- 3.3: Antibiotic Susceptibility patterns of Enteric bacteria isolates comparing with CLSI standard breakpoints and negative).

Source	Isolate	Antibiotics	Inhibition zone	R	I	S	% R	% I	% S
		<b>GM (10mg)</b>	16mm	-	-	+			
Cow3 milk	<i>Citrobacter freundii</i>	<b>CIP (5mg)</b>	30mm	-	-	+			
		<b>AMC (30mg)</b>	8mm	+	-	-	1(20%)	0(0%)	4(80%)

		<b>CRO (30mg)</b>	28mm	-	-	+			
		<b>C (30mg)</b>	28mm	-	-	+			
		<b>GM (10mg)</b>	17mm	-	-	+			
		<b>CIP (5mg)</b>	34mm	-	-	+			
Cow4	<i>Salmonella</i>	<b>AMC (30mg)</b>	6mm	+	-	-			
milk	<i>typhi</i>						1(20%)	0(0%)	4(80%)
		<b>CRO (30mg)</b>	23mm	-	-	+			
		<b>C (30mg)</b>	25mm	-	-	+			
<b>KEY:</b> R,I,S represent( Resistant, Intermediate, Sensitive);(+),(-) represent(positive									

As mentioned in table 3, Cow3 isolate of *Citrobacter freundii* was 1(20%) resistant, 0(0%) intermediate and 4(80%) Sensitive to the five tested antibiotics, while Cow4 isolate of *Salmonella typhi* was 1(20%) resistant, 0(0%) intermediate and 4(80%) Sensitive to the five tested antibiotics.

## DISCUSSION

Culturing and identification of enteric bacteria from cow’s milk using macConkey and hicrome agar, determined in this study showed the identification of *Citrobacter freundii* cultured on MacConkey agar with morphology pink shape and *Salmonella typhi* on Hicrome agar with morphology pink rod shape, similar research was done(Mariam et al.,2021) reported similar pink, lactose-fermenting colonies of *Citrobacter* on MacConkey agar from milk samples, emphasizing its utility for presumptive identification of enteric Gram-negatives, with bile salts inhibiting Gram-positives to enhance selectivity. Biochemical test for identifying enteric bacteria, the tests were Kligler iron agar, citrate, motility-indole-ornithine and urea to identified *Citrobacter freundii* and *Salmonella typhi* and such research was conducted (Gebeyehu et al., 2022) applied identical biochemical panels (including KIA, citrate, MIO, and urea) to confirm *Salmonella* isolates. Antibiotics susceptibility test using the CLSI standard breakpoint.

The research result showed that of the five antibiotics tested( Gentamicin, Ciprofloxacin, Amoxicillin, Ceftriaxone and Chloramphenicol) *Citrobacter freundii* and *Salmonella typhi* isolated from Cowmilk 3 and 4 were 1(20%) resistant, 0(0%) intermediate and 4(80%) sensitivity, similar research was done (Temesgen et al.,2025) tested *Salmonella* from Ethiopian raw cow milk using CLSI disk diffusion on Mueller-Hinton agar, finding 76.5% sensitivity to ciprofloxacin and ceftriaxone, with amoxicillin resistance predominant (up to 98.8% in related Enterobacteriaceae), and no intermediate, aligning with this research.

## CONCLUSION

This study successfully isolated and identified two important enteric pathogens, *Citrobacter freundii* and *Salmonella typhi*, from raw cow’s milk using standard microbiological techniques. The use of MacConkey agar and HiCrome agar provided reliable presumptive identification through characteristic colony morphologies, while a battery of biochemical tests (Kligler iron agar, citrate utilization, motility-indole-ornithine, and urease) confirmed the identity of both organisms with high accuracy. Antibiotic susceptibility testing using the CLSI disk diffusion method revealed that both isolates exhibited a favorable sensitivity profile, remaining fully susceptible to gentamicin, ciprofloxacin, ceftriaxone, and chloramphenicol, with resistance observed only to amoxicillin. This low level of antimicrobial resistance (20%) is encouraging and suggests that, at present, these critical drugs remain effective against *Citrobacter freundii* and *Salmonella typhi* contaminating raw cow’s milk in the study area. The presence of these potentially pathogenic enteric bacteria in raw cow’s milk underscores a significant public health risk, particularly in communities where unpasteurized milk is consumed or used in



traditional dairy products. The findings highlight the importance of strict hygienic practices during milking, storage, and handling, as well as the need for routine microbiological monitoring of raw milk.

In conclusion, while the isolated enteric bacteria currently show limited antibiotic resistance, the detection of *Citrobacter freundii* and *Salmonella typhi* in raw cow's milk emphasizes the urgent need for improved farm hygiene, proper pasteurization, and public awareness to prevent milk-borne infections and safeguard consumer health.

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