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Street-Vended Wara as a Reservoir of Foodborne and Antibiotic-Resistant Bacteria: A Microbiological Assessment in Owo, Nigeria

Arijeniwa, O.C.¹, Jose, A.R.², Ogundare, A.O.³

Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria

Corresponding author: michaelococcus@gmail.com

ABSTRACT :

Street-vended wara (traditional soft cheese) is a popular ready-to-eat dairy product in southwestern Nigeria. However, poor hygienic handling during production and retail may predispose the product to contamination by foodborne and antimicrobial-resistant bacteria. This study evaluated the microbiological quality and antibiotic resistance profile of bacteria isolated from street-vended wara sold in Owo, Ondo State, Nigeria. Nine freshly prepared wara samples were collected from three major vending locations in Owo and analyzed using standard microbiological techniques. Total heterotrophic, enteric, coliform, and staphylococcal bacterial counts were determined by the pour plate method. Bacterial isolates were identified using cultural, morphological, Gram staining, and biochemical characteristics. Antibiotic susceptibility was assessed using the Kirby–Bauer disc diffusion method, and multiple antibiotic resistance indices (MAR) were calculated. Total heterotrophic bacterial counts ranged from 9.00×10^6 to 33.67×10^6 CFU/g, while enteric counts ranged from 4.33×10^6 to 18.67×10^6 CFU/g. Coliforms and staphylococci were detected in most samples, indicating inadequate hygienic quality. Seven bacterial genera were identified including *Escherichia*, *Staphylococcus*, *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Bacillus* and *Micrococcus*. *Pseudomonas*, *Staphylococcus*, *E. coli*, and *Micrococcus* occurred across all sampling locations. Most isolates were susceptible to gentamicin, ciprofloxacin, ofloxacin, perfloracin, and levofloxacin, whereas resistance was common against ampiclox, azithromycin, rifampicin, and amoxicillin. *Pseudomonas* and *E. coli* exhibited the highest multiple antibiotic resistance indices of 0.5 and 0.4, respectively, suggesting exposure to environments with considerable antibiotic selection pressure. Street-vended wara sold in Owo harbors substantial microbial contamination and clinically important antibiotic-resistant bacteria, posing potential food safety and public health risks. Strengthening hygienic production practices, routine microbiological surveillance, and prudent antibiotic stewardship is essential to improve the safety of traditional dairy products in Nigeria.

Keywords: Wara, antibiotic resistance, food safety, street-vended foods, dairy microbiology

Introduction

Food safety remains one of the most important public health concerns globally because contaminated foods serve as major vehicles for the transmission of pathogenic microorganisms and antimicrobial-resistant bacteria (Adesokan *et al.*, 2023). The World Health Organization (WHO, 2023) estimates that hundreds of millions of people develop foodborne illnesses annually, resulting in substantial morbidity, mortality, and economic losses, particularly in low- and middle-income countries where informal food vending is widespread. Ready-to-eat foods sold under inadequate hygienic conditions are especially vulnerable to microbial contamination because they are often consumed without further heat treatment, thereby increasing the risk of exposure to pathogenic bacteria and their antimicrobial resistance determinants (Akinyele *et al.*, 2024).

In Nigeria, traditional dairy products constitute an important component of household nutrition and local commerce. Among these products, wara, a fresh unripened soft cheese produced by coagulating cow milk with the extract of *Calotropis procera* (Sodom apple), is widely consumed throughout southwestern Nigeria (Ogunleye *et al.*, 2024). Wara is valued because of its high-quality protein, essential amino acids, calcium, phosphorus, and other micronutrients that contribute significantly to human nutrition. The product is inexpensive, readily available, and commonly sold by roadside vendors, markets, schools, and motor parks, making it one of the most accessible ready-to-eat dairy foods in the region. However, its high moisture content, near-neutral pH, and rich nutrient composition also provide an excellent substrate for microbial growth, thereby reducing its shelf life and increasing its susceptibility to contamination (Alao *et al.*, 2024).

The production and distribution of wara are predominantly carried out using traditional cottage-scale methods with minimal regulatory oversight. Fresh milk is frequently obtained from local cattle without pasteurization, while processing equipment, water, utensils, and packaging materials may not undergo adequate sanitation (Birgen *et al.*, 2022). Following production, the cheese is commonly displayed in open trays or immersed in whey during transportation and retail, exposing it to dust particles, insects, environmental aerosols, contaminated water, and repeated handling by vendors and consumers. These practices substantially increase the likelihood of contamination by spoilage microorganisms as well as clinically significant foodborne pathogens (Ibrahim and Adeola, 2024).

Several bacterial species have previously been isolated from traditional dairy products, including *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter* spp., *Bacillus* spp. and *Micrococcus* spp. These organisms differ considerably in their public health significance. *Escherichia coli* is widely recognized as an indicator of fecal contamination and poor sanitary practices during food processing. *Staphylococcus aureus* is commonly introduced through human skin, nasal secretions, or improper handling and is capable of producing heat-stable

enterotoxins responsible for food poisoning (Idris and Mohammed, 2025). *Pseudomonas* species contribute primarily to spoilage through enzymatic degradation of proteins and lipids, whereas *Bacillus* species produce resistant endospores that survive adverse environmental conditions and may persist during food processing. Consequently, the microbiological quality of wara serves as an important indicator of both food hygiene and consumer safety (Mazi *et al.*, 2023).

Beyond microbial contamination, the emergence of antimicrobial resistance (AMR) has transformed food safety into a multidimensional One Health challenge linking human, animal, food, and environmental health. The inappropriate use of antibiotics in livestock production, veterinary medicine, agriculture, and human healthcare has accelerated the selection of resistant bacterial populations (Nwaiwu and Odeyemi, 2023). These resistant organisms may subsequently enter the food chain through contaminated raw milk, processing environments, food handlers, and environmental reservoirs. Consumption of foods contaminated with resistant bacteria may facilitate colonization of the human gastrointestinal tract and contribute to the dissemination of resistance genes within microbial communities. The WHO continues to identify antimicrobial resistance as one of the leading global health threats requiring coordinated surveillance across the food production continuum (Ogunleye *et al.*, 2024).

Recent investigations in Nigeria have demonstrated that ready-to-eat foods frequently harbor multidrug-resistant Gram-positive and Gram-negative bacteria possessing clinically important resistance determinants Okoruwa and Onuigbo-Chatta, 2022). A nationwide concern has emerged regarding the occurrence of extended-spectrum β -lactamase-producing Enterobacteriaceae, methicillin-resistant staphylococci, and other resistant foodborne pathogens in commonly consumed street foods. Similarly, studies on Nigerian traditional dairy products have reported high levels of resistance among enteric bacteria isolated from wara and nono, particularly against amoxicillin, trimethoprim-sulfamethoxazole, chloramphenicol, and related antibiotics. These findings emphasize the growing importance of routine antimicrobial susceptibility testing in food microbiology and the need for improved hygienic practices among food vendors (Ogunleye *et al.*, 2026).

Despite the widespread consumption of street-vended wara in Ondo State, information regarding its microbiological quality and antibiotic resistance profile remains limited, particularly within Owo metropolis where roadside food vending constitutes a major source of affordable nutrition for students, traders, commuters, and residents. Local epidemiological data are essential for identifying potential food safety hazards, guiding public health interventions, and supporting antimicrobial resistance surveillance programs in accordance with Nigeria's Second One Health National Action Plan on AMR (2024–2028).

Therefore, this study investigated the microbiological quality and antibiotic resistance profile of bacteria isolated from street-vended wara sold in Owo, Ondo State, Nigeria. Specifically, the study determined the total bacterial load, identified the bacterial species associated with the product, assessed their distribution across different vending locations, and evaluated their susceptibility to commonly used antibiotics. The findings provide valuable baseline information for improving food hygiene, protecting consumer health, and strengthening antimicrobial resistance monitoring within traditional dairy food systems.

2. Materials and Methods

Study area

The study was conducted in Owo, Ondo State, Nigeria.

Sample collection

Nine freshly prepared wara samples were purchased from different street vendors, aseptically packaged in sterile polythene bags, and transported immediately to the microbiology laboratory for analysis.

Microbiological analysis

Five grams of each sample were homogenized in sterile distilled water, serially diluted, and cultured using the pour plate technique. After incubation, colonies were counted and expressed as colony-forming units per gram (CFU/g).

Identification of isolates

Pure cultures were characterized using colony morphology, Gram staining, catalase, coagulase, citrate, urease, motility, carbohydrate fermentation, and indole tests (CLSI, 2024).

Antibiotic susceptibility testing

The Kirby–Bauer disc diffusion method on Mueller–Hinton agar was used following preparation of a 0.5 McFarland bacterial suspension. Zones of inhibition were measured after 18 hours of incubation (CLSI, 2024).

3. Results

Table 1. Microbial counts of street-vended wara ×10⁶ CFU/g.

Sample	THC	TEC	TCC	TSC
GG1	17.33±1.00 ^a	15.00±0.50 ^a	7.00±0.01 ^b	23.00±1.15 ^a
GG2	16.00±1.00 ^a	8.00±0.00 ^a	5.00±0.00 ^a	10.00±0.09 ^b
GG3	18.00±1.07 ^b	10.33±0.10 ^b	6.33±0.00 ^c	6.67±0.08 ^b
GT1	11.33±1.25 ^c	4.33±0.50 ^c	0.00±0.00 ^c	6.33±0.00 ^c
GT2	9.00±0.07 ^c	6.67±0.01 ^b	5.00±0.01 ^c	5.00±0.10 ^c
GT3	13.33±0.58 ^d	6.00±0.08 ^c	5.33±0.05 ^d	5.00±0.00 ^d
GOB1	33.67±1.42 ^f	18.67±1.50 ^f	7.67±0.05 ^d	7.00±0.05 ^b
GOB2	29.00±2.00 ^e	11.00±1.00 ^d	5.50±0.08 ^e	13.67±1.00 ^c
GOB3	22.67±1.20 ^d	12.33±0.10 ^e	3.67±0.00 ^e	8.33±0.02 ^a

Key: GG= sample from first gate, GT= sample from second gate, GOB= sample from Oja Oba, THC= Total heterophilic count, TEC= Total enteric bacteria count, TCC= Total coliform count, TSC= Total staphylococcal count.

Table 2: Biochemical Test Results

ORG	BIOCHEMICAL TEST RESULTS										
	Gram	OXID	COAGU	CATAL	URE	MOTI	CITRA	GLUC	LACTO	MALT	INDO
		ASE	LASE	ASE	ASE	LITY	TE	OSE	SE	OSE	LE
<i>Klebsiella sp.</i>	- rod	-	-	+	+	-	+	+	+	+	-
<i>Pseudomonas sp</i>	- rod	+	-	+	-	+	-	-	+	-	-
<i>Staphylococcus sp</i>	+ cocci	-	+	+	-	-	-	-	-	-	-
<i>E. coli</i>	- rod	-	-	-	-	+	-	+	+	-	+
<i>Enterobacter spp.</i>	- rod	+	-	+	-	-	-	+	+	+	-
<i>Bacillus spp.</i>	+ rod	-	-	+	-	-	-	+	+	+	-
<i>Micrococcus spp</i>	+ cocci		-	+	-	-	-	+	+	+	-

Table 3: Occurrence of the isolates in each Sample Location

ORGANISM	Gate1	Oja Oba	Gate2
<i>Klebsiella sp.</i>	-	+	-
<i>Pseudomonas sp</i>	+	+	+
<i>Staphylococcus sp</i>	+	+	+
<i>E. coli</i>	+	+	+
<i>Enterobacter spp.</i>	+	-	+
<i>Bacillus spp.</i>	+	-	-
<i>Micrococcus spp</i>	+	+	+

Key: + = present, - = absent

Table 4A: Antibiotics susceptibility Test Results (Gram negatives)

ORG	Test Antibiotics										MARI
	AV	CN	PEF	OFX	AZ	LEV	CF	SP	CPX	AM	
<i>Klebsiella sp.</i>	S	S	S	S	S	S	S	R	S	R	0.2
<i>Pseudomonas sp</i>	R	S	S	S	R	S	R	R	S	R	0.5
<i>E. coli</i>	R	S	S	S	R	S	S	S	R	R	0.4
<i>Enterobacter spp.</i>	S	S	S	S	S	S	R	S	S	S	0.1

Key: I = S = sensitive, R = resistant

Table 4B: Antibiotics susceptibility Test Results (Gram positives)

ORG	Test Antibiotics										MARI
	PEF	CN	APX	Z	AM	R	CPX	AZ	LEV	E	
<i>Staphylococcus sp</i>	S	S	R	R	R	S	S	S	S	S	0.3
<i>Bacillus spp.</i>	S	S	S	S	R	S	R	S	S	S	0.2
<i>Micrococcus spp</i>	S	S	S	S	S	S	S	S	S	S	0.0

Key: S = sensitive, R = resistant

DISCUSSION

This study evaluated the microbiological quality and antibiotic resistance profile of bacteria isolated from street-vended wara sold in Owo, Ondo State. The findings demonstrated substantial bacterial contamination, the occurrence of foodborne bacterial genera, and the presence of clinically important antimicrobial resistance among isolates, confirming that traditionally processed and street-vended dairy products may constitute important vehicles for the transmission of antimicrobial-resistant bacteria. These findings support the growing One Health concern that foods of animal origin contribute to the environmental and human dissemination of resistant microorganisms.

The total heterotrophic bacterial counts (THC) obtained in this study ranged from 9.00×10^6 to 33.67×10^6 CFU/g, while enteric bacterial counts varied between 4.33×10^6 and 18.67×10^6 CFU/g across the sampled locations. The highest microbial loads were recorded in samples obtained from Oja-Oba market, whereas comparatively lower counts occurred in samples collected around Gate Two. These variations most likely reflect differences in environmental sanitation, handling practices, exposure time during vending, and the hygienic conditions under which the cheese was displayed.

The bacterial loads observed are considerably high for a ready-to-eat dairy product and suggest inadequate hygienic practices during production and retail. Wara possesses high moisture content and abundant nutrients, making it highly susceptible to rapid microbial proliferation, particularly under ambient tropical temperatures. Similar observations have been reported for traditionally processed wara and nono sold in southwestern Nigeria, where prolonged exposure to open markets, contaminated utensils, and repeated handling significantly increased microbial loads (Adesokan *et al.*, 2023; Bello *et al.*, 2024). The WHO also recognizes informal ready-to-eat foods as important sources of foodborne disease in low- and middle-income countries due to limited temperature control and inadequate sanitation.

Enteric bacterial counts were particularly elevated in several samples, indicating possible fecal contamination during processing or vending. Enteric bacteria are widely regarded as indicators of poor environmental hygiene and contamination arising from water, equipment, handlers, or animal-derived raw materials (Onyeka *et al.*, 2023). Their presence raises concern because contaminated dairy products can serve as reservoirs for opportunistic pathogens capable of causing gastrointestinal infections, especially among children, elderly individuals, and immunocompromised consumers (Omokpariola, 2026).

The microbial count table revealed measurable total coliform counts ranging from 3.67×10^6 to 7.67×10^6 CFU/g in most samples, with only one sample recording zero counts. The presence of coliforms is a significant microbiological indicator because it reflects possible fecal contamination or inadequate sanitary handling during cheese production. Similarly, total staphylococcal counts ranged from 5.00×10^6 to 23.00×10^6 CFU/g, with the highest value recorded in Gate One samples.

High staphylococcal loads are particularly important from a public health perspective because *Staphylococcus aureus* is commonly carried on the skin, hands, and nasal passages of healthy individuals. During manual processing and packaging, poor personal hygiene permits direct contamination of ready-to-eat foods. Certain strains produce heat-stable enterotoxins capable of causing acute food poisoning even when bacterial cells are subsequently destroyed. Recent investigations on traditional dairy products in Nigeria similarly reported *Staphylococcus* species as one of the predominant contaminants associated with inadequate vendor hygiene and poor storage conditions (Ogunleye *et al.*, 2024).

Biochemical characterization identified seven bacterial genera, namely *Klebsiella*, *Pseudomonas*, *Staphylococcus*, *Escherichia coli*, *Enterobacter*, *Bacillus*, and *Micrococcus*. These organisms represent both environmental contaminants and clinically important foodborne bacteria. Their occurrence confirms that street-vended wara provides a favorable ecological niche for diverse microorganisms introduced during multiple stages of production and distribution (Owolabi *et al.*, 2023).

Pseudomonas, *Staphylococcus*, *Escherichia coli*, and *Micrococcus* occurred across all sampling locations, whereas *Klebsiella* was restricted to Oja-Oba and *Bacillus* occurred only in Gate One samples. The widespread distribution of *Pseudomonas* and *E. coli* suggests persistent environmental contamination rather than isolated point-source contamination. *Pseudomonas* species are psychrotrophic spoilage organisms capable of producing proteases and lipases that rapidly deteriorate dairy products, while *E. coli* serves as a recognized indicator of fecal pollution (Samapundo, 2022). The detection of *Klebsiella* and *Enterobacter* further indicates contamination originating from water, soil, or handling equipment.

The isolation of *Bacillus* species also deserves attention because members of this genus produce heat-resistant endospores that survive harsh environmental conditions and may persist throughout traditional food processing. Their presence may therefore reflect inadequate cleaning of processing utensils or contamination from dust and soil during open-air vending (Uzeh *et al.*, 2022).

The antimicrobial susceptibility results revealed encouraging susceptibility of most Gram-negative isolates to gentamicin, pefloxacin, ofloxacin, levofloxacin, and ciprofloxacin, whereas resistance was predominantly observed against ampiclox, azithromycin, rifampicin, and amoxicillin. *Pseudomonas* exhibited the highest multidrug resistance pattern with a Multiple Antibiotic Resistance Index (MARI) of 0.5, followed by *Escherichia coli* (0.4). MARI values above 0.2 are generally interpreted as evidence that bacterial populations originate from environments where antibiotics are frequently used or misused. Consequently, the observed resistance profiles suggest previous exposure of these bacteria to antimicrobial selection pressure within livestock production, contaminated environments, or human activities (Yusuf *et al.*, 2022, WHO, 2025).

The continued effectiveness of fluoroquinolones and aminoglycosides observed in this study is consistent with recent Nigerian reports showing relatively high susceptibility of foodborne Enterobacterales to ciprofloxacin and gentamicin. However, increasing resistance to β -lactam antibiotics has become widespread due to indiscriminate antibiotic use in veterinary and human medicine. The detection of resistant *Escherichia coli* and *Pseudomonas* in ready-to-eat dairy products therefore represents a potential public health concern because these organisms may transfer resistance genes to other bacteria within the human gastrointestinal tract.

Among the Gram-positive isolates, *Staphylococcus* demonstrated resistance to ampiclox, amoxicillin, and azithromycin, with a MARI value of 0.3, while *Bacillus* showed intermediate resistance (0.2). In contrast, *Micrococcus* remained susceptible to all tested antibiotics (MARI = 0.0). The relatively higher resistance observed in *Staphylococcus* may be attributed to its frequent exposure to antibiotics within human communities and dairy production systems. Resistant staphylococci are increasingly recognized in milk and dairy products and may possess mobile genetic elements that facilitate dissemination of resistance determinants (Zhang *et al.*, 2023). Although methicillin resistance was not specifically investigated in this study, the resistance pattern observed emphasizes the importance of continuous surveillance of staphylococci isolated from foods of animal origin.

The complete susceptibility of *Micrococcus* suggests limited antimicrobial exposure or lower intrinsic resistance compared with pathogenic staphylococci. Nevertheless, its presence still reflects inadequate hygienic handling because *Micrococcus* is commonly associated with human skin and environmental surfaces. The combination of high microbial counts and antimicrobial resistance has important implications for food safety in Owo metropolis. Street-vended wara is widely consumed without further cooking, meaning that consumers are directly exposed to viable bacteria and potentially resistant strains. The occurrence of *Escherichia coli*, *Klebsiella*, and *Staphylococcus* indicates risks of gastrointestinal infections, food poisoning, urinary tract infections, and opportunistic infections among vulnerable populations (Zhao *et al.*, 2023).

From a One Health perspective, antimicrobial resistance should no longer be regarded solely as a hospital problem but also as an environmental and food-chain challenge. Resistant bacteria circulating within dairy foods may originate from untreated animal infections, contaminated water, indiscriminate veterinary antibiotic use, or poor waste management practices. Their transmission through ready-to-eat foods contributes to the broader environmental resistome and complicates future treatment of bacterial infections (Zhou *et al.*, 2022). Recent global reports have emphasized that integrated surveillance of food, humans, animals, and the environment is essential for combating antimicrobial resistance effectively (Zwietering *et al.*, 2023). Overall, the findings indicate that the microbiological quality of street-vended wara sold in Owo falls below desirable food safety standards and underscore the urgent need for improved hygiene throughout production, transportation, storage, and retail. Regular microbiological monitoring, vendor education, and enforcement of food hygiene regulations would substantially reduce consumer exposure to pathogenic and antimicrobial-resistant bacteria.

Conclusion

This study demonstrated that street-vended wara sold in Owo, Ondo State harbours substantial bacterial contamination and serves as a potential reservoir of antimicrobial-resistant bacteria. High total heterotrophic, enteric, coliform, and staphylococcal counts indicate inadequate hygienic practices during production and retail. The isolation of clinically important genera, including *Escherichia coli*, *Klebsiella*, *Pseudomonas*, *Staphylococcus*, *Enterobacter*, *Bacillus*, and *Micrococcus*, further confirms the microbiological risks associated with the consumption of traditionally processed ready-to-eat dairy products. Antimicrobial susceptibility testing revealed encouraging activity of fluoroquinolones and gentamicin, but significant resistance to ampiclox, azithromycin, rifampicin, and amoxicillin was observed, particularly among *Pseudomonas* and *E. coli*, indicating prior exposure to antibiotic selection pressure. The findings highlight the need for improved sanitary practices among wara producers and vendors, routine microbiological surveillance of traditional dairy products, prudent antibiotic use in livestock production, and strengthened food safety regulations. Implementing these measures will contribute to reducing foodborne diseases and limiting the dissemination of antimicrobial resistance through the food chain.

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