

## Antibiotic Susceptibility of Contaminated Cheese “Wara” in Owo, Ondo State

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### Abstract

**Background:** Cheese is one of the products gotten from milk. The manufacturing process of soft cheese in Nigeria is lagging behind and as a result, there is difficulty in production, storage (extending shelf life) and preserving the nutritious components of milk. Food safety is a global concern and must be taken seriously therefore this study was designed to investigate possible bacterial contamination of locally made cheese (Wara) and antibiotic susceptibility pattern of the bacterial isolates. **Methodology:** A total number of twenty samples of “Wara” were purchased from four different locations in Owo, Ondo State, Nigeria. Bacteriological culture was done using standard methods. The identification of the isolates was based on morphological and biochemical characteristics exhibited by the isolates. **Results:** Four bacteria were isolated from locally made cheese sold in Owo. They were Citrobacter (20%), *Escherichia coli* (50%), Enterobacter (15%) and Klebsiella (15%) respectively. The Total viable count of Citrobacter was  $6.2 \times 10^8$  CFU/ml, *Escherichia coli* was  $4.8 \times 10^8$  CFU/ml, Enterobacter was  $3.3 \times 10^8$  CFU/ml and Klebsiella was  $5.8 \times 10^8$  CFU/ml respectively. **Conclusion:** The majority of isolates were susceptible to Imipenem, Ofloxacin, Gentamycin, Levofloxacin and Ceftriaxone, but was resistant to Nitrofuranton. Cefuroxime and Cefexime. There is a need to standardise the production methods so that the cheese will be free from any form of contamination.

**Keywords:** Cheese, bacterial contamination, antibiotics, culture, isolation.

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### Introduction

Soft cheese also called wara. It was named ‘wara’ by one of the ethnic languages in Nigeria (Yoruba). Soft cheese is a very nutritious food obtained from cow milk. Milk is an aqueous suspension of proteins, fats, and carbohydrates that contains numerous vitamins and minerals. Cheese is one of the products gotten from milk (1). Cheeses are milk-based food that is

consumed all over the world ranging from Africa, Asia and European countries. The large acceptance of cheeses by consumers worldwide can be attributed to their pleasant sensorial characteristics, good nutritional properties, and versatility and by the advent of innovative products with novel ingredients, packaging, and sale formats (2). Cheese is common in many countries (3) because of the associated health associated

advantages and the taste. One of the health associated advantages includes probiotics that can be used for therapeutic reasons and anti-tumor properties. Additionally, cheese is a rich source of dietary calcium, phosphorus, and proteins (4), and has been shown to reduce the incidence of type II diabetes (5). The manufacturing process of soft cheese in Nigeria is insufficient and its left for the natives to manufacture locally, hence there is difficulty in prolonging shelf life and preserving the nutritious components of milk. Process of cheese-making, ripening and storage can be a cause of cheese contamination. Transmission of bacterial can also be as a result of contamination during processing. Cheese however has been shown to have antimicrobial properties and prevent disease. It has been used as drug for certain infection when common antimicrobial agents failed. It has also been found that soft cheese has growth inhibitory activity against common bacteria that causes diarrhoea in Southwest Nigeria (6). Due to the high consumption rate of soft cheese and in view of manner of cheese production in Nigeria, there was need to determine the microbial quality which will be of utmost public health importance.

## **Materials and Methods**

### **Study Area**

The study was carried out in Owo, Ondo State; a rural community in Owo Local Government Area (LGA) located in Ondo-South District, South-West Nigeria. The major occupations of the inhabitants are farming, timber felling and lumbering.

### **Sample Collection and processing**

Twenty samples of locally made cheese 'wara' were collected from different locations in Owo.

### **Methodology**

Twenty samples of soft cheese with the whey (water portion) were bought from hawkers and point of production at different locations in Owo, Ondo state. The soft cheese and the whey were put inside a

sterile plastic universal container and labeled accordingly.

### **Cheese samples**

Twenty samples of soft cheese with the whey (water portion) were bought from hawkers and point of production at different locations in Owo, Ondo state; Ikare junction, local government, Rufus Giwa polytechnic and point of production. The soft cheese and the whey were put inside a sterile plastic universal container. The samples were transported immediately to the laboratory for processing or kept inside a refrigerator for few minutes if delay was envisaged.

### **Microbial Count and Analysis**

Viable microbial count analyses were performed on samples of cheese as follows; a 10-fold serial dilution of up to 10<sup>-10</sup> for each sample was prepared in 0.1% peptone water. For viable bacterial count, each dilution was subsequently plated onto standard plate count agar (PCA). The PCA plates were incubated at 37°C for 48 hours. The colony forming units (CFU) were counted on plates having between 30 and 300 colonies using Quebec colony counter. The enumeration of viable bacteria count was carried out in duplicate on each sample and the isolated bacteria was identified using standard bacteriological procedures.

One gram proportion of cheese samples was aseptically taken (after thorough mixing) and weighed into a beaker containing 9 ml of 0.1% sterile peptone water (w/v). Ten-fold serial dilution was subsequently prepared by transferring 1ml aliquot of the mixture into 9ml sterile peptone water as diluent. Further serial dilution was carried out and thereafter, 1ml of appropriate dilution was aseptically plated using pour plate technique for total viable bacterial count on plate count agar, chocolate agar, mannitol salt agar (Oxoid), McConkey agar (Oxoid). The media were prepared according to the manufacturers' instructions.

### **Isolation, Characterization, and Identification of microorganisms**

Isolation and identification of bacterial pathogens was determined following aseptic

sampling techniques. Briefly: a loop full (0.01ml) of cheese sample was streaked on 7% blood agar and incubated at 37°C. The plate was checked for bacteria growth after 24, 48 and 72 hours to rule out slow growing microorganisms and sub-cultured on blood agar at 37°C for 24 hours to get pure culture. A single colony from a pure culture was subjected to Gram's stain to observe morphological characteristics and it was transferred to MacConkey agar.

The bacteria isolates were identified phylogenetically using both morphology and biochemical reactions. Biochemical test such as catalase, coagulase test (tube and slide method), indole, citrate, urease, and oxidase tests.

### Colonial characteristics

Some isolates appeared singly, in pairs, tetrads, short chain. Parameters like size, shape, odour, colour, elevation, edges, pigmentation and their lactose fermenting ability was used in the identification of the organisms.

### Results

Table 1 showed the bacteria isolated from locally made cheese "Wara" sold in Owo, Ondo State. The results obtained showed that four bacteria were isolated from locally made cheese sold in Owo. They were Citrobacter (20%), *Escherichia coli* (50%), Enterobacter (15%) and Klebsiella (15%) respectively. Table 2 showed the prevalence of bacteria found in locally made cheese "Wara" sold in Owo based on location. Four locations were used in this study namely; Ikare junction, Local government, Rufus Giwa Poly and Point of Production. The results obtained showed that at Ikare Junction, the organisms isolated were Citrobacter (15%) and *Escherichia coli* (10%). At the Local Government, the organisms isolated were Citrobacter (5%), *Escherichia coli* (10%), and Enterobacter (10%). At Rufus Giwa Polytechnic, only *Escherichia coli* (25%) were isolated.

Finally, at the point of production *Escherichia coli* isolated was 1 (5%), Enterobacter isolated was 1 (5%) and Klebsiella isolated were 3 (15%) respectively. Table 3 showed the Total Viable Counts (TVC) of the bacteria isolated from locally made cheese "Wara" in Owo. The results obtained showed that the TVC of Citrobacter was  $6.2 \times 10^8$  CFU/ml, *Escherichia coli* was  $4.8 \times 10^8$  CFU/ml, Enterobacter was  $3.3 \times 10^8$  CFU/ml and Klebsiella was  $5.8 \times 10^8$  CFU/ml respectively. The antibiotic susceptibility of bacteria isolates from "Wara" was presented in Table 4. The results obtained showed that the antibiotics susceptibility for Citrobacter were as follows; IMP-Imipenem (0%), OFL-Ofloxacin (100%), GEN-Gentamycin (100%), CRX-Cefuroxime (0%), CEF-Cefexime (0%), LEV-Levofloxacin (100%), NAC-Nalidixic Acid (75%), NIT-Nitrofuraxon (0%) and CTR-Ceftriaxone (100%). Similarly, the antibiotics susceptibility for *Escherichia coli* were; IMP-Imipenem (10%), OFL-Ofloxacin (90%), GEN-Gentamycin (90%), CRX-Cefuroxime (20%), CEF-Cefexime (10%), LEV-Levofloxacin (60%), NAC-Nalidixic Acid (10%), NIT-Nitrofuraxon (40%) and CTR-Ceftriaxone (100%) respectively. Furthermore, the antibiotics susceptibility for Enterobacter were as follows; IMP-Imipenem (0%), OFL-Ofloxacin (66.7%), GEN-Gentamycin (66.7%), CRX-Cefuroxime (0%), CEF-Cefexime (66.7%), LEV-Levofloxacin (33.3%), NAC-Nalidixic Acid (66.7%), NIT-Nitrofuraxon (33.3%) and CTR-Ceftriaxone (100%). Finally, the antibiotics susceptibility of Klebsiella were as follows; IMP-Imipenem (66.7%), OFL-Ofloxacin (100%), GEN-Gentamycin (66.7%), CRX-Cefuroxime (33.3%), CEF-Cefexime (0%), LEV-Levofloxacin (0%), NAC-Nalidixic Acid (0%), NIT-Nitrofuraxon (0%) and CTR-Ceftriaxone (100%) respectively.

**Table 1: Prevalence of bacteria found in locally made cheese “Wara” in Owo**

Bacteria Isolated	Number Isolated	Percentage (%)
Citrobacter	4	20.00
<i>Escherichia coli</i>	10	50.00
Enterobacter	3	15.00
Klebsiella	3	15.00
<b>Total</b>	<b>20</b>	<b>100</b>

**Table 2: Prevalence of bacteria found in locally made cheese “Wara” sold in Owo based on location**

Location	Citrobacter	<i>Escherichia coli</i>	Enterobacter	Klebsiella sp
Ikare Junction	3 (15.0%)	2 (10.0%)	0(0.0)	0(0.0)
Local Government	1 (5.0%)	2 (10.0%)	2 (10.0%)	0(0.0)
Rufus Giwa Poly	0(0.0)	5 (25.0%)	0(0.0)	0(0.0)
Point of Production	0(0.0)	1 (5.0%)	1 (5.0%)	3 (15.0%)
<b>Total</b>	<b>4 (20.0%)</b>	<b>10 (50.0%)</b>	<b>3 (15.0%)</b>	<b>3 (15.0%)</b>

**Table 3: Total Viable Counts (TVC) of the Organisms isolated from locally made cheese “Wara” in Owo**

Bacteria Isolated	Number Isolated	Counts of microorganisms (CFU/ml)	
		Mean	Range
Citrobacter	4	6.2x10 <sup>8</sup>	3.6 – 9.5
<i>Escherichia Coli</i>	10	4.8 x 10 <sup>8</sup>	1.09 – 9.6
Enterobacter	3	3.3 x 10 <sup>8</sup>	1.6 – 4.7
Klebsiella	3	5.8 x 10 <sup>8</sup>	4.9 – 7.2

**Key:** CFU – Colony Forming Unit

**Table 4 Percentage Antibiotics Susceptibility of Bacterial Isolates from “Wara”**

Organisms	IMP	OFL	GEN	CRX	CEF	LEV	NAC	NIT	CTR
Citrobacter (n=4)	0.0%	100%	100%	0.0%	0.0%	100%	75.0%	0.0%	100%
<i>Escherichia Coli</i> (n=10)	10.0%	90.0%	90.0%	20.0%	10.0%	60.0%	10.0%	40.0%	100%
Enterobacter (n=3)	0.0%	66.7%	66.7%	0.0%	66.7%	33.3%	66.7%	33.3%	100%
Klebsiella (n=3)	66.7%	100%	66.7%	33.3%	0.0%	0.0%	0.0%	0.0%	100%

**Keys:** IMP – Imipenem, OFL – Ofloxacin, GEN – Gentamycin, CRX – Cefuroxime, CEF – Cefexime, LEV - Levofloxacin, NAC - Nalidixic Acid, NIT – Nitrofuraxon, CTR – Ceftriaxone

## Discussion

Locally made cheese called “Wara” gets spoiled when attacked by pathogenic bacteria, as a result of poor hygiene practice by the producer and handlers (7). The main reasons for cheese-related outbreaks of food poisoning may include poor starter activity due to bacteriophage or the presence of antibiotics, poor hygiene, gross environmental contamination, or faulty pasteurization of the cheese milk, poor packaging and storage.

This study was carried out to identify some common bacteria present in locally produced cheese in Owo, Ondo state. The results obtained showed that four bacteria were isolated from locally made cheese sold in Owo. They are; *Citrobacter* (20.0%), *Escherichia coli* (50.0%), *Enterobacter* (15.0%) and *Klebsiella* species (15.0%) respectively. This finding is in agreement with previous study by (8) who reported the presence of *Salmonella*, *Klebsiella*, *E. coli* and *Staphylococcus* in Wara. This finding is similar to the report of (7) that Fulani could contaminate the product through the unhygienic practice which may have contributed to the high level of bacteria found in this study.

The results of this study also showed that the total viable counts (TVC) of *Citrobacter* was  $6.2 \times 10^8$  CFU/ml, *Escherichia coli* was  $4.8 \times 10^8$  CFU/ml, *Enterobacter* was  $3.3 \times 10^8$  CFU/ml and *Klebsiella* was  $5.8 \times 10^8$  CFU/ml respectively. The high TVC observed in some Wara samples in this study agrees with the findings of (9). The high total bacterial count in this study might be attributed to the use of low quality milk. According to (10), the microbial quality of raw milk is crucial for the production of any high quality dairy food. It could also be as result of unsanitary conditions by the producers and vendors during processing and handling of the cheese. Mishandling and disregard of hygienic measures on the part of the food vendors may enable pathogens to come into contact with foods and in some cases to survive and multiply in

sufficient numbers to cause illness in the consumer (11).

Although there was no significant difference ( $p > 0.05$ ) in the microbial load of organisms isolated from locally made cheese (Wara), the samples consistently contained *E. coli* and *Klebsiella* species; this is similar to the study of (12) where 98% of milk samples grew *E. coli*. Also, retail surveys by (13) in Italy of soft and semi cheeses found *E. coli* in 40–50% of these cheeses demonstrating that a mode of contamination of *E. coli* exists during the chain of production or processing. *E. coli* is a consistent inhabitant of the human intestinal tracts and regular presence of the bacterium in the human intestine and faeces has led to tracking the bacterium in nature as an indicator of faecal pollution. Through this, it means wherever *E. coli* was found, there may be faecal contamination (14).

The occurrence of Enterobacteriaceae family such as *Escherichia*, *Klebsiella* and *Enterobacter* as found in this study with cheese could be relatively safe food, if boiled before consumption but can cause serious health condition through consumption of the contaminated cheese product as reported by (15) that contamination of cheese by coliform and other pathogenic bacteria could constitute health hazard. The methods of transportation, handling and sale of cheese or cheese products are not hygienic or sterile enough. One major observable problem is that the producers or vendors are really not educated and locals which consequentially affect the methods and handling of these products. It had earlier opined by (16) that unclean hands of workers, poor quality of materials used and water supplied for washing utensils could be the source of accelerating the bacterial contamination of milk products.

In this study, *E. coli* was susceptible to Imipenem, Ofloxacin, Gentamycin, Levofloxacin and Ceftriaxone, but was resistant to Imipenem, Cefuroxime, Cefexime Nalidixic Acid and Nitrofuraxon respectively which is similar to the work of

(17). *Enterobacter spp.* was susceptible to Ofloxacin, Gentamycin, Cefexime, Nalidixic Acid and Ceftriaxone but resistant to Imipenem, Cefuroxime, Levofloxacin and Nitrofuraxon which is consistent with the study by (18). *Klebsiella spp.* showed susceptibility to Imipenem, Ofloxacin, Gentamycin, and Ceftriaxone, but grossly resistant to Cefuroxime, Cefexime, Levofloxacin, Nalidixic Acid and Nitrofuraxon which is similar to report of (19). *Citrobacter spp.* was susceptible to Ofloxacin, Gentamycin, Levofloxacin,

Nalidixic Acid and Ceftriaxone, but resistant to Imipenem, Cefuroxime, Cefexime and Nitrofuraxon which is in agreement with the study by (17).

### Conclusion

The study concludes that four pathogenic bacteria were isolated from locally made cheese sold in Owo which were *Citrobacter*, *Escherichia coli*, *Enterobacter* and *Klebsiella* respectively. All the organisms were sensitive to Ofloxacin, Gentamycin and Ceftriaxone but resistant to Cefuroxime and Nitrofuraxon.

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