Prevalence and Antibiotic Sensitivity Patterns of *Salmonella enterica* serovar *typhimurium* Isolated from Local Cheese in Ondo State, Nigeria

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Authors’ contributions

This work was carried out in collaboration between both authors. Author ODO carried out the experiments, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AKO designed and managed the analyses of the study. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/SAJRM/2019/v5i3013

Editor(s):
(1) Dr. Obafemi Yemisi Dorcas, Assistant Lecturer, Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria.

Reviewers:
(1) Devrim Dundar, Kocaeli University, Turkey.
(2) Kálmán Imre, Banat's University of Agricultural Sciences and Veterinary Medicine, Romania.

Complete Peer review History: http://www.sdiarticle4.com/review-history/54537

Received 06 December 2019
Accepted 12 February 2020
Published 19 February 2020

ABSTRACT

**Aims:** To determine the prevalence and current sensitivity status of *Salmonella enterica* serovar *typhimurium*. Typhimurium to commercial antibiotics in cheese at the Southern Western part of Nigeria.

**Study Design:** Experimental design.

**Place and Duration of Study:** Cheese samples were bought from nomad cheese vendors across various suburbs in three major districts (Akure, Ikare and Owo) of Ondo state.

**Methodology:** A total of two hundred and sixty (260) cheese samples were vend from various suburbs across the aforementioned senatorial districts of Ondo State. Isolation of *Salmonella enterica* serovar typhimurium. Typhimurium from cheese samples were carried out on Salmonella-shigella (S.S) and were identified biochemically through standard microbiological techniques. The identified strains were subjected to ten (10) conventional antibiotics for their sensitivity patterns.

**Results:** The distribution patterns of the *S. enterica* serovar typhimurium. Typhimurium isolated were 9(47.4%), 8(42.10%) and 2(10.5%) in Ikare, Akure, and Owo respectively. The degree of resistance of the *Salmonella enterica* serovar *typhimurium*. Typhimurium to the various antibiotics

*Corresponding author: Email: lololadapo@gmail.com;
The presence of *Salmonella enterica* serovar *typhimurium* poses serious threat to human health and food antimicrobial sensitivity. *Salmonella enterica* serovar *typhimurium* is a primary enteric pathogen infecting both humans and animals. Infection begins with ingestion of contaminated food or water which allows the passage of *Salmonellae* into intestinal epithelium of the human system and trigger gastrointestinal disease [1]. However, enteric organisms like *Salmonella, E. coli* and *Shigella* have dominated in destroying the antimicrobial importance of Cheese. However, *Salmonella* is the most common etiological agent of cases and outbreak of food-borne diarrheal illnesses. The emergence and spread of *Salmonella* spp which has become multi-drug resistant and potentially more pathogenic, have increased the concern with this pathogen [2]. For *Salmonella enterica* serovar *typhimurium* to cause any infection, the presence of O and H antigens is essential which indicate the somatic and the flagella strains of genes respectively, i.e. the invasion and the spread of the pathogen [3]. Mostly, *Salmonella enterica* serovars are found worldwide in warm-blooded animals. They are intracellular pathogens which means that they reside inside the body of living host. Salmonellosis is associated with severe morbidity and even mortality in farm animals representing a major economic productivity loss in the food and animal industries. Hence, it is systematic infection caused by *Salmonella* species and serotypes. The most common clinical symptoms range from headache, fever, abdominal cramps and vomiting. Young and Old individuals especially those with weakened immune system, either they are immunocompromised or immunosuppressed, are bound to develop severe form of salmonellosis like reactive arthritis, irritable bowel syndrome.

Enteric infections caused by *Salmonella enterica* still continues to be a major public health problem in developing countries like Nigeria. It is expedient for all living organisms to carry out all actions for growth and survival. However, if this survival is based on natural selection, it becomes harmful to other organisms. These actions are encoded and directed in the gene inside or outside the chromosome passed on from one generation to another, which can either be vicious or edifying trait. Due to increased risk of gastro intestinal tract (GIT) infections associated with dairy product, there is a need to review the sensitivity of *Salmonella enterica* serovar *typhimurium* stating the virulence genes associated with site of infection, growth of organisms, point of establishing infection and spread of organisms.

Cheese is a popular safe food, produced in many countries because of its health benefits and flavour. The health benefit revolves around its natural probiotic 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. However, the prevalence of foodborne pathogens in milk product is influenced by factors such as farm animal populations, hygiene, farm management practices and geographical location. Lactic acid bacteria strengthen the quality of cheese by playing a key role in the prevention of coliform. However, studies have shown that various enteric pathogens such as *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans*, are found to proliferate in cheese, despite the lactic acid concentrations present in cheese, [5]. Therefore, this study is planned to check the prevalence and antibiotic sensitivity patterns of *Salmonella enterica* serovar *typhimurium* isolated from cheese in Southern Western Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The Areas of study are Ikare, Owo and Akure which are located at the Northern, and Central part of Ondo state respectively. Ondo State is a

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**Keywords:** *Salmonella enterica* serovar *typhimurium*; cheese; antibiotics; multidrug resistant.

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**INTRODUCTION**

The presence of *Salmonella enterica* serovar *typhimurium* poses serious threat to human health and food antimicrobial sensitivity. *Salmonella enterica* serovar *typhimurium* is a primary enteric pathogen infecting both humans and animals. Infection begins with ingestion of contaminated food or water which allows the passage of *Salmonellae* into intestinal epithelium of the human system and trigger gastrointestinal disease [1]. However, enteric organisms like *Salmonella, E. coli* and *Shigella* have dominated in destroying the antimicrobial importance of Cheese. However, *Salmonella* is the most common etiological agent of cases and outbreak of food-borne diarrheal illnesses. The emergence and spread of *Salmonella* spp which has become multi-drug resistant and potentially more pathogenic, have increased the concern with this pathogen [2]. For *Salmonella enterica* serovar *typhimurium* to cause any infection, the presence of O and H antigens is essential which indicate the somatic and the flagella strains of genes respectively, i.e. the invasion and the spread of the pathogen [3]. Mostly, *Salmonella enterica* serovars are found worldwide in warm-blooded animals. They are intracellular pathogens which means that they reside inside the body of living host. Salmonellosis is associated with severe morbidity and even mortality in farm animals representing a major economic productivity loss in the food and animal industries. Hence, it is systematic infection caused by *Salmonella* species and serotypes. The most common clinical symptoms range from headache, fever, abdominal cramps and vomiting. Young and Old individuals especially those with weakened immune system, either they are immunocompromised or immunosuppressed, are bound to develop severe form of salmonellosis like reactive arthritis, irritable bowel syndrome.

Enteric infections caused by *Salmonella enterica* still continues to be a major public health problem in developing countries like Nigeria. It is expedient for all living organisms to carry out all actions for growth and survival. However, if this survival is based on natural selection, it becomes harmful to other organisms. These actions are encoded and directed in the gene inside or outside the chromosome passed on from one generation to another, which can either be vicious or edifying trait. Due to increased risk of gastro intestinal tract (GIT) infections associated with dairy product, there is a need to review the sensitivity of *Salmonella enterica* serovar *typhimurium* stating the virulence genes associated with site of infection, growth of organisms, point of establishing infection and spread of organisms.

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## 2. MATERIALS AND METHODS

### 2.1 Study Area

The Areas of study are Ikare, Owo and Akure which are located at the Northern, and Central part of Ondo state respectively. Ondo State is a
Onifade and Omololu; SAJRM, 5(3): 1-10, 2019; Article no.SAJRM.54537

western state of Nigeria created on February 3, 1976 where Akure is the state capital which lies between latitudes 7.25° N and longitude 5.19° E. Ikare location lies between latitudes, 7.2° N; 7.0° N and longitudes 5.59° E. Owo, on the other hand, is located between latitude, 7.2° N and longitude, 5.59° E. Ondo state is densely populated with land mass of an approx. of 15,500 km². The Consensus of Ondo state was measured to be 3,441,024 residents as at 2006 i.e. 1,761,263 males and 1,679,761 females.

2.2 Collection of Cheese Samples

Local soft cheese samples, indigenously known as Wara, were bought from vendors at suburbs, streets and counties in Akure, Ikare and Owo district of Ondo state, Nigeria. They were stored in a cooler, averagely filled with ice packs and thereafter, it is being transferred to the laboratory. However, Fresh raw milk of a healthy cow was the positive control experiment while water source which were used for the cooking of cheese product was the negative control experiment.

2.3 Isolation of Salmonella enterica serovar typhimurium

The samples were processed in sterile conditions. Salmonella enterica serovar typhimurium were isolated using Salmonella-Shigella agar through Standard microbiological techniques. The media was prepared according to the manufacturers’ instruction and sterilized by autoclaving at 121°C for 15 min [6]. One gram (1 g) of each food sample was comminute in 9 mL of sterile 0.1% peptone water, shaken vigorously for a minute and serially diluted. One milliliter of each sample was plated in duplicates using the pour-plate method on Salmonella Shigella (S.S) Agar and the plates were incubated aerobically at 37°C for 24 hours. Colonial growth on the plates were enumerated and recorded after 24 hours while the representative S. enterica serovar Typhimurium cells were also purified by repeated streaking on fresh Salmonella Shigella (SS) agar plates. Each Salmonella species was later stored on double strength nutrient agar slants at 4°C until when needed [7].

2.4 Enumeration of Bacterial Colony

Colony counting was carried out visually by counting the number of visible colonies that appeared on the plates, plate that has a distinct colony was used. Calculation of colony forming unit (CFU) per gram for the bacteria was based on the form. The number of colonies on each plate was recorded [8].

2.5 Confirmation of Bacterial Isolates

Cultural, morphological and biochemical characteristics of bacterial isolates obtained from the cheese samples were carried out as described by Abhishek [9]. Colonial characteristics observed were colour, edge, shape, surface, elevation. The bacterial isolates were confirmed through API 20E kit.

2.6 Standardization of the Inoculum

Method modified by Cheesbrough [7], was used to prepare the McFarland 0.5 turbidity standard which was used to measure the density of bacterial cells. This was done by preparing a 1% solution of anhydrous barium chloride (BaCl₂). 1% solution of sulfuric acid (H₂SO₄) was prepared. Thereafter, both barium chloride and sulfuric acid solutions were completely mixed together in a sterile conical flask to form a turbid suspension of BaSO₄ in a specific proportion for each McFarland turbidity standard. Hence, the resulting mixture was stored in a foil-covered screw-cap test tube as a MacFarland standard at room temperature (25°C) when not in use.

2.7 Antibiotic Sensitivity Test

The standardized Salmonella isolates were subjected to susceptibility testing using the disk diffusion techniques as recommended by Clinical and Laboratory Standard Institute (CLSI) guidelines [10]. All isolates were grown on Mueller-Hinton agar plates for the antimicrobial susceptibility test and adjusted to 0.5 MacFarland. The following antimicrobial agents were used; Septrin (30 µg), Chloramphenicol (30 µg), Perflloxacin (10 µg), Tarivid.Olfloxacin (10 µg), Sparfloxacin (10 µg), Streptomycin (30 µg) Ciprofloxacin (10 µg), Amoxicillin (30 µg), Gentamycin (10 µg). Resistance was determined by placing on Mueller-Hinton agar plate without NaCl and incubated for 24 hrs at 35°C. The resistance was confirmed using CLSI published guidelines for the agar screen test [10,11].

2.8 Data Analysis

Data obtained were subjected to two-way Analysis of Variance (ANOVA) and treatment means were separated using Duncan’s New
Multiple Range Test (DMRT) at p≤0.05 level of significance with the aid of Statistical Package for Social Sciences (SPSS) version 23.

3. RESULTS AND DISCUSSION

3.1 Results

The cheese samples were obtained from three (3) senatorial district of Ondo state. A total of 260 cheese samples were studied for the presence and the virulence patterns of Salmonella enterica serovar typhimurium as described in (Table 1). Fifty (50) Cheese samples were vended from Ikare provinces with varying locations existing from transit garages, and market square. At Akure, one hundred and thirty (130) cheese samples were vended from bus stops, Car parks, suburbs, as well as market square. The remaining cheese samples were vended across towns in Owo province, as illustrated in the table. A Total of 20 pure strains were analyzed for further investigations. The mean colony forming units of enteric pathogen in local dairy products are shown in Table 2. Ikare exhibited highest mean pure strains of Salmonella enterica spp of 2.25±0.95b.

From Tables 4 and 5, sixteen (16) pure strains of supposed Salmonella enterica spp were analysed for morphological and biochemical characteristics. This enteric pathogen was isolated from Salmonella – Shigella (S.S) agar and Salmonella spp were identified immediately by their black colour and sulphuric odour as the agar is selective for the said organism. They are mostly gram negative with short rod formed in either scattered or in clusters. Notwithstanding, series of biochemical test were carried out to confirm the presence of Salmonella enterica spp. Out of the Sixteen (16) bacterial isolates tested, fourteen (14) were positive for Salmonella enterica spp. The remaining two (2) isolates are E. coli and Shigella.

Table 1. The total plate count of enteric pathogens in cheese samples on Salmonella-Shigella (SS) agar (CFU/ML)

| Town/City | Week | Samples (Cheese) | Number of enteric pathogens (CFU/ML) | Pure strains of Salmonella enterica spp |
|-----------|------|------------------|--------------------------------------|----------------------------------------|
| Ikare     | 1    | 10               | 81 x 10^3                             | 1                                      |
|           | 2    | 10               | 50 x 10^3                             | 5                                      |
|           | 3    | 10               | 57 x 10^3                             | 2                                      |
|           | 4    | 20               | 86 x 10^3                             | 1                                      |
| Akure     | 5    | 20               | 190 x 10^3                            | Nil                                    |
|           | 6    | 20               | 133 x 10^3                            | Nil                                    |
|           | 7    | 20               | 157 x 10^3                            | 2                                      |
|           | 8    | 20               | 177 x 10^3                            | 2                                      |
|           | 9    | 10               | 183 x 10^3                            | 1                                      |
|           | 10   | 15               | 127 x 10^3                            | 2                                      |
|           | 11   | 15               | 90 x 10^3                             | 1                                      |
| Owo       | 12   | 10               | 109 x 10^3                            | Nil                                    |
|           | 13   | 21               | 187 x 10^3                            | 1                                      |
|           | 14   | 20               | 115 x 10^3                            | Nil                                    |
|           | 15   | 19               | 134 x 10^3                            | Nil                                    |
|           | 16   | 20               | 145 x 10^3                            | 1                                      |
| Grand Total|      | 2021             | 19                                    |                                        |

Table 2. The mean total plate count of enteric pathogen in Local dairy products across selected towns in Ondo State

| Town/City | Samples (Cheese) | Number of colonies (CFU/ML) | Pure strains of Salmonella enterica spp |
|-----------|------------------|----------------------------|----------------------------------------|
| Ikare     | 12.50±2.50a      | 68.500±8.84a               | 2.25±0.95b                             |
| Akure     | 16.25±1.57ab     | 145.75±12.97b              | 1.00±0.32ab                            |
| Owo       | 20.00±0.00c      | 126.31±11.36b              | 0.50±0.28a                             |

Data are presented in mean ±standard deviation (where n=3). Values carrying the same alphabet in the same column are not significantly different (p>0.05) using Duncan’s New Multiple Range test.
Table 3. The Percentage frequency of occurrence of *Salmonella enterica* spp in Cheese samples across towns

| Town/City | All Cheese samples studied | Number of Cheese tested | Number positive (%) |
|-----------|----------------------------|-------------------------|---------------------|
| Akure     | 130                        | 8(0.68%)                |
| Ikare     | 50                         | 9(3.28%)                |
| Owo       | 80                         | 2(0.34%)                |

Table 4. Morphological characterization of pure isolates from Cheese samples

| Isolates | Morphological Characteristics | Gram reaction | Cell shape | Cell arrangement | Reaction |
|----------|-------------------------------|---------------|------------|------------------|----------|
| IK1      | Round, Undulate, Raised, Smooth, Black | Cocci | Clusters | - | - |
| IK2      | Irregular, Undulate, Flat, Smooth, Black | Rod | Scattered | - | - |
| IK3      | Irregular, Lobate, Raised, Smooth, Brown | Rod | Scattered | - | - |
| IK4      | Round, Undulate, Flat, Smooth, Black | Rod | Scattered | - | - |
| IK5      | Irregular, Undulate, Flat, Smooth, Black | Rod | Clusters | - | - |
| A1       | Irregular, Undulate, Flat, Smooth, Black | Rod | Clusters | - | - |
| A2       | Round, Undulate, Raised, Smooth, Black | Rod | Scattered | - | - |
| A3       | Round, Undulate, Flat, Smooth, Black | Rod | Clusters | - | - |
| A4       | Round, Lobate, Flat, Smooth, Black | Rod | Clusters | - | - |
| A5       | Irregular, Lobate, Round, Smooth, Black | Rod | Clusters | - | - |
| A6       | Irregular, Lobate, Flat, Smooth, Black | Rod | Clusters | - | - |
| A7       | Irregular, Lobate, Raised, Smooth, Black | Rod | Clusters | - | - |
| A8       | Irregular, Undulate, Flat, Smooth, Black | Rod | Clusters | - | - |
| W1       | Irregular, Lobate, Flat, Smooth, Black | Rod | Clusters | - | - |
| W2       | Round, Undulate, Raised, Smooth, Black | Rod | Scattered | - | - |
| W3       | Round, Undulate, Flat, Smooth, Black | Rod | Clusters | - | - |

*Key: A – Akure; Ik – Ikare; O – Owo; -(Negative)*

3.2 Discussion

The curiosity of traditionally produced raw milk cheeses over the years by many researchers showed that the safety of these products is extremely important and ought to be questioned frequently. The aim of this study was to examine the presence of potential food pathogenic bacteria after the process of small-scale cheese production in Ondo state, Nigeria. The prevalence of food-borne *Salmonella* was reported 81% from which, 50 samples were *S. enterica* serovar enteritidis and only 3 samples were *S. enterica* serovar *typhimurium*. *Typhimurium* [12,13].

Most food pathogenic organisms are psychrophilic and mesophilic in nature which cannot survive the temperature involved in Cheese making which ranges from 80°C to 100°C. However, the bacterial isolates gotten were derived based on the hygienic impact by the vendors. *Salmonella enterica* serovar *typhimurium* were isolated from selective media as fifteen (15) pure isolates was determined to be *S. enterica* serovar *typhimurium*. On the S.S agar plate, the bacteria appeared to be black and opaque. A positive result would show colonies with a black sheen, meaning the organism could ferment lactose to produce Hydrogen sulphide, while a negative, pink or creamy colour result would indicate the inability to do so.
Table 5. Biochemical characteristics of *Salmonella enterica* spp from cheese samples

| Isolates | Catalase | Motility | Indole test | Triple sugar Iron Test | Citrate | Urease | Methyl Red | Probable organism |
|----------|----------|----------|-------------|------------------------|---------|--------|------------|-------------------|
| W1       | +        | +        | -           | +                      | +       | +      | -          | Salmonella enterica serovar typhimurium |
| W2       | +        | +        | -           | +                      | +       | +      | -          | Salmonella enterica serovar typhimurium |
| W3       | +        | +        | -           | +                      | +       | +      | -          | Salmonella enterica serovar typhimurium |

| Key: A – Akure; Ik – Ikare; O – Owo; - (Negative); + (Positive). |

Table 6. Antimicrobial sensitivity profile of *Salmonella enterica* serovar typhimurium. Typhimurium strains

| CPX | AM | AU | GN | PEF | OFX | S | SXT | CH | SP |
|-----|----|----|----|-----|-----|---|-----|----|----|
| K1  | 27.33±0.67d | 22.00±1.15e | 22.67±1.33f | 24.00±1.15f | 26.87±0.67f | 29.33±0.67f | 24.67±0.67fg | 23.33±0.67de | 28.00±2.00e | 29.33±0.67f |
| K2  | 23.33±33c | 18.00±1.15d | 0.00±0.00a | 17.33±0.67c | 24.00±1.15cde | 29.33±0.67f | 16.67±0.67b | 14.00±0.00b | 26.67±1.33de | 24.67±0.67d |
| K3  | 28.00±1.15e | 13.33±0.67b | 14.00±0.00c | 20.67±0.67ode | 20.00±1.15c | 21.33±0.67cde | 17.33±0.67c | 25.33±0.67de | 28.00±1.15ef |
| K4  | 30.00±0.00f | 22.00±0.00e | 20.00±0.67e | 0.00±0.00a | 25.33±0.67de | 28.00±1.15ef | 0.00±0.00a | 20.67±2.67c | 28.00±1.15ef |
| K5  | 17.33±0.67b | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 18.67±0.67b | 13.33±0.67b | 0.00±0.00a | 0.00±0.00a | 16.67±0.67b |
| A1  | 28.00±2.00def | 15.00±1.00bc | 0.00±0.00a | 19.00±1.00cde | 22.00±0.00cd | 23.00±1.00d | 20.00±0.00cd | 16.00±0.00c | 15.00±1.00bc | 25.00±1.00de |
| A2  | 7.67±0.33a | 16.00±0.00cd | 0.00±0.00a | 20.67±0.67de | 21.33±0.67bc | 23.33±0.67d | 24.00±0.00f | 23.67±0.88de | 21.33±0.67c | 29.33±0.67 |
| A3  | 29.33±0.67f | 29.33±0.67f | 0.00±0.00a | 19.00±1.00cde | 23.33±0.67cd | 25.00±1.15de | 25.33±0.67gh | 28.00±1.15f | 29.33±0.67 | 27.33±0.67def |

Data are presented as mean ± S.E. Standard error (where n=3). Values carrying the same alphabet in the same column are not significantly different (p>0.05).

Legend: IK - Ikare, W - Owo, A - Akure, SXT - Septin (30 µg), CH - Chloramphenicol (30 µg), PEF - Perfoxacin (10 µg), CPX - Ciprofloxacin (10 µg), AM - Amoxicillin (30 µg), AU - Augmentin (30 µg), CN - Gentamycin (10 µg), SP - Streptomycin (30 µg).
Table 6 (Cont’d). Antimicrobial sensitivity profile of *Salmonella enterica* serovar *typhimurium*. *typhimurium* strains

|    | CPX | AM  | AU  | CN  | PEF | OFX | S  | SXT | CH  | SP  |
|----|-----|-----|-----|-----|-----|-----|----|-----|-----|-----|
| A4 | 28.67±0.67ef | 13.33±0.67b | 15.33±0.67c | 24.67±0.67f | 27.33±2.67f | 26.00±1.15de | 19.33±0.67c | 21.33±0.67d | 25.33±0.67de | 25.33±0.67def |
| A5 | 27.33±1.76de | 20.67±1.33e | 18.67±1.33d | 19.33±0.67cde | 22.00±0.00cd | 20.00±1.15c | 26.67±0.67hi | 23.33±0.67de | 28.00±1.15f | 28.00±1.15ef |
| W1 | 22.67±1.33cd | 17.33±1.76cd | 0.00±0.00a | 20.00±0.00de | 25.33±0.67de | 26.00±1.15de | 28.00±0.00i | 24.00±0.00e | 25.33±0.67de | 28.00±1.15ef |
| A6 | 29.33±0.67f | 0.00±0.00a | 0.00±0.00a | 14.67±0.67b | 24.00±1.15de | 19.33±1.76c | 23.33±0.67ef | 0.00±0.00a | 15.33±0.67b | 0.00±0.00a |
| A7 | 29.33±0.67f | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 23.33±0.67cd | 29.33±0.67f | 20.67±0.67cd | 13.33±0.67b | 24.00±1.15cd | 28.00±1.15ef |
| W2 | 28.67±0.67ef | 0.00±0.00a | 19.33±0.67e | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 20.67±0.67cd | 25.33±0.67de | 23.33±0.67cd | 29.33±0.67ef |
| W3 | 20.00±5.03bc | 16.67±0.67cd | 12.00±0.00b | 24.00±1.15f | 24.67±0.67f | 25.33±0.67de | 23.33±0.67f | 27.33±0.67f | 28.00±1.15f | 27.33±1.76def |
| A8 | 29.33±0.67f | 15.33±0.67bc | 15.33±0.67c | 21.33±0.67e | 14.67±0.67b | 29.33±0.67f | 22.67±1.33f | 24.00±1.15e | 25.67±0.33de | 21.67±0.33c |

Data are presented as mean ± S.E. Standard error (where n=3). Values carrying the same alphabet in the same column are not significantly different (p>0.05).

Legend: IK - Ikare, W - owo, A – Akure, SXT- Septrin (30 µg), CH - Chloramphenicol (30 µg) PEF- Perflroxacin (10 µg), OFX – Tarivid. Ofloxacin (10 µg), SP - Sparfloxacin (10 µg), S - Streptomycin (30 µg), CPX -Ciproflaxacin (10 µg), AM - Amoxicillin (30 µg), AU - Augmentin (30 µg), CN – Gentamycin (10 µg)

Table 7. Antimicrobial sensitivity screening of *Salmonella* isolates against commercially available antibiotics having their respective zones of inhibition across cities

|    | CPX | AM  | AU  | CN  | PEF | OFX | S  | SXT | CH  | SP  |
|----|-----|-----|-----|-----|-----|-----|----|-----|-----|-----|
| Ikare | 25.20±1.23a | 15.07±2.21a | 11.47±2.63a | 12.40±2.77a | 24.27±0.91b | 24.00±1.73b | 12.53±2.82a | 10.93±2.52a | 20.13±2.84a | 25.33±1.28a |
| Akure | 26.21±1.49a | 13.67±1.93a | 6.17±1.68a | 22.58±2.63b | 24.58±0.80b | 22.67±0.57b | 18.71±1.75b | 23.04±1.06a | 23.13±1.89a |
| Owo | 23.78±1.98a | 11.33±2.89a | 10.44±2.82a | 14.67±3.73a | 16.67±4.18a | 17.11±4.29a | 24.00±1.11b | 25.56±0.56c | 25.56±0.80a | 28.22±0.70a |

Legend: IK - Ikare, W - owo, A – Akure, SXT- Septrin (30 µg), CH - Chloramphenicol (30 µg) PEF- Perflroxacin (10 µg), OFX – Tarivid. Ofloxacin (10 µg), SP - Sparfloxacin (10 µg), S - Streptomycin (30 µg), CPX -Ciproflaxacin (10 µg), AM - Amoxicillin (30 µg), AU - Augmentin (30 µg), CN – Gentamycin (10 µg)
Table 8. Interpretations of Antibiotic sensitivity profiles of bacterial isolates

| Isolate | CPX | AM | AU | CN | PEF | OFX | S | SXT | CH | SP |
|---------|-----|----|----|----|-----|-----|---|-----|----|----|
| IK1     | I   | S  | S  | S  | S   | S   | S | S   | S  | S  |
| IK2     | I   | S  | R  | S  | S   | S   | S | I   | S  | S  |
| IK3     | I   | I  | I  | S  | S   | S   | S | S   | S  | S  |
| IK4     | I   | S  | S  | R  | S   | R   | R | R   | S  | S  |
| IK5     | R   | R  | R  | R  | R   | I   | R | R   | R  | I  |
| A1      | I   | I  | R  | S  | R   | S   | S | I   | S  | S  |
| A2      | R   | I  | R  | S  | R   | S   | S | S   | S  | S  |
| A3      | I   | S  | R  | S  | S   | S   | S | S   | S  | S  |
| A4      | I   | R  | I  | S  | S   | S   | S | S   | S  | S  |
| A5      | I   | S  | S  | R  | S   | S   | S | S   | S  | S  |
| W1      | I   | S  | R  | S  | S   | S   | S | S   | S  | S  |
| A6      | I   | R  | R  | I  | S   | S   | S | R   | I  | R  |
| A7      | I   | R  | R  | R  | R   | S   | S | I   | S  | S  |
| W2      | I   | R  | S  | R  | R   | R   | S | S   | S  | S  |
| W3      | R   | S  | R  | S  | S   | S   | S | S   | S  | S  |
| A8      | I   | I  | I  | S  | R   | S   | S | S   | S  | S  |

Resistance (%) 18.8 31.3 56.3 25 37.5 6.3 12.5 18.8 6.3 6.3

R = Resistance, I = Intermediate, S = Sensitive

Clinical and Laboratory Standards Institute; 2018

Table 9. Selected Multidrug resistant bacterial isolates and their resistance pattern

| Isolates code | Multidrug resistant isolates | Resistant pattern |
|---------------|-----------------------------|-------------------|
| IK4           | Salmonella enterica serovar | CN S SXT          |
|               |     typhimurium. Typhimurium  |                  |
| IK5           | Salmonella enterica serovar | CPX AM AU CN PEF S SXT CH |
|               |     typhimurium. Typhimurium  |                  |
| A2            | Salmonella enterica serovar | CPX AU PEF        |
|               |     typhimurium. Typhimurium  |                  |
| A6            | Salmonella enterica serovar | AM AU SXT SP      |
|               |     typhimurium. Typhimurium  |                  |
| A7            | E. coli, Shigella            | AM AU CN          |
| W2            | Salmonella enterica serovar | AM CN PEF OFX     |
|               |     typhimurium. Typhimurium  |                  |

Legend: IK - Ikare, W - owo, A - Akure, SXT- Septrin (30 µg), CH - Chloramphenicol (30 µg) PEF- Perfoxacin (10 µg), OFX – Tarivid.Ofoxacin(10 µg), SP- Sparfloxacin (10 µg), S - Streptomycin (30 µg), CPX - Ciprofloxacin (10 µg), AM - Amoxicillin (30 µg), AU - Augmentin (30 µg), CN - Gentamycin (10 µg)

Table 10. Multiple antibiotics resistance index (MARI) of multidrug resistant isolates

| Isolate code | Resistant (a) | Tested (b) | MARI |
|--------------|---------------|------------|------|
| IK4          | 3             | 10         | 0.3  |
| IK5          | 8             | 10         | 0.8  |
| A2           | 3             | 10         | 0.3  |
| A6           | 4             | 10         | 0.4  |
| A7           | 3             | 10         | 0.3  |
| W2           | 4             | 10         | 0.4  |

Legend: IK - Ikare, W - owo, A - Akure

a - represents the number of antibiotics to which the isolates were resistant
b - represents the total number of antibiotics to which the isolates was exposed.

From Table 3, Ikare showed the highest percentage frequency of occurrence of 3.28% compared to Akure and Owo whose frequencies are 0.68% and 0.34% respectively. Ikare districts are known to be recreational centre for tourist and in yesteryears has friendly people whose
hospitality knows no bound for all tribes of Nigeria. The Fulani are the main vendors of cheese samples in Ondo state and 90% are young girls with piece of personal hygiene. This skill revolves around the water used in the process of production. Hence, Ikare has the highest frequency of occurrence due to the behavioural pattern of the vendors and the water used at the final stage of production i.e. cleaning of utensils, washing of hands and packaging baskets. The source of water supply from polluted wells and streams whose locations are at sewer channels and tanks.

From the result (Table 8), All isolates (n =16, 100%) were screened against ten commercial antibiotics. About 93.8% of the bacterial isolates were sensitive to Ofloxacin (10 µg), Chloramphenicol (30 µg). Only fourteen (14) bacterial isolates (87.5%) are sensitive to Streptomycin (30 µg) as well as 75% are sensitive to gentamycin (10 µg), 31.3% - 56.3% of the bacterial isolates are resistant to Amoxicillin (30 µg), Augmentin (30 µg) and perflaxacin (10 µg). This also shows that eight (8), i.e. 50%, of the isolates showed multiple resistance to at least two (2) antibiotics. This is worrisome, considering the global threat of antibiotic resistance. In fact, a related research carried out by Beshiru [11] in South-South, Nigeria, showed that 27.3% of the S. enterica serovar typhimurium. Typhimurium from ready-to-eat food samples were resistance to 11 commonly antibiotics. The highest level of resistance was found in S. enterica serovar typhimurium. Typhimurium strain isolated from cheese bought at Ikare, precisely IK5. This indicates the gradual emergence of multidrug resistant S. enterica serovar typhimurium. Typhimurium and subsequent intake of cheese contaminated with these strains might lead to unforeseen risk to public health where interventions will be very scarce and expensive.

The multiple antibiotics resistance (MAR) index of the selected multiple drug resistant isolates which indicates the use and misuse of antibiotics in the study areas is shown in Tables 9 and 10. MAR index of Salmonella isolates ranged from 0.14 to 0.45 for different ready-to-eat foods in a study by Budiai [14] in Malaysia. From Brazil, Carvalho [15] reported that 23% of Salmonella serovars were resistant to ≤ 1 antibiotic, 20% were resistant to ≤ 2 antibiotics while 3 strains showed multi-resistance characteristics. From the result obtained from table, the MAR index ranges from 0.3 to 0.8 which is very high. MAR index of 0.2 or higher indicates high risk sources of contamination where antibiotics are frequently used. Prior to the existence of antibiotics, invention of herbs was used by these sellers. These herbs have no optimal therapeutic dose. Therefore, subsequent intake of these herbs can also lead to the existence of multidrug resistant organisms. However, the vendors are local sellers who lived in villages and discrete suburbs around the city. They may be healthy carrier with no symptoms of the infection.

4. CONCLUSION

Cheese creates a favourable environment for a variety of microorganisms and can be important source of foodborne pathogens. This study has been able to investigate and identify the presence of antibiotic resistant Salmonella enterica serovar typhimurium. Typhimurium in cheese in Ondo State, Nigeria. It also showed that high level of contamination and microbial resistance is seen in soft cheese samples bought. Therefore, cheese can be an important food medium via which not only diarrhoeagenic Salmonellae may be spread, but also aid in the spread of antibiotic resistant Salmonella spp.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Amiri S, Moradli GH. Molecular identification of virulence genes (agfA and mgtC) in Salmonella Typhimurium strains isolated from children with gastroenteritis using multiplex PCR method. J Babol Uni Med Sci. 2016;18(10):40-45.
2. Jessica LF, David G, Ludovic V, Nicholas H, Christine H, Derek P, Subhankar M, Gordon D. The interaction of Salmonella enterica serovar Typhimurium with intestinal organoids derived from human induced pluripotent stem cells. Infect Immun. 2015;00161-15.
3. Larrisa B, Moonjely S, Behie SW, Bidochka MJ. Fungi with multifunctional lifestyles: Endophytic insect pathogenic fungi. Plant M. Biol. 2016;90(6):657-664.
4. USDA/ARS (U.S. Department of Agriculture/Agricultural Research Service). National Nutrient Database for Standard Reference Release 27; 2011. [Accessed January 5, 2015]
5. Ogbolu DO, Terry AAO, Oluemzi AS, Olanrewaju AA. Microbial contamination of locally produced cheese and determination of their antimicrobial potential in Nigeria. Afri. J. Clin. Exper. Microbiol. 2014;15(2): 76-83.

6. Sandle T, Satyada R. Determination of the cleaning efficiency for glassware in the pharmaceutical microbiology laboratory. Eur J Parent Pharma Sci. 2016;21(1):16-22.

7. Cheesbrough M. District laboratory practice in tropical countries. 2nd Ed., Cambridge University Press, Cambridge, UK. 2006;50:75-77.

8. McHugh AJ, Feehily C, Hill C, Cotter PD. Detection and enumeration of spore-forming bacteria in powdered dairy products. Front. Microbiol. 2017;8:109.

9. Abhishek V, Kumari N, Saina P, Menghani E. Isolation, identification and characterization of oil-degrading bacteria isolated from the contaminated sites of Barmer, Rajasthan. Int J Biotechnol Bioeng Res. 2013;4(5):429-436.

10. CLSI. Performance standards for antimicrobial disk susceptibility tests. 13th Edition. CLSI Standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

11. Beshiru AT, Igbinosa EO, Igbinosa IH. Prevalence of antimicrobial resistance and virulence gene elements of Salmonella serovars from ready-to-eat (RTE) shrimps. Front. Microbiol. 2019;10:1613.

12. Frasson I, Bettanello S, De Canale E, Richter SN, Palù G. Serotype epidemiology and multidrug resistance patterns of Salmonella enterica infecting humans in Italy. Gut Pathog. 2016;8(1):26.

13. Scuderi G, Fantasia M, Filetici E, Anastasio MP. Foodborne outbreaks caused by Salmonella in Italy, 1991–4. Epidemiol Infect. 1996;116(3):257-265.

14. Budiati T, Rusul G, Wan-Abdullah WN, Arip YM, Ahmad R, Thong KL. Prevalence, antibiotic resistance and plasmid profiling of Salmonella in catfish (Clarias gariepinus) and tilapia (Tilapia mossambica) obtained from wet markets and ponds in Malaysia. Aquaculture. 2013;372:127-132.

15. Carvalho FC, Sousa OV, Carvalho EM, Hofer E, Vieira RH. Antibiotic resistance of Salmonella spp. isolated from shrimp farming freshwater environment in Northeast Region of Brazil. J. Pathog; 2013. DOI: 10.1155/2013/685193

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