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

Anthony Kayode Onifade[1] and Oladapo Daniel Omololu[1*]

[1]Department of Microbiology, Federal University of Technology, Akure (FUTA), Ondo State, Nigeria.

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.

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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 vended 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: loluoladapo@gmail.com;*

used was 6.3% to Chloramphenicol (30 µg); 6.3% to Ofloxacin (10 µg); 6.3% of Sparfloxacin (10 µg); 12.5% to Streptomycin (30 µg); 18.8% to Sceptrin; 18.8% to Ciprofloxacin (10 µg); 25% to Gentamycin (10 µg); 31.3% to Amoxicillin (30 µg); 37.5% to Pefloxacin (10 µg) and 56.3% to Augmentin (30 µg).

Conclusion: Cheese creates a favourable environment for a variety of microorganisms and can be important source of foodborne pathogens.

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

1. 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.

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

western state of Nigeria created on February 3, 1976 where Akure is the state capital which lies between latitudes 7.25' N and longitude 5.195' E. Ikare location lies between latitudes, 070.27'N; 070.31'N and longitudes 0050.43'E; 0050.47'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 km^2. 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 ($BaCl_2$). 1% solution of sulfuric acid (H_2SO_4) 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), Perfloxacin (10 µg), Tarivid.Ofloxacin (10 µg), Sparfloxacin (10 µg), Streptomycin (30 µg) Ciprofloxacin (10 µg), Amoxicillin (30 µg), Augmentin (30 µg) and 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 (DNMRT) 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	81×10^{-3}	1
	2	10	50×10^{3}	5
	3	10	57×10^{3}	2
	4	20	86×10^{3}	1
Akure	5	20	190×10^{3}	Nil
	6	20	133×10^{3}	Nil
	7	20	157×10^{3}	2
	8	20	177×10^{3}	2
	9	10	183×10^{3}	1
	10	15	127×10^{3}	2
	11	15	90×10^{3}	1
	12	10	109×10^{3}	Nil
Owo	13	21	187×10^{3}	1
	14	20	115×10^{3}	Nil
	15	19	134×10^{3}	Nil
	16	20	145×10^{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 Cheese samples were bought from different vendors situated at various local towns and senatorial districts of Ondo State, Nigeria. Sixteen pure bacterial isolates were identified as resident *Salmonella* strains in the cheese samples. The numbers of the resident microorganisms identified as *Salmonella* enterica serovar *typhimurium.* Typhimurium are relatively small, due to the processes involved in the cheese making which affects the growth of microorganisms. Also, in a 1996 report from Italy, 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
				Slant	Butt	H_2S	Gas			Methyl	Voges	
IK1	+	+	-	+	+	+	+	+	-	+	-	*Salmonella enterica serovar typhimurium*
IK2	+	+	-	+	+	+	+	+	-	+	-	*Salmonella enterica serovar typhimurium*
IK3	+	+	-	+	+	+	+	+	-	+	-	*Salmonella enterica* serovar *typhimurium*
IK4	+	+	-	+	+	+	+	+	-	+	-	Salmonella enterica serovar typhimurium
1K5	+	+	-	+	+	+	+	+	-	+	-	"
A1	+	+	-	+	+	+	+	+	-	+	-	"
A2	+	+	-	+	+	+	+	+	-	+	-	"
A3	+	+	-	-	-	-	-	+	-	-	-	*Shigella, E. coli*
A4	+	+	-	+	+	+	+	+	-	+	-	*Salmonella enterica serovar typhimurium*
A5	+	+	-	+	+	-	-	+	-	+	-	*Shigella, E. coli*
A6	+	+	-	+	+	+	+	+	-	+	-	*Salmonella enterica*
A7	+	+	-	+	+	-	+	+	-	+	-	*E. coli, Shigella*
A8	+	+	-	+	+	+	+	+	-	+	-	*Salmonella enterica serovar typhimurium*
W1	+	+	-	+	+	+	+	+	-	+	-	*Salmonella enterica serovar typhimurium*
W2	+	+	-	+	+	+	+	+	-	+	-	*Salmonella enterica* serovar typhimurium
W3	+	+	-	+	+	+	+	+	-	+	-	"

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	28.67±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±0.67de	24.67±0.67cde	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	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	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±1.00b	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±0.00fg	23.67±0.88de	21.33±0.67c	29.33±0.67f
A3	29.33±0.67f	29.33±.67f	0.00±0.00a	19.33±0.67cde	23.33±0.67cd	25.00±1.15de	25.33±0.67gh	28.00±1.15f	29.33±0.67f	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- Septrin (30 µg), CH - Chloramphenicol (30 µg), PEF- Perfloxacin (10 µg), OFX- Tarivid. Ofloxacin (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 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±1.15cde	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±0.67f	23.33±0.67cd	29.33±0.67f
W3	20.00±5.03bc	16.67±0.67cd	12.00±0.00b	24.00±1.15f	24.67±0.67cde	25.33±0.67de	23.33±0.67fg	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	18.67±1.33b	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- Perfloxacin (10 µg), OFX – Tarivid. Ofloxacin (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 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	17.42±1.49a	22.58±0.63b	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- Perfloxacin (10 µg), OFX-Tarivid. Ofloxacin (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 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	S	R	R	S	S
IK5	R	R	R	R	R	I	R	R	R	I
A1	I	I	R	S	R	S	S	S	I	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	S	R	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	S	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 typhimurium. Typhimurium	CN S SXT
IK5	*Salmonella enterica* serovar typhimurium. Typhimurium	CPX AM AU CN PEF S SXT CH
A2	*Salmonella enterica* serovar typhimurium. Typhimurium	CPX AU PEF
A6	*Salmonella enterica* serovar typhimurium. Typhimurium	AM AU SXT SP
A7	*E. coli, Shigella*	AM AU CN
W2	*Salmonella enterica* serovar typhimurium. Typhimurium	AM CN PEF OFX

Legend: IK - Ikare, W - owo, A – Akure, SXT- Septrin (30 µg), CH - Chloramphenicol (30 µg) PEF- Perfloxacin (10 µg), OFX – Tarivid.Ofloxacin(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 perfloxacin (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 Budiati [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.

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