



## Prevalence of *Shigella* spp and Methicillin-Resistant *Staphylococcus aureus* in Select Commercially Processed Meat Products

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### Authors' contributions

This work was carried out in collaboration among both authors. Author IMO designed the study, supervised the research, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author HIA co-designed the study and wrote part of the manuscript. All authors read and approved the final manuscript.

### Article Information

DOI: 10.9734/AFSJ/2021/v20i530298

#### Editor(s):

(1) Dr. Mindaugas Liaudanskas, Lithuanian University of Health Sciences, Lithuania.

#### Reviewers:

(1) Hammuel, Chrinius, Federal University Wukari, Nigeria.

(2) Hafiz Shahbaz, University of Veterinary and Animal Sciences (UVAS), Pakistan.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/67494>

Received 07 February 2021

Accepted 14 April 2021

Published 22 April 2021

Original Research Article

### ABSTRACT

**Aim:** The aim of this study was to determine the prevalence of *Shigella* spp and methicillin resistant *Staphylococcus aureus* in some selected commercially processed ready-to-eat snail (*Archachatina marginata*) and edible worm (*Rhynchophorus phoenicis*).

**Study Design:** Cross-sectional analysis.

**Place and Duration of Study:** Samples were obtained from vendors along the Benin-Sapele express road, South-South Nigeria, over a two months period (November to December, 2019).

**Methodology:** The enumeration of total heterotrophic counts, total *Shigella* counts and total *Staphylococcus aureus* counts were done using nutrient agar, *Salmonella Shigella* agar and mannitol salt agar respectively. All isolates were further identified by their cultural, morphological and biochemical characteristics. Methicillin resistant *Staphylococcus aureus* were identified using methicillin-oxacillin agar, while the antibiogram profile of selected isolates and their multidrug resistant profile were done according to the CSLI guideline.

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**Results:** The mean heterotrophic bacterial counts (THCs) obtained in *Rhynchophorus phoenicis* ranged from  $0.00 \times 10^3 \pm 0.00$  cfu/g to  $500.00 \times 10^3 \pm 0.00$  cfu/g, while the mean THCs obtained in *Archachatina marginata* ranged from  $13.3 \times 10^3 \pm 1.15$  cfu/g to  $500 \times 10^3 \pm 0.00$  cfu/g. The total *Staphylococcus aureus* obtained in *Rhynchophorus phoenicis* was between  $0.00 \times 10^3 \pm 0.00$  cfu/g to  $294 \times 10^3 \pm 4.7$  cfu/g and the *Shigella* bacteria counts from  $0.00 \times 10^3 \pm 0.00$  cfu/g to  $258 \times 10^3 \pm 14.64$  cfu/g. The antibiogram of *Staphylococcus aureus* isolated from both *Rhynchophorus phoenicis* and *Archachatina marginata*, found the majority of isolates (96.67%) to be resistant to ceftazidime and cefuroxime, while 83.33% of the isolates were sensitive to ofloxacin. All *Staphylococcus* isolates were resistant to ceftriaxone, cloxacillin and amoxicillin clavulanate. The multidrug resistant index recorded was between 37.5 and 100. All *Staphylococcus* (9) isolates tested for methicillin resistance was observed to be positive.

**Conclusion:** This study demonstrated that RTE vended meat products sold in along Benin City By-pass, contains *Shigella* and methicillin resistant *Staphylococcus* species, which are potential foodborne pathogens and efforts should be made at eliminating them from these vended meat products.

**Keywords:** Food safety; public health; *Rhynchophorus phoenicis*; *Archachatina marginata*; Methicillin Resistant *Staphylococcus aureus*.

## 1. INTRODUCTION

In most developing nations especially in Africa, protein malnutrition is a major challenge. This has brought about the need to explore the use of other sources in the wild, which includes snail, edible worms and others in order to meet the requirements for protein.

Snail (*Archachatina marginata*) and edible worm (*Rhynchophorus phoenicis*) are served as food delicacies of protein sources in Nigeria, and can also be an inadvertent source for the transfer of multi-drug resistant (MDR) bacteria [1] as well as become causative agents of diarrhea infections [1]. *Staphylococcus aureus* is recognized as one of the major foodborne pathogens in RTE meat products and a major matrix for the transfer of this pathogen coming second after fresh vegetables [2]. The presence of *S. aureus* in food usually indicate poor personal hygiene of the handler or vendor and poor manufacturing practices of the producer according to Musa and Akande [3]. This bacterium multiplies quickly at room temperature to produce toxin that cause illness [4], this becomes a problem when food is prepared under a poor hygienic environment [5] and among those who live in areas with poor food storage and preparation facilities being the most vulnerable to staphylococcal foodborne disease [3]. Food borne diseases (FBD) are endemic in most developing countries including Nigeria, partly due to the customary poor food handling and hygiene practices, derisory food safety laws, weak regulatory systems, poor capital resources to devote to safer equipment and lack of education on food safety practices for

food handlers in addition to the warm tropical temperatures ideal for microbial proliferation [6,7].

*Shigella* species on the other hand is a highly infectious agent that causes shigellosis, also known as bacillary dysentery [8]. *Shigella* often causes foodborne outbreaks involving infected food handlers [9]. Unlike other bacterial foodborne pathogen, humans are the only natural hosts of *Shigella* spp. [8]. Food borne diseases (FDBs) remain a major public health problem globally, but are often worse in low and medium income countries (LMICs) often because of lack of knowledge about good hygiene practices by vendors [7]. An estimated 70% of diarrheic cases in developing countries for example are associated with the consumption of contaminated foods alone [10]. The extensive consumption of ready-to-eat meat protein products especially foods like Edible worm (palm weevil larvae, *Rhynchophorus phoenicis* and West African giant snail (*Archachatina marginata*) amongst travellers along the highways trans versing one state to another is a main concern for this study. The study aimed at examining the prevalence of two potential foodborne pathogens: *Shigella* and methicillin resistance strains of *Staphylococcus aureus* (MRSA) in random samples of commercially processed Snail (*Archachatina marginata*) and Edible worm (*Rhynchophorus phoenicis*), it also aimed at determining the microbiological quality level of compliance by the vendors who sold along Benin-Sapele road, Benin City, Edo State and Sapele junction Amukpe, Delta State, Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Study Site

Three different traffic jam points within Benin City (Adesuwa Junction and Benin-Sapele Road Bypass) in Edo State and Sapele roundabout Amukpe in Delta State were used in this study.

### 2.2 Collection of Samples

A total of 24 commercially processed meat (12 Edible worms (*Rhynchophorus phoenicis*) and 12 Snails (*Archachatina marginata*) products were purchased from different vendors over a 30-day period. Samples were purchased randomly from vendors in the identified vicinity from November to December, 2019. All samples were obtained aseptically in sterile sealable cellophane bags and transported in ice pack to the Microbiology Laboratory of Benson Idahosa University for immediate analysis.

### 2.3 Microbiological Analysis

#### 2.3.1 Sample preparation and isolation of microorganisms

Ten gram (10 g) each of the meat products were weighed into a beaker, and 90 ml of sterile distilled water was added and homogenized for about 2 minutes. Thereafter, a 10-fold serial dilution was made from the supernatant and 1 ml of the appropriate dilutions were cultured on the respective media.

#### 2.3.2 Total heterotrophic count

Aliquot amount (1 ml) of the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> dilutions were aseptically transferred onto already prepared nutrient agar plates (Titan biotech ltd India) by the pour plate method, and incubated at 37°C for 24 hrs. All samples were cultured in triplicates, and the mean heterotrophic count recorded based on the number of colonies on the nutrient agar plates, determined after 24 hrs of incubation.

#### 2.3.3 Total *Staphylococcus aureus* count

One millimeter (1 ml) of the appropriate dilution (10<sup>3</sup>) of each sample was collected using an automatic sterile pipette and diluted into 9 ml of sterile deionized water in a test tube. Three further dilutions of this were made and 1ml each of the third dilution were cultured in triplicates onto mannitol salt agar (MSA) plates. The plates

were then incubated at 37°C, total number of colonies were determined using a Labtech (England) Colony counter after 24 hrs of incubation.

#### 2.3.4 Total *Shigella* count

One millimeter (1 ml) of the appropriate dilution of 10<sup>3</sup> of each sample was collected using an automatic sterile pipette and diluted into 9ml of sterile deionized water in a test tube. Three further dilutions of this were made and 1ml each of the third dilution were cultured in triplicates on *Salmonella-Shigella* agar (SSA) plates. The plates were then incubated at 37°C for 24 hours. Total number of colonies was counted using Colony counter (Labtech England).

### 2.4 Identification of Bacterial Isolates

The bacteria isolates were identified using their cultural, morphological and biochemical characteristics (catalase, citrate, motility, indole, coagulase and oxidase).

### 2.5 Phenotypic Identification of Methicillin-Resistant *Staphylococcus aureus*

From the biochemical test conducted, isolates presumptively identified as *Staphylococcus aureus* were further screened for their resistance and/or susceptibility to methicillin, using mannitol-oxacillin agar. Briefly, 1 g of oxacillin was added to 250 ml of already prepared mannitol salt agar, before the inoculation of *Staphylococcus aureus* and then incubated for 24 hours at 37°C *Staphylococcus aureus* that grew on the said medium were indicative of methicillin-resistance.

### 2.6 Antibiotic Susceptibility Test

The antibiotics susceptibility test was performed using standard disc diffusion method as described by the Clinical and Laboratory Standard Institute [11]. Antibiotics used included: ceftazidime (30 µg), gentamicin (10 µg), cefuroxime (30 µg), ceftriaxone (30 µg), ofloxacin (5 µg) amoxicillin clavulanate (30 µg), erythromycin (5 µg) cloxicillin (5 µg) and oxacillin (1 µg). The results were recorded and interpreted according to the description of CLSI.

### 2.7 Statistical Analysis

The mean and standard deviation were calculated using Microsoft Excel, 2010 model.

### 3. RESULTS

The results obtained from the total heterotrophic bacterial count show that heterotrophic bacteria, *Staphylococcus* and *Shigella* species are prevalent in RTE *Rhynchophorus phoenicis* from the sampled area. The mean heterotrophic bacterial count obtained from Sapele ranged from  $0.00 \times 10^3 \pm 0.00$  cfu/g (VEDW05) to  $291.66 \times 10^3 \pm 3.79$  cfu/g (VEDW01) as shown in Table 1. Interestingly, as with the total heterotrophic bacterial counts, (THCs) VEDW05 also had no *Staphylococcus* count recorded. The THCs obtained from *Rhynchophorus phoenicis* samples collected from Benin-Sapele road ranged from  $4.00 \times 10^3 \pm 1.73$  cfu/g to  $500.00 \times 10^3 \pm 0.00$  cfu/g (Table 1).

The total *Staphylococcus* count were fairly low in all the samples investigated, except for 3 samples obtained from Sapele (Delta State); VEDW01, VEDW02 and VEDW03 with mean *Staphylococcus* counts of  $290.67 \times 10^3 \pm 8.5$  cfu/g,  $279.66 \times 10^3 \pm 13.65$  cfu/g and  $294.33 \times 10^3 \pm 4.7$  cfu/g respectively. The total *Shigella* counts (TSCs) obtained were also low in Benin Sapele road (range =  $1.00 \times 10^3 \pm 0.00$  cfu/g to  $35.00 \times 10^3 \pm 22.52$  cfu/g), when compared with the TSCs obtained from Sapele (Delta State) ( $0.00 \times 10^3 \pm 0.00$  cfu/g to  $258.6 \times 10^3 \pm 14.64$  cfu/g) as shown in Table 1.

Table 2 shows that the total heterotrophic bacteria (THC), *Staphylococcus* and *Shigella* species are prevalent in RTE *Cryptomphalus aspersus* sold both along Benin-Sapele express way, Benin City, Edo State and at Sapele Round About, Delta State. The mean heterotrophic bacterial count obtained from Sapele ranged from  $13.3 \pm 1.15$  cfu/g (S02) to  $500 \times 10^3 \pm 0.00$  cfu/g (S05) as shown in Table 2. Also, no *Staphylococcus* counts were recorded in S04 and S01 samples collected. The THCs obtained from *Cryptomphalus aspersus* samples collected from Benin-Sapele road ranged from  $115.33 \times 10^3 \pm 29$  cfu/g to  $500.00 \times 10^3 \pm 0.00$  cfu/g.

The total *Staphylococcus* count were fairly low in all the samples investigated, with mean *Staphylococcus* counts ranging between  $0.00 \times 10^3$  from Sapele to  $51.33 \times 10^3 \pm 2.51$  cfu/g and the Total mean *staphylococcus* of  $0.00 \times 10^3$  from Benin to  $20 \times 10^3 \pm 12.49$  cfu/g. The total *Shigella* counts (TSCs) obtained were a little higher in Benin Sapele road ( $2.67 \times 10^3 \pm 1.52$  cfu/g to

$80.67 \times 10^3 \pm 23.25$  cfu/g), when compared with the TSCs obtained from Sapele, Delta State ( $4.00 \times 10^3 \pm 1.00$  cfu/g to  $45.00 \times 10^3 \pm 5.29$  cfu/g).

A total of 90 anatomically distinct bacterial isolates were obtained and screened based on their cultural, morphological and biochemical characteristics as presented in Table 3. Based on their presumptive identity, 38 isolates were reported to be *Shigella* species while 52 isolates were observed to be *Staphylococcus* species.

Table 4 shows the antibiotics susceptibility profile of *Staphylococcus* isolated in Edible worm from Sapele roundabout Amukpe, showing seven (7) of the isolates to be resistant to ceftazidime, cefuroxime, ceftriaxone, erythromycin, cloxacillin and amoxicillin clavulanate. Interestingly, among all the isolates obtained from Benin-Sapele road only one (1) of the isolated organisms was reported to be resistant to all the antibiotics used; ceftazidime (30 µg), gentamicin (10 µg), cefuroxime (30 µg), ceftriaxone (30 µg), ofloxacin (5 µg) amoxicillin clavulanate (30 µg), erythromycin (5 µg) cloxacillin (5 µg) and oxacillin (1 µg), with the majority of the isolates showing multi-drug resistant properties (Table 5).

Table 6 shows the antibiotics susceptibility profile of *Staphylococcus* isolated from *Archachatina marginata* against 8 commonly used antibiotics; ceftazidime (30 µg), gentamicin (10 µg), cefuroxime (30 µg), ceftriaxone (30 µg), ofloxacin (5 µg) amoxicillin clavulanate (30 µg), erythromycin (5 µg) cloxacillin (5 µg). Majority of the isolates (96.67%) were observed to be resistant to Cloxacillin and Amoxycillin-clavulanate, while 83.33% of the isolates were sensitive to ofloxacin. Interestingly, all *Staphylococcus* isolates from *Archachatina marginata* were resistant to cloxacillin and amoxycillin clavulanate.

Phenotypic identification of methicillin resistant strains of *Staphylococcus aureus* (MRSA) revealed all *Staphylococcus* isolates tested (7) were methicillin resistant. The antibiotics susceptibility profile of *Staphylococcus* isolated from RTE Snail (*Archachatina marginata*) from Benin-Sapele road and Sapele metropolis. Two (2) of the isolates were observed to be resistant to ceftazidime, cefuroxime, ceftriaxone, erythromycin, cloxacillin, amoxicillin-clavulanate and oxacillin (Table 7).

**Table 1. Total heterotrophic, total *Staphylococcus aureus* and total *Shigella* counts obtained from Edible worm (*Rhynchophorus phoenicis*)**

Sample Code	Sample Location	THCs (cfu/g) (x10 <sup>3</sup> )	TSACs (cfu/g) (x10 <sup>3</sup> )	TSCs (cfu/g) (x10 <sup>3</sup> )
VEDW01	Sapele	291.66 ± 3.79	290.67 ± 8.5	258.6 ± 14.64
VEDW02	Sapele	286.66 ± 14.47	279.66 ± 13.65	92.33 ± 3.5
VEDW03	Sapele	286.00 ± 6.24	294.33 ± 4.7	30.33 ± 2.08
VEDW04	Sapele	20.06 ± 7.02	159.66 ± 12.89	57.06 ± 5.51
VEDW05	Sapele	0.00 ± 0.00	0.00 ± 0.00	63.66 ± 6.35
VEDW06	Sapele	290.00 ± 4.00	29.66 ± 4.04	0.00 ± 0.00
VEDW07	Benin-SR	74.00 ± 26.63	7.33 ± 3.21	1.00 ± 0.00
VEDW08	Benin-SR	4.00 ± 1.73	4.00 ± 3.00	0.66 ± 0.50
VEDW09	Benin-SR	500.00 ± 0.00	62.66 ± 17.79	3.00 ± 1.00
VEDW10	Benin-SR	500.00 ± 0.00	50.00 ± 13.89	7.33 ± 5.00
VEDW11	Benin-SR	249.33 ± 38.79	31.06 ± 9.50	45.00 ± 10.8
VEDW12	Benin-SR	149.33 ± 8.02	12.66 ± 2.23	6.30 ± 3.50

Key: THCs: Total Heterotrophic Counts; TSACs: Total *Staphylococcus aureus* counts; TSCs: Total *Shigella* Counts; VEDW: Edible worm; Benin-SR: Benin-Sapele Road

**Table 2. Total heterotrophic, total *Staphylococcus aureus* and total *Shigella* counts obtained from Snail (*Archachatina marginata*)**

Sample Code	Sample Location	THCs (cfu/g) (x10 <sup>3</sup> )	TSACs (cfu/g) (x10 <sup>3</sup> )	TSCs (cfu/g) (x10 <sup>3</sup> )
S01	Sapele	159.66 ± 87.01	0.00 ± 0.00	8.00 ± 3.61
S02	Sapele	13.03 ± 1.15	2.00 ± 1.00	4.00 ± 1.00
S03	Sapele	250.33 ± 48.16	48.00 ± 1.00	22.33 ± 2.08
S04	Sapele	418.00 ± 142.02	0.00 ± 0.00	45.00 ± 5.29
S05	Sapele	500.00 ± 0.00	51.33 ± 2.51	38.66 ± 6.4
S06	Sapele	145.00 ± 47.69	9.33 ± 0.57	6.33 ± 1.53
S07	Benin-SR	500.00 ± 0.00	19.33 ± 2.83	21.66 ± 6.03
S08	Benin-SR	152.00 ± 8.72	1.33 ± 0.58	14.00 ± 6.55
S09	Benin-SR	115.33 ± 29.96	0.00 ± 0.00	18.66 ± 23.75
S10	Benin-SR	233.67 ± 53.57	11.33 ± 7.51	80.67 ± 23.25
S11	Benin-SR	209.00 ± 60.85	3.00 ± 1.00	7.66 ± 4.04
S12	Benin-SR	283.67 ± 6.51	20.00 ± 12.49	2.67 ± 1.52

Key: THCs: Total Heterotrophic Count; TSACs: Total *Staphylococcus aureus* count; TSCs: Total *Shigella* Count; S: Snail; Benin-SR: Benin-Sapele Road

**Table 3. Cultural, morphological and biochemical characteristics of bacterial isolates**

Characteristics								Presumptive Identity	No. of Isolates
Gram Reaction	Catalase	Oxidase	Citrate	Motility	Indole	Coagulase			
-ve	+ve	-ve	-ve	-ve	-ve	ND		<i>Shigella</i> spp.	38
+ve	+ve	-ve	+ve	-ve	ND	+ve		<i>Staphylococcus aureus</i>	52

Key: -ve: Negative; +ve: Positive; ND: Not determined

**Table 4. Antibiotics susceptibility profile of *Staphylococcus aureus* isolated from Edible worm (*Rhynchophorus phoenicis*)**

Isolate Code	ANTIBIOTICS									MRSA/MSSA	MDR INDEX (%)
	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG	OXA		
EWst01S	R	R	I	R	R	R	S	R	R	MRSA	75
EWst02S	R	R	R	R	R	R	S	R	R	MRSA	87.5
EWst03S	R	R	S	R	R	R	S	R	ND		75
EWst04S	R	R	I	R	R	R	S	R	R	MRSA	75
EWst05S	R	R	S	R	R	R	S	R	ND		75
EWst06S	R	R	S	R	R	R	S	R	ND		75
EWst07S	R	R	S	R	R	R	S	R	R	MRSA	75
EWst08S	R	R	S	R	R	R	S	R	ND		75
EWst09S	R	R	S	R	R	R	S	R	R	MRSA	75
EWst10S	R	R	S	R	R	R	S	R	ND		75
EWst11S	R	R	S	R	R	R	S	R	R	MRSA	75
EWst12S	R	R	S	R	R	R	S	R	ND		75
EWst13S	R	R	R	R	R	R	S	R	R	MRSA	87.5
EWst14S	R	R	S	R	R	R	S	R	ND		75
EWst15S	R	R	S	R	R	R	S	R	ND		75
EWst01B-SR	R	R	S	R	R	R	S	R	ND		75
EWst02B-SR	R	R	S	R	R	R	S	R	ND		75
EWst03B-SR	R	R	S	R	R	R	S	R	ND		75
EWst04B-SR	R	R	S	R	R	R	S	R	ND		75
EWst05B-SR	S	R	S	R	S	R	S	R	ND		37.5
EWst06B-SR	R	R	R	R	R	R	I	R	R	MRSA	87.5
EWst07B-SR	R	R	S	R	S	R	S	R	ND		62.5
EWst08B-SR	R	S	S	R	I	R	S	R	ND		50
EWst09B-SR	R	R	S	R	I	R	S	R	ND		62.5
EWst10B-SR	R	R	R	R	R	R	R	R	R	MRSA	100

Isolate Code	ANTIBIOTICS									MRSA/MSSA	MDR INDEX (%)
	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG	OXA		
EWst11B-SR	R	R	R	R	R	R	I	R	ND		87.5
EWst12B-SR	R	R	I	R	R	R	S	R	ND		75
EWst13B-SR	R	R	R	R	R	R	R	R	ND		100
EWst14B-SR	R	R	R	R	R	R	R	R	ND		100
EWst15B-SR	R	R	S	R	R	R	S	R	ND		100

Key: CAZ: Ceftazidime CRX: Cefuroxime, GEN: Gentamicin, CXM: Cefixime, OFL: Ofloxacin, AUG: Augmentin, NIT: Nitrofurantion, CPR: Ciprofloxacin, OXA: Oxacillin, R: Resistance; S: Sensitive; I: Intermediate; MRSA: Methicillin-resistant *Staphylococcus aureus*, MDR: Multi drug resistance

**Table 5. Multidrug Resistant (MDR) profile of *Staphylococcus aureus* isolated from Edible worm (*Rhynchophorus phoenicis*)**

No. of isolates	MDR profile									Isolate code
1	CTR	CXC	AUG							EWst05S
2	CAZ	CTR	CXC	AUG						EWst08S;, EWst19S
1	CAZ	CRX	CTR	CXC	AUG					EWst07S
1	CRX	CTR	ERY	CXC	AUG					EWst01S
7	CAZ	CRX	CTR	ERY	CXC	AUG				EWst2S; EWst3S; EWst4S; EWst12S; EWst10S; EWst14S; EWst15S
2	CAZ	CRX	CTR	ERY	CXC	AUG	OXA			EWst9S; EWst11S
1	CAZ	CRX	CTR	ERY	CXC	AUG	GEN			EWst11B-SR
3	CAZ	CRX	CTR	ERY	CXC	AUG	GEN	OXA		EWst06B-SR; EWst2S; EWst13S
1	CAZ	CRX	CTR	ERY	CXC	AUG	GEN	OFL		EWst14B-SR
1	CAZ	CRX	CTR	ERY	CXC	AUG	GEN	OFL	OXA	EWst10B-SR

KEY: CAZ: Ceftazidime CRX: Cefuroxime, GEN: Gentamicin, CXM: Cefixime, OFL: Ofloxacin, AUG: Augmentin, NIT: Nitrofurantion, CPR: Ciprofloxacin, OXA: Oxacillin, R: Resistance; S: Sensitive; I: Intermediate; MRSA: Methicillin-resistant *Staphylococcus aureus*, MDR: Multi drug resistance

**Table 6. Antibiotics susceptibility profile of *Staphylococcus aureus* isolated from Snail (*Archachatina marginata*)**

Isolate Code	ANTIBIOTICS									MRSA/MSSA	MDR INDEX (%)
	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG	OXA		
Sst01S	R	R	S	R	R	R	S	R	ND		75
Sst02S	R	R	S	R	R	R	S	R	ND		75
Sst03S	S	I	S	I	S	R	S	R	R	MRSA	25
Sst04S	R	R	S	R	R	R	S	R	NA		75
Sst05S	R	R	R	R	S	R	S	R	NA		75
Sst06S	R	R	R	R	R	R	S	R	R	MRSA	87.5
Sst07S	R	R	S	R	I	R	S	R	ND		62.5
Sst08S	R	R	I	R	R	R	S	R	R	MRSA	75
Sst09S	R	R	S	R	S	R	S	R	ND	MRSA	62.5
Sst10S	R	R	S	R	S	R	S	R	ND		62.5
Sst01B-SR	R	R	S	R	R	R	S	R	R	MRSA	75
Sst02B-SR	R	R	S	R	S	R	S	R	ND		62.5
Sst03B-SR	R	R	S	R	R	R	S	R	ND		62.5
Sst04B-SR	R	R	I	R	R	R	S	R	R	MRSA	75
Sst05B-SR	R	R	S	R	I	R	S	R	ND		62.5
Sst06B-SR	I	S	S	S	S	R	S	R	R	MRSA	25
Sst07B-SR	S	I	S	I	S	R	S	R	ND		25
Sst08B-SR	I	I	S	I	S	R	S	R	ND		25
Sst09B-SR	I	R	S	I	S	R	S	R	ND		37.5
Sst10B-SR	R	R	S	R	S	R	S	R	ND		62.5
Sst11B-SR	R	R	S	R	S	R	S	R	ND		62.5
Sst12B-SR	R	R	S	R	I	R	S	R	ND		62.5

KEY: CAZ: Ceftazidime; CRX: Cefuroxime; GEN: Gentamicin; CTR: Ceftriaxone; ERY: Erythromycin; CXC: Cloxacillin; OFL: Ofloxacin; AUG: Amoxycillin clavulanate, OXA: Oxacillin; R: Resistance; S: Sensitive; I: Intermediate; MRSA: Methicillin-resistant *Staphylococcus aureus*; MDR: Multi drug resistance



**Table 7. Multidrug Resistant profile of *Staphylococcus aureus* isolated from snail (*Cryptomphalus aspersus*)**

No. of isolates	AMDR PROFILE							Isolate code
1	CXC	AUG	OXA					Sst06B-SR
1	CRX	CXC	AUG					Sst09B-SR
1	CXC	AUG	OXA					Sst03S
3	CAZ	CRX	CTR	CXC	AUG			Sst07S; Sst09S; Sst10S
6	CAZ	CRX	CTR	CXC	AUG			Sst02B-SR; Sst3B-SR; Sst5B-SR; Sst10B-SR; Sst11 B-SR; Sst12B-SR
4	CAZ	CRX	CTR	CXC	AUG	ERY		Sst01S; Sst2S; Sst4S; Sst5S
2	CAZ	CRX	CTR	CXC	AUG	ERY	OXA	Sst06S; Sst8S
2	CAZ	CRX	CTR	CXC	AUG	ERY	OXA	Sst01B-SR; Sst4B-SR

Key: CAZ: Ceftazidime; CRX: Cefuroxime; GEN: Gentamicin; CTR: Ceftriaxone; ERY: Erythromycin; CXC: Cloxacillin; OFL: Ofloxacin; AUG: Amoxicillin clavulanate, OXA: Oxacillin; R: Resistance; S: Sensitive; I: Intermediate; MRSA: Methicillin-resistant *Staphylococcus aureus*; MDR: Multi drug resistance

**Table 8. Antibiotics susceptibility profile of *Shigella* isolated from Edible worm**

Isolate code	Antibiotics									MDR index (%)
	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG	OXA	75
EWsh01S	R	R	I	R	R	R	S	R	ND	62.5
EWsh02S	R	R	S	R	I	R	S	R	ND	62.5
EWsh03S	R	R	S	R	I	R	S	R	ND	62.5
EWsh04S	R	R	S	R	S	R	S	R	ND	62.5
EWsh05S	R	R	S	R	S	R	S	R	ND	75
EWsh06S	R	R	S	R	R	R	S	R	ND	62.5
EWsh07S	R	R	S	R	S	R	S	R	ND	62.5
EWsh08S	R	R	S	R	I	R	S	R	ND	75
EWsh09S	R	R	S	R	R	R	S	R	ND	75
EWsh10S	R	R	S	R	R	R	S	R	ND	75
EWsh11S	R	R	S	R	R	R	S	R	ND	75
EWsh12S	R	R	S	R	R	R	S	R	ND	75
EWsh13S	R	R	S	R	R	R	S	R	ND	75
EWsh14S	R	R	I	R	R	R	S	R	ND	62.5
EWsh15S	R	R	S	R	S	R	S	R	ND	75
EWsh16S	R	R	S	R	R	R	S	R	ND	75
EWsh17S	R	R	S	R	R	R	S	R	ND	75
EWsh18S	R	R	S	R	R	R	S	R	ND	75
EWsh19S	R	R	S	S	R	R	S	R	ND	75

Isolate code	Antibiotics									MDR index (%)
	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG	OXA	
EWsh20S	R	R	S	R	R	R	S	R	ND	75
EWsh21S	R	R	R	R	R	R	S	R	ND	87.5
EWsh22S	R	R	R	R	R	R	S	R	ND	87.5
EWsh23S	R	R	S	R	R	R	S	R	ND	87.5
EWsh01B-SR	R	R	I	R	R	R	I	R	ND	75
EWsh02B-SR	R	R	S	R	R	R	S	R	ND	75
EWsh03B-SR	R	R	S	R	R	R	S	R	ND	75
EWsh04B-SR	R	R	S	R	R	R	S	R	ND	75
EWsh05B-SR	R	R	S	R	R	R	S	R	ND	75
EWsh06B-SR	R	R	S	R	R	R	S	R	ND	75
EWsh07B-SR	R	R	S	R	R	R	S	R	ND	75
EWsh08B-SR	R	R	S	R	S	R	S	R	ND	62.5
EWsh09B-SR	R	R	S	R	S	R	S	R	ND	62.5
EWsh10B-SR	R	R	S	R	R	R	S	R	ND	75
EWsh11B-SR	R	R	S	R	R	R	S	R	ND	75
EWsh12B-SR	R	R	S	R	S	R	S	R	ND	62.5
EWsh13B-SR	R	R	R	R	R	R	S	R	ND	87.5
EWsh14B-SR	R	R	S	R	R	R	S	R	ND	75
EWsh15B-SR	R	R	R	R	S	R	S	R	ND	75
EWsh16B-SR	R	R	S	R	R	R	S	R	ND	87.5
EWsh17B-SR	R	R	S	R	S	R	S	R	ND	25
EWsh18B-SR	R	R	S	R	S	R	S	R	ND	62.5
EWsh19B-SR	R	R	S	R	R	R	S	R	ND	75
EWsh20B-SR	R	R	S	R	R	R	S	R	ND	75

Key: CAZ: Ceftazidime; CRX: Cefuroxime; GEN: Gentamicin; CTR: Ceftriaxone; ERY: Erythromycin; CXC: Cloxacillin; OFL: Ofloxacin; AUG: Amoxicillin clavulanate, OXA: Oxacillin; R: Resistance; S: Sensitive; I: Intermediate; MRSA: Methicillin-resistant *Staphylococcus aureus*; MDR: Multi drug resistance

**Table 9. AMDR profile of *Shigella* spp isolated from Edible worm (*Rhynchophorus phoenicis*)**

No. of isolates	AMDR profile							Isolate code
1	CTR	CXC	AUG					EWsh17 B-SR
8	CAZ	CRX	CTR	CXC	AUG			EWsh2S; EWsh3S; EWsh4S; EWsh5S; EWsh7S; EWsh8S; EWsh9S; EWsh15S
4	CAZ	CRX	CTR	CXC	AUG			EWsh8B-SR; EWsh9B-SR; EWsh12 B-SR; EWsh18 B-SR
13	CAZ	CRX	CTR	CXC	ERY	AUG		EWsh01S; EWsh6S; EWsh9S; EWsh10S; EWsh11S; EWsh12S; EWsh13S; EWsh14S; EWsh16S; EWsh17S; EWsh18S; EWsh19S; EWsh20S;
13	CAZ	CRX	CTR	CXC	ERY	AUG		EWsh01B-SR; EWsh2B-SR; EWsh3B-SR; EWsh4B-SR; EWsh5B-SR; EWsh6B-SR; EWsh7B-SR; EWsh10B-SR; EWsh11B-SR; EWsh12B-SR; EWsh15B-SR; EWsh19B-SR; EWsh20B-SR
3	CAZ	CRX	CTR	CXC	ERY	GEN	AUG	EWsh21S; EWsh22S; EWsh23S
1	CAZ	CRX	CTR	ERY	CXC	GEN	AUG	EWshB-SR13

KEY: CAZ: Ceftazidime; CRX: Cefuroxime; GEN: Gentamicin; CTR: Ceftriaxone; ERY: Erythromycin; CXC: Cloxacillin; OFL: Ofloxacin; AUG: Amoxicillin clavulanate, OXA: Oxacillin; R: Resistance; S: Sensitive; I: Intermediate; MRSA: Methicillin-resistant *Staphylococcus aureus*; MDR: Multi drug resistance

**Table 10. Antibiotics susceptibility profile of *Shigella* spp isolated from Snail (*Archachatina marginata*)**

Isolate code	antibiotics										MDR index (%)
	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG	OXA		
Ssh01S	R	R	I	R	R	R	S	R	ND	75	
Ssh02S	R	R	S	R	R	R	S	R	ND	75	
Ssh03S	R	R	I	R	R	R	S	R	ND	75	
Ssh04S	R	R	S	R	R	R	S	R	ND	75	
Ssh05S	R	R	S	R	R	R	S	R	ND	75	
Ssh06S	R	R	S	R	R	R	S	R	ND	75	
Ssh07S	R	R	S	R	R	R	S	R	ND	75	
Ssh08S	R	R	S	R	S	R	S	R	ND	62.5	
Ssh09S	R	R	S	R	R	R	S	R	ND	75	
Ssh10S	R	R	S	R	R	R	S	R	ND	75	
Ssh01B-SR	R	R	S	R	R	R	S	R	ND	75	
Ssh02B-SR	R	R	S	R	R	R	S	R	ND	75	
Ssh03B-SR	R	R	S	R	R	R	S	R	ND	75	
Ssh04B-SR	R	R	S	R	R	R	S	R	ND	75	
Ssh05B-SR	R	R	S	R	R	R	S	R	ND	75	
Ssh06B-SR	R	R	S	R	R	R	S	R	ND	75	

Isolate code	antibiotics									MDR index (%)
	CAZ	CRX	GEN	CTR	ERY	CXC	OFL	AUG	OXA	
Ssh07B-SR	R	R	I	R	R	R	S	R	ND	75
Ssh08B-SR	R	R	S	R	R	R	S	R	ND	75

KEY: CAZ: Ceftazidime; CRX: Cefuroxime; GEN: Gentamicin; CTR: Ceftriaxone; ERY: Erythromycin; CXC: Cloxacillin; OFL: Ofloxacin; AUG: Amoxicillin clavulanate, OXA: Oxacillin; R: Resistance; S: Sensitive; I: Intermediate; MRSA: Methicillin-resistant *Staphylococcus aureus*; MDR: Multi drug resistance

**Table 11. MDR profile of *Shigella* spp isolated from Snail (*Archachatina marginata*)**

No. of isolates	AMDR PROFILE						ISOLATE CODE
1	CAZ	CRX	CTR	CXC	AUG		Ssh08S
9	CAZ	CRX	CTR	CXC	ERY	AUG	Ssh01S; Ssh02; Ssh03S; Ssh04S; Ssh05S; Ssh06S; Ssh07S; Ssh09S; Ssh10S
8	CAZ	CRX	CTR	CXC	ERY	AUG	Ssh01B-SR; Ssh02B-SR; Ssh03B-SR; Ssh04B-SR; Ssh05B-SR; Ssh06B-SR; Ssh07B-SR; Ssh08B-SR

KEY: CAZ: Ceftazidime; CRX: Cefuroxime; GEN: Gentamicin; CTR: Ceftriaxone; ERY: Erythromycin; CXC: Cloxacillin; OFL: Ofloxacin; AUG: Amoxicillin clavulanate, OXA: Oxacillin; R: Resistance; S: Sensitive; I: Intermediate; MRSA: Methicillin-resistant *Staphylococcus aureus*; MDR: Multi drug resistance

Table 8 show the antibiotics susceptibility profile of *Shigella* isolated from Edible worm against 8 commonly used antibiotics; ceftazidime (30 µg), gentamicin (10 µg), cefuroxime (30 µg), ceftriaxone (30 µg), ofloxacin (5 µg) amoxicillin clavulanate (30 µg), erythromycin (5 µg) cloxacillin (5 µg). Most of the isolates (97.67%) were observed to be resistant to ceftazidime, cefuroxime, ceftriaxone, Cloxacillin and Amoxicillin clavulanate, while 98.33% of the isolates were sensitive to ofloxacin and 85.36% of gentamicin. Table 9 shows the antibiotics susceptibility profile of *Shigella* isolated in Edible worm from Benin-Sapele road, 13 of the isolates were observed to be resistant to ceftazidime, cefuroxime, ceftriaxone, erythromycin, cloxacillin and amoxicillin clavulanate while from Sapele metropolis 3 of the isolates obtained were reported to be resistant to ceftazidime, cefuroxime, ceftriaxone, erythromycin, cloxacillin and amoxicillin clavulanate and gentamicin. Table 10 shows the antibiotics susceptibility profile of *Shigella* isolated from Snail against 8 commonly used antibiotics; ceftazidime (30 µg), gentamicin (10 µg), cefuroxime (30 µg), ceftriaxone (30 µg), ofloxacin (5 µg) amoxicillin clavulanate (30 µg), erythromycin (5 µg) cloxacillin (5 µg). Majority of the isolates (96.67%) were observed to be resistant to ceftazidime, ceftriaxone, cefuroxime, cloxacillin, erythromycin and amoxicillin/clavulanate, while all of the isolates were sensitive to ofloxacin and (95.33%) to gentamicin.

The antibiotics susceptibility profile of *Shigella* isolated from Snail from Benin-Sapele road is shown in Table 11. Eight (8) of the isolates were observed to be resistant to ceftazidime, cefuroxime, ceftriaxone, erythromycin, cloxacillin and amoxicillin clavulanate while nine (9) from Sapele metropolis isolates obtained were also reported to be resistant to ceftazidime, cefuroxime, ceftriaxone, erythromycin, cloxacillin and amoxicillin clavulanate and gentamicin.

#### 4. DISCUSSION

The presence of *Shigella Staphylococcus* species in these RTE meat product is an indicator of faecal contamination and improper handling [12] which ranks high on the list of food borne hazards listed by WHO [13]. Havelaar et al. [14] reported that low and medium income countries (LMICs) in Africa and South East Asian sub-regions particularly suffer from a significant burden of foodborne diseases (FBDs) which

results in huge economic losses. Food borne disease is a major impediment to growth and development in LMICs and they pose a threat to a number of the Sustainable Development Goals [13,15]. It is also a pointer of possible presence of associated enteric pathogens which raises a suspicion of poor food handling, poor hygiene during preparation, handling and storage, lack of reheating and improper vending temperatures practice and may represent a risk to the consumers if related to pathogenic strains.

The contamination of these ready to eat protein meats sold along the highways can be attributed to a number of factors. Firstly, during sample collection, it was observed that the RTE meat products were poorly covered and this caused insects infestation. Additionally, the non-availability of proper water systems on the highways could further pose as a challenge to food vending practices requiring lots of water for hygienic operations, as food vendors are left to manage very little water taken to the sales point (SP), thereby undermining some sanitary practices such as frequent washing of hands especially after contact with suspicious matter like faeces. A comparable study on the risk factors for contamination of RTE street vended poultry dishes in Dakar, Senegal found that most of the vendors used dirty buckets, sinks dishes and tongs (to serve food to their customers) in dirty washing water [16], Thereby exposing consumers to the risk of eating poultry products that are contaminated with coliforms.

Another major challenge with the microbiological safety of RTE foods sold on the high way is the poor sanitary conditions in which the food are processed and stored prior to sales. Interestingly, this could be attributed to the poor socioeconomic status of these RTE food vendors and or producers. Most vendors were also observed to re-use disposable nylons and polythene bags for sales either to save cost or because of their poor knowledge of proper sanitary practices. Earlier reports show that 41.6% and 31.5% of food vendors in Ogun State, Nigeria had poor knowledge of foodborne infection and poor food safety practices respectively [17]. Improper waste disposal have also been implicated as a contributing factor in the contamination of RTE meat products [18].

The result of this study shows that heterotrophic bacteria, *Staphylococcus* and *Shigella* spp are prevalent in two common RTE meat products

sold along Benin Sapele express way. The presences of these organisms are worrisome, owing to their potential in causing FBDs and its outbreaks, particularly staphylococcal food poisoning and shigellosis. The absence of *Staphylococcus* species reported in one of the samples obtained from Sapele Junction Amukpe could be attributed to adherence to good hygiene practices and good manufacturing practices all through the RTE food value chain for that particular batch. Interestingly, the said sample also did not have any heterotrophic bacteria or *Shigella* species present in it. The result of the current study is in keeping with that of Amadi et al. [19], who also reported a high prevalence of similar microorganisms in Rivers State, Nigeria. They reported the presence of *S. aureus*, *Shigella spp* and other pathogenic bacteria from similar RTE meat products (suya and smoked fishes). Similar studies conducted in Nigeria by Egbebi and Muhammed [20] and Chukwura and Mojekwu [21], also reported comparably high level of *S. aureus* and other pathogenic bacteria isolated from RTE meat products sold by vendors. The presence of these isolates in RTE meat product is a confirmation that during food preparation and processing, pathogenic bacteria may be transferred to such food items by food handlers both directly or by cross contamination through hands, surfaces, utensils and equipment that have been inadequately cleaned and disinfected between the preparation of different types of food as initially asserted by Toit and Venter [22]. Antibiotics resistance is currently a major challenge globally, but is worse in developing countries where self-medication and inappropriate use of antibiotics is a major practice [23]. In the current study, 99% of all *Staphylococcus aureus* isolates and *Shigella* species investigated were multidrug resistant with relatively high MDR index. This finding is similar to the results recently reported [23,24], who reported high prevalence of multidrug resistant *Staphylococcus aureus* from abattoir facilities and poultry respectively. The high antibiotics resistance profile could also be attributed to the high use of antibiotics in animals; for therapeutic purposes, as growth promoters and for prophylactic use [25].

Methicillin Resistant *Staphylococcus aureus* (MRSA)-contaminated meat could serve as vehicle for the dissemination of antibiotic-resistant bacteria associated with risks to public health. Also, the incidence of methicillin-resistant staphylococci from meat in open markets confirms that methicillin-resistant staphylococci

are no longer a problems for only hospitals because they have entered the food chain, suggesting the dissemination of these resistant traits through horizontal gene transfer [26,27,28,29].

In this study, all *Staphylococcus aureus* isolates tested for methicillin resistance was observed to be resistant. As in the current study, Vanegas-Lopez et al. [28] reported all (149/149) staphylococci isolates from Columbian foods to be methicillin resistant. Methicillin-resistant staphylococci have also been reported in animal-derived food products worldwide [27,28,29], and are estimated to cause around 185,000 cases of food poisoning each year [30]. It is worthy of note that human epidemic clones of MRSA have been reported in meat products [31,32], suggesting that retail meat products can be contaminated by human source.

In a somewhat contrary study, van Loo et al. [33] reported RTE meat products (pork and beef) in the Netherlands to harbor high amount of methicillin-sensitivity with low methicillin-resistant *Staphylococcus aureus*, their result was probably due to the surveillance program on antibiotics use in animal husbandry started in 1999 by the Netherland government. Also, Yang et al. [34] revealed that, of the reasonable number of RTE food samples investigated in China, they were positively low for methicillin-resistant *S. aureus*.

## 5. CONCLUSION AND RECOMMENDATION

Methicillin resistant *Staphylococcus aureus* and *Shigella* species are prevalent in commercially processed ready to eat meat products sold along Benin Sapele express way, both on the Benin City wing and in Delta State. The high prevalence of methicillin-resistant *Staphylococcus aureus* in RTE meat products further lend credence to the fact that MRSA is not just associated with health care facilities, and that livestock associated MRSA can go through the food chain and persist on RTE food. Ready to eat Edible worms and Snails vending along Benin-Sapele road to Amukpe, Sapele junction axis are therefore unsafe for human consumption and could be a threat to public health. Hence, the need for control measures to protect the consumption of these delicious products which is becoming increasingly popular for their nutrition health benefits. Therefore, there is need for some legislative regulations, installation of proper facilities at strategic points along the highways to

encourage adherence to Good Hygiene Practices by the commercial food vendors. Enlightenment programmes and training of vendors plus monitoring of adherence to hygiene practices will help to prevent the looming threat to public health.

## ACKNOWLEDGEMENT

We acknowledge the contributions of Late Mrs. Vera Kalu, who was originally a co-author, but passed on at the verge of submitting this manuscript for publication. May her gentle soul rest in peace, Amen.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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