



## **Prevalence and Antibiogram of *Salmonella* Species Isolated from Snail (*Archachatina marginata*) Sold in Port Harcourt, Rivers State, Nigeria**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author VD designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript, managed the analyses of the study and literature searches under the strict supervision of authors DNO, LOA and NNO. All authors read and approved the final manuscript.*

### **Article Information**

DOI: 10.9734/EJNFS/2020/v12i1230336

Editor(s):

(1) Dr. Kristina Mastanjevic, Josip Juraj Strossmayer University of Osijek, Croatia.

Reviewers:

(1) Anna Carla Alberto-Silva, Universidade Federal Rural do Rio de Janeiro (UFRRJ), Brazil.

(2) Josiane Moreira da Costa, Universidade Federal de Minas Gerais, Brazil.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/64037>

**Original Research Article**

**Received 20 October 2020**  
**Accepted 26 December 2020**  
**Published 31 December 2020**

### **ABSTRACT**

Increase in microbial population especially *Salmonella* species in food due to improper handling, storage and exposure to contaminants can raise public health concerns when consumed without adequate processing. This study evaluates the prevalence and antibiogram of *Salmonella* species associated with the giant land snail (*Archachatina marginata*) sold in markets around Port Harcourt metropolis. A total number of seventy two (72) samples of land snail were collected from three markets; Creek Road, Mile one and Rumuokoro. The samples were labelled and transported in an ice packed coolers to the laboratory for analyses. Standard microbiological protocols were employed to determine the microbial load and species of the various parts (intestine, meat and fluid) of the snail samples after shucking. Antibiotics sensitivity profile testing of the isolated and identified *Salmonella* species were carried out as recommended by Clinical Laboratory Standard

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Institute (CLSI) and statistical analyses using one way ANOVA and all pairs Turkey-Kramer. Results from the study showed that the highest total heterotrophic bacteria count (THBC) of  $8.6 \times 10^6$  CFU/g was obtained in the snail intestine sourced from Creek road market while THBCs of  $8.2 \times 10^6$  CFU/g and  $7.3 \times 10^6$  CFU/g were from Mile one and Rumuokoro markets respectively. THBCs of meat from the markets ranged from  $4.3$ - $5.4 \times 10^6$  CFU/g and  $3.7$ - $4.9 \times 10^6$  CFU/ml in fluid with Rumuokoro having the least occurrence respectively. Mean *Salmonella* counts (MSCs) ranged from  $0.4$ - $3.6 \times 10^3$  CFU/g, with least count obtained from Rumuokoro and Mile 1 markets. Similarly, least MSCs in fluid and intestine were obtained from Rumuokoro and Mile 1 whereas Creek road Market had the highest respectively. Three species of *Salmonella*; *S. arizonae*, *S. gallinarum* and *S. typhi* were predominant in addition to other species such as *Vibrio* spp., *Bacillus* spp., *Staphylococcus* spp., *Shigella* spp., *Pseudomonas* spp., *Enterobacter* spp., *E. coli*, *Micrococcus* spp., *Acinetobacter* spp., *Klebsiella* spp. and *Listeria* spp identified using both conventional and molecular method. Antibiogram profile revealed that all the identified *Salmonella* species were susceptible to Ofloxacin and Ciprofloxacin but strongly resistance to Cetazidime and Gentamicin. The diversity and elevated microbial load observed from this study calls for caution in handling and processing of snails since most of these bacteria may become aetiologic agents of several food-borne diseases and other pathological conditions. As a necessity, good quality control measures and proper chemotherapy should be administered to patients with signs and symptoms of food borne illness emanating from consumption of snail.

**Keywords:** *Salmonella* spp; *Archachatina marginata*; antibiogram; aetiologic; ciprofloxacin; cetazidime.

## 1. INTRODUCTION

Snails are popular protein sources consumed by people worldwide, Apart from being a protein source, they are a good source of iron, calcium, and phosphorus, and are said to contain almost all the amino acids needed by humans [1]. The species of land snails commonly sold in Port Harcourt and other coastal towns in Nigeria is *Archachatina marginata*, also known as the African giant land snail [2,3]. The African giant land snails are mostly found in the forest, farms and gardens where they have unlimited vegetation to feed on. They are also bred in snail farms. The close contact of wild snails with soil and organic debris, and their uncontrolled feeding pattern make them susceptible to microbial contamination. Snails are regarded to have high populations of indigenous bacteria and coliforms, and also suspected to contain certain poisonous substances which they ingest from their environment [1].

The meat of snails can be easily contaminated by microbial pathogens thereby serving as a vehicle for transmission of infectious agents to consumers. Microorganisms that have been isolated in snails include *Escherichia coli*, *Pseudomonas* spp., *Shigella* spp., *Enterobacter* spp., *Salmonella* spp., and *Klebsiella* spp. [4]. The presence of *Listeria monocytogenes* have also been reported in snails [5]. Meat sourced

from snail as a delicacy in most local foods constantly gulp bacteria from the soil in Southern part of Nigeria and are susceptible to microbial infestation and heavy metal contaminations from the environment [6,7,8]. Unpreserved snails are highly perishable and can develop suitable breeding Sites for aerobic microorganisms especially as it concerns improper postharvest handling, processing and storage. Snails have been reported to have been implicated as vehicles for human infections [9] including *Salmonella* related infections. Most cases of persistent fever reported in clinics in Nigeria today are linked to typhoid caused by *Salmonella typhumurium* which may be due to ingestion of infested food due to handling and the way of harvesting snails from unhygienic areas [10]. Causes of food contamination can be enormous, as *Salmonella* pathogens can colonize the gastro intestinal tracts (GITs) of live stocks as normal floras of healthy carriers [11]. Besides in animals and animal products, *Salmonella* can adhere well to the work surfaces, and from there spread to other foodstuff by cross-contaminations [12]. When chronic complications from salmonellosis such as ocular and urinary disorders set in, they are hard to treat even with common antibiotics [13] Hence, this study aim at determination of the prevalence and antibiogram of *Salmonella* species isolated from snail (*Archachatina marginata*) sold in Port Harcourt, Rivers state.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

A total of seventy two (72) samples of land snail were purchased from three prominent markets in Port Harcourt metropolis (Creek Road, Mile one and Rumuokoro). Twenty four (24) specimens were collected from each locations. The samples were labeled and transported in an ice packed coolers to the laboratory for analyses. This study was carried for a period of one year.

### 2.2 Sample Preparation

The snail samples (Plate 1) were prepared for bacteriological analysis as described by Nyoagbe et al. [1] and Bhandare et al. [14] with added modifications; the snail samples were scrubbed and rinsed with water to remove surface dirt, and then washed with sterile distilled water and scrubbed with ethanol to remove external microorganisms. The snails were aseptically shucked and meat samples homogenized while the fluids were carefully collected with sterile universal bottles.



Plate 1. Land snail (*Archachatina marginata*)

### 2.3 Isolation and Resuscitation of *Salmonella* Species

Ten gram (10 g) of the respective snail parts were agitated manually in 90 ml sterile peptone broth and incubate at 37°C for 48 h., for enrichment, then 10 ml of the incubated peptone containing the sample were transferred into Selenite F broth at 37°C for 24 hr.

### 2.4 Identification and Bacteria Enumeration

Bacterial colonies enumerated as colony forming units (CFUs) were sub-cultured after streaking on solidified surface-dried nutrient/plate count agar for purity purposes. Identification of species followed cultural and morphological characteristics and various biochemical tests including Methyl red, Motility, Indole, Oxidase, Catalase, Voges-Proskauer, Citrate and Sugar fermentation according to methods adopted by Ogbonna and Inana [15].

The bacteria count of the snail was obtained by adding 9.0 ml of peptone water to 1 ml of each snail sample to obtain a 10 fold serial dilution. 0.1 ml of the appropriated dilution was spread on Plate Count Agar (PCA) for total heterotrophic bacteria and *Salmonella-Shigella* agar (SSA) for *Salmonella* spp. and incubated for 24 hr at 37°C. The colonies present on the plate were counted and the total viable number was calculated in colony forming units per ml/gram (CF/ml; g) using the formula;

$$CFU = \frac{N}{D \times VP}$$

Where N = Number of Colonies  
D = Dilution  
VP = Volume Plated

### 2.5 Antibiotics Susceptibility Testing

Peptone water was prepared; five discrete colonies of the different identified isolates were inoculated into 5 ml of the broths and incubated at 35°C for 4-6 hours after which the inoculum for primary sensitivity testing was prepared from the broth that has been incubated for 4-6 hours. The density of the suspension was adjusted by adding the bacterial suspension to a sterile saline tube to match the density of the desired 0.5 McFarland standard. Each of the isolates was uniformly and aseptically inoculated into a different Mueller-Hinton agar plates by spread plate method using sterile swap strikes. The antibiotic sensitivity test was performed by disc diffusion technique using commercially available discs on Mueller Hinton agar plates [16], (Iroha et al., 2009). The appropriate antibiotic discs were aseptically placed on the agar using sterile forceps. The plates were then incubated at 37°C for 24 h. Interpretation of results was done using the zones of inhibition sizes (Cheesbrough, 2006; Okonko et al., 2009).

### 3. RESULTS AND DISCUSSION

The total heterotrophic bacteria count ranged from  $3.7 - 8.6 \times 10^6$  cfu/g in all snail parts studied across the different markets with snail intestine having the highest rate of contamination compared to its fluid with the least. *Salmonella* counts ranged from  $1.0 \times 10^3$  cfu/ml to  $3.0 \times 10^3$  cfu/g with the least recorded in snail fluid and highest in snail meat (Table 1). It has been reported previously that snail fluid contains antimicrobial properties [17] which could be the reason for the lowest microbial load observed in snail fluid in this study.

The prevalence of *Salmonella* spp. in the three markets studied was 27(37.5%) with 11(45.83%), 10(41.66%) and 6(25.0%) percentage occurrence recorded for Creek road, Mile one

and Rumuokoro respectively (Fig. 1). This data is comparable to the 3(6.6%) and 21(17.5% prevalence of *Salmonella* spp. in snail sold in markets published by Adagbada et al., (2011) and Nwiyia and Amaechi (2011) in Nigeria respectively.

Table 2 shows the result of morphological and biochemical characteristics of the isolates. Three species of *Salmonella*; *S. arizonae*, *S. gallinarum* and *S. typhi* identified were identified using molecular method in addition to other bacteria genera belonging to *Vibrio* spp., *Bacillus* spp., *Staphylococcus* spp., *Shigella* spp., *Pseudomonas* spp., *Enterobacter* spp., *E. coli*, *Micrococcus* spp., *Acinetobacter* spp., *Klebsiella* spp. and *Listeria* spp were isolated and identified in this study. while the results of antibiogram of the isolates are presented in Table 3 respectively.

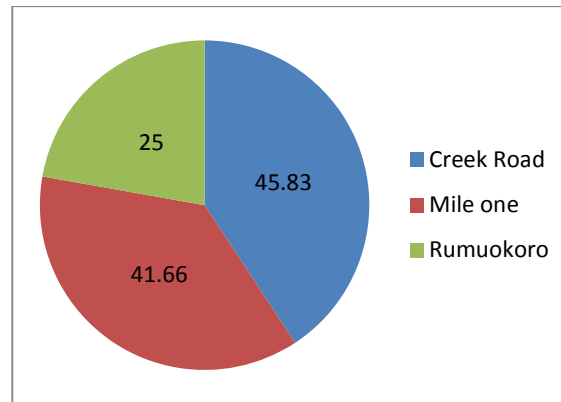
**Table 1. Mean bacteriological counts for snail parts collected from the three sampled markets**

	Snail Fluid		
	THB	<i>Salmonella</i> spp.	Unit
Rumuokoro	$3.7 \times 10^6 \pm 0.52$	$0.4 \times 10^3 \pm 0$	cfu/ml
Mile 1	$4.4 \times 10^6 \pm 0.87$	$0.4 \times 10^3 \pm 0.96$	cfu/ml
Creek Road	$4.9 \times 10^6 \pm 0.96$	$1.0 \times 10^3 \pm 0$	cfu/ml
Snail Meat			
Rumuokoro	$4.3 \times 10^6 \pm 2.32$	$1.0 \times 10^3 \pm 1.0$	cfu/g
Mile 1	$5.0 \times 10^6 \pm 2.56$	$1.5 \times 10^3 \pm 1.0$	cfu/g
Creek Road	$5.4 \times 10^6 \pm 3.01$	$1.7 \times 10^3 \pm 0.96$	cfu/g
Snail Intestine			
Rumuokoro	$6.7 \times 10^6 \pm 0.99$	$1.5 \times 10^3 \pm 1.15$	cfu/g
Mile 1	$8.2 \times 10^6 \pm 1.23$	$1.7 \times 10^3 \pm 0.96$	cfu/g
Creek Road	$8.6 \times 10^6 \pm 1.24$	$3.6 \times 10^3 \pm 1.58$	cfu/g

Key: Total Heterotrophic Bacteria count

**Table 2. Prevalence of *Salmonella* species in snail parts tested at different markets**

Source Market	Sample Type	Number tested (n=8)	Prevalence of <i>Salmonella</i> spp.
Creek Road	Snail fluid	8	2 (25%)
	Snail meat	8	5 (62.5%)
	Snail intestine	8	4 (50%)
Mile One	Snail fluid	8	1 (12.5%)
	Snail meat	8	4 (50%)
	Snail intestine	8	5 (62.5%)
Rumuokoro	Snail fluid	8	1 (12.5%)
	Snail meat	8	2(25%)
	Snail intestine	8	3(37.5%)
Total		72	27(37.5%)



**Fig. 1. Prevalence of *Salmonella* species in snail in relation to the different markets studied**

The recalcitrance of bacterial strains to antimicrobials could be explained by the possibility of the heavy use of these compounds in aquaculture and agriculture, several of which are non-biodegradable, thus increasing antibiotic selective pressure in soil and water, facilitating the transfer of antibiotic-resistant determinants between aquatic and terrestrial bacteria, including fish, snail and human pathogens, and allowing the presence of residual antibiotics in commercialized fish and snail [18,19]. Antibiotics susceptibility testing carried out on *Salmonella* spp. isolated in this study showed one hundred percent 105(100%) resistance to at least one antibiotic across the three locations studied.

All tested *Salmonella* spp showed a 105(100%) resistance to Cetazidime (CAZ) across the markets with resistance of isolates from Rumuokoro contributing 23(100%); mile one 35(100%) and Creek road 47(100%). These records differs from report by Bulbu et al., (2011) who published 16(100%) sensitivity of all *Salmonella* isolated in their study without any being resistant to CAZ. Contrarily, Islam et al. [20] reported a 5(31.25) resistance to CAZ in their study. Similar to records taken for Cetazidime, a 105(100%) level of resistance to Gentamycin (GEN) was revealed Isolates from Rumuokoro market showed 23(100%) resistance while creek road and mile one contributed 35(100%) and 47(100%) respectively to the overall 105(100%) resistance to gentamycin recorded. This result is in sharp disagreement with previous studies that revealed a 14(100%) susceptibility to *Salmonella* spp. to the antibiotic reported by Tamba et al. [21] in Zaria, Nigeria. Islam et al. [20] also reported an alteration to the findings in this study with their 16(100%)

susceptibility of *Salmonella* pp. to gentamycin without any resistance recorded. Notwithstanding, our study partly corroborates studies done by Jambo et al. [22] who recorded a low 6.9% susceptibility to gentamycin with a 93.1% resistance to the antibiotics. Level of resistance against gentamycin was very much in agreement to the findings of Samanta et al. [23] who found 100% of their *Salmonella* isolates resistant gentamycin in India.

Isolates from Rumuokoro market showed a 23(100%) resistance to cloxacillin (CXC) but varying resistance pattern of 38(80.8%) and 18(51.4%) for *Salmonella* spp. isolated from samples sourced from Creek road and Mile one markets respectively. However, the overall resistance to CXC was 79(75.2%) with an intermediate pattern of 26(24.8%) suggesting a sharp variation from the 43% resistance of *Salmonella* to cloxacillin reported by Claudious et al. [24]. None of the isolates from all the source studied were susceptible to cloxacillin.

Antibiotics susceptibility pattern of *Salmonella* isolates to other antibiotics showed varying pattern of susceptibility. An overall 19(18.1) resistance to Augmentin with a 53(50.5%) susceptibility was recorded with Rumuokoro contributing 5(21.7%), Creek road 10(21.3%) and mile one 4(11.4%) of the resistance reported. Ciprofloxacin (CPR) and (OFL) were 100% potent against all *Salmonella* species isolated from the three locations. This does not agree with the 15(46.87) low susceptibility and 5 (15.62%) resistance to ciprofloxacin published by Irfan et al. [25]. However the overall 100% susceptibility of *Salmonella* spp. recorded corroborates report by Selvaraj et al. [26].

**Table 3. Morphological and biochemical characteristics of bacteria isolated from the three sampled market**

Isolate Code	Texture	Colour	Elevation	Translucent	Shape Gram reaction	Oxidase	Indole	Catalase	Motility	Coagulase	Citrate	MR	VP	Glucose	Lactose	Maltose	Sucrose	Fructose	Probable Identification
Pc A	Mucoid	Creamy	Raised	Opaque	+ve Rod	+	-	+	+	-	+	+	-	+	-	-	-	-	<i>Bacillus sp</i>
Pc B	Moist	Yellow	Smooth	Translucent	+ve Cocci	-	-	+	-	+	+	+	+	+	+	+	+	+	<i>Staphylococcus sp</i>
Pc C	Moist	Creamy	Raised	Opaque	-ve Rod	+	-	+	+	-	-	+	-	+	-	+	-	-	<i>Salmonella sp</i>
Pc D	Smooth	Clear	Raised	Translucent	-ve Rod	-	-	+	-	-	-	+	-	+	-	-	+	-	<i>Shigella sp</i>
Pc E	Dried	Green	Flat	Opaque	-ve Rod	+	+	+	+	-	+	+	-	+	-	+	+	-	<i>Pseudomonas sp</i>
Pc F	Moist	Clear	Raised	Opaque	-ve Rod		-	+	+	-	-	+	-	+	-	+	+	+	<i>Enterobacter sp.</i>
Pc	Moist	Creamy	Raised	Translucent	-ve Rod	+	+	+	+										<i>E.coli</i>
Pc	Moist	Yellow	Raise	Translucent	+ve Cocci	-	-	+	-	-	+	+	+	+	+	+	+	+	<i>Micrococcus sp.</i>
Pc	Dried	Milky	Flat	Opaque	-ve Rod	-	-	+	+	+	-	+	-	+	+	+	+	-	<i>Acinetobacter sp.</i>
Pc	Moist	Pale	Raised	Opaque	-ve Rod	-	-	+	+	+	-	+	-	+	+	+	+	-	<i>Klebsiella sp</i>
SSA 1	Moist	Black center	Raised	Opaque	-ve Rod		-	+	+	-	-	+	-	+	-	+	-	-	<i>Salmonella sp</i>
SSA 2	Smooth	Pinkish	Raised	Translucent	-ve Rod		-	+	-	-	-	+	-	+	-	-	+	-	<i>Shigella sp</i>

**Table 4. Percentage of antibiotics sensitivity values (%) of *Salmonella* spp isolated from the three markets**

Anti biotics	Rumuokoro (n=23)			Mile One (n=35)			Creek Road (n=47)			Overall sensitivity report across all markets (N=105)		
	R	I	S	R	I	S	R	I	S	R	I	S
CRX	0(0)	7(30.4)	16(69.6)	2(5.7)	5(14.3)	28(80)	5(10.6)	10(21.3)	32(68.1)	7(6.7)	22(21)	76(72.4)
AUG	5(21.7)	12(52.2)	6(26.1)	4(11.4)	11(31.4)	20(57.1)	10(21.3)	10(21.3)	27(57.4)	19(18.1)	33(31.4)	53(50.5)
NIT	7(30)	3(13.4)	13(56.6)	4(11.4)	18(51.4)	13(37.2)	0(0)	24(51.1)	23(48.9)	11(10.5)	45(42.9)	49(46.6)
CPR	0(0)	0(0)	23(100)	0(0)	0(0)	35(100)	0(0)	0(0)	47(100)	0(0)	0(0)	105(100)
CAZ	23(100)	0(0)	0(0)	35(100)	0(0)	0(0)	47(100)	0(0)	0(0)	105(100)	0(0)	0(0)
GEN	23(100)	0(0)	0(0)	35(100)	0(0)	0(0)	47(100)	0(0)	0(0)	105(100)	0(0)	0(0)
CXM	5(21.7)	7(30.5)	11(47.8)	4(11.4)	21(60)	10(28.6)	9(19.1)	14(29.8)	24(51.1)	18(17.1)	42(40)	45(42.9)
OFL	0(0)	0(0)	23(100)	0(0)	0(0)	35(100)	0(0)	0(0)	47(100)	0(0)	0(0)	105(100)
CTR	5(21.7)	8(34.8)	10(43.5)	4(11.4)	21(60)	10(28.6)	5(10.6)	19(40.5)	23(48.9)	14(13.3)	48(48.7)	43(40.9)
ERY	0(0)	14(60.9)	9(39.1)	4(11.4)	14(40)	17(48.6)	10(21.3)	14(29.8)	23(48.9)	14(13.3)	42(40)	49(46.7)
CXC	23(100)	0(0)	0(0)	18(51.4)	17(48.6)	0(0)	38(80.8)	9(19.1)	0(0)	79(75.2)	26(24.8)	0(0)

Key: AUG; Augmentin, NIT; Nitrofurantion, CPR; Ciprofloxacin, CAZ; Cetazidime,; GEN.; Gentamicin, CXM.; Cefixime, OFL.; Ofloxacin, CTR.; Cftriaxone, ERY; Erythromycin, CXC.; Cloxacillin. CRX; Cefuroxi

Data revealed for levels of antibiotic resistance form Erythromycin (ERY) showed total resistance of 14(13.3%) with the bulk resistance percentage recorded for samples sourced from Creek road 10(21%). However, Mile one contributed 4(11.4%) while data from Rumuokoro only had intermediate 14(60.9%) and sensitive 9(39.1) levels of anti-erythromycin susceptibility pattern. Previous studies by Claudious et al. [24] revealed a (65%) resistance to ERY contradicting our record in this study. There were no significant difference for levels of resistance, intermediate and sensitivity to Nitrofurantoin (NIT) by *Salmonella* spp. isolates in the three markets studied at  $p < 0.005$ . However the overall resistance level of isolates to NIT was 11(10.5%) while 45(42.9%) and 49(46.6%) were recorded for intermediate and sensitive levels respectively. *Salmonella* spp. isolated in this study were more resistant to the cephalosporins and aminoglycosides in terms of classes.

#### 4. CONCLUSION AND RECOMMENDATIONS

The values of bacteriological counts obtained in this study were significantly higher compared to the permissible limit as recommended by regulatory bodies According to the World Health Organization (WHO) directives on microbial limits, total bacterial count should not exceed  $5 \times 10^5$  colonies per gram of sample. Three species of *Salmonella*; *S. arizonae*, *S. gallinarum* and *S. typhi* in addition to other bacteria genera belonging to *Vibrio* spp., *Bacillus* spp., *Staphylococcus* spp., *Shigella* spp., *Pseudomonas* spp., *Enterobacter* spp., *E. coli*, *Micrococcus* spp., *Acinetobacter* spp., *Klebsiella* spp. and *Listeria* spp were isolated and identified in this study.

The prevalence of *Salmonella* spp. in the three markets studied shows that Creek road had the highest percentage occurrence of *Salmonella* spp. Followed by Mile 1 while Rumuokoro had the least percentage respectively. Antibigram profile revealed that all the identified *Salmonella* species were susceptible to Ofloxacin and Ciprofloxacin but strongly resistance to Cetazidime and Gentamicin. The diversity and elevated microbial load observed from this study calls for caution in handling and processing of snails since most of these bacteria may become aetiological agents of several food-borne diseases and other pathological conditions. Hence, Proper blanching and heating methods should be employed during preparations of and snail to avoid cross contamination and food intoxication / poisoning before consumption and also It is

important that all hazard analysis critical control point be adhered to for good production processes.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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