Proximate Analysis and Occurrence of Antibiotic Resistant*Escherichia coli* in Street Vended Ready To Eat Edible Larvae of *Rynchophorus phoenicis* in Port Harcourt, Nigeria

Abstract

Rhynchophorusphoenicis, known as the African palm tree larva weevil or edible worm, is a popular edible insect in southern Nigeria and other African countries. These larvae, by-products of oil palm and palm trees, are consumed roasted or boiled and are known for their nutritional and medicinal properties. In Nigeria, they are widely sold along highways in states like Bayelsa, Delta, Edo, Imo, Rivers, and the upper Cross River basin. Preparation methods include consuming them raw, boiled, fried, smoked, or in stews and soups. Typically, the larvae are washed, skewered, sprinkled with salt and pepper, and grilled. They are juicy and have a taste reminiscent of Torzo or cow intestines.

Despite their popularity, edible worms pose safety and health concerns due to contamination and the emergence of antimicrobial-resistant bacteria. The indiscriminate use of antimicrobials in animals and humans has led to the development of resistant microorganisms, including multidrug-resistant *Escherichia coli*, which has been increasingly reported in food poisoning outbreaks. There is limited data on the incidence and antibiotic resistance of *Escherichia coli* in ready-to-eat foods in Nigeria, prompting this study.

The study aimed to determine the proximate composition, occurrence, and antibiotic-resistant pattern of *Escherichia coli* from edible larvae (*Rhynchophorusphoenicis*). Objectives included determining the proximate composition of the larvae, the occurrence of Escherichia coli in them, and their antibiotic susceptibility pattern.

Fifty ready-to-eat food samples of edible larvae were randomly collected from hawkers. These samples, often partially exposed and poorly packaged, were transported to the microbiology laboratory for analysis. Sterilized glassware and media were used, including Nutrient Agar, Eosin Methylene Blue (EMB) Agar, and Mueller Hinton Agar. Samples were diluted using peptone water, and bacteriological determination involved homogenizing 25 grams of the sample in 225 ml of peptone water, followed by serial dilutions. Aliquots were inoculated on EMB Agar plates, and incubated at 37°C for 24 hours, and colonies were counted and recorded as colony-forming units per gram (cfu/g). Isolates were identified using standard microbiological methods and biochemical tests.

Proximate analysis revealed moisture (35.49%), carbohydrate (6.01%), protein (20.73%), ash (1.25%), lipid (22.3%), and fiber (14.23%) content. Bacterial counts ranged from 8.1 x 10^7 to 6.34 x 10^8 cfu/g, with the highest count at the Checkpoint sample and the lowest at Elele-Alumini. Of the 27 isolates, 15 (55.56%) were confirmed as E. coli, with 18.52% of samples exceeding the acceptable microbiological limit (≥10^7 CFU/g). Antibiotic susceptibility tests showed all isolates resistant to augmentin, penicillin, cephalosporin, and ofloxacin, with 46.67% resistance to sulfamethoxazole and streptomycin.

Keywords: Occurrence, *Escherichia coli*, *Rhynchophorus phoenicis*, Ready-To-Eat Edible Larvae, Antibiotic resistance.

Introduction

Ready-to-eat (RTE) foods are a convenient culinary option, encompassing a range of items that necessitate minimal or no further preparation before consumption, aside from potential reheating. These foods, spanning raw, minimally cooked, or fully cooked varieties, hot or cold, are readily available for instant consumption without additional handling. In various African countries, including Nigeria, the prevalence of RTE foods sold by street vendors has surged (*Makelele et al., 2015*), offering an assortment of snacks, meats, salads, fruits, and beverages. Their popularity has extended to markets, roadsides, and restaurants, with the emergence of migratory food vending, particularly along highways, exposing RTE foods to microbial hazards.

"In Nigeria, *Rhynchophorusphoenicis* (F.) (Coleoptera: Curculionidae), commonly known as the edible worm or African palm weevil, is a traditional delicacy consumed either roasted or boiled, especially in southern Nigeria and other African regions where palm trees thrive. These larvae which feed on palms and are a by-product of palm trees used in palm wine production, hold nutritional and medicinal significance. Widely sold along major Nigerian highways and in states like Bayelsa, Delta, Edo, Imo, Rivers, and the upper Cross-River basin" (*Ekrakene&Igeleke, 2007*), they are also available in local markets and restaurants. Embraced by various Nigerian tribes, such as the Ibibio, Ilesha, Urboho, and Isoko, these larvae are sometimes recommended to pregnant women for their nutrient content (*Ekpo & Onigbinde, 2005*).In certain instances, the medicinal value of the larvae in aiding treatments of ailments like women's infertility, rashes and wounds, coughs, and colds among others have been reported by *Fogoh et al., (2015).*

"Despite the nutritional value of *R. phoenicis,* detailed data on its nutritional composition remain scarce. Previous studies in Nigeria have overlooked key nutritional aspects, such as vitamin content, amino acids, fatty acids, and minerals" (*Braide &Nwaoguikpe, 2011; Elemo et al., 2011; Ekpo & Onigbinde, 2005*). "However, palm weevil larvae generally offer valuable nutrients like iron, zinc, essential amino acids, and fat, with reported average protein, fat, fiber, and ash contents" (*Rumpold & Schluter, 2013*).

Escherichia coli (*Escherlich*) (*Enterobacterales; Enterobacteriaceae*), a prevalent foodborne pathogen, poses significant health risks, causing urinary tract infections and gastrointestinal diseases. Widely distributed in various environments, including soil, water, sewage, and foods, *E. coli* transmission primarily occurs through contaminated foods. While few studies have

reported E. coli in edible worms, other microorganisms like *Staphylococcus aureus, Bacillus species, and Salmonella species* have been detected (*Ramashia et al., 2020; Ngoka et al., 2021*). The emergence of multidrug-resistant (MDR) bacterial strains worldwide presents a grave public health concern. Increasing antibiotic resistance among *Escherichia coli* strains contributes to morbidity, mortality, and substantial healthcare costs, exacerbated by indiscriminate antibiotic use in veterinary medicine and agriculture.

The growing prevalence of contaminated ready-to-eat foods correlates with increased mobility, urbanization, and reliance on ready-to-eat foods for sustenance and employment. This shift has transferred food safety responsibilities from individuals to vendors, many of whom lack awareness of proper food handling practices, leading to unsanitary conditions and susceptibility to bacterial contamination. Additionally, open-air vending exposes ready-to-eat foods to environmental pollutants like flies and dust.

Given these challenges, there is a pressing need to assess the *Escherichia coli* contamination and antimicrobial resistance profiles of ready-to-eat foods like *Rhynchophorusphoenicis*, particularly along major transit routes such as the Emuoha Toll Gate East-West Road in Port Harcourt, Nigeria. This study aims to evaluate *Escherichia coli* levels in RTE edible worms of *Rhynchophorusphoenicis* and their resistance to clinically relevant antimicrobial agents, shedding light on potential health risks associated with their consumption.

Materials and Methods

Study Location

Samples were obtained from vendors and mobile hawkers by the roadside from different locations (Local Government Areas and Markets) along Emuoha Toll Gate East-West Road, Port-Harcourt, Nigeria. They were: Choba Market, Checkpoint, Ogbakiri Market, Elele-Alimini, and Akpor-Ndele Market.

Collection Of Samples

A total of 50 ready-to-eat food samples of edible larvae were brought randomly from hawkers. These RTE edible larvae were partially exposed and poorly packaged (Plate 1). The sample was aseptically stored in well-labeled and sealed sterile transparent bags and transported immediately to the microbiology laboratory at the Department of Microbiology, University of Port Harcourt for analyses.

Plate 1. Roasted ready-to-eat edible larvae of *Rhynchophorus phoenicis*.

Media Preparation

The following media were employed during this study. All media was prepared according to the manufacturer's specifications.

- Nutrient Agar
- Eosin Methylene Blue (EMB) Agar
- Mueller Hinton Agar

Each of the media was prepared by measuring out the appropriate weight of the agar and dissolving them in 1000ml of distilled water according to the manufacturer's instructions. It was whirled to mix well and mixture was pre-heated to 100^{o}C to completely dissolve the powder. It was sterilized by autoclaving at 121^{0}C for 15 mins at 15 psi. After sterilization, the medium was allowed to cool at 45^{0}C. On cooling, 20ml of the sterile medium was aseptically dispersed into sterile petri dishes and allowed to solidify. It was then inverted immediately to avoid vapor falling on the medium.

Preparation of Diluents

The diluents used for sample analysis were peptone water. Peptone water is used for the cultivation of non-fastidious microorganisms. During the study, Peptone water was used to carry out a 10-fold serial dilution in duplicate for the analysis of the RTE edible worm sample. The medium was prepared by weighing out 15g of peptone medium and transferringit to a conical flask. About 1000ml of distilled water was added and pre-heated to 100^{0}C to dissolve completely. Then 9ml of the diluent was aseptically transferred into test tubes. The test tubes were corked with non-absorbent cotton wool and then sterilized by autoclaving at 121^{0}C for 15 mins at 15psi and allowed to cool.

Preparation of the Sample/Isolation Procedure

The bacteriological determination of ready-to-eat edible larvae samples was carried out by placing individually the samples in a sterile petri dish. A 25g of the composite food sample was weighed out with the aid of a foil paper using a digital weighing balance (*Philip, HR2001, China*) into 225ml of peptone water (*Oxoid, Basingstoke, UK*) in labeled stomacher bags. The sample was homogenized using a stomacher machine for 2 mins. The homogenized sample was then transferred into a sterile conical flask and corked with cotton wool. This served as the stock culture and gave a 10^1 dilution.

Serial Dilution

Ten-fold serial dilution were made from the stock culture to obtain dilution factors of 10^2, 10^3, 10^4, 10^5 and 10^6 respectively to reduce the microbial load as described by *Makelele et al*. (2015). With the aid of a 1ml pipette, 1ml aliquot of the homogenized sample was transferred

from the stock culture which gave a 10^1 dilution into the first test tube containing 9ml peptone water giving a 10^2 dilution factor which was further serially diluted up to 10^6 dilution factor. That is, another 1ml sample from 10^2 to 10^3, 10^3 to 10^4, 10^4 to 10^5, 10^5 to 10^6. For each dilution, a new pipette was used to avoid contamination. This was repeated for all samples.

Isolation and Identification of Microorganisms

From the dilution factors mentioned above, 0.1ml of the aliquots was inoculated in duplicate plates of Eosin Methylene Blue (EMB) Agar for isolation of *Escherichia* spp. using the spread plate method. Using a hockey stick dipped in 70% ethyl alcohol, flamed using the Bunsen burner, and cooled, the inoculum was spread around the plates. The inoculated plates were then inverted and incubated aerobically at 37^0C for 24 h.

Determination of Total Viable Plate Counts

Aliquot (0.1 ml) of each of the selected sample dilutions was aseptically spread plated on the sterile solidified nutrient agar (NA:TM, Rajasthan, India) in duplicate. The inoculum was allowed to be absorbed into the agar plate, inverted, and incubated at 30^0C for 24 h. After incubation, plates with 30-300 colonies were selected and counted and results recorded. Counts were expressed as colony-forming units per gram of sample homogenized (cfu/g) and expressed as logarithm (log10 CFU/g) (Harrigan & Mc-Cance, 1990; Cheesbrough, 2010). The cfu/g was calculated using the average number of colonies for the duplicate plates of the sample.

The following formula was used to calculate the CFU/g:

CFU/g = (Number of Colonies X Dilution Factor) / Volume of Culture (ml)

Variables:

CFU/g is the colony-forming units per gram

The number of Colonies is the count of colonies observed on a plate

The dilution Factor is the factor by which the original sample was diluted

The volume of Culture is the volume of the diluted sample plated (in mL)

Subculture of the Isolates

Discrete colonies on the Eosin Methylene Blue agar were plated and streaked on freshly prepared nutrient agar based on their morphology which includes color, size, shape, texture, elevation, edge to obtain pure colonies. The plates were then incubated at 37^0C for 24 h. Isolates from the nutrient agar were used for biochemical testing and identification.

Identification and Characterization of Isolates

The isolates were identified based on standard microbiological methods. This includes observation of the different characteristics of the colonies such as colony pigmentation, colony elevation, texture, shape, and size. Biochemical tests were also carried out for further identification and characterization.

Colony Morphology

The colonies of the isolates give a distinctive metallic green sheen which is due to the metachromatic property of dyes (eosin and methylene blue in the ratio of 6:1) and the lactose fermenting property of *E. coli*, which changes the pH of the medium to acidic. Hence, making the medium more selective for *E. coli* makes the identification much easier.

Confirmation of Isolate (IMViC Test)

All the isolate was subjected to various biochemical tests to characterize and identify them. *Escherichia coli* was confirmed biochemically using a traditional method called IMViC tests. This is a set of four tests that are used to differentiate members of Enterobacteriaceae. IMViC is an abbreviation that stands for the Indole, Methyl-red, Voges-Proskauer, and Citrate utilization tests. These tests detect the presence or lack of a particular enzyme by the test organism.

Indole Test

In the indole test, the bacteria were tested for their ability to produce indole from tryptophan (amino acid) using the enzyme tryptophanase. The indole reacts with the aldehyde in Kovac's reagent to give a red or pink ring at the top of the tube. Peptone water in a tube thatcontained tryptophan was inoculated with bacteria isolate. The mixture was then incubated overnight at

37^0C. Then, a few drops of Kovac's reagent were added to the mixture and the formation of a red or a pink colored ring at the top was seen as a positive reaction. *E. coli are* indole-positive bacteria (Cheesbrough, 2010).

Methyl Red Test

The methyl red test detects the ability of a bacterium to produce acid from glucose fermentation. Methyl red, a PH indicator, remains red at a PH less or equal to 4.4. The isolate was inoculated into glucose phosphate (MRVP) broth, which contains glucose and a phosphate buffer and was incubated at 37^0C for 48 h. Three to five drops of MR reagent were added to the tube. The red color development is a positive reaction that occurs when the bacteria have produced enough acid to neutralize the phosphate buffer. Yellow discoloration occurs in MR-negative bacteria. *E. coli* are MR-positive bacteria.

Voges-Proskauer Test

Voges-Proskauer test was used to detect the presence of acetoin in the bacteria-containing media. Acetoin is oxidized to diacetyl in the presence of air and sodium hydroxide. Diacetyl in the presence of alpha-naphthol reacts with guanidine to produce a red color. To perform the VP test, the test bacterium was inoculated into glucose phosphate (MRVP) broth in a tube and incubated for 72 h. Then 15 drops of alpha-naphthol were added to the test broth followed by shaking. Then five drops of 40% potassium hydroxide (KOH) were added to the broth and shaken well. The tubes were then allowed to stand for 15 min to see a positive red discoloration. After 1 h of no color change the isolate was categorized as VP-negative*E. coli*.

Citrate Utilization Test

The citrate utilization test detects the ability of the bacteria to use citrate as its sole source of carbon and energy. Citrate agar media contains a PH indicator called bromothymol blue. The agar media changes from green to blue at an alkaline PH.

A loopful of the bacteria was streaked onto a citrate agar slant without stabbing the butt and incubated at 37^0C for 24 h with a loose cap. Citrate in the media breaks down to oxaloacetate and acetate due to the action of the enzymecitritase. Oxaloacetate was further broken down into pyruvate and CO2. Production of Na2C03 from sodium citrate changes the media into alkaline pH, and hence color change from green to blue. The blue color formation is a positive reaction, whereas the slant remaining green colored is a feature for a negative test. *E. coli* is citrate-negative (Cheesbrough, 2010).

Proximate Analyses of the RTE Food Sample of *Rhynchphorus phoenicis*

The proximate composition of ash content, moisture content, crude protein, crude lipid, crude fiber, and total carbohydrate of the RTE sample was determined as described by the Association of Official Analytical Chemists (AOAC, 2000). The crude protein content was determined by the Kjedahi method, adopted from the procedures of James (1995) and Chang (2003). The crude fat content was determined by solvent extraction using the Soxhlet technique. The food sample was first hydrolyzed with boiling HCL before extraction with petroleum ether. Each extraction group was then dried in a forced air oven at 105^0C for 3 h (to constant mass), cooled in desiccator and then weighed to obtain the dry solids content. To determine ash content, the food sample (1g) were minced, weighed, and ignited in the crucible. Then it was transferred in the muffle furnace held at dark red at a rate of 550^oC for 6-8 h until the residue was white. Moisture content was determined by drying the sample overnight at 105^oC. Each parameter was conducted in duplicates.

Calculation of moisture:

$$Moisture\ (\%) = \frac{Weigh\ loss\ x\ 100}{Original\ weigh\ of\ the\ sample\ 1}$$

Calculation of crude protein:

$$N2\ (\%) = \frac{0.00014\ x\ Vol\ of\ acid\ x\ Filtration\ flask\ for\ preparation\ of\ digest\ x\ 100}{Weigh\ of\ sample\ x\ Amount\ of\ digest\ introduced\ into\ the\ flask}$$

$$Protein\ (\%)\ = \%\ of\ total\ N2\ x\ 6.25$$

Calculation of crude fat:

$$Fat\ (\%)\ = \frac{Weight\ of\ the\ oil\ x\ 100}{Weight\ of\ the\ samples\ taken\ 1}$$

Calculation of ash:

$$Ash\ (\%) = \frac{Weight\ of\ ash\ x\ 100}{Original\ weight\ of\ the\ sample\ taken 1}$$

Calculation of Carbohydrate:

$$Carbohydrate\ (\%) = 100\% - (\%\ Water\ +\ Ash\ +\ \%\ Proteins\ +\ \%\ Lipids)$$

Antibiotics Susceptibility Assay

A Kirby-Bauer method as described by Cheesbrough (2010) was employed to test the antibiotic susceptibility pattern of confirmed *Escherichia* spp. The antibiotics used were gentamycin (10ug GEN), augmentin (30ug AU), ciprofloxacin (5ug CPX), trimethoprim/sulfamethoxazole (30ug SXT), streptomycin (10ug S), penicillin (6ug PN), cephalosporin (30ug CEP), ofloxacin (5ug OFX), nalidixic acid (30ug NA) and pefloxacin (5ug PEF). The tests were performed by standard disk diffusion technique on Muller Hinton Agar (TM, Rajasthan, India) with the turbidity standard (0.5 McFarland). The plates were incubated aerobically at 35c for 16 to 18h. After incubation, test plates were examined for confluent growth, and the diameter of each zone of inhibition was measured in mm. Each inhibition zone diameter was interpreted using an interpretive chart from the World Health Organization (CLSI, 2015).

RESULT

Proximate Values of RTE Edible Larvae

The value of moisture content is 35.49%, carbohydrate is 6.01%, protein is 20.73%, ash is 1.25%, lipid is 22.3% and fiber is 14.23%. The composition of moisture was the highest, followed by lipid, protein, fiber, carbohydrate, and ash (Table 1).

Mean Counts of RTE Edible Larvae

The results showed that TVC (log_{10}cfu/g) in Checkpoint was the highest (6.34×10^8), followed by Choba Market (4.49×10^8), Ogbakiri (1.14×10^8), Akpor-Ndele (9.4×10^7) while Elele-Alumini had the lowest count (8.1×10^7) (Table 2).

Distribution of *Escherichia* spp. On EMB Agar

Escherichia spp. are Gram-negative, rod-shaped bacteria when viewed under a microscope. When cultured on Eosin Methylene Blue agar, which is a selective medium for gram-negative bacteria, they appear as a metallic sheen green colony. Out of the total of 50 samples, 27 were positive for *Escherichia spp* (Table 3).

Interpretation of Sanitary Quality of the RTE Edible Larvae

The sanitary quality of the RTE edible larvae was interpreted with consideration to microbiological guidelines for ready-to-eat foods; category 5 (cooked foods chilled but with some handling before sale or consumption). All 27 confirmed *Escherichia* spp. of the edible larvae revealed that 81.48% (22 of 27) of *Escherichia* spp. in the edible larvae were below the acceptable limit (10^5 to $<10^7$) CFU/g while 18.52% (5 of 27) of the *Escherichia* spp. in the edible larvae were unsatisfactory ($\geq 10^7$ CFU/g). The RTE edible larvae from checkpoint were the most unsatisfactory (Table 4).

Confirmation of *Escherichia coli*.

The result of the confirmation test done to identify *E. coli* from *Escherichia*spp through the biochemical test was positive for Indole, Methyl red, Voges-Proskauer and Citrate test. Out of the 27 *Escherichia* spp., 15 were positive for *E. coli* (Table 5).

Antibiotic Resistance Pattern of Tested *Escherichia coli* in RTE Edible Larvae

The *E. coli* from the RTE edible larvae were tested with 10 antibiotics. All isolates were resistant to augmentin, penicillin, cephalosporin, and nalidixic acid at 100%. Other isolates showed varying antibiotic resistance patterns with 46.67% resistance to sulfamethoxazole and streptomycin (Figure 1).

Table 1. Proximate analysis of a composite sample of RTE edible larvae of *Rhynchophorus phoenicis*.

S/No	Sample identity	% Moisture	% CHO	% Protein	% Ash	% Lipid	% Fibre
1	RTE Edible Larvae (*Rhynchophorus phoenicis*)	35.49	6.01	20.73	1.25	22.3	14.23

Table 2. Mean count of *Escherichia coli* in RTE edible larvae of *Rhynchophorus phoenicis.*

Location of samples	Sample location/Mean count (log_{10} CFU/g)		
	No of colonies	CFU/g	Log_{10} CFU/g
Akpor-Ndele	94	9.4×10^7	7.9731
Ogbakiri Market	114	1.14×10^8	8.0569
Checkpoint	634	6.34×10^8	8.8021
Choba Market	449	4.49×10^8	8.6522
Elele-Alumini	81	8.1×10^7	7.9085

Table 3. Distribution of *Escherichia spp*. on EMB agar from different location samples.

Location of samples	Number of samples	Number of confirmed *Escherichia spp.* n (%)
Choba Market	10	5 (18.52)
Checkpoint	10	5 (18.52)
Elele-Alumini	10	5 (18.52)
Akpor-Ndele	10	6 (22.22)
Ogbakiri Market	10	6 (22.22)

Table 4. Interpretation of total Escherichia counts of RTE edible larvae of *Rhynchophorus phoenicis* with microbiological guidelines for RTE foods – category 5.

Sample location	**No of samples (n)**	**No of Escherichia spp.(n)**	**Satisfactory**	**Borderline**	**Unsatisfactory**
	N = 50	N = 27	$< 10^5$	10^5 - $<10^7$	$\geq 10^7$
Choba Market	10	5	-	3	2
Checkpoint	10	5	-	2	3
Elele-Alumini	10	5	-	5	-
Akpor-Ndele	10	6	-	6	-
Ogbakiri	10	6	-	6	-
Total				22 (81. 48%)	5 (18.52%)

Table 5. Biochemical test (IMViC) result for the identification of *E. coli.*

Sample location /Sampling point	**Biochemical Test (IMViC) result**				**Identification of *E. coli*** **+ *E. coli* present** **- *E. coli* absent**
	Indole	Methyl Red	Voges-Proskauer	Citrate	
Choba Market					
1	+	+	-	-	+ *E. coli*
2	+	+	-	-	+ *E. coli*
3	+	+	-	-	+ *E. coli*
4	+	+	-	+	- *E. coli*
5	-	-	-	+	- *E. coli*
Checkpoint					
1	+	-	-	-	- *E. coli*
2	+	+	-	-	+ *E. coli*
3	+	+	-	-	+ *E. coli*
4	+	-	-	-	- *E. coli*
5	+	+	-	-	+ *E. coli*
Elele-Alumini					
1	-	-	+	+	- *E. coli*
2	+	+	-	-	+ *E. coli*
3	-	-	+	+	- *E. coli*
4	+	+	-	-	+ *E. coli*
5	+	+	-	-	+ *E. coli*
Akpor-Ndele					
1	+	+	-	-	+ *E. coli*
2	+	-	-	+	- *E. coli*
3	+	+	-	-	+ *E. coli*
4	-	-	-	+	- *E. coli*

5	-	-	-	+	- *E. coli*
6	+	+	-	-	+ *E. coli*
Ogbakiri Market					
1	+	-	-	-	- E. coli
2	+	+	-	-	+ *E. coli*
3	+	+	-	-	+ *E. coli*
4	+	-	-	+	- *E. coli*
5	+	+	-	-	+ *E. coli*
6	+	-	-	-	- *E. coli*

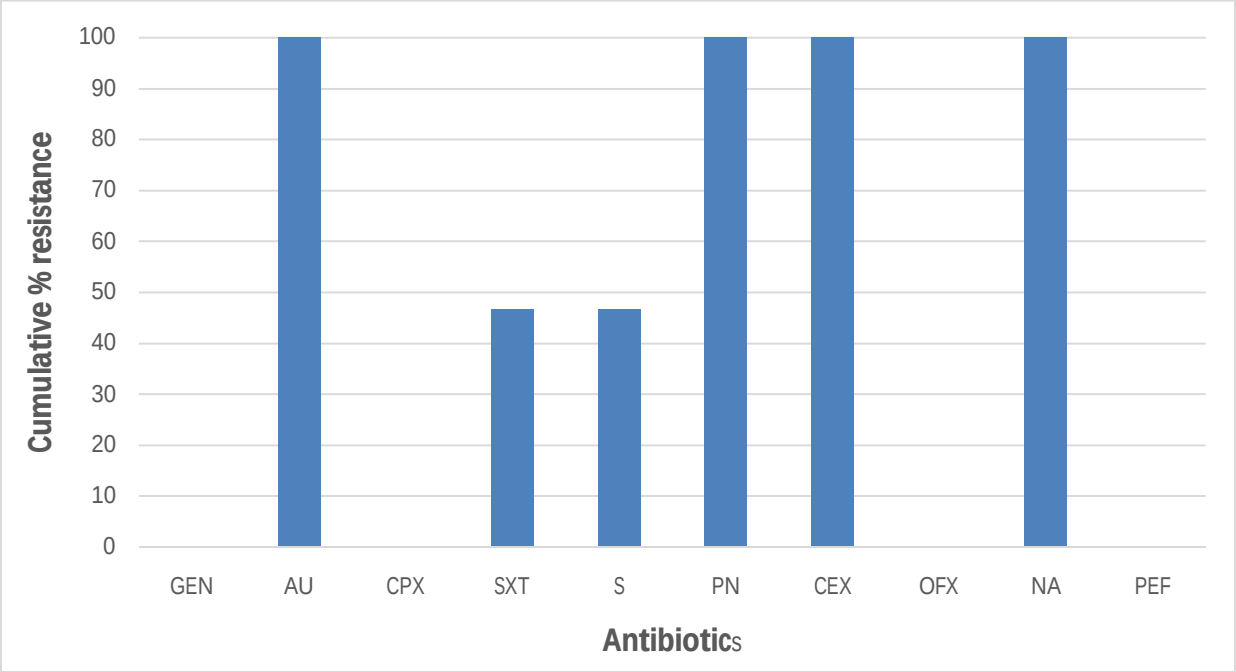

Figure 1. Antibiotic resistance pattern of *Escherichia coli* isolated from RTE edible larvae of *Rhynchophorus phoenicis*. GEN = Gentamycin, AU = Augmentin, CPX = Ciprofloxacin, SXT = Sulfamethoxazole, S = Streptomycin, PN = Penicillin, CEX = Cephalexin, OFX = Ofloxacin, NA = Nalidixic acid, and PEF = Pefloxacin.

DISCUSSION

Ready-to-eat (RTE) food products are considered high-risk foods since no additional action such as re-heating is required before eating. These foods are liable to be contaminated from different sources during production and market handling making them unfit for consumption and constituting a public health hazard. Improper handling of RTE food may cause contaminations and display at improper temperature favors the rapid growth of pathogens and may result in foodborne outbreaks. It is common to hear travelers complaining of stomach upset which might be linked to RTE foods consumed along the route because of varying incubation periods of food-borne pathogens such as bacteria. In this study, microbiological qualities of the ready-to-eat edible larvae of *R. phoenicis*, proximate analysis and antibiotics sensitivities were investigated.

The proximate compositions of ready-to-eat edible larvae of *R. phoenicis* indicated that it is sufficient in nutrients. Several authors have reported that edible larvae are very excellent sources of protein (*Banjo et al., 2006; Opara et al., 2012; Amadi et al., 2014; Ebenebe & Okpoko, 2015*) in comparison with proteins in chicken (24.96%), pork (16.57%), goat meat (20.14%) and beef (18.81%) (*Afolabi et al., 2017*). This study showed 20.73% proximate value for protein in the *R. phoenicis* larvae which truly can be compared to the other above-mentioned sources of protein. The proximate value of lipid in this study (22.3%) is compared to 19.54% reported for the same kind of larvae by Okaraonye & Ikewuchi (2008). The carbohydrate content of edible larvae of *R. phoenicis* in this study was also low (6.01%). This supports the report by *Opara et al., (2012*) that edible larvae are poor energy-giving foods. The moisture content of the edible larvae evaluated in this study was relatively high (35.49%) but found to be slightly lower than that previously reported for the larva (*Edijala et al., 2009; Ekpo & Onigbinde 2005; Womeni et al., 2012*). The moisture content evaluated in the present reveals their stability and susceptibility to contamination by microorganisms. The larvae of *R. phoenicis*have been reported to have a relatively low ash content, ranging from 0.60% to 5.76% (*Siddiqui et al., 2024*). This is in agreement with the findings of this study as the ash content was 1.25%. The low fiber content of RTE Edible larvae of *R. phoenicis* is an adequate source of diet roughage for bowel peristalsis (*Okaraonye & Ikewuchi, 2008*).

It is evident from the results that out of the 50 samples, a total of 27 *Escherichia* spp. was isolated of which 15 (55.56%) were identified as *E. coli* (Table 3 & 5). The highest prevalence

and occurrence of *Escherichia-positive*samples were from those obtained at Akpor-Ndele 6 (22.22%) of which 4 (25.00%) represented *E. coli* and Ogbakiri 6 (22.22%) of which 3 (18.75%) represented *E. coli*. This was followed by Checkpoint, Choba, and Elele-Alumini, which had an occurrence of 5 (18.52%) of which 3 (18.75%) represented *E. coli*. The mean total viable bacterial count (TVC) of RTE edible larvae results showed that TVC (log_{10}cfu/g) in Checkpoint was the highest (6.34×10^8), followed by Choba (4.49×10^8), Ogbakiri (1.14×10^8), Ndele (9.4×10^7) while Elele-Alimini had the lowest count (8.1×10^7) (Table 2). *Escherichia coli* was recovered from every sampling point in this study implying that contamination may arise during or after processing and these larvae could be a potential vehicle for the transmission of diarrheagenic infection. The prevalence of *E. coli* can be attributed to handling, processing, and storage. Also, the protein nature of the larvae can support the growth of microorganism. *Ikenebomeh & Elohor (2005*), reported the isolation of *Staphylococcus aureus*, *Bacillus cereus,* and *E. aerogenes* from fresh and roasted edible larvae from 5 locations in Delta and Edo states of Nigeria. Also, Ekrakene and Igeleke (2007), isolated *S. aureus* (100%), *B. cereus* (30%), *E. coli* (20%), *Enterococcus faecalis* (45%) and *Pseudomononas aeruginosa* (35%) from roasted larva of the palm weevil, *R. phoenicis*. Ngoka *et al*. (2021) also reported the occurrence of *Staphylococcus* species in RTE edible larvae of *R. phoenicis* vended along the Port Harcourt-Bayelsa route, Nigeria.

In an attempt to determine the antimicrobial resistance pattern of *E. coli* isolates, all the isolates showed 100% resistance to four antibiotics tested (augmentin, penicillin, cephalosporin, and nalidixic acid). Other isolates showed varying antibiotic resistance patterns with 46.67% for sulfamethoxazole and streptomycin. Overall, they were 100% sensitive to gentamycin, ciprofloxacin, ofloxacin, and pefloxacin. *Harakeh et al. (2005)* reported that *E. coli* of RTE meats origin were 100% resistant to trimethoprim/sulfamethoxazole and Cephalexin and 88.9% resistant to erythromycin, augmentin, ciprofloxacin, and oxacillin. This is similar to the findings in this study as this study also found high resistance to cephalexin and augmentin but lower resistance to sulfamethoxazole. *Rahman et al. (2017)* reported that *E. coli* from RTE meats were resistant to amoxicillin (76.00%), sulfonamide-trimethoprim (84.00%), and oxytetracycline (92%) but susceptible to gentamycin (100%) and ciprofloxacin (100%). This correlates with this present study as it also found a higher susceptibility to gentamycin and ciprofloxacin while it showed a high resistance to sulfamethoxazole. A high degree of sensitivity to gentamycin and

ofloxacin has been previously reported (*Okonko et al., 2008; Mordi & Momoh, 2009; Umofia, 2012; Afolabi et al., 2017*). The resistance shown by E. coli strains in this and previous studies to common antibiotics of choice may be due to abuse of drugs used in animal husbandry or mutation and the appearance of new bacterial strains.

CONCLUSION

The study suggests that RTE edible larvae (*Rhynchophorus phoenicis*) available to consumers are excellent sources of essential nutrients. However, they can also transmit bacterial pathogens that could cause food poisoning when ingested. The study showed that 18.52% of samples tested had total *Escherichia* counts above the acceptable microbiological limit ($\geq 10^7$ CFU/g) for RTE-vended foods. *Escherichia coli* isolated from the RTE edible larvae of *Rhynchophorus phoenicis* showed various dimensions of resistance patterns to the commercial antibiotics with multi-resistance which may lead to human infection as reported in several countries. These data should be valuable for assessing human health risks due to the consumption of RTE foods from the community impacted by inadequate cooking or contamination by inadequate handling of post-processing. This study thus emphasized the need for intensive surveillance by local health authorities of E. coli and other pathogenic bacteria in RTE foods variety to avert food-borne infection and spot emerging anti-microbial resistance phenotypes since anti-microbial resistant strains may colonize the human population via these RTE foods. Hence, unhygienic practices during processing and sales should be discouraged, and the requirement for a superior and realistic approach to ensure roadside food vendors comply with standard food safety practices.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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Details of the AI usage are given below:

1.

2.

3.

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Appendix A

CONFIRMATION OF *Escherichia coli*

Table 1. Incidence of *Escherichia spp*. and *Escherichia coli* in RTE edible larvae

Sample location	*Escherichia spp.* n (%)	*Escherichia coli* n (%)
Choba Market	5 (18.52)	3 (18.75)
Checkpoint	5 (18.52)	3 (18.75)
Elele-Alumini	5 (18.52)	3 (18.75)
Akpor-Ndele	6 (22. 22)	3 (18.75)
Ogbakiri Market	6 (22.22)	4 (25.00)
Total	27	16 (59.26)

Appendix B

ANTIBIOTIC RESISTANCE PATTERN OF TESTED *Escherichia coli* IN RTE EDIBLE LARVAE

Table 2. The varying antibiotics resistance pattern of all isolate.

Isolate code	**GEN**	**AU**	**CPX**	**SXT**	**S**	**PN**	**CEP**	**OFX**	**NA**	**PEF**
A	15mm	(R)	20mm	(R)	15mm	(R)	(R)	16mm	(R)	14mm
B	25mm	(R)	20mm	14mm	20mm	(R)	(R)	25mm	(R)	20mm
C	20mm	(R)	25mm	(R)	(R)	(R)	(R)	30mm	(R)	20mm
D	15mm	(R)	23mm	(R)	(R)	(R)	(R)	25mm	(R)	22mm

E	25mm	(R)	35mm	20mm	13mm	(R)	(R)	30mm	(R)	12mm
F	12mm	(R)	25mm	20mm	(R)	(R)	(R)	16mm	(R)	22mm
G	16mm	(R)	23mm	17mm	10mm	(R)	(R)	20mm	(R)	15mm
H	10mm	(R)	25mm	(R)	(R)	(R)	(R)	16mm	(R)	16mm
I	17mm	(R)	20mm	(R)	10mm	(R)	(R)	20mm	(R)	17mm
J	11mm	(R)	25mm	11mm	(R)	(R)	(R)	15mm	(R)	19mm
K	15mm	(R)	30mm	12mm	(R)	(R)	(R)	12mm	(R)	16mm
L	13mm	(R)	20mm	16mm	22mm	(R)	(R)	10mm	(R)	17mm
M	12mm	(R)	22mm	(R)	(R)	(R)	(R)	28mm	(R)	20mm
N	9mm	(R)	28mm	(R)	12mm	(R)	(R)	13mm	(R)	12mm
0	13mm	(R)	24mm	14mm	20mm	(R)	(R)	14mm	(R)	10mm

Table 3. Susceptible and resistance pattern of different antibiotics from the RTE edible larvae.

Antibiotics	**Susceptible n (%)**	**Resistance n (%)**
Gentamycin (GEN)	15 (100.00)	0 (0.00)
Augmentin (AU)	0 (0.00)	15 (100.00)
Ciprofloxacin (CPX)	15 (100.00)	0 (0.00)
Sulfamethoxazole (SXT)	8 (53.33)	7 (46.67)
Streptomycin (S)	8 (53.33)	7 (46.67)
Penicillin (PN)	0 (0.00)	15 (100.00)
Cephalosporin (CEP)	0 (0.00)	15 (100.00)

Ofloxacin (OFX)	15 (100.00)	0 (0.00)
Nalidixic acid (NA)	0 (0.00)	15 (100.00)
Pefloxacin (PEF)	15 (100.00)	0 (0.00)

Appendix C

ISOLATION AND IDENTIFICATION OF *Escherichia coli*

The presence of *Escherichia coli* was detected by spread plates and incubated at 37^0C for 48 hours. Samples that were positive showed a greenish metallic sheen which is the appearance of *Escherichia coli*.

Plate 1. Appearance of greenish metallic sheen *Escherichia coli* on EMB Agar