Occurrence Of Antibiotic Resistant *Escherichia Coli* in Ready-To-Eat Edible Larvae Sold Along Emuoha Toll Gate East West Road Port Harcourt

Abstract

This study was aimed at determining the occurrence of *Escherichia coli* in ready-to-eat (RTE) *Rhynchophorusphoenicis* (edible larvae) vended along Emuoha Toll Gate East-West Road, Port-Harcourt. Fifty (50) samples from five locations were analysed employing standard techniques for proximate, microbiological analyses, using Nutrient and Eosine methylene blue agar and antibiotics susceptibility. The mean proximate results of ready-to-eat edible larvae revealed the presence of moisture (35.49%), carbohydrate (6.01%), protein (20.73%), ash (1.25%), lipid (22.3%) and fibre (14.23%). The results obtained indicated that the mean values of bacteria count ranged from 8.1 x 10^7 to 6.34 x 10^8 cfu/g. The sample from the Checkpoint had the highest bacterial count of 6.34 x 10^7cfu/g while Elele-Alumini had the lowest bacterial count of 8.1 x 10^7. A total of 15 (55.56%) of the 27 isolates were confirmed biochemically as *Escherichia coli,* of which 18.52% samples tested had total *Escherichia* counts above the acceptable microbiological limit ($\geq 10^7$ CFU/g). Antibiotics susceptibility pattern of *Escherichia coli* showed that all isolate were resistant to augmentin, penicillin, cephalosporin and ofloxacin. Other isolate show varying antibiotics resistance pattern with 46.67% resistance to sulfamethoxazole and streptomycin. The study showed that ready-to-eat edible larvae (*Rhynchophorusphoenicis*) is a potential source of *Escherichia* food poisoning in commuters; hence, food vendors need to conform to standard practices.

Keywords: Occurrence, *Escherichia Coli*, Ready-to-eat (RTE) Edible Larvae, Antibiotic, Resistant.

Introduction

Ready-to-eat foods (RTE) 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, commonly known as the edible worm, is a traditional delicacy consumed either roasted or boiled, especially in southern Nigeria and other African

UNDER PEER REVIEW

regions where palm trees thrive. These larvae, 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 and 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 Rhynchophorusphoenicis, 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; *Edijala et al.,* 2009; *Elemo et al.,* 2011; Ekpo&Onigbinde, 2015; Omotoso&Adedire, 2007). 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 (Rumpoid&Schluter, 2013).

Escherichia coli, 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. The emergence of multidrug-resistant (MDR) bacterial strains worldwide presents a grave public health concern. Increasing antibiotic resistance among *E. 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 RTE foods correlates with increased mobility, urbanization, and reliance on RTE 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

UNDER PEER REVIEW

to contamination. Additionally, open-air vending exposes RTE foods to environmental pollutants like flies and dust.

Given these challenges, there is a pressing need to assess the *E. coli* contamination and antimicrobial resistance profiles of RTE 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 E. coli levels in RTE edible worms and their resistance to clinically relevant antimicrobial agents, shedding light on potential health risks associated with their consumption.

Materials And Methods

Study Location

Samples was gotten 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. They are: 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 ready-to-eat edible larvae was partially exposed and poorly packaged (Plate 1). The sample were aseptically stored in well labelled and sealed sterile transparent bags and transported immediately to the microbiology laboratory for analyses.

UNDER PEER REVIEW

Plate 1. Roasted ready-to-eat edible larvae (*Rhynchophorusphoenicis*).

UNDER PEER REVIEW

Media Sterilization

The experiment was conducted at the University of Port Harcourt Microbiology Laboratory. The glassware's such as test tubes, glass rod, pipette, measuring cylinder, beakers and conical flasks required for this research work were soaked and washed with detergent and rinsed with distilled water. They were wrapped with aluminum foil paper and dried in the oven in inverted position at 80^0C for 45-60 mins. The working area was swabbed with ethanol. Contamination by microorganisms from the external environment was reduced by closing windows and putting off fans in the laboratory.

Media Preparation

The following media were employed during this study. All media was prepared according to the manufacturer's specification. Composition of the media is shown in the appendix.

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

Nutrient Agar

Nutrient Agar was prepared by weighing twenty-eight grams of the nutrient agar using a digital weighing balance and transferred to a sterile conical flask. 1000ml of distilled water was added and whirled to mix well. The mixture was pre-heated to 100^{0}C to completely dissolve the power since agar does not dissolve in cold water, after which it was sterilized by autoclaving at 121^{0}C for 15 minutes at 15 psi. After sterilization, the medium was allowed to cool to 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.

Eosin Methylene Blue (Emb) Agar

Eosin Methylene Blue Agar was prepared by weighing thirty-five grams of the eosin methylene blue using a digital weighing balance and transferred to a sterile conical flask. 1000ml of distilled water was added and whirled to mix well. The mixture was pre-heated to 100^{0}C to completely dissolve since agar does not dissolve completely in cold water, after which it was sterilized by autoclaving at 121^{0}C for 15 minutes at 15 psi. After sterilization, the medium was allowed to cool to 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.

Mueller Hinton Agar

This agar was prepared by suspending 38 gm of the medium in one liter of distilled water. The medium was then heated with frequent agitation and allow to boil for one minute to completely dissolve the medium. The medium was then autoclaved at 121^{0}C for 15 minutes and allowed to cool to room temperature. The Mueller Hinton agar was poured into sterile petri dishes on a level, horizontal surface to give uniform depth 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 was peptone water. Peptone water is used for the cultivation of non-fastidious microorganisms. During study, Peptone water was used to carry out

UNDER PEER REVIEW

10-fold serial dilution in duplicate for the analysis of the ready-to-eat edible worm sample. The medium was prepared by weighing out 15g of peptone medium and transferred to a conical flask. 1000ml of distilled water was added and pre-heated to 100^0C 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 121^0C for 15 minutes at 15psi and allowed to cool.

Preparation Of the Sample/Isolation Procedure

The bacteriological determination of ready-to-eat edible larvae sample was carried out by placing the samples in a sterile petri dish. Twenty-five grams (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 labelled stomacher bags. The sample was homogenized using a stomacher machine for 2 minutes. 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% alcohol, flamed using the Bunsen burner and cooled, the inoculum was spread round the plates. The inoculated plates were then inverted and incubated aerobically at 37^0C for 24 hours.

UNDER PEER REVIEW

Determination Of Total Viable Plate Counts

Aliquot (0.1 ml) of each of the selected sample dilution 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 for 30^0C for 24 hours. 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 and 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 is used to calculate the CFU/g:

CFU/g = (NumberofColonies x DilutionFactor)/VolumeofCulture(ml)

Variables:

CFU/g is the colony-forming units per gram

Number of Colonies is the count of colonies observed on a plate

Dilution Factor is the factor by which the original sample was diluted

Volume of Culture is the volume of the diluted sample plated (in milliliters)

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 hours.

Preservation Of Isolates

With the aid of a sterile wire loop, discrete colonies were picked from the nutrient agar plate and inoculated on nutrient agar slant in bijou bottle which were prepared the same way as nutrient agar dispended in bijou bottles and kept in a slanting position to form a slant. After inoculation

UNDER PEER REVIEW

the bijou bottles were incubated at 37^{0}C for 24 hours. Afterwards, they were stored in the refrigerator at 4^{0}C until they were ready to be used.

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 the family 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 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 the Kovac's reagent to give a red or a pink ring at the top of the tube. Peptone water in a tube which contain tryptophan was inoculated with bacteria isolate. The mixture was then incubated overnight at 37^{0}C. Then, few drops of Kovac's reagent were added to the mixture and formation of a red or a pink colored ring at the top was seen as a positive reaction. *E. coliare* indole-positive bacteria.

Methyl Red Test

UNDER PEER REVIEW

Methyl red test detects the ability of a bacterium to produce acid from glucose fermentation. Methyl red, a PH indicator, remains red in color 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. Red color development is a positive reaction that occurs when the bacteria have produced enough acid to neutralize the phosphate buffer. Yellow discoloration occurs to 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 red color. In order to perform 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 shake 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

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 action of an enzyme citritase. Oxaloacetate was further broken down to pyruvate and CO2. Production of Na2C03 from sodium citrate changes the media into alkaline Ph, and hence color change from green to blue. Blue color formation is a positive reaction, whereas the slant remaining green colored is a feature for negative test *E. coli*, citrate negative.

Proximate Analyses of The Ready-To-Eat Food Sample (*RhynchphorusPhoenicis*)

UNDER PEER REVIEW

The proximate composition for ash content, moisture content, crude protein, crude lipid, crude fibre and total carbohydrate of the ready-to-eat sample were determined as described by Associated of Official Analysis 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 hours (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 hours 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:

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

Calculation of crude protein:

$$N_2\text{ (\%)} = \frac{\text{0.00014 x Vol of acid x Filtration flask for preparation of digest x 100}}{\text{Weigh of sample x Amount of digest introduced into the flask.}}$$

Protein (%) = % of total N_2 x 6.25

Calculation of crude fat:

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

Calculation of ash:

$$\text{Ash (\%)} = \frac{\text{Weight of ash x 100}}{\text{Original weight of the sample taken1}}$$

UNDER PEER REVIEW

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 millimeter (mm). Each inhibition zones diameter was interpreted using interpretive chart from WHO (CLSI, 2015).

RESULT

Proximate Values of RTE Edible Larvae

The proximate compositions of edible larvae showed in Table 1 The values of moisture content is 35.49%, carbohydrate is 6.01%, protein is 20.73%, ash is 1.25%, lipid is 22.3% and fibre is 14.23%. The composition of moisture was the highest, followed by lipid, protein, fibre, carbohydrate, and ash.

Mean Counts of RTE Edible Larvae

The mean total viable bacterial count (TVC) of RTE edible larvae is shown in Table 2. The results showed that TVC ($\log_{10}$cfu/g) in Checkpoint was the highest (6.34×10^8), followed by

UNDER PEER REVIEW

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).

Distribution Of *Escherichia* Spp. On Emb Agar

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

Interpretation Of Sanitary Quality of The Rte Edible Larvae

In Table 3, 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 prior to 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.

Confirmation Of *Escherichia Coli*.

Table 4. and Appendix A shows the result of the confirmation test done to identify *E. coli* from the *Escherichia* spp. The biochemical test was positive for Indole, Methyl red, Voges-Proskauer and Citrate test. Out of the 27 *Escherichia* spp., 15 were positive for *Escherichia coli*.

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 resistance to Augmentin, penicillin, cephalosporin and ofloxacin at 100% (see Figure 1 and Appendix B). Other isolate showed varying antibiotics resistance pattern with 46.67% resistance to sulfamethoxazole and streptomycin.

Multiple-Antibiotics Resistance Profile of *Escherichia Coli* Isolated from RTE Edible Larvae

UNDER PEER REVIEW

Escherichia coli showed varying multiple resistances to antibiotics as presented in table 5. Code isolate C, D, H, and M had the highest percentage resistance of 60% to multiple antibiotics, followed by code isolate A, F, I, J, K and N with 50%. Code isolate B, E, G, L and O showed the least multiple resistances of 40%.

Table 1. Proximate analysis of composite sample of edible larvae (*Rhynchophorusphoenicis*)

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

Table 2. Mean count of *Escherichia coli* in ready-to-eat edible larvae.

	Sample location/Mean count (log_{10} CFU/g)		
Location of samples	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

UNDER PEER REVIEW

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 with microbiological guidelines for ready-to-eat 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%)

UNDER PEER REVIEW

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

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

UNDER PEER REVIEW

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

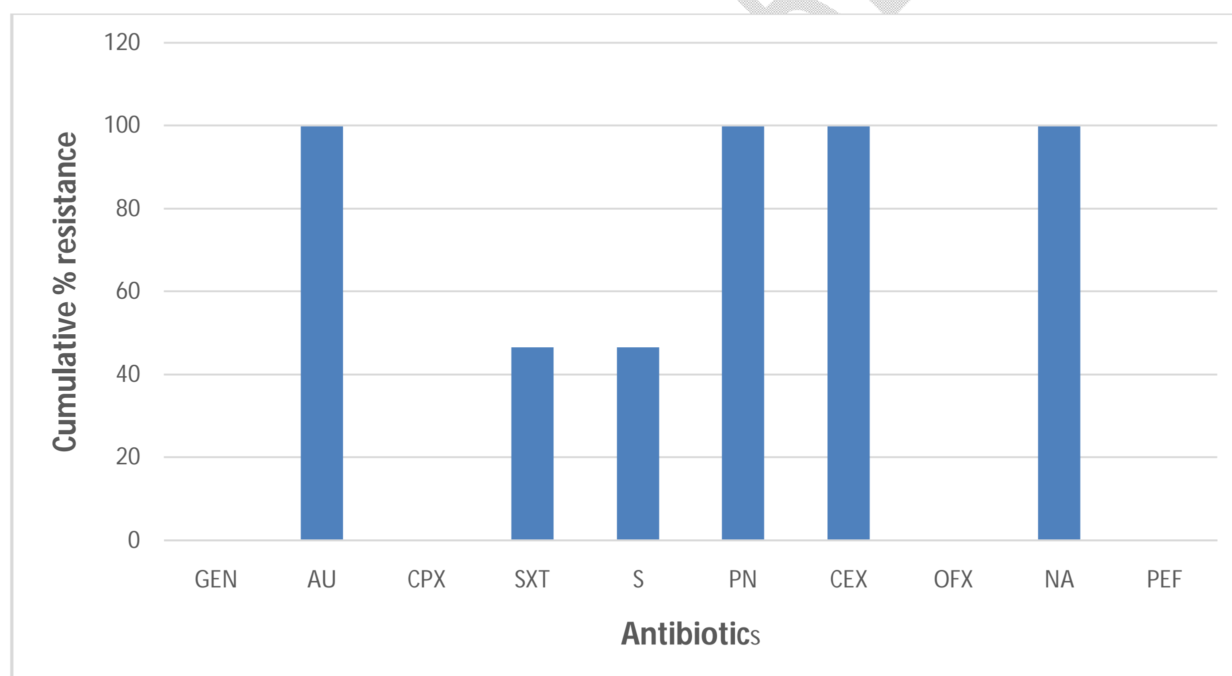

Figure 1. Antibiotic resistance pattern of *Escherichia coli* isolated from ready-to-eat edible larvae. GEN = Gentamycin, AU = Augmentin, CPX = Ciprofloxacin, SXT = Sulfamethoxazole, S = Streptomycin, PN = Penicillin, CEX = Cephalexin, OFX = Ofloxacin, NA = Nalidixic acid, and PEF = Pefloxacin.

DISCUSSION

Ready-to-eat (RTE) food products are considered as 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, contamination, and their presence in food lead to be unfit for consumption and constitute 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 ready-to-eat foods consumed along the route because of varying incubation periods of food borne pathogens. In this study, microbiological qualities of the ready-to-eat edible larvae, proximate analysis and antibiotics sensitivities were investigated.

The proximate compositions of ready-to-eat edible larvae indicated that it is sufficient in nutrients. Several authors have reported that edible larvae are very excellent sources of protein (Banjo *et al*., 2016; Opara *et al*., 2012; Amadi *et al*., 2014; Ebenebe and Okpoko, 2015) in comparison with proteins in chicken (24.96%), pork (16.57%), goat meat (20.14%) and beef (18.81%) (Afolabi*etal*., 2017). The proximate value of lipid in this study is compared to 19.54% reported for the same kind of larvae by Okaraonye and Ikewuchi (2008). The lipid contents of pork (36.87%) showed great disparity to lipid contents of edible larvae (Afolabi *et al*., 2017). Reports by Opara *et al*., (2012) that edible larvae are a poor energy giving foods, the carbohydrate content of edible larvae in this study was also low (6.01%). 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 reveals their stability and susceptibility to contamination by organisms. The low fibre content of RTE Edible larvae is adequate source of diet roughage for bowel peristalsis (Okaraonye and Ikewuchi, 2008).

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

UNDER PEER REVIEW

of RTE edible larvae is shown in Table 2. The results showed that TVC (log_{10}cfu/g) in Checkpoint was the highest (6.34 x 10^8), followed by Choba (4.49 x 10^8), Ogbakiri (1.14 x 10^8), Ndele (9.4 x 10^7) while Elele-Alimini had the lowest count (8.1 x 10^7). *Escherichia coli* was recovered from every sampling point in this study implying that contamination may arise 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 proteineous nature of the larvae can support the growth of microorganism. Although no *E. coli* have been reported from *Rhynchophorusphoenicis*larvae in previous work, other bacteria of public health importance have been isolated. Ikenebomeh and Elohor (2005), reported the isolation of *Staphylococcusaureus*, *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 *Staphylococcus aureus* (100%), Bacillus cereus (30%), *Escherichia coli* (20%), *Enterococcus faecalis* (45%) and *Pseudomononas aeruginosa* (35%) from roasted larva of the palm weevil. Ngoka*et al*., 2021 also reported the occurrence of *Staphylococcus* species in ready-to-eat edible larvae vended along the Port Harcourt-Bayelsa route.

In attempt to determine the antimicrobial resistance pattern of *E. coli* isolates, all the isolates showed 100% resistance to four antibiotics tested (augumentin, penicillin and cephalosporin and nalidixic acid). Other isolate showed varying antibiotics resistance pattern with 46.67% for sulfamethoxazole and streptomycin. The resistance shown by *E. coli* strains in this study to common antibiotics of choice maybe due to abuse of drugs use in animal husbandry or mutation and appearance of new strains. 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% resistance to erythromycin, augumentin, ciprofloxacin and oxacillin. This study also found high resistance to cephalosporin and augumentin but a lower resistance to sulfamethoxazole. Rahman *et al*., 2017 reported that *E. coli* from RTE meats were resistance to amoxicillin (76.00%), sulfonamide-trimethoprim (84.00%) and oxytetracycline (92%) but susceptibility to gentamycin (100%) and ciprofloxacin (100%). This study found also found higher susceptibility to gentamycin and ciprofloxacin while it showed a high resistance to sulfamethoxazole. High degree of sensitivity to gentamycin and ofloxacin has been previously reported (Okonko*et al*., 2008; Mordi and Momoh, 2009; Umofia, 2012; Afolabi*et al*., 2017).

UNDER PEER REVIEW

CONCLUSION

The study suggests that RTE edible larvae (*Rhynchophorusphoenicis*) available to consumers are excellent sources of essential nutrients. The study showed that 18.52% samples tested had total *Escherichia* counts above the acceptable microbiological limit ($\geq 10^7$ CFU/g) for ready-to-eat vended foods. *Escherichia coli* isolated from the RTE edible larvae showed various dimensions of resistance pattern to the commercial antibiotics with multi resistant 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 post-processing contamination. This study thus emphasized the need for intensive surveillance of this isolate in RTE foods variety to avert food-borne infection and spot emerging anti-microbial resistance phenotypes since Anti-microbial resistance strain may colonize the human population via these RTE foods. Hence, unhygienic practices during processing and sales should be discourage and the requirement for a superior and realistic approach to ensure road side food vendors comply with standard food safety practices.

RECOMMENDATION

Proper food handling, food safety and sanitation practices should be followed diligently for those offering ready-to-eat foods. Food handlers must wash their hands frequently; not just after using the restroom, eating, smoking, or touching the face or mouth. Food handling employees must wear gloves or use some other barriers so that bare hands do not directly contact the food. Suitable utensils such deli paper, spatulas, tongs, and dispensing equipment must be used. Temperatures for hot holding and cold holding must be taken and recorded at regular intervals to assure the food is maintaining safe temperature. Lastly, there should be an enforcement from the government that would help prevent transmission among food handlers selling foods in retail outlets.

UNDER PEER REVIEW

REFERENCE

Afolabi FT, Arowesebe AR, Adeyemi SM (2017). Antibiotic Susceptibility pattern and Plasmid Profilling of some isolates obtained from raw meats sold in Ibadan, Metropolis. Journal of Microbiology and Biotechnology Research 7(4): 13 - 22.

Akinyemi K.O., Fashola M.O., Nene H., Akinwande E. Vended foods in Lagos, Nigeria: A potential reservoir for the spread of emerging strains of drug resistant. Health. 2013; 5:675–680. doi: 10.4236/health.2013.54089. - DOI

Amadi EN, Kiin-Kabari DB, Kpormon LB, Robinson VKK (2014). Microbial flora and Nutritional Composition of Adult Palm wine Beetle (*Rhynchophorusphoenicis*). International Journal of Current Microbiology and Applied Science 3(11):189-192.

Amare, A., Worku, T., Ashagirie, B., Adugna, M., Getaneh, A. and Dagnew, M. (2019) Bacteriological profile, antimicrobial susceptibility patterns of the isolates among street vended foods and hygienic practice of vendors in Gondar town, Northwest Ethiopia: a cross sectional study. BMC Microbiol 19, 1-9.

Association of Official Analytical Chemists (AOAC) (2000). Official methods of Analysis of AOAC International, 17th Edition, II. Washington, DC, USA.

Asiegbu C.V., Lebelo S.L., Tabit F.T. The food safety knowledge and microbial hazards awareness of consumers of ready-to-eat street-vended food. Food Control. 2016; 60:422–429. doi: 10.1016/j.foodcont.2015.08.021. – DOI

Association of Analytical Chemists (AOAC) In: Official Methods of Analysis. eighteenth ed. Horowitz W., editor. AOAC Publisher; Gaithersburg, USA: 2005. (Official Methods of Analysis).

Banjo A.D, Lawal O.A &Songonuga E.A. (2006). The nutritional value of fourteen species of edible insects in southwestern Nigeria. African Journal of Biotechnology 5(3): 298 - 301.

Beshiru A, Okoh AI, Igbinosa EO (2022). Processed ready-to-eat (RTE) foods sold in Yenagoa Nigeria were colonized by diarrheagenic Escherichia coli which constitute a probable hazard to human health.*PLoS One*, 17(4): e0266059.

Braide, W &Nwaoguikpe, R.N. (2011). Assessment of microbiology quality and nutritional values of a processed edible weevil caterpillar (Rhynchophorusphoenicis) in Port Harcourt, Southern Nigeria. International Journal of Biological and Chemical Science 5(2): 410 - 418.

Chang SK (2003). Protein analysis in Food. 3rd Edition. Kluwe Academic Plenum Publishers: New York, USA pp. 134-135.

Cheesbrough M (2010). District Laboratory Practice in Tropical Countries, Part 1. Cambridge University Press.

UNDER PEER REVIEW

Clinical and Laboratory Standards Institute (CLSI) Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI; Wayne, PA, USA: 2020. [(accessed on 26 June 2020)]. (M100-ED30). Available online: http://em100.edaptivedocs.net/dashboard.aspx

CLSI (2015). Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standards-12th Edition. Clinical and Laboratory Standards Institute. Wayne, USA.

Edijala, J.K., Egbogbo O. &Anigboro, A.A. (2009). Proximate composition and cholesterol concentrations of Rhynchophorusphoenicis and Oryctes monoceros larvae subjected to different heat treatments. African Journal of Biotechnology 8(10): 2346 - 2348.

EFSA Scientific Committee Risk profile related to production and consumption of insects as food and feed. EFSA J. 2015; 13:4257. doi: 10.2903/j.efsa.2015.4257.

Ebenebe and Okpoko (2015), Edible insect consumption in the South Eastern Nigeria. *International Journal of Science and Engineering.* vol 6, pp. 171-177.

Ekpo and Onigbinde. (2005), Nutritional potentials of the larva of Rhynchophorusphoenicis (F). Pakistan Journal of Nutrition, vol. 4, pp. 287-290.

Ekrakene and Igeleke (2007), Microbial isolates from the roasted larva of the palm weevil (Rhynchophorusphoenicis [F]) from Edo and Delta states of Nigeria. Australian Journal of Basic and Applied Sciences, 2007, Vol. 1, No. 4, 763-768 ref. 13.

Elemo B.O, Eleme G.N, Makinde M. and Erukainure O.L (2011). Chemical evaluation of African palm weevil, Rhychophorusphoenicis, larvae as a food source. Journal of Insect Science 11: 146. DO1: 10.1673/031.011.14601.

Eruteya O.C, Odunfa S.A, Lahor J. Listeria spp. in raw cow and goat meat in Port Harcourt, Nigeria. Br. Biotechnol. J. 2014;4(2):204–214.

European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC). (2019). The European Union One Health 2018 Zoonoses Report. EFSA J.17, e05926.

Fogoh JM, Aurele AA, Philippe L, Patrice L (2015). Exploitation, Trade and Farming of Palm weevil grubs in Cameroon. Working paper 178, Bogor, Indonesia; CIFOR.

Harakeh S, Yassine H, Gharios M, Barbour E, Hajjar S, El-Fadal M, Toufeili I, Tannous R (2005). Isolation, molecular characterization and antimicrobial resistance of *Salmonella* and *Escherichia coli* isolates from meat-based fast food in Lebanon. Science of the Total Environment 341(1-3):33-44.

Health Protection Agency (2009) Guidelines for Assessing the Microbiological Safety of Ready-to-eat Foods. London: Health Protection Agency.

UNDER PEER REVIEW

Igbinosa IH, Beshiru A, Egharevba NE, Igbinosa EO. Distribution of Enterobacteria in ready-to-eat food in cafeterias and retail food outlets in Benin City: Public health implications. J Comm Med Pri Health Care. 2020; 32(2):80–94.

Ikenebomeh MJ, Elohor O (2005). Microbiology of fresh and roasted edible worms' larvae (Rhynchophorusphoenics). In: the Book of Abstract of the 29th Annual Conference & General Meeting (Abeokuta2005) on Microbes as Agents of Sustainable Development, organized by Nigerian Society for Microbiology (NSM), University of Agriculture, Abeokuta, from 6-10th November, p. 26.

Kim S.K., Weaver C.M., Choi M.K. Proximate composition and mineral content of five edible insects consumed in Korea. CyTA - J. Food. 2017;15(1):143–146.

Luu-Thi H, Michiels CW. Microbiological safety of ready-to-eat foods in Hospital and University canteens in Hanoi, Vietnam. J Food Prot. 2021; 84(11):1915–1921. doi: 10.4315/JFP-20-324 - DOI - PubMed

Makelele LK, Kazadi ZA, Oleko RW, Mpalang NK, Ngbolua NK, Gedeon BN (2015). Microbiological quality of food sold by street vendors in Kisangani, Democratic Republic of Congo. African Journal of Food Science 9(5):285-290.

Makinde O.M., Adetunji M.C., Ezeokoli O.T., Odumosu B.T., Ngoma L., Mwanza M., Ezekiel C.N. Bacterial contaminants and their antibiotic susceptibility patterns in ready-to-eat foods vended in Ogun state, Nigeria. Lett. Appl. Microbiol. 2020; 72:187–195. doi: 10.1111/lam.13407. - DOI - PubMed

Moshood A.Y. and TengkuHaziyamin, A.A. Isolation and identification of Listeria sp from ready-to-eat (RTE) kilishi in retailed outlet in Bauchi, Nigeria. Int J Pharm Sci Invent. 2013; 2: 22–25.

Mordi RM, Momoh MI. Incidence of Proteus species in wound infections and their sensitivity pattern in the University of Benin Teaching Hospital. African Journal of Biotechnology, 2009; 8 (5): 725-730

Ngoka C,Eruteya O.C and Ibiene A.A. (2021). Occurrence of virulent and antibiotic resistant Staphylococcus species in ready-to-eat Rhynchophorusphoenicis and Archachatina marginata vended along the Port Harcourt-Bayelsa route. African Journal of Microbiology Research, Vol.15(2), pp. 107-119.

Ogogouri G, Maxwell Beugre G, Brou K, Atchibri O & Linder M. 2013. Rhynchoporuspalmarum L. larva, an edible insect in Cote D'Ivore: Nutritional value and characterization of the lipid fraction. International Journal of Chemical Science 11: 1692 - 1704.

Oje O. J, Ajibade V. A, Fajilade O. T, and Ajenifuja A. (2018). Microbiological analysis of RTE foods vended in mobile outlet catering units from Nigeria. *Journal of Advances in Food Science & Technology*, vol. 5, no. 1, pp. 15–19.

UNDER PEER REVIEW

Okaraonye CC, Ikewuchi JC (2008). Rhynchophorusphoenicis (F) Larvae Meal: Nutritional value and health implications. Journal of Biological Sciences 8(7):1221-1225.

Okonko IO, Adejoye OD, Ogunnusi TA, Fajobi, EA, Shittu OB. (2008). Microbiological and physicochemical analysis of different water samples used for domestic purposes in Abeokuta and Ojota, Lagos State, Nigeria. Afr. J. Biotechnol. 7(3): 617-621.

Ologhobo A, et al. Safety of street vended meat products-chicken and beef suya. Afr J Biotechnol. 2010;9(26):4091–4095.

Omotoso O.T &Adedire C.O. (2007). Nutrient composition, mineral content, and the solubility of the proteins of palm weevil Rhynchophorusphoenicis F. (Coleoptera: Curculionidae). Journal of Zhejiang University Science B 8:318 - 322.

Opara, FT Sanyigha, IP Ogbuewu, IC Okoli. (2012). Studies on the production trend and quality characteristics of palm grubs in the tropical rainforest zone of Nigeria. Journal of Agricultural Technology, vol 8, pp. 851-860.

Rahman M.A., Rahman A.K., Islam M.A., Alam M.M. Antimicrobial resistance of Escherichia coli isolated from milk, beef and chicken meat in Bangladesh Bangl. J. Vet. Med. 2017;2 [Google Scholar].

Rumpold B.A &Schluter O.K. (2013) a. Nutritional composition and safety aspects of edible insects. Molecular Nutrition& Food Research 57: 802 - 823.

Rumpold B.A &Schluter O.K. (2013) b. Potential and challenges of insects as an innovative source for food and feed production. Innovative Food Science & Emerging Technologies 17: 1 - 11.

Somda, N. S., Bonkoungou, Q. J., and Zongo, C. (2018). Safety of ready-to- eat chicken in burkinafaso: microbiological quality, antibiotic resistance, and virulence genes in Escherichia coli isolated from chicken samples of Ouagadougou. *Food Science & Nutrition*, vol. 6, no. 4, pp. 1077–1084.

Umofia I (2012). Antibiotic Susceptibility studies of some bacterial isolates from packaged milk of marketed in Zaria, Nigeria. M.Sc. Thesis. Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Nigeria.

WHO (2015) Food safety. http://www.who.int/mediacentre/factsheets/fs399/en/. Accessed 1 Mar 2016

Womeni HM, Tiencheu B, Linder M, Nabayo EMC, Tenyang N, Mbiapo FT, Villeneuve P, Fanni J, Parmentier M (2012). Nutritional value and effect of cooking, drying and storage process on some functional properties of Rhynchophorusphoenicis. International Journal of Life Science and Pharma Research 2(3):203-219.

UNDER PEER REVIEW