



## Research article

# Culture-dependent examination of the bacteriological quality of ready-to-eat African salads in Enugu metropolis, Nigeria and antibiotic resistance profile of associated bacteria



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## ABSTRACT

This study investigated the bacteriological quality of ready-to-eat (RTE) African salads in Enugu metropolis, Enugu, Nigeria. A total of 10 samples of African salad were purchased from 10 different vendors in Enugu metropolis. The samples were purchased from Agbani Road, Ogbete, Mayor, Uwani, Kenyatta, Achara Layout, Obiagu and Timber. Isolation and enumeration of bacterial isolates were done using Nutrient agar, Eosin Methylene Blue (EMB) agar, Thiosulphate-citrate-bile salts-sucrose (TCBS) agar, Salmonella-Shigella Agar (SSA) and MacConkey agar, following standard methods. Identification of the bacterial isolates were done through biochemical tests and the Analytical Profile Index (API 20E) test kit. The antibiotic sensitivity of the bacterial isolates was also done using the Kirby Bauer disc diffusion method. Total culturable heterotrophic count was above 300 colonies across the samples. The highest bacterial counts recorded on EMB, SSA and TCBS across the samples were  $6.3 \times 10^6$  CFU/g,  $7.4 \times 10^6$  CFU/g and  $1.21 \times 10^7$  CFU/g respectively. The identities of the organisms were; *Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli*, *Vibrio mimicus*, *Vibrio fluvialis*, *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio hollisae*. The prevalent organism across the samples was *Vibrio* spp. The antibiotic sensitivity test suggested that *Vibrio* spp. was resistant to Ampiclox and Amoxycillin but sensitive to Erythromycin, Pefloxacin and Septrin. From this study, it was discovered that consumers of RTE African salad from majority of the vendors across Enugu metropolis are at risk of severe food poisoning.

## 1. Introduction

Ready-to-Eat (RTE) African Salads are edible foods usually consumed in its raw state (Eluu et al., 2018). African salad (popularly called “Abacha, Abacha Ncha, Abacha and Ugba” by Igbo tribe of Nigeria) is a delicacy of African origin, discovered to be mostly loved by the Igbos (South Eastern part) in Nigeria. The name “African salad” is thought to have originated from the idea of the Igbo’s that salad contains lots of raw, fresh vegetables and some other ingredients consumed without further cooking, hence it is a salad and of African origin (Oranusi et al., 2013).

African salad is made from cassava, garnished with vegetables [Garden egg (*Solanum melongena*), Garden egg leaves, Utazi leaves (*Gononema latifolium*), Okazi (Ukazi) leaves (*Gnetum africana*), Ozeza (Uzeza) leaves (*Piper guineense*)] and other ingredients such as, oil beans (*Pentaclethra macrophylla*), pigeon peas (*Cajanus cajan*) (also known as fiofio), Palm oil, Potash, Onions, Nutmeg, Crayfish, Salt, Pepper, Maggi, Ogiri (*Ricinus communis*), Kpomo (cow skin), meat and stockfish/fish

(Abadias et al., 2008; Miriam and Anthonio, 2011; Maky, 2013; Osewa, 2013). It is a low-calorie food, rich in fiber and with great variety of phyto-nutrients, vitamins and minerals, prepared with little or no heat involved, thus, making it a high-risk food as it could be infected easily by coliforms and other food borne pathogens.

In Nigeria, the poor economic state and unemployment rate in the country has led to the development of survival tactics by its citizens, which has led to a social pattern characterized by increased mobility, increased number of workers on scheduled duties, with a consequent decline in home activities, making majority of the populace to be dependent on RTE foods. Therefore, the services of food vendors are on the rise, thereby transferring the responsibility of ensuring hygienic practices during food processing and proper food handling from families to food vendors who hardly enforce or adhere to such practices (Afolabi et al., 2012). Also, there is insufficient knowledge and awareness regarding food borne illnesses and how they are transmitted among food handlers/vendors, there is lack of stipulated rules by appropriate

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regulatory agencies and most of these food vendors are not licensed, thereby predisposing consumers to the risk of food poisoning daily. Furthermore, due to poor access to potable water supply and the high cost of clean water in Enugu State, residents are forced to depend on polluted water sources for domestic activities including cooking/food processing (Ajala, 2022). These challenges therefore demand more attention and surveillance on food vendors and their products (RTE foods) by way of investigating the microbiological quality of RTE foods in order to reduce the risk of possible food poisoning and create more awareness about ensuring hygienic practices among food vendors.

Studies have revealed the presence of different microbial pathogens in RTE foods which include *Aeromonas hydrophila*, *Shigella sonnei*, *Vibrio* spp., *Escherichia coli*, Coliforms, *Staphylococcus aureus*, *Enterobacter* spp., *Klebsiella* spp., *Salmonella typhi*, *Serratia* spp., *Providencia* spp., *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, and *Listeria monocytogenes* (Ali et al., 2011; Guardiola-Avila et al., 2015), with enteropathogenic *E. coli*, *Salmonella*, *Vibrio* and *Shigella*, being important enteric pathogens, then *Vibrio* spp., *Salmonella* spp. and *E. coli* O157:H7, being the most dangerous food borne bacterial pathogens of public health concern (Xanthopoulos et al., 2009).

*Vibrio cholera* is the bacterium that causes cholera. It is often present in water contaminated by faeces from a person with the infection or in foods. It is abundant in marine and freshwater ecosystems. The disease is occasionally spread through eating raw or undercooked shellfish that are naturally contaminated (Beshiru et al., 2020). *Vibrio mimicus* is a *Vibrio* specie that mimics *V. cholera*. It has been recognized as a cause of gastroenteritis transmitted by crayfish, raw oysters, fish, prawns, squid, and turtle eggs (Guardiola-Avila et al., 2015). *Vibrio parahaemolyticus* infection can be acquired by drinking contaminated water, eating raw or undercooked shellfish. Its symptoms include gastroenteritis, watery diarrhea, abdominal cramps, nausea, fever, vomiting and headache (Igbinsosa et al., 2021). *Vibrio fluvialis* has been reported as an emerging food borne pathogen and has been implicated in sporadic cases of diarrhoea and outbreaks (Igbinsosa and Okoh, 2010).

World Health Organization (WHO) reported that every day more than 5000 children die in the world owing to consumption of contaminated water and food (Oguwike et al., 2014). The incidence of antibiotic-resistant commensals and antibiotic resistance genes in RTE retailed foods, and their role in transmitting these antibiotic resistance genes via horizontal gene transfer has been reported (Van et al., 2007; Verreaes et al., 2013). Food contaminated with antibiotic-resistant pathogenic bacteria is an important threat to public health (Gajaraj et al., 2012). Apart from causing human infection, they have the capacity to serve as host for antimicrobial resistance and these pathogens easily transfer antibiotic-resistant elements to unrelated and related bacterial species (Odu and Akano, 2012).

RTE fast food is one of the most liked and preferred quick-bites in Enugu metropolis and African salad is one of the most popular RTE fast-foods in Enugu metropolis. However, it has been noticed that in Enugu metropolis, fresh vegetables used for preparation of salads are often openly displayed for sale in an unhygienic state on floors, tables, trays, wheelbarrows and handled by intending buyers with bare hands which is suspected to serve as reservoir for microbial consortium (Eluu et al., 2018). Antimicrobial susceptibility profile of various microorganisms obtained from African salads has revealed that salads may serve as vehicles for the transfer of antimicrobial resistance to pathogenic bacteria from environments to humans and from one place to another.

Although there are several studies on the microbiological quality of some of the component ingredients of African salads, there is little information on microbiological composition of African salads as consumed. Also, the incidence of emerging infections has been associated with food borne transmission (Parmley et al., 2012), thus, it informed broadening the scope of this research to include more clinically relevant culture media (like TCBS), in order to recover possible emerging pathogens associated with African salads. This study thus evaluated the bacteriological quality of ready-to-eat (RTE) African salads sold across Enugu

metropolis and determined the antibiotic sensitivity profile of the microorganisms.

## 2. Materials and methods

### 2.1. Study area

This study was carried out in Enugu metropolis, which is situated in Enugu South, Enugu North and Enugu East Local Government Area of Enugu State, Nigeria. It is the present capital of Enugu State and is located between latitudes 6° 20'N and 6° 30'N and longitude 7° 20'E and 7° 30'E and lies within 221 m–317 m above mean sea level of the Udi plateau. Samples were collected from Agbani road located between latitude 5.40932 and longitude 7.49577, Ogbete located between latitude 6.434956, and longitude 7.484803, Mayor located between latitude 6.40911 and longitude 7.49609, Uwani located between latitude 6.42404, and longitude 7.49411, Kenyatta located between latitude 6.41442, and longitude 7.50252, Achara layout located between latitude 6.41147, and longitude 7.498339, Timber located between latitude 6.41330 and longitude 7.50469, and Obiagu located between latitude 6.43794, and longitude 7.50163.

### 2.2. Sample collection

A total of 10 RTE African salads were purchased from 10 different vendors in; Agbani road, Ogbete (2 samples), Mayor, Uwani, Kenyatta (2 samples), Achara Layout, Obiagu, Timber, in November, 2020. This study included African salads with different ingredients; Pigeon peas, Oil beans, Utazi, Onions. The samples were labeled based on their location of purchase, packed separately and kept in an ice box and transferred to the Microbiology laboratory of Renaissance University, Ugbawka, Enugu State, for further analysis. Sample location and designated sample codes are shown in Table 1.

### 2.3. Bacteriological analysis

#### 2.3.1. Sample preparation

Samples were processed according to Roland et al. (2012) with some modifications. Twenty-five grams (25 g) of each sample were homogenized by blending in 225 ml of sterile buffered peptone water. One millilitre (1 ml) of the homogenate was introduced into 9 ml of the buffered peptone water in a test tube labeled 1:10 ( $10^{-1}$ ) dilution and serially diluted to three other test tubes labeled  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$ . The procedure was repeated for each sample and the blender was carefully washed, cleansed and disinfected in between sample preparation to prevent any cross contamination.

#### 2.3.2. Isolation and enumeration of bacteria

Isolation and enumeration of microbial species were conducted according to the method of Igbinsosa et al. (2020). An aliquot (0.1 ml) from the  $10^{-4}$  dilution for each sample was plated on MacConkey agar (TM MEDIA, India), Thiosulfate-citrate-bile salts-sucrose (TCBS) agar (TM MEDIA, India), Eosin Methylene Blue (EMB) agar (TM MEDIA, India) and

Table 1. Sample location and codes.

S/N	Sample location	Sample code
1.	Agbani Road	Agb
2.	Ogbete market	Ogb
3.	Mayor	May
4.	Kenyatta	Ken
5.	Uwani	Uwa
6.	Obiagu	Obi
7.	Achara layout	Ach
8.	Timber	Tim

**Table 2.** Bacterial counts from RTE African Salads across sampled locations in Enugu.

Location	Mean <i>Staphylococcal</i> spp. Counts on NA (CFU/g) ± SD	Mean counts on EMB (CFU/g) ± SD	Mean counts on TCBS (CFU/g) ± SD	Mean counts on SSA (CFU/g) ± SD
Agbani Rd.	$2.4 \times 10^7 \pm 5.65$	TNC	TNC	$7.4 \times 10^6 \pm 5.65$
Ogbete	$1.3 \times 10^6 \pm 2.82$	Negligible	$8.5 \times 10^6 \pm 2.82$	$5.7 \times 10^6 \pm 2.82$
Mayor	Nil	$3.1 \times 10^6 \pm 1.41$	$1.21 \times 10^7 \pm 4.24$	$2.3 \times 10^6 \pm 2.82$
Kenyataa I	Negligible	Negligible	$3.7 \times 10^6 \pm 4.24$	Negligible
Kenyatta II	Nil	Negligible	Negligible	$6.5 \times 10^5 \pm 4.24$
Obiagu	Nil	$6.3 \times 10^6 \pm 5.65$	$4.0 \times 10^6 \pm 1.41$	$4.2 \times 10^6 \pm 7.0$
Timber	Nil	TNC	TNC	$1.21 \times 10^7 \pm 1.41$
Achara layout	Negligible	Negligible	Negligible	Negligible

**Key:** Negligible = < 30 colonies; TNC (Too numerous to count) = > 300 colonies.

Nil = no observed colonies; SD = Standard deviation.

Salmonella-Shigella Agar (SSA) (TM MEDIA, India) in duplicates. The plates were incubated aerobically at 37 °C for 24 h. After incubation, the inoculated plates were examined for growth and then the morphological characteristics of the isolates were noted. All discrete colonies were counted and expressed in colony forming units per gram (CFU g<sup>-1</sup>).

### 2.3.3. Biochemical characterization and identification of the isolates

Pure cultures were obtained by streaking a portion of an isolated colony on nutrient agar plate and incubated aerobically at 37 °C for 24 h. These were subsequently inoculated on agar slants, incubated at 37 °C for 24 h and preserved for further tests. Gram staining was carried out on the bacterial isolates and further characterization and identification was done using the Analytical Profile Index (API 20E) (Biomerieux, France) test strips following the method of Igbiosa et al. (2020). The test was performed following manufacturer's instructions at the Department of Biotechnology, Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Nigeria, and results were read and interpreted accordingly using API catalog or apiweb: <https://apiweb.biomerieux.com>.

**2.3.3.1. Preparation of the strips.** Incubation box (tray and lid) was prepared and about 5 ml of distilled water was distributed into the honey combed wells of the tray to create a humid atmosphere. The strain reference number was recorded on the elongated flap of the tray. The strip was removed from its packaging and was placed in the incubation box.

**2.3.3.2. Preparation of the inoculum.** With a pipette (sterile), a single well-isolated colony was removed from an isolation plate of 18–24 h old and placed in an opened ampoule of API NaCl 0.85% medium aseptically. The suspension was carefully emulsified to achieve a homogenous bacterial suspension.

**2.3.3.3. Inoculation of the strip.** With sterile micropipette and tips, the bacterial suspension was distributed into the tubes of the strip by tilting the strip slightly forward after placing the tip of the pipette against the side of the cupule. For the CIT, VP and GEL tests, the tube and cupule are both filled up and for the other tests, only the tubes were filled and for the tests ADH, LDC, ODC, H<sub>2</sub>S and URE were all overlaid with mineral oil to create anaerobiosis condition. The incubation box was closed with the lid and all boxes were incubated at 36 °C for 18–24 h.

The various tests are represented thus; Orthro-Nitrophenyl-beta-D-GalactoPyranosidase (ONPG), Arginine DiHydrolase (ADH), Lysine DeCarboxylase (LDC), Ornithine DeCarboxylase (ODC), Citrate (CIT), Hydrogen sulphide Production (H<sub>2</sub>S), Urease (URE), Tryptophan DeAminase (TDA), Indole production (IND), Voges Proskauer (VP), Gelatinase (GEL), D-glucose (GLU), D-mannitol (MAN), Inositol (INO), D-Sorbitol (SOR), L-Rhamnose (RHA), Saccharose (D-Sucrose) (SAC), D-melibiose (MEL), Amygdalin (AMY), L-Arabinose (ARA), Cytochrome-Oxidase (OX), Motility (MOB), MacConkey medium (McC), Fermentation – under mineral oil (OF-F), Oxidation – exposed to the air (OF-O).

**2.3.3.4. Reading the strip.** After incubation period, the strip tests which required the addition of reagents such as TDA test, one drop of TDA reagent was added. With a reddish brown colour indicates a positive reaction. IND test (Indole) a drop of JAMES reagent was added. A pink colour was positive reaction. VP test, a drop each of VP1 and VP2 reagents with a pink or red colour indicates a positive reaction. While NO<sub>3</sub>–NO<sub>2</sub> was added a drop of NIT1 and NIT2 reagents to the GLU tube. A red colour indicates a positive reaction. Motility (MOB) ampule of API medium were inoculated, growth on MacConkey agar medium, (McC). MacConkey agar plates were streaked and incubated at 36 °C ± 2 for 24 h. The positive reactions were all recorded on the recording sheet.

**2.3.3.5. Interpretation.** Identification was obtained with the numerical profile. On the result sheet, the tests are separated into groups of 3 and a value of 1.2 × 4 was indicated for each tray adding together the values corresponding to positive reactions. Within each group, a 7-digit profile number was obtained for the 20 tests on the API 20E strips which were later identified with apiweb identification software.

### 2.3.4. Antibiotic sensitivity test

Antibiotic sensitivity of the isolates was done using the Kirby Bauer disc diffusion technique described by Devi et al. (2009) and interpreted by adopting the breakpoints of Clinical and Laboratory Standard Institute (CLSI document M100-S24, 2014 and CLSI guideline M45, 2015). Mueller-Hinton agar (MHA) was prepared according to the manufacturer's instructions. The medium was cooled to 45–50 °C and poured into plates. Plates were allowed to set on a level surface to a depth of approximately 4 mm. When the agar had gelled, plates were allowed to dry before use. An 18–24 h-old broth culture of the isolates were standardized by diluting to 0.5 McFarland's standard. A sterile cotton swab stick was inserted into the standardized inoculums (1 × 10<sup>8</sup> CFU/ml) each, drained to remove excess inoculum load and inoculated by spreading on the surface of prepared MHA plates. The inoculated MHA plates were subsequently allowed to dry for a few minutes at room temperature with the lid closed; thereafter the antibiotic impregnated discs of known concentrations were aseptically placed on the inoculated MHA plates, 15 mm away from the edge of the plates with the aid of sterile forceps. The plates were then incubated at 37 °C for 18–24 h. After which, the diameters of the zones of inhibition were measured with a metre rule and recorded in millimetres. In this study, Maxi disc antibiotic sensitivity disc by Maxicare laboratory was used. Antibiotic impregnated discs of known concentrations included; **Gram-negative:** Septrin (SXT) (30 µg), Chloramphenicol (CH) (30 µg), Sparfloxacin (SP) (10 µg), Ciprofloxacin (CPX) (30 µg), Amoxicillin (AM) (30 µg), Augmentin (AU) (10 µg), Gentamycin (CN) (30 µg), Pefloxacin (PEF) (30 µg), Tarivid (OFX) (10 µg), Streptomycin (S) (30 µg).

**Gram-positive:** Pefloxacin (PEF) (10 µg), Gentamycin (CN) (10 µg), Ampiclox (APX) (30 µg), Zinnacel (Z) (20 µg), Amoxicillin (AM) (30 µg), Rocephin (R) (25 µg), Ciprofloxacin (CPX) (10 µg), Streptomycin (S) (30 µg), Septrin (SXT) (30 µg), Erythromycin (E) (10 µg).

Characterization of the resistance (R), intermediate (I) or sensitive (S) profile of the isolates was determined by measuring the diameters of the zones of inhibition, then compared with the interpretative chart to determine the resistant, intermediate or sensitive nature of the isolates

**Table 3.** Biochemical characterization and identities of the isolates using the Analytical Profile Index (API 20E) Kit.

Isolate code	Biochemical tests																	
	ONPG	ADH	LDC	ODC	CIT	H2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL
Tim 218	+	-	+	+	-	-	-	-	+	-	-	+	+	-	+	+	+	+
May 216	+	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	+	-
May 212A	+	+	-	-	-	-	-	-	+	-	+	-	+	-	-	-	+	-
Agb 217	-	-	+	+	+	-	-	-	+	-	+	+	+	-	-	-	-	-
May 212B	+	-	+	+	+	-	-	-	+	-	+	+	+	-	-	-	-	-
May 211	+	-	-	+	+	-	-	-	-	-	-	+	+	-	+	-	+	-
Agb 208	-	+	-	-	+	-	+	-	-	+	+	+	+	-	+	-	+	-
Agb 210, Tim 207, Ken 201	-	-	+	+	-	+	-	-	-	-	-	+	+	-	+	+	-	+
Ken 211, Obi 202, Uwa 201	+	-	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+	+
Uwa 204, Ogb 201	+	+	-	+	+	-	-	-	-	+	-	+	+	-	+	+	+	+

to the antibiotics used, with the aid of the interpretative chart by CLSI (Appendix). It is noteworthy that following standard by CLSI document M100-S24, 2014, Ampicillin is representative for Amoxicillin. Results for Ampicillin can be used to predict results for Amoxicillin.

### 3. Results and discussion

#### 3.1. Bacterial counts for RTE African salads from some sampled locations in Enugu metropolis

Bacterial counts recorded for RTE African salads across the sampled locations are expressed as counts on the various microbiological media used in the study and presented in Table 2. Total culturable heterotrophic bacteria (TCHB) counts across sampled locations were too numerous to count (TNC). On EMB agar, the highest bacterial count recorded was  $6.3 \times 10^6$  CFU/g from Obiagu. On TCBS, the highest bacterial count recorded was  $1.21 \times 10^7$  CFU/g from Mayor. The highest bacterial count recorded on SSA was  $7.4 \times 10^6$  CFU/g from Agbani Rd.

#### 3.2. Biochemical characterization and identification of bacterial isolates using the Analytical Profile Index (API 20E) test kit

The Gram reaction, biochemical characterization and subsequent identities of the bacterial isolates from the RTE African salads across the sampled locations in this study are presented in Table 3. Majority of the bacterial isolates were Gram negative rods except *Staphylococcus* sp. that was Gram positive cocci. The identities of the bacterial isolates were; *E. coli*, *S. aureus*, *Salmonella* spp., *V. cholerae*, *V. fluvialis*, *Vibrio hollisae*, *V. mimicus*, *V. parahaemolyticus*, *Klebsiella* spp., *Enterobacter* spp.

#### 3.3. Distribution and frequency of bacterial isolates across sampled locations

The distribution of the bacterial isolates from the RTE African salads, across the sampled locations as displayed in Table 4 are as follows: *Vibrio* spp. [10 (from all sampled locations)], *E. coli* [Agbani (1) Mayor (1), Ogbete (1), Uwani (1), Kenyatta (1), Achara layout (1), Timber (1)], *Salmonella* spp. [Agbani road (1), Ogbete (1), Kenyatta (2), Achara layout (1), Obiagu (1), Timber (1)], *S. aureus* [Agbani road (1), Ogbete (1), Kenyatta (1)], *Klebsiella* sp. [Agbani road (1), Ogbete (1), Mayor (1), Uwani (1), Kenyatta (2), Obiagu (1), Timber (1)], *Enterobacter* spp. [Ogbete (1), Mayor (1), Uwani (1)].

The frequency of occurrence of the bacterial isolates as shown in Figure 1 includes *Vibrio* spp. 10(26.3%), *E. coli* 7(18.4%), *Salmonella* spp. 7(18.4%), *S. aureus* 3(7.8%), *Klebsiella* spp. 8(21.0%), *Enterobacter* spp.

3(7.8%). Thus, the most frequently isolated species from RTE African salad across sampled locations was *Vibrio*.

#### 3.4. Antibiotic sensitivity of the isolates

The antibiotic sensitivity profiles of the bacterial isolates are presented in Table 5 *Vibrio cholerae* was susceptible to Chloramphenicol, Ciprofloxacin, Streptomycin & Erythromycin but resistant to Amoxicillin. *V. parahaemolyticus* and other *Vibrio* spp. were susceptible to all the antibiotics tested. *E. coli*, *Salmonella* sp. and *S. aureus*, were all susceptible to the antibiotics tested in this study.

### 4. Discussion

This study focused on evaluating the bacteriological quality of RTE African salads across Enugu metropolis, Nigeria. Bacterial contamination is an indicator of the extent of safe food handling, which is known globally as vehicle for pathogen transmission. Measure for scoring bacteriological quality used in this study was bacterial counts (load). The RTE African salads from all the sampled locations had varying degrees of bacterial contamination (mostly coliforms), which proved unsatisfactory or unacceptable as far as bacteriological quality is concerned. *E. coli*, *S. aureus*, *V. mimicus*, *V. fluvialis*, *V. cholerae*, *V. parahaemolyticus*, *V. hollisae*, *Klebsiella* spp., *Enterobacter* spp. and *Salmonella* spp. were the bacterial species isolated from the various RTE African salads sampled in this study. Research frontiers have reported similar findings from their studies on street foods in some African and low-income countries (Mensah et al., 2002; Afolabi et al., 2012; Igbinosa and Beshiru, 2019; Beshiru et al., 2020; Igbinosa et al., 2021).

The isolation of *E. coli*, *Klebsiella* spp., *Enterobacter* spp. and *Salmonella* spp., from the RTE African salads, suggests that most food borne enteric infection and diseases occur because of unhygienic handling of the foods and or the poor sanitary condition of the environment during preparation/processing of the food. It is also indicative of possible faecal contamination from the water used in food processing, improper washing of the vegetables used or from the fertilizer/manure used in cultivating the vegetables used. Corresponding reports were made by Wogu and Iwezua (2013) and Igbinosa et al. (2020).

Isolation of *S. aureus* is suggestive of contamination (carriage in nasal passages) by food handlers or infected workers, as it has been reported that food handlers are the main source of food contamination in *S. aureus* food poisoning. *S. aureus* contamination can also arise from infected wounds, stroking hair, scalp, burns and dirty fomites of food handlers (Yeboah-Manu et al., 2010; Afolabi et al., 2012).

The presence of *Vibrio* species in the RTE African salads suggests that the water used in the preparation of the African salad is unsafe for human

Biochemical tests									API % similarity	Identity	Organisms' Tentative Identity
AMY	ARA	OX	NO2	N2	MOB	MoC	OF-O	OF-F			
-	+	-	+	-	+	+	+	+	99.5		<i>E. coli</i>
-	-	+	+	-	+	+	+	+	99.9		<i>V. cholera</i>
+	+	+	+	-	+	+	+	+	90.0		<i>V. fluvialis</i>
-	+	+	+	-	+	+	+	+	99.9		<i>V. parahaemolyticus</i>
-	-	+	+	-	+	+	+	+	94.6		<i>V. mimicus</i>
+	+	-	+	-	+	+	+	+	92.5		<i>V. hollisae</i>
-	+	-	+	-	-	-	-	+	99%		<i>S. aureus</i>
-	+	-	-	-	+	+	+	+	90.5%		<i>Salmonella</i> spp.
-	+	-	+	-	-	-	-	-	90.0%		<i>Klebsiella</i> spp.
+	+	-	+	-	+	+	+	+	95.0%		<i>Enterobacter</i> spp.

consumption as it is contaminated with such water borne pathogens. The presence of *Vibrio* spp. in African salad is a rare finding across other studies in this area, however, species of *Vibrio* were isolated from RTE shrimps, crabs, oysters, clams and African Salad by (Adeleye et al., 2010; Saad et al., 2013; Guardiola-Avila et al., 2015; Beshiru et al., 2020; Igbinsosa et al., 2021). Apart from Nigeria, these *Vibrio* species have also been associated with human diseases in different countries like the United States, Japan, Brazil, Venezuela, Mexico and others (Munoz et al., 2012; Guardiola-Avila et al., 2015).

These organisms are harmful to humans when their population in the human body is equal to or greater than their infectious dose. Bacterial pathogens tend to require a larger dose to cause infection ( $>10^5$  CFU), except for *Shigella* spp. ( $10^1$ – $10^2$  CFU), non-O1 strain of *V. cholera* ( $10^6$  CFU) and Toxigenic *V. cholera* ( $10^3$ – $10^4$  CFU) (Mahendra et al., 2006). The risk of infection by these organisms is also dependent on the strain/serotype of the organism involved. For instance, there are many serogroups of *V. cholerae*, but only two – O1 and O139 – produce the cholera toxin that cause outbreaks. *V. cholerae* O1 has caused all recent outbreaks. *V. cholerae* O139 – first identified in Bangladesh in 1992 – caused outbreaks in the past, but recently has only been identified in sporadic cases (Lutz et al., 2013). This means that though the isolates surpass the infectious dose, there may be no outbreak of cholera if the two serogroups mentioned are absent. This could be the reason there was no cholera outbreak reported in Enugu metropolis, as at the time of this study (year 2020), despite the presence of *V. cholerae* in the RTE African salads.

The most frequently isolated species from RTE African salad across sampled locations in this study was *Vibrio* spp. 10(27%), followed closely by *Klebsiella* spp. 8(21.6%). The *Vibrionaceae* family comprise diverse significant organisms, of which 12 species are clinically relevant as etiologic agents of human diseases. The principal human pathogens are *Vibrio vulnificus*, *V. cholerae*, and *V. parahaemolyticus* (Guardiola-Avila et al., 2015), which were isolated in this study. The high level of *Vibrio* spp. present in the sampled RTE African salads could be explained by the presence of the crustacean (crayfish) which is one of the ingredients used in its processing. The interaction between *Vibrio* species and Crustaceans (crayfish, shrimps, lobster, shellfish) have been reported. The chitin present in the shells of Crustaceans gives *Vibrio* species a protective effect, thus, their abundance in the RTE African salads (Guardiola-Avila et al., 2015).

The highest *Vibrio* count recorded in this study was  $1.21 \times 10^7$  CFU/g. In a similar study by Igbinsosa et al. (2021), 63 *V. parahaemolyticus* isolates were recovered from African salad samples, in eight (8) different states across Nigeria, nine (9) of which (14.29%), was recovered from Enugu State. Cell density of *V. parahaemolyticus* across their samples

ranged from 1.5 to 1000 MPN/g. Also, Guardiola-Avila et al. (2015) reported that 9% of the ready-to-eat fish and sea food in their study were positive for *V. mimicus*.

The highest coliforms count (on EMB media) across the samples in this study was  $6.3 \times 10^6$  CFU/g or 6.7  $\log_{10}$  CFU/g. This is within the infectious dose range for food borne infection/illness ( $>4.0 \log_{10}$  CFU/g for *Enterobacteriaceae*) (Mahendra et al., 2006; Afolabi et al., 2012). The report by Afolabi et al. (2012) corroborates this finding. They reported total coliform counts recovered from ready-to-eat foods (jollof rice and others) sold in primary schools in Abeokuta, Nigeria, ranging from 6.00  $\log_{10}$  CFU/g to 6.28  $\log_{10}$  CFU/g. Also, amongst other bacterial species isolated from their study, *Klebsiella pneumoniae* was recovered from the ready-to-eat foods, and this agrees with findings from this study.

Therefore, there is the need to control enteric infections and gastroenteritis in Enugu metropolis and Nigeria as a nation. This can only be achieved by having a clear understanding of the frequency of occurrence of the organisms responsible for these diseases and their survival in RTE foods.

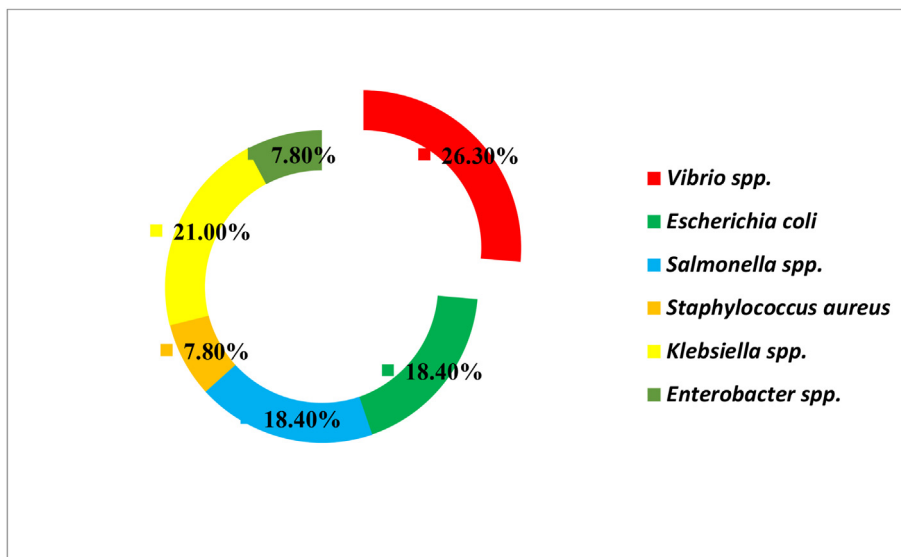
The antibiotic sensitivity profiling of the isolates recovered from the RTE African Salads revealed that all the organisms were sensitive to antibiotics used except for *V. cholerae* which was resistant to two of the antibiotics (Ampicillin and Amoxycillin). However, in 2007, Van et al. reported 83.3% of the *E. coli* recovered from RTE retailed foods was resistant to at least one antibiotic used in their study. Das et al. (2020) reported that *V. cholerae* acquired resistance functions against almost all the commonly used antibiotics over time. Contrary to findings by Das et al. (2020), in this study, *V. cholerae* was sensitive to all the antibiotics used except Ampicillin and Amoxycillin. Mandal et al. (2012) also reported *V. cholerae* resistance to ampicillin and tetracycline in the two outbreaks that occurred in India in May and November, 2010. Ampicillin and Amoxycillin are a broad-spectrum penicillin that has been in use for a long period of time and is active against numerous bacterial species. It is affordable and people can easily self-medicate with it, as it is easily accessed without doctor's recommendation. The resistance of *V. cholerae* to Ampicillin and Amoxycillin could be attributed to its production of beta-lactamases. These enzymes have the capacity to breakdown or degrade the antibiotics.

In addition, sensitivity/resistance of *V. cholerae* to these antibiotics is determined by the strains (virulent or non-virulent) of *V. cholera* involved. Poor patient adherence to prescribed medications, self-medication with previously unused antibiotics, use of sub-standard medications amongst others, increase the development of drug (antibiotic) resistance.

In recent years, treatment failures are often seen with the recurrent emergence of antimicrobial resistant (AMR) *V. cholerae*. A drastic increase in its resistance against Ampicillin, Nalidixic acid,

**Table 4.** Distribution of the bacterial isolates across sample locations.

Bacterial isolates	Agbani road	Ogbete I & II	Mayor	Uwani	Kenyatta I & II	Achara layout	Obiagu	Timber
<i>Vibrio</i> spp.	2	1	1	1	1	1	1	2
<i>E. coli</i>	1	1	1	1	1	1	0	1
<i>Salmonella</i> spp.	1	1	0	0	2	1	1	1
<i>S. aureus</i>	1	1	0	0	1	0	0	0
<i>Klebsiella</i> spp.	1	1	1	1	2	0	1	1
<i>Enterobacter</i> spp.	0	1	1	1	0	0	0	0



**Figure 1.** Frequency of occurrence of the bacterial isolates.

**Table 5.** Antibiotic susceptibility profile of some of the bacterial isolates.

Antimicrobial class	Antibiotics	<i>E. coli</i> (mm)	<i>V. cholerae</i> (mm)	<i>V. parahaemolyticus</i> (mm)	<i>Salmonella</i> spp. (mm)	<i>S. aureus</i> (mm)	<i>Klebsiella</i> spp. (mm)	<i>Enterobacter</i> spp. (mm)
Sulfonamides	SXT	28 (S)	30 (S)	30 (S)	30 (S)	30 (S)	27 (S)	27 (S)
Phenicol	CH	29 (S)	28 (S)	31(S)	30 (S)	29 (S)	25 (S)	26 (S)
Fluoroquinolones	CPX	30 (S)	29 (S)	30 (S)	30 (S)	28 (S)	27 (S)	27 (S)
	SP	29 (S)	28 (S)	32 (S)	28 (S)	28 (S)	26 (S)	28 (S)
	OFX	28 (S)	23 (S)	27 (S)	29 (S)	28 (S)	23 (S)	25 (S)
	PEF	29 (S)	20 (S)	26 (S)	29 (S)	28 (S)	27 (S)	22 (S)
Macrolides	E	29 (S)	31 (S)	27 (S)	28 (S)	30 (S)	26 (S)	NA
Cephalosporins	R	29 (S)	29 (S)	26 (S)	29 (S)	27 (S)	28 (S)	NA
	(ceftriaxone)	27 (S)	28 (S)	23 (S)	27 (S)	28 (S)	17 (I)	NA
	Z (cefuroxime)							
Aminoglycosides	CN	27 (S)	20 (S)	26 (S)	29 (S)	28 (S)	28 (S)	24 (S)
	S	29 (S)	29 (S)	26 (S)	30 (S)	30 (S)	27 (S)	25 (S)
Amoxicillin-clavulanate	AU	28 (S)	18 (S)	19 (S)	29 (S)	28 (S)	23 (S)	24 (S)
Penicillinase-labile penicillins	AM	29 (S)	8 (R)	22 (S)	28 (S)	28 (S)	20 (S)	23 (S)

\*Zone of inhibition represented in millimeters (mm); Septrin (SXT), Chloramphenicol (CH), Ciprofloxacin (CPX), Sparfloxacin (SP), Tarivid (OFX), Pefloxacin (PEF), Erythromycin (E), Rocephin (R), Zinnacel (Z), Gentamycin (CN), Streptomycin (S), Augmentin (AU), Amoxicillin (AM); NA(Not Available), (S) = Sensitive, (R) = Resistant.

Chloramphenicol and Tetracycline started to appear from early 1990 (Yu et al., 2012). However, in this study, *V. cholerae* was sensitive to Chloramphenicol but resistant to Ampicillin. Although chromosomal mutations can contribute to antimicrobial resistance (AMR), the frequent acquisition of extra-chromosomal mobile genetic elements (MGEs) from closely/distantly related bacterial species are major players in *V. cholerae* drug resistance (Das et al., 2020).

### 5. Conclusion

This study has provided evidence that the RTE African salads sold by vendors in the sampled parts of Enugu metropolis pose high-risk to its consumers as they are potential reservoirs for coliforms, drug resistant *V. cholerae* and other *Vibrio* species, and *S. aureus*. The recovery of these organisms from the RTE African salads gives a robust

understanding concerning the possible occurrence of pathogenic and drug resistant organisms in African salads, which is a pointer to the possibility of horizontal dissemination of antibiotic resistance genes amongst these bacterial species. Findings from this study therefore is significant for decision making on key monitoring programs, and provides information for risk and exposure assessment of *V. cholerae* and other *Vibrio* spp. Good hygiene practices and the use of potable water and clean kitchen utensils is therefore encouraged amongst food handlers.

### Declarations

#### Author contribution statement

Chinyere Augusta Ajuzieogu, Ph.D: Conceived and designed the experiments; Performed the experiments; Analysed and interpreted the data; Wrote the paper.

Irene Chidinma Dyboh, B.Sc: Performed the experiments; Analysed and interpreted the data; Contributed reagents, materials and analysis tools.

### Appendix

Table A1: Standards for Antibiotic Susceptibility of Enterobacteriaceae group and *Vibrio* species.

Antibiotic	Susceptibility		
	Resistant (mm)	Intermediate (mm)	Sensitive (mm)
<u>Pefloxacin (PEF)</u>	≤12	13-16	≥17
<u>Gentamycin (GN)</u>	≤12	13-14	≥15
<u>Ampicillin (AMP)</u>	≤13	14-16	≥17
<u>Zinnaclef (Z)</u>	≤14	15-22	≥23
<u>Amoxicillin (AM)</u>	≤13	14-17	≥18
<u>Rocephin (R)</u>	≤14	15-19	≥20
<u>Ciprofloxacin (CPX)</u>	≤15	16-20	≥21
<u>Streptomycin (S)</u>	≤11	12-14	≥15
<u>Septtrin (SXT)</u>	≤10	11-15	≥16
<u>Erythromycin (E)</u>	≤15	16-22	≥23
<u>Chloramphenicol (CH)</u>	≤12	13-17	≥18
<u>Sparfloxacin (SP)</u>	≤15	16-18	≥19
<u>Augmentin (AU)</u>	≤13	14-17	≥18
<u>Tarivid (OFX)</u>	≤12	13-15	≥16

David Chinemerem Nwobodo, M.Sc: Contributed reagents, materials and analysis tools.

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#### Data availability statement

Data included in article/supp. material/referenced in article.

#### Declaration of interest's statement

The authors declare no conflict of interest.

#### Additional information

No additional information is available for this paper.

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