FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 8 No. 2, April, 2024, pp 5 - 14
DOI: https://doi.org/10.33003/fjs-2024-0802-2326

OCCURRENCE OF VIRULENCE GENES OF *Staphylococcus aureus* AND *Escherichia coli* IN VENDED FOODS IN SELECTED MOTOR-PARKS IN OBIO/AKPOR, RIVERS STATE

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ABSTRACT

The study aimed at evaluating the microbiological safety of vended foods in selected motor parks in Obio/Akpor Local Government Area, Rivers State, Nigeria. Forty-eight samples obtained from four motor parks (Choba, Rumuokoro, Eliozu and Oil-mill Park) were analyzed using standard microbiological methods for total heterotrophic bacteria count, virulent and antibiotic resistant profiles of *Staphylococcus aureus* and *Escherichia coli*. Total heterotrophic bacteria count ranged from 4.60 to 8.39 $\log_{10}$CFU/g. A total of 10 (20.83%) samples were positive for *S. aureus* while 14 (29.17%) were positive for *E. coli*. Total staphylococcal and *Escherichia coli* counts ranged from 4.89 to 7.01 and 2.70 to 5.31 $\log_{10}$cfu/g, respectively. Both bacteria were 100% resistant to augmentin. The confirmed *E. coli* showed varying resistant to cefixime (50%), ceftazidime (42.86%), cefuroxim (14.29%) and ofloxacin (7.14%); while *S. aureus* also showed varying resistant to erythromycin (10.00%), ceftriaxone (90.00%), cloxacillin (100.00%) and gentamycin (0.00%) The confirmed *E. coli* produced the expected bands for the *E. coli* attaching and effusing (*eaeA*), aggregative adherence fimbriae (*aggR*) and antimicrobial sensitivity testing (*astA*) virulence genes while *S. aureus* did not produce the expected bands with staphylococcal enterotoxin A (*sea*) and staphylococcal enterotoxin B (*seb*) genes. The detection of virulence gene bearing *E. coli* and multiple antibiotic resistant *E. coli* and *S. aureus* portends danger for commuters who patronizes these foods, hence the need for urgent public health interventions.

Keywords: Microbiological safety, Vended foods, Antibiotic resistance, Virulence gene, Public health

INTRODUCTION

Food is a fundamental and crucial requirement for humans to stay nourished and sustain life. The Food and Agricultural Organization (FAO) defined vended foods as ready-to-eat foods and any beverage that is prepared and sold by vendors and hawkers especially in streets and other public places like motor parks (FAO, 1989). They do not have any need of being prepared or processed before they can be ingested since they have already been prepared by the vendors (Oranusi and Olorunfemi, 2011). These ready-to-eat foods could be cooked or uncooked, cold or hot and can be ingested without any further processing or heat treatment (Clarence *et al.,* 2009). These foods have gained popularity in the developing countries due to increasing population, change in the country's economy and urbanization (Akuzi *et al.,* 2016). World Health Organization (2002) reported that lack of social programs as well as the affordability of snack meals and refreshments made available by vendors provide working class persons, shoppers, travelers and low-income earners with essential services. Massive food vending has been ascribed to factors like high rate of unemployment, poor living conditions and apartheid, increasing poverty, inability to cook, urbanization that causes congestion, increase in population, pressure from work, change in lifestyle which results in a temporarily mobile group of individuals, distance between workplace and homes as well as lack of storage or total absence of business establishment that make food available to individuals close to their place of work at a reasonable price (Maxwell *et al.,* 2000; Martin, 2006).

Ready-to-eat vended foods can be prepared from one or multiple raw materials such as meat, fish, cereal, nuts, and spices, into liquid, semi-solid and solid food. They could be industrially processed or traditionally fermented (Adebayo-Oyetoro *et al.,* 2017; Ceyhun-Sezgin and Sanher, 2016). Ready-to-eat street vended foods could be snacks such as Bambara nut meal (okpa), meat pie, fish pie, small chops, buns, pan cake, plantain chips, doughnut, egg roll, sausage roll; nuts such as groundnut, walnut, coconut, tiger nut, date, cashew nut; Fruits such as watermelon, sliced pineapple, pealed orange, pealed paw-paw, apple, banana, or foods such as fried rice, plain rice, bean pudding (Moi-moi), maize meal (Agidi), beans porridge, roasted plantain, yam, African salad, roasted fish, fried fish, vegetable salad and many others.

Nigeria previously had developed industries in form of supermarkets prior to the early 1980's when social and economic changes had a toll on the food system of the middle class. From then on, most Nigerians obtain their food from mobile food vendors, traders or open-air traditional markets (Nzeka, 2011). Food vendors vary due to their difference in population, social and economic conditions and as a result, they do not constitute a single group but are rather classified into the mobile or immobile vendors (Drapper, 1996).

The rising demand and supply of vended ready-to-eat foods in the world has increased the emergence of foodborne diseases mediated by pathogenic microorganisms. Consumption of street vended foods and fruits has been implicated for the increase in the risk of foodborne diseases transmission as most are readily contaminated with pathogens carrying virulence genes from different sources during preparation and some fail to apply good hygiene practices with little or no consideration for the safety of food to the consumers (Tambekar *et al.,* 2008).

Consuming food free of contaminants is an essential and basic need for one to be healthy, active and productive. Inadequate amount of safe food results in a harmful cycle of illness particularly among persons with compromised immune system, children and the elderly (WHO, 2020). There has been an increase in the preparation, sale and consumption of

ready-to-eat foods in many African countries including Nigeria. Illnesses resulting from consumption of foods contaminated with pathogens is a global health issue and an important factor that leads to minimized economic growth (Makelele *et al.,* 2015).

Staphylococcus aureus and *Escherichia coli* have been implicated for many foodborne diseases. A potential health issue associated with the occurrence of *Staphylococcus aureus* in food is the bacteria's ability to create enterotoxin and the danger of future food poisoning that it possesses. Although there are nine identified staphylococcal enterotoxins, designated as A, B, C1, C2, C3, D, E, F, and G, types A and D are accountable for the majority of the outbreaks. The Centre for Disease Control estimates that, in the US, staphylococcal food poisoning causes around 241,188 illnesses, 1,064 hospitalizations, and 6 deaths each year (FDA, 2012).

Most pathogenic variants of *E. coli* are responsible for high mortality and morbidity rate worldwide and have public health importance as they have low infectivity dose and are transmitted through food and water (Croxen *et al.,* 2013). The transmission of *E. coli* takes place when water or food contaminated by human or animal faeces is consumed (Garcia *et al.,* 2010).

Pathogenic *E. coli* due to their mechanism of action have been grouped into six categories: Enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* also called shiga-toxin producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), enteroaggressive *E. coli* (EAggEC), enteroinvassive *E. coli* (EIEC) and attaching and effacing *E. coli* (AEEC) (Garcia *et al***.,** 2013; Croxen *et al.,* 2013).

Annually, STEC strain 0157:H7 is estimated to be responsible for 63, 000 illnesses, 2100 hospitalizations and 20 deaths. Their infection causes mild to severe diarrhoea with about 5-10% generate hemolytic Uremic Syndrome (HUS) a condition that causes excess bleeding that leads to kidney failure and death (Scallan *et al.,* 2011).

Many who patronize these vended foods experience stomach ache afterwards and some discomfort together with other symptoms like diarrhea depending on the incubation period of the associated Microorganisms. They just go to any nearby drug store, lay their complain or just purchase tetracycline, ingest it and feel a little better without realizing that they have suffered a foodborne illness associated with the vended food they consumed along the way capable of causing symptoms that are severe or could even lead to death. Hence, this work is geared towards investigating the safety of selected vended foods in motor parks in Obio/Akpor LGA, Rivers State. The specific objective includes: (i) to determine the microbiological safety of vended foods in motor parks (ii) to examine the presence of virulence genes *sea* and *seb* in *Staphylococcus aureus* as well as *eaeA*, *astA* and *aggR* in *Escherichia coli* (iii) to conduct antibiotic susceptibility test on isolated *Staphylococcus aureus* and *Escherichia coli*.

MATERIALS AND METHODS

Study Area

The research was conducted in Obio/Akpor Local Government Area, Rivers State, Nigeria (Fig. 1). It is located between latitude 4º 45'N and 4º 60'N and longitude 6º 50'E and 8º 00N and covers land area of 260km^2 with a population of 464, 789 in line with the 2006 census (Obio/Akpor Embassy, 2012).

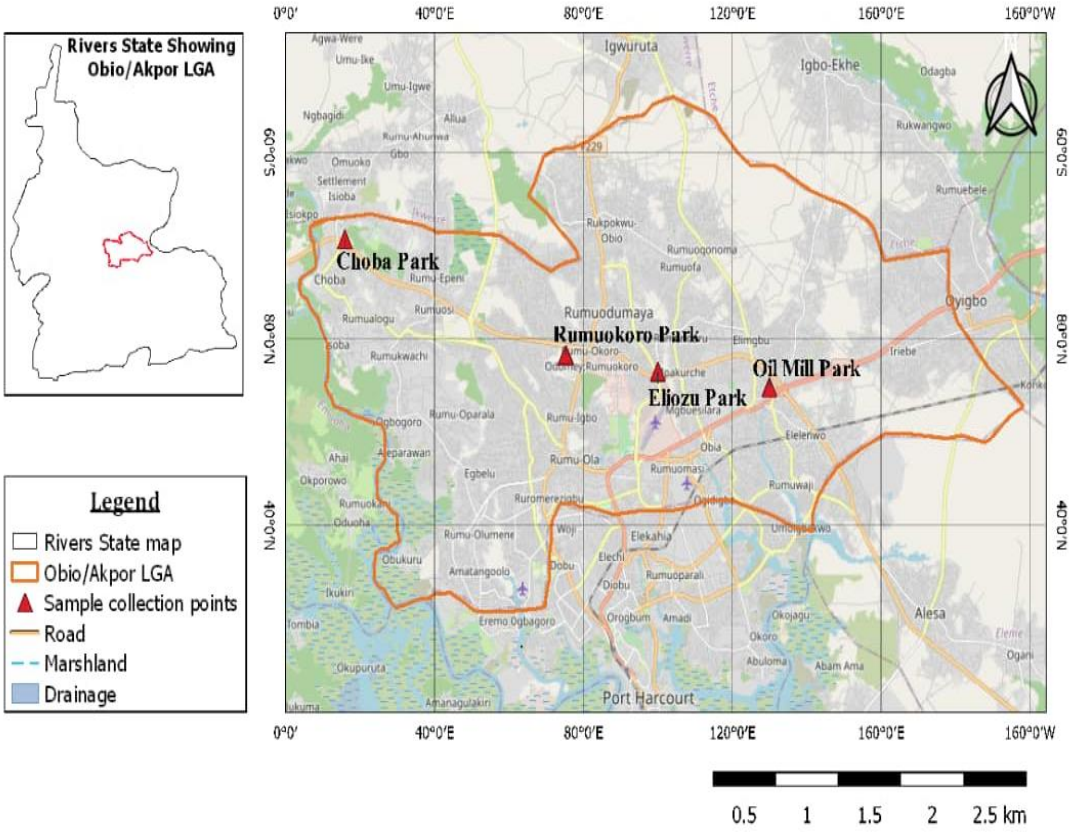

Figure 1: Sample collection points in Obio/Akpor

Sample collection
Forty-eight (48) samples, comprising 12 each of ready-to-eat foods [vegetable salad (S), African salad (A), fried fish (F) and meat pie (M)] were randomly purchased from both mobile and stationary food vendors in each study location. Sampling was done weekly for three months. The samples were wrapped in sterile Ziploc bags, labeled appropriately and transported to the laboratory within 2 hours of purchase for analysis.

Sample preparation and enumeration for bacteria species
Twenty-five grams (25g) of each food sample was aseptically weighed and transferred to 225ml of sterile peptone water (Biolab, South Africa) in a stomacher bag and macerated to homogenize (Nina Japan) for 1-2 min. Thereafter, 10-fold serial dilution was carried out on the stock up to 10^5 as described by Makele *et al.* (2015). Aliquots (0.1ml) of appropriate dilutions were spread on sterile Nutrient agar, Eosin methylene blue (EMB) agar and Mannitol salt agar (MSA) (TM, Rajasthan, India) aseptically in duplicates. The inoculated Petri plates were inverted and incubated at 37ºC for 24-48 h in an incubator. Distinct colonies on nutrient agar representing the total heterotrophic bacteria were counted and recorded as coliform forming units (cfu). Colonies with green metallic sheen on EMB agar was counted and recorded as presumptive *Escherichia species* while a change in MSA colour from red to yellow (mannitol fermentation) indicated presumptive *Staphylococcus species* colonies. Purified isolates were confirmed on the bases of cultural morphology, physiological and biochemical characteristics (Cheesebrough, 2006; Lancette and Bernett, 2001).

Determination of the presence of virulence genes
Deoxyribonucleic acid (DNA) Extraction
The DNA of the isolates were extracted using the boiling method. A loopful of each isolate was suspended into 100µl of sterile distilled water in an Eppendorf tube and centrifuged (Dragon Lab Microcentrifuge M- D3024, USA) for 3 min at 10,000g. The supernatant was discarded and the harvested cells vortexed (vortexer VWR G-560, Scientific industries, USA); re-centrifuge in normal saline and supernatant discarded. DNA elution buffer (500µl) was added. The suspension was heated in a water bath (HH-1 numerical show constant temperature water bathing boiler) for 10 min at 90º C and was fast cooled on ice at -20ºC for 5 min. The cells were centrifuged at 10,000 g for 5 min. The supernatant was transferred into fresh Eppendorf tubes as described by Ngoka *et al.* (2021).

Amplification of virulence genes
The DNA was subjected to the following cocktail mix and condition for the PCR at a final volume of 25 µL for 35 cycles. 2.5µL of 10x PCR buffer, 1.0µL 5pMol forward primer, 1.0µL of 5pMol reverse primer (Table 1), 1.0µLof 25Mm $Mgcl_2$, 1.0µL of DMSO, 2.0µL of 2.5Mm DNTPs, 0.1µL of Taq 5µ/µL, 3.0µL of 10ng/µL DNA and 13.4µL of water were mixed together in an Eppendorf tube. The touch down PCR protocol used for the primers involve initial denaturation at 95°C for 5 min.; denaturation at 94°C for 15 s; annealing at 65°C for 20 s; extension at 72°C for 30 s for 9 cycles and denaturation at 94ºC for 15 s, annealing at 55ºC for 20 s and extension for 30 s at 72ºC for the 35th cycle then final extension at 72°C for 7 min in the MyGene series palter thermal cycler Model MG96G as described by Bisi-Johnson *et al.* (2011).

Table 1: Primer sequences for target virulence genes in isolated *Staphylococcus aureus* (Kamarehei et al., 2013) and *Escherichia coli* (Bisi-Johnson et al., 2011)

Target genes	Primer Nucleotide Sequences (5'- 3')	Amplicon sizes (bp)
sea	TGCAGGGAACAGCTTTAGGC GTGTACCACCCGCACATTGA	250bp
seb	ATTCTATTAAGGACACTAAGTTAGGG ATCCCGTTTCATAAGGCAAACTTG	400bp
aggR	GTATACACAAAAGAAGGAAGC ACAGAATCGTCAGCATCAGC	254bp
eaeA	ATGCTTAGTGCTGGTTTAGG	248bp
astA	GCCTTCATCATTTCGCTTTC GCCATCAACACAGTATATCC GAGTGACGGCTTTGTAGTCC	106bp

Gel electrophoresis
The DNA products of PCR were assessed using gel electrophoresis on a portable gel hood built in Blue LED (470nm); using 1.5% agarose gel at a constant voltage of 120V and 1X TBE for approximately 1 h. They were visualized by Ethidium bromide staining and photographed under ultraviolet transilluminator (CL 3120 Brevity desktop Analysis) for each target gene. The ladder used is 1kb base pair ladder from thermo scientific.

Antibiotics susceptibility patterns
Antibiotics sensitivity test was performed using the standard disc diffusion method by Kirby-Bauer as described by Cheesebrough (2006). The test isolate was introduced into 5ml of sterile distilled water and its turbidity was standardized to 0.5 McFarland standard. Aliquot (0.1 ml) was dispensed on a pre-solidified Mueller Hinton agar. The antibiotic disc was aseptically place on the seeded plates and incubated at 29±2°C for 24 h. The zones of inhibition were recorded in millimetre (mm) and classified as resistant or sensitive based on the interpretative chart of Clinical laboratory standard Institute (CLSI) performance standards for antimicrobial disk susceptibility tests (CLSI, 2015).

Data analysis
Statistical Package for Social Sciences (SPSS) version 20 for the Analysis of variance (ANOVA), post hoc test was employed to obtain significant $p< 0.05$ and Descriptive statistics were computed using Cross tabulation to obtain percentages.

RESULTS AND DISCUSSION

The total heterotrophic bacteria count

The result for the total heterotrophic bacteria count showed that there was a statistically significant difference in the mean total heterotrophic bacteria count for African salad (8.00 ± 0.54) and fried fish (6.81 ± 0.26) from Choba at P<0.05 with African salad having the highest mean 8.00±0.54 and meat pie having the lowest mean of 4.97±0.35. There was also a significant difference in mean total heterotrophic bacteria count for samples from Rumuokoro with Fried fish having the highest mean (7.96±0.31) while Meat pie had the lowest mean (6.05±0.66). Samples from Eliozu were significantly different with African salad having the highest mean (7.82±0.56) while vegetable salad had the lowest mean (6.08±0.13). There was no significant difference in samples from Oil Mill (Table 2).

Table 2: Mean total heterotrophic bacteria count (log_{10} cfu/g)

Sample	Choba	Rumuokoro	Eliozu	Oil Mill
African salad	8.00 ± 0.54^{a}	7.70 ± 0.29^{a}	7.82 ± 0.56^{a}	6.51 ± 0.61^{ab}
Meat pie	4.97 ± 0.35^{c}	6.05 ± 0.66^{b}	6.60 ± 0.32^{b}	6.93 ± 0.10^{a}
Fried fish	6.81 ± 0.26^{b}	7.96 ± 0.31^{a}	6.42 ± 0.49^{b}	6.69 ± 0.33^{ab}
Vegetable salad	6.16 ± 0.13^{b}	6.70 ± 0.56^{a}	6.08 ± 0.13^{a}	6.17 ± 0.13^{a}

Mean with the same letters in the same location are not significantly different at p <0.05

Means were compared across columns and means with the same superscripts are equal. Mean counts ± SE (standard error).

Percentage occurrence of *E. coli* and *S. aureus* in samples

The result obtained from the use of selective media showed that out of the forty-eight samples examined, 10 (20.83%) samples were positive for *Staphylococcus aureus* while 14 (29.17%) food samples were positive for *Escherichia coli* (Fig. 2).

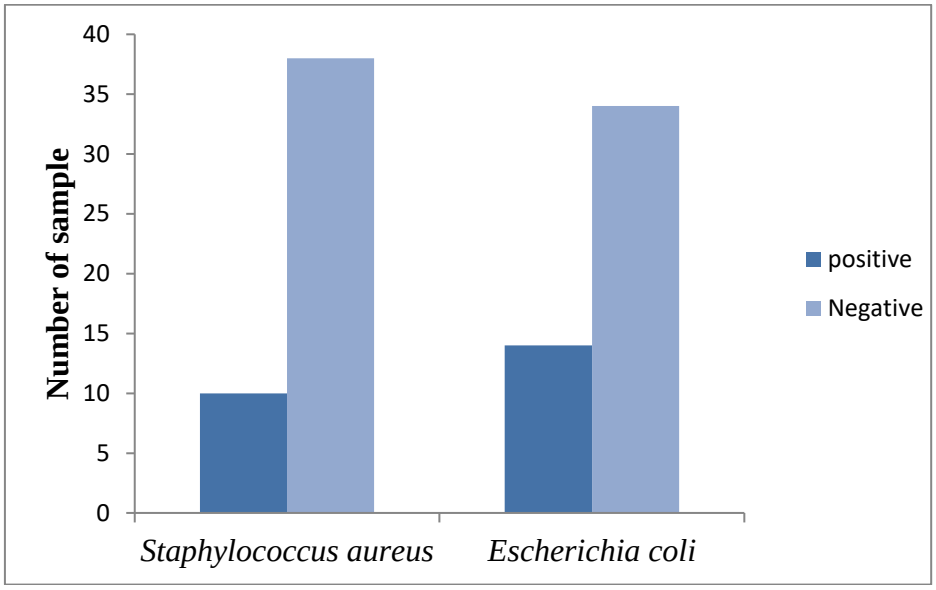

Figure 2: Percentage positive and negative of *E. coli* and *S. aureus*.

Occurrence of *Escherichia coli* and *Staphylococcus aureus* in the examined samples

The result showed that *E. coli* had the occurrence of 5 (35.71%) at Oil mill and Rumuokoro while Eliozu and Choba had 2 (14.28%) occurrences (Fig 3). The highest occurrence of *E. coli* was observed in African salad which had 6 (42.86%), followed by vegetable salad with 5 (35.71%), fried fish had a prevalence of 2 (14.28%). The lowest occurrence was in meat pie with 1 (7.14%) isolate (Fig 4).

The results for the occurrence of *Staphylococcus aureus* in examined samples based on location showed that Choba had the highest occurrence of 4 (40%) while each of Rumuokoro, Eliozu and Oil Mill had 2 (20%) occurrences (Fig 5). African salad had 4 (40%) occurrences followed by vegetable salad with 3 (30%), 20% (2) occurrence was noticed in fried fish samples while meat pie had 1 (10 %) occurrence (Fig 6).

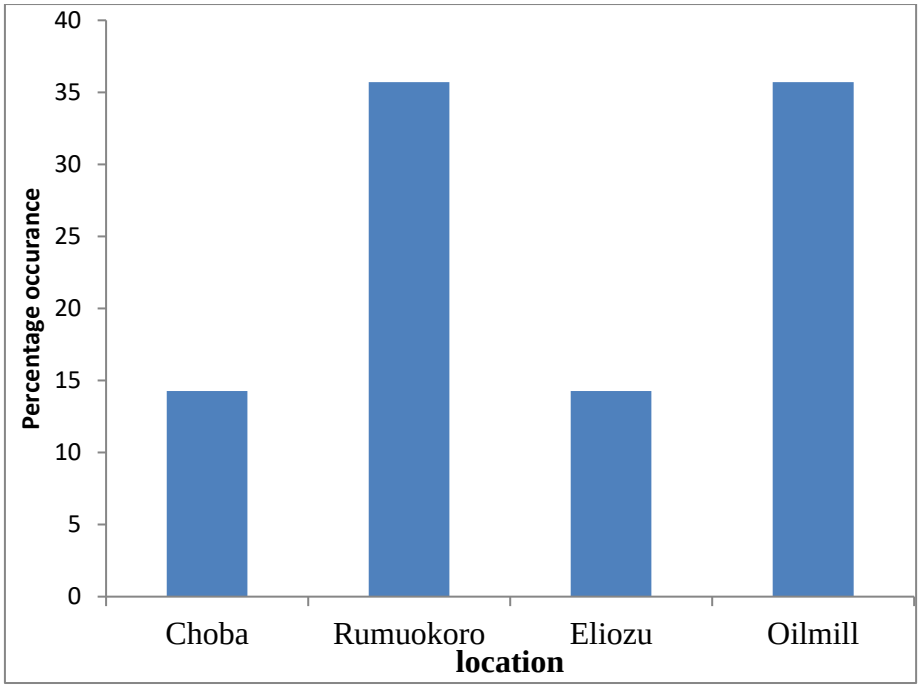

Figure 3: Percentage occurrence of *E. coli* based on location

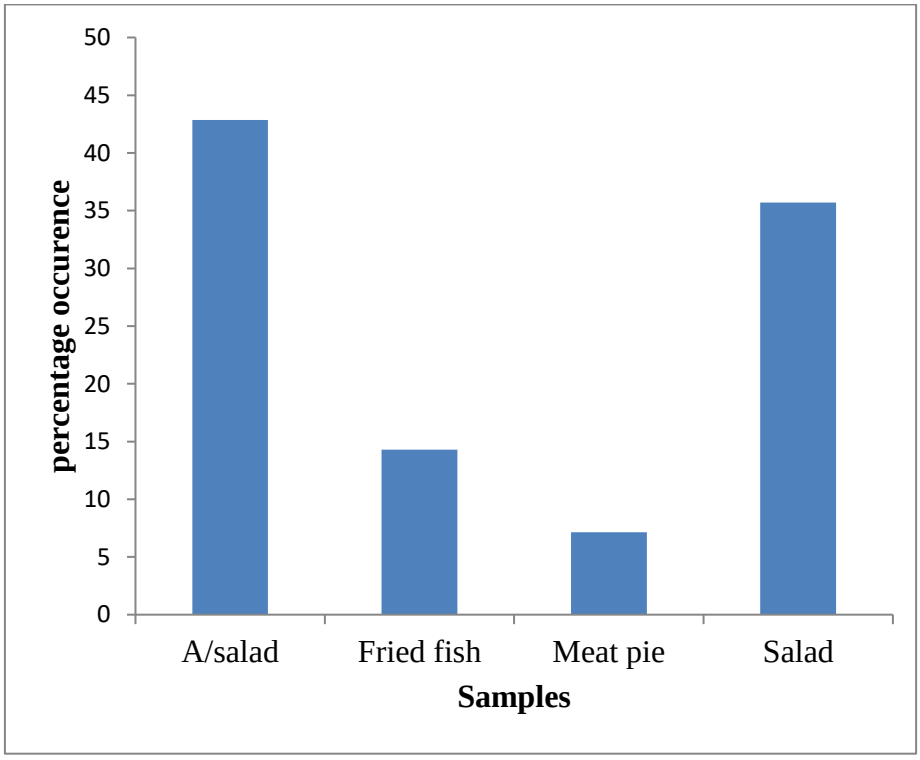

Figure 4: Percentage occurrence of *E. coli* in the various food samples

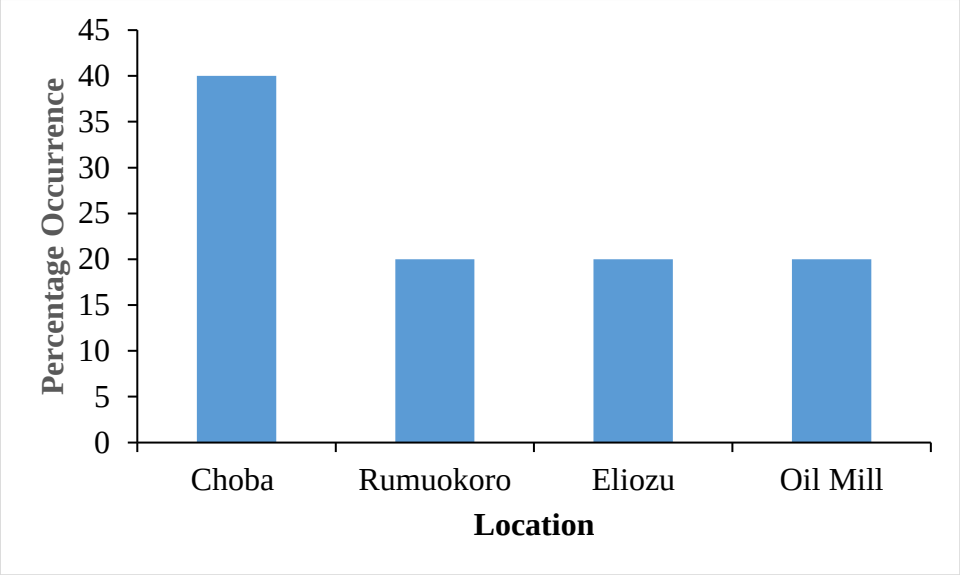

Figure 5: Percentage occurrence of *staphylococcus aureus* based on location

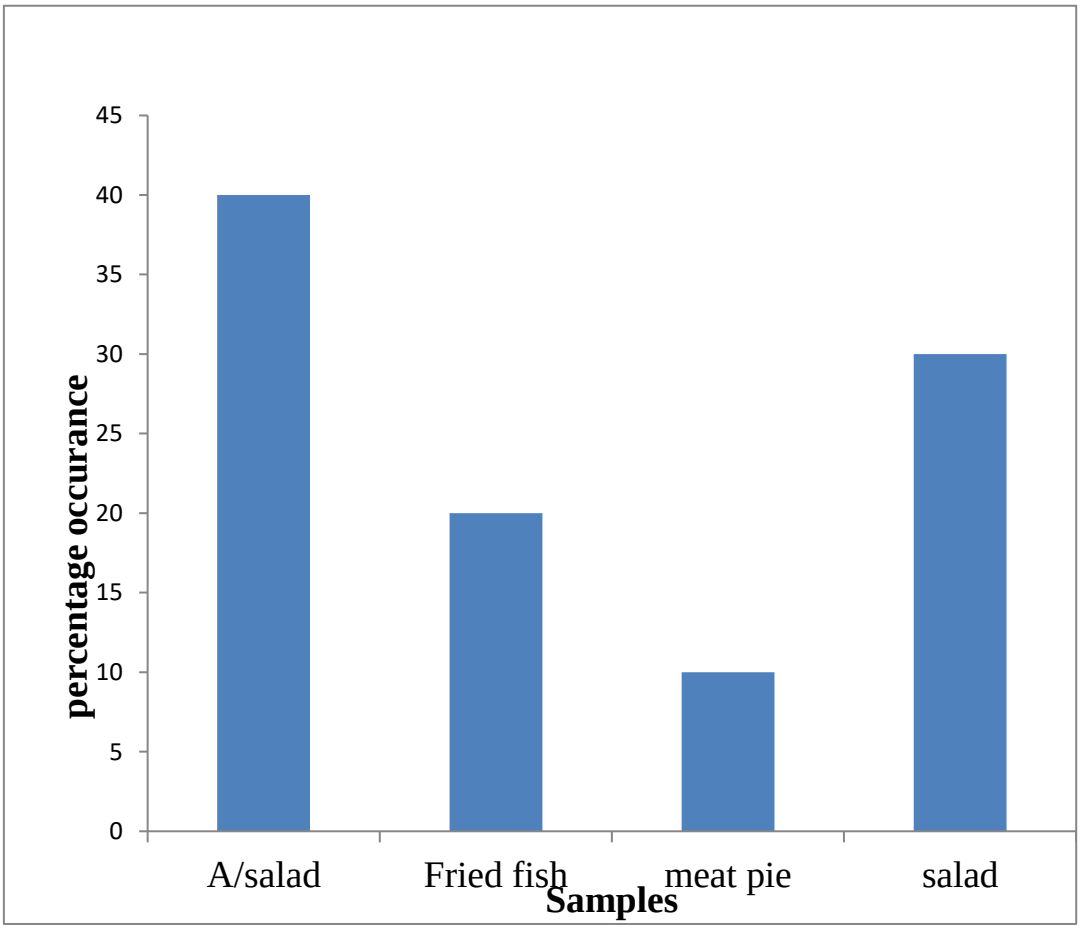

Figure 6: Percentage Occurrence of *S. aureus* in various food samples

Antibiotic sensitivity test for *Escherichia coli* and *Staphylococcus aureus*

The result of the antibiotic sensitivity tests for *Escherichia coli* is presented in Figure 7. The result showed that *E. coli* showed 100% resistance to augmentin followed by cefixime with 50% resistance, ceftazidime (42.86%), gentamycin and cefuroxime had 14.29% resistance while nitrofuraton, ciprofloxacin and ofloxacin each had 7.14% resistance.

The result for the antibiotic sensitivity test for *Staphylococcus aureus* is presented in Figure 8. The result showed that gentamycin, erythromycin and ofloxacin were the most sensitive agents for *Staphylococcus aureus* with erythromycin showing only 10.0% resistances, ceftriazone showed 90.0% resistance while augmentin, cefuroxime, ceftazidine and cloxacillin had 100.0% resistance.

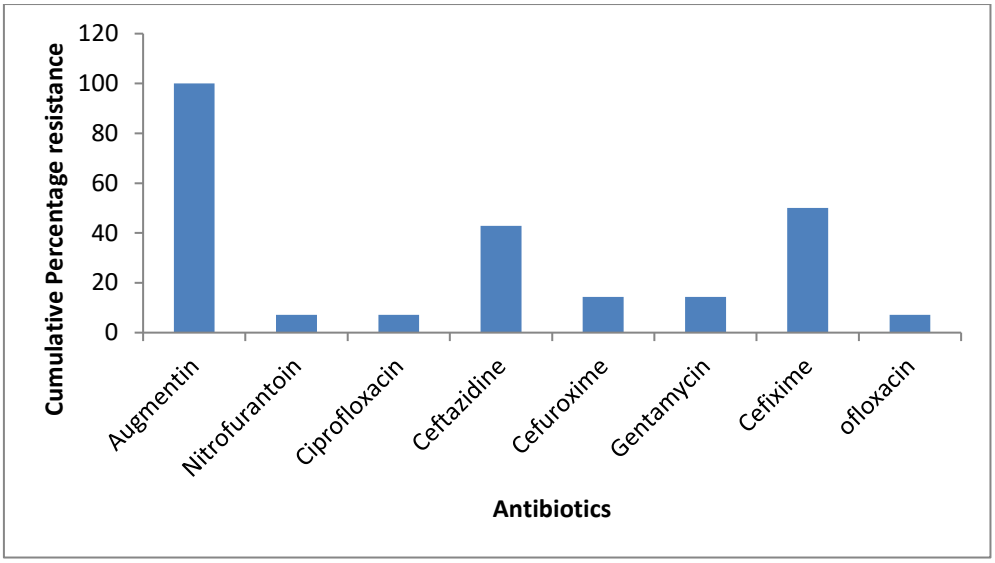

Figure 7: Cumulative percentage antibiotics resistance of the isolated *E. coli.*

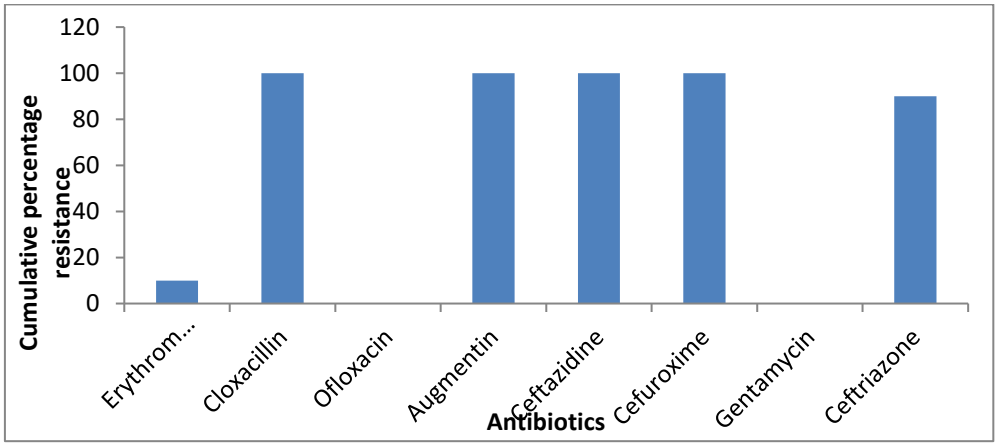

Figure 8: Cumulative percentage antibiotics resistance of isolated *S. aureus.*

Prevalence of virulence genes

The result of the PCR for detection of virulence genes in *S. aureus* showed that none of the amplified genes were presence in any of the *S. aureus.*

The result of the PCR for detection of virulence genes in *E. coli* showed the presence of all three tested genes. The agarose gel electrophoresis of the amplified *astA* genes obtained from subjecting the isolated *E. coli DNA* to PCR using *astA* primers is shown in Plate 1.

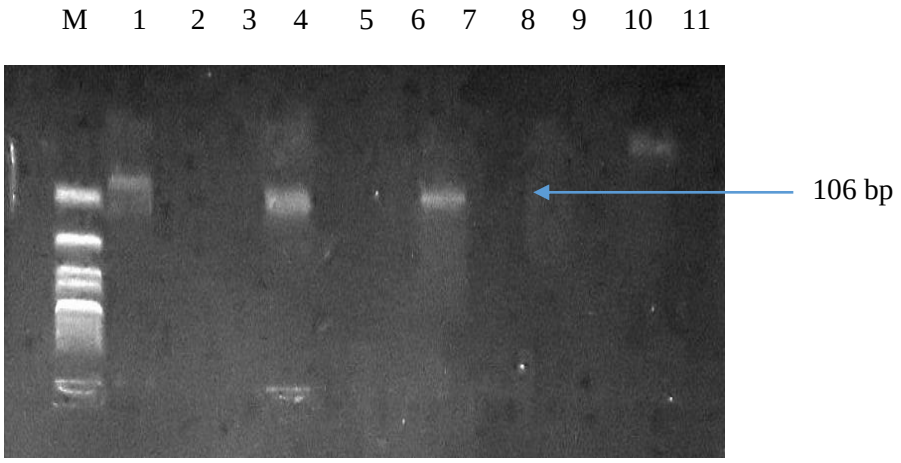

Plate 1: Amplified ***astA*** genes from the isolated *E. coli*.

Discussion

The fact that microorganisms are everywhere cannot be overemphasized except when they occur in unusual numbers that are capable of causing harm if consumed. Foodborne diseases mediated by potentially pathogenic microorganism is the main public health problem related to ready-to- eat vended foods sold daily in most parts of the globe.

The results from this study revealed that all foods sampled were contaminated with microorganisms. The mean total heterotrophic bacteria count for African salad ranged from 6.03 to 8.39 $\log_{10}$CFU/g, meat pie had the range of 4.60 to 7.03 $\log_{10}$CFU/g, Fried fish had the range 6.04 to 8.21 $\log_{10}$CFU/g while vegetable salad had the range 5.96 to 7.05 $\log_{10}$ CFU/g. The result showed that most of the samples' mean total heterotrophic bacteria count was at the borderline level (5.0 to 7.0 $\log_{10}$CFU/g) according to the International Commission on Microbiological Specification for Foods category 5. This is in agreement with reports by Amadi and Nwankwo (2021) and Salamandane *et al.* (2021).

It has been revealed that the prime indicators of microbial activity in food include the type of ingredients used for the preparation of the food, hygiene level as well as time and temperature of holding. Higher microbial counts were reported for African salad when fermented food ingredients such as *Pentaclethra macrophylla* (ugba) and *Ricinus communis* (ogiri) were incorporated into the African salad (Nwamaka *et al.,* 2010). Indigenous foods that are usually served cold, be it snack or staple food are often contaminated with higher microbial count due to preparation methods, handling, preservation and storage as the temperature allows microbial proliferation with no further heating before serving. The use of water obtained from sources that are untreated in food preparation and production process predisposes the resultant food to high microbial load (Muinde and Kuria, 2005). The vegetables used for vegetable salad and African salad production could be hazardous to the health of consumers of street food if not wash at all, poorly washed or washed with contaminated water as these may have come in contact with soil or contaminants from other possible sources. High microbial count could also be due to the unhygienic conditions where food preparation takes place as most of the food vendors prepare their food in open space; some of which are close to areas where waste is deposited. It is pertinent then for street food vendors to adhere to the principles of Good Manufacturing Practices (GMP) in order to produce foods that are safe as well as provide required nutrient. Aluko *et al.* (2014) reported that a several of street-food vendors lack basic knowledge about the principle of GMP due to lack of knowledge on basic food hygiene. Campos *et al.* (2015) also reported that food-handlers' hygienic status is contributing to the poor microbiological quality and safety of the street foods examined in Porto, Portugal.

The isolation of *E. coli* and *S. aureus* from the vended food sampled is consistent with those found in indigenous ready-to-eat foods and those from other countries as reported by Ngoka *et al.* (2020), Nkere *et al.* (2011) and Clarence *et al.* (2008) who reported high contamination level for *S. aureus* and *E. coli. Escherichia coli* count ranged from 2.85 to 5.31 $\log_{10}$CFU/g. The result of this study disagrees with *E. coli* count of 7.51 $\log_{10}$CFU/g reported by Ologhobo *et al.* (2010) in spiced beef which was attributed to deplorable state of hygiene employed in the processing and packaging of the food. The level of exposure of these ready-to-eat food to dirty surroundings around the motor parks is also a factor (Oje *et al.,* 2018). The presence of *E. coli* in the food sample is an indication of faecal contamination of water used in the food preparation process and the raw materials.

Total staphylococcal count ranged from 4.89 to 7.01 $\log_{10}$CFU/g. *Staphylococcus aureus* is a normal flora of human and may have been introduced through the vendors since the materials used for packing food are usually opened by hand squeezing or blowing air into it with the mouth. *Staphylococcus aureus* is an opportunistic pathogen and possess enterotoxigenic strains known for its ability to inflict serious food borne disease (Balaban and Rasooly, 2000).

The antibiotic sensitivity test for *E. coli* showed a 100% resistance against augmentin, followed by cefixime with 50% resistance, ceftazidime 42.86%, gentamycin and cefixime had 14.29% resistance while nitrofurantoin, ciprofloxacin and ofloxacin had 7.14% resistance. Antibiotic sensitivity test for *S. aureus* showed that the most active antimicrobial agent for which the *S. aureus* strains were sensitive are erythromycin, ofloxacin and gentamycin. These can be administered for treatment of *Staphylococcus* mediated illness. The *S. aureus* strains were 100% resistant against augmentin, cloxacin and ceftadizine This is in agreement with previous study by Ngoka *et al*. (2021). Other studies have reported higher resistance to gentamycin and ofloxacin by different microorganisms (Afolabi *et al.,* 2017). Higher degree of resistance for erythromycin (87.5%) was also reported by Kumar *et al*. (2009) while lower resistance of 0.83% was reported by Achi and Madubuike (2007) which is comparable to that obtained from this study. The result of the antibiotic sensitivity of this study disagrees with the report by Agbo et al. (2016) that *S. aureus* strains isolated from street ready-to-eat foods in Calabar had varying resistance to gentamycin (10%), ciprofloxacin (20%) and levofloxacin (5%).

The rate at which strains of *S. aureus* and *E. coli* isolated from food samples are resistant to certain antimicrobial agents and their overall occurrence in food poses an issue of public health concern as the trend may heighten; thus, exposing consumers of street food to the danger of antibiotic resistance associated with food borne pathogens. Antibiotic resistance has been attributed to gene transfer that yields mutant strains that may contaminate raw materials that are consumed from contaminated foods (Mirzaei *et al*., 2012). Sasidharan *et al*. (2011) opined that careless use of antibiotics by man on the farm has been implicated as possible cause of resistance to antimicrobial agent in food borne pathogens.

The presence of antibiotic resistant strains of *E. coli* and *S. aureus* in the ready-to-eat food samples poses serious public health concerns compounded with the detection of the presence of virulence genes. The polymerase chain reaction results for detection of the virulence genes *sea* and *seb* in *S. aureus* showed that all *S. aureus* strains did not possess both virulence genes. The result from this study is in contradiction to the finding of Ngoka *et al*. (2021) who reported presence of *sea* and *seb* genes in *S. aureus* strains isolated from ready-to-eat *Rhynchophorus phoenicis* and *Archachatina marginata* vended along the Port Harcourt-Bayelsa route. The PCR result for amplification of the *aggR*, *eaeA* and *astA* virulence genes showed that *aggR* had the highest prevalence of 4 (28.58%) positives, followed by *astA*, 3 (21.44%) while *eaeA* had the lowest of 14.29%. This is in agreement with reports by Bii *et al*. (2005), that *aggR* and *astA* genes were the most frequently detected among the members of the EAggEC and ETEC isolates in multidrug-resistant diarrhoeagenic *E. coli* isolates from Kenya and Japan. However, Gonzalez-Escalona, and Kase (2019) reported *eaeA* showing 25% occurrence in their study of phylogeny of Shiga toxin-positive *E. coli* strains isolated from FDA regulated foods during 2010-2017 while Carvalho *et al.* (2016) reported zero occurrence for *eaeA* and *aggR* in their studies of Mangrove

Crabs (*Ucides cordatus*) from Guanabara Bay, Rio de Janeiro, Brazil.

CONCLUSION

The study revealed that strains of *E. coli* and *S. aureus* occurred in the four vended foods in motor parks in Obio-Akpor Local Government Area. The Polymerase chain reaction protocol for detection of virulence genes in *Escherichia coli* was positive for the *astA*, *eaeA* and *aggR* virulence genes. The major source of food borne disease in Nigeria is the ingestion of food contaminated by bacteria pathogens. The occurrence of the two potentially pathogenic microbes in a ready-to-eat food samples and the detection of virulence gene bearing *E. coli* and antibiotic resistant strains of *E. coli* and *S. aureus* portends danger for commuters who patronizes these foods, hence the need for urgent public health interventions.

ACKNOWLEDGEMENTS

The authors appreciate the assistance of the Technical Staff of Emadavistic Laboratory and Prof. B. O. Agaviezor.

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