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INCIDENCE OF *Staphylococcus aureus* IN STREET VENDED FOOD SOLD IN CALABAR MUNICIPALITY, NIGERIA

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ABSTRACT

Street vended foods are the major source of food borne diseases in most parts of sub-Saharan Africa including Nigeria. In this study, six samples each of six different vended foods obtained in Calabar municipality were evaluated for bacteriological quality and presence of *Staphylococcus aureus*using standard microbiological techniques. Each food was examined macroscopically for evidence of spoilage, followed by culture isolation for *Staphylococcus aureus.* The organisms isolated were subjected to antibiotic assay test using the disc diffusion techniques. The mean bacterial count for fried yam was 7.4 x 10^4cfu/ml while that of fried fish was 7.4 x 10^4cfu/ml. Moi-moi had a mean count of 1.0 x 10^4cfu/ml, suya 9.3 x 10^4cfu/ml, meat pie 6.4 x 10^4cfu/ml and fried plantain 7.6 x 10^4cfu/ml respectively. The highest mean count was recorded at Akai-effa and IkotIshie. Out of the 36 food samples analyzed, 25(64.4%) showed growth on nutrient agar, 20(55.6%) showed growth on Staphylococcus medium 110 and was confirmed as *Staphylococcus aureus* through several biochemical tests. Distribution of *Staphylococcusaureus* isolates with respect to food type analysed showed that out of the six (6) samples from each of the food type collected and examined 3(50.0%) of fried yam, 1(16.0%) of fried fish, 5(83.3%) of moi-moi, 4(66.0%) of suya 5(83.3%) of meat pie and 2(33.3%) of fried plantain were contaminated by *Staphylococcus aureus.* The distribution of *Staphylococcus aureus* isolates in food with respect to location showed that out of the six locations examined, Eta-Agbo road and Akim market had 2(33.3%), Akai-effa and IkotIshie had 5(83.3%) while Marian and Effio-ette had 3(50.0%) contaminated with *Staphylococcus aureus*. Result of the antibiotic susceptibility revealed that the isolated *Staphylococcus aureus* was highly resistant to Norfloxacin (50.0%), Ampiclox (45.0%), Erythromycin (40.0%), Amoxil (35.0%) resistance was least to ciprofloxacin (20.0%), Gentamycin (10.0%) and Levofloxacin (5.0%). Contamination of these food samples is an indication of poor personal hygiene and poor sanitation among food handlers. To prevent outbreak of food poisoning and possible spread of antibiotic resistant *Staphylococcus aureus*, healthcareand other institutions should enforce proper handling of foods in the Canaan city-Calabar municipality, Nigeria.

Key words: Ready-to-eat foods,vended foods, suya, food poisoning, moi-moi.

1. INTRODUCTION

The level of poverty in rural and urban communities coupled with demographic expansion as a result of urbanizationin a lot of developing countries like Nigeria have brought about the springing up of a novel and most abundant but crude form of restaurants known as “Street Vended Foods” (Omemu and Adeoju, 2008; Sina *et al.,* 2011).Street vended foods are ready-to-eat foods or finger foods prepared and sold by vendors on street, similar public places and parks (Mosupye *et al.,* 2002). It provides readily available, inexpensive meals, which might be more nutritional than the ones in the main restaurants as well as provide income for the vendors. Food vendors are group of people that sells ready-to-eat food (finger foods) or drink in a street, other public places or parks such as a market or fair (Artemis & Ramesh 2011). The type of street vended foods differs greatly between countries and cultures. Street food industry however, plays a very vital role in meeting food requirement of commuters and urban dwellers in many cities and towns mostly in developing countries as it feeds thousands of people daily with a large range of foods that are relatively cheap and easily accessible (Tambeker *et al.,* 2008*)*.According to Ghosh *et al.*(2007) and Nyenje *et al.,* (2012), street sold foods are appreciable for their convenience and unique flavors. They also assure food availability for low income urban population and livelihood for a significant proportion of the population in many developing countries (Nyenje *et al.,* 2012).

Despite the advantage that street vended food provides in terms of inexpensiveness and convenience, concerns for the quality and safety

of such vended foods has been raised (Mosupye *et al.,* 2002). This is owing to the fact that most street food vendors lackadequate understanding of food safety regulations. The hygienicof street vended foods is a major source of concern and according to Mensah *et al.* (2002) a relationshipbetween streets vended food and illnesses have been established.

Additionally, the unhygienic and unsafe conditions in which these foods are prepared, stored and served raises another important question regarding their microbiological safetyand quality.Food borne illnesses that arises from microbial infection or intoxication of food are a major health problem associated with the consumption of street vended foods (Mensah *et al*., 1997; Kaneko *et al*., 1999; Mensah *et al.,* 2002; Feglo and Sakyl, 2012; Nyenye *et al.,* 2012). *Staphylococcal* food poisoning is caused by an infection with *Staphylococcus aureus* bacterium (Case, 2004). This bacterium *Staphylococcus aureus,* which can be carried by food causes food poisoning and other food-borne diseases (Foskett *et al.,* 2003).*Staphylococcal* food poisoning is an illness caused by a toxin or poison released by bacteria from the *staphylococcus* group (Lennox *et al*., 2012). It is a food borne intoxication that develops in people who ingest food that has been improperly stored or cooked (particularly food such as salad,ice cream,ham, processed meats, chicken, pastries, and hollandaise sauce) in which *Staphylococcus aureus* has grown (Prescott *et al.,* 2008).

The Staphylococci are spherical cells, usually arranged in grape like irregular clusters. They are gram-positive and grow readily on many types of media and are active metabolically, fermenting carbohydrates and producing pigments that vary from white to deep yellow. Some are members of the normal flora of themucous membranesand skin of humans; others causes abscess formation, suppuration, a variety of pyogenic infections and even fatal septicemia. The genus *Staphylococcus* has at least 40 species. The three most frequently encountered species of clinical importance are *Staphylococcus aureus, staphylococcus epidermis* and *Staphylococcus saprophyticus* (Brooks *et al.,* 2010). *Staphylococcus aureus* is the most predominantly virulent bacteria responsible for a wide range of human diseases (Lopez *et al.*, 1993; Sina *et al.,* 2011), its strains can be pathogenic and relatively nonpathogenic.

Food safety is a public health concern, most importantly the food served to students and pupils at schools by vendors.Children in the African region usually experience five episode of diarrhea per year and about 800,000 children die each year from diarrhea and dehydration (Mead *et al.,* 1999; Esena & Owusu, 2013). Diarrhea remains the third cause of death among children under age five accounting for 10,000 death annually (Mwangi, 2002; Esena and Owusu, 2013). Although international agencies and the government of Nigeria are making efforts to improve the safety of the food supply, the occurrence of food borne disease remains a significant health issue in both developed and developing countries (Nyenje *et al.,* 2012).

Consumers who depend on such food are more interested in its convenience but pays little or no attention to its safety, quality and hygiene (Mensah *et al.,* 2002; Barron *et al.,* 2006; Tambekar *et al.,* 2008). Ingesting such foods brings about food infection. Symptoms typically begin several hours to several days after consumption of infected food such as abdominal pain, gastroenteritis, nausea, vomiting, fatigue, diarrhea, headache or fever, which also depend on the agent involved.

In most cases, the body is able to permanently recover after a short period of acute discomfort and illness. However, food borne illness can result in permanent health problem or even death, especially for people at high risk, including babies, young children, pregnant women, elderly people, sick people and others with weak immune system (Lennox *et al.,* 2012). Therefore the fact cannot be over emphasized that ready-to-eat food sold by vendors in our environment might pose a direct health risk if appropriate measures are not taken to sensitize and educate them on the health implications. The obvious is that they cannot be totally eliminated from the chain. Therefore, there is need to study the strain, distribution and pathogenicity of presumptive food pathogens

and the relationship between their occurrence and the hygiene practices in Calabar Municipality, Nigeria.

2. MATERIALS AND METHODS

Aseptically samples of ready-to-eat food, which included fried yam, fried fish, moi-moi, fried plantain, suya and meat pie were collected from six different locations within Calabar Municipality, Nigeria. These locations included: Eta Agbo road, Akim market, Akai-Efa, Effio-ette junction, Ikot-ishie market and Marian market respectively in Calabar Municipality which lies between latitude $4^{0}15$' Nand longitude $8^{0}25$' E. At the north, the Municipal city is bounded by Odukpani Local Government Area, East by the great Qua River. Its southern shores are bounded by the Calabar River and Calabar south local government area. It has an area of 332, 551 square kilometers and a population of 179, 392 at the 2006 census (Ekpo, 2005)

Each food sample was wrapped in aluminum foil and put in a sterile polythene bag to prevent contamination. They were then transported to the laboratory within one hour for microbiological analysis.

2.1 Sample Processing

Ten grams of each food sample was weighed out and homogenized into 90ml of sterile distilled deionized water using a sterile blender (Nana *et al.,* 2013; Abubakari *et al.,* 2015). 1ml of each sample (aliquot) was then transferred to 9ml of sterile distilled water contained in the test tube (diluents) and then 10-fold serial dilution was carried out. 1ml of the dilution was plated in Petri-dish (in triplicate) according to the method of Mboto *et al.,* 2012.

2.2 Enumeration and Identification:

Pour plate technique was used for plating the samples, 1ml of the sample was poured into petri-dish (in triplicate) and the media used was Staphylococcus medium No 110 for Staphylococcal isolates & Nutrient agar (for general bacterial count) was poured into the plate for each sample and then mixed well and allow to solidify, then incubated at 37^{o}C for 24-48 hours (Muhammad *et al.,* 2013).

- After incubation, the colonies were observed and then counted and recorded, and then identification of pure culture from each sample was done as described by Cheesbrough, (2002) and Muhammad *et al.,* (2013).

- Golden yellow colonies were characterized as presumptive of *Staphylococcus aureus*then confirmation was done as described by Ghosh *et al.,* 2007.

- The colonies were streaked on blood agar to check its hemolytic property, zone of clearance was classified as β-hemolysis which is always presumptive of *Staphylococcus aureus* (El-jakee *et al.,* 2008; Brooks *et al.,* 2010; Ikpoh *et al.,* 2013).

2.3 Antibiotics Susceptibility Testing: The susceptibility of *S. aureus* isolates to various conventional antibiotics was determined using Mueller Hinton agar while employing the disk diffusion method of Agbo and Mboto, (2012).The antibiotics used included: Amoxil (*AMX*), Chloramphenicol (*CHL*), Ciprofloxacin (*CPX*), Erythromycin (*ERY*), Gentamycin (*GEN*), Norfloxacin (*NFX*), Rifampicin (*RFP*), Streptomycin (*STR*), Ampiclox (*APX*) and Levofloxacin (*LEV*).

The commercial antibiotics disks were introduced on an agar plates previously seeded with 18hrs broth culture of the test organism. The plates were incubated at 37^{o}C for 24hrs. The inhibition zones were measured in millimeters and data collated for analysis (Mboto *et al.,* 2009; Bello *et al.,* 2013).

Data obtained from the aerobic plate count and measurement of zones of inhibition of each antibiotics on isolates from each food sample was expressed as mean of duplicates and as percentages.

3. RESULTS

A total of 36 samples were analyzed out of the 36 samples, 25(64.4%) showed growth on nutrient agar, 20(55.6%) showed growth on staphylococcus medium 110 and thus was confirmed to be *Staphylococcus aureus* through several biochemical tests such as motility, indole, oxidase, carbohydrate fermentation and

Gram's reaction. Other plates displayed no growth.

3.1 Biochemical characterization of the bacteria isolates

After purification and sub-culturing, the bacterial isolates were further identified using the method described by Cheesbrough, 2002. The isolates were identified using the keys provided in Holt *et al.,* 1994.The isolates obtained were *Staphylococcus aureus*, *Escherichia coli, Salmonella spp., Klebsiella spp., Pseudomonas spp.*, and *Shigella spp*.

3.2 Bacterial Enumeration

The bacteria count gotten from the various food samples analyzed comprised of the mean bacteria count (cfu/ml), the minimum and maximum bacteria count (cfu/ml) for each of the samples. The mean, minimum and maximum bacteria count gotten from fried yam was $1.0x10^4$, $3.0x10^3$, $2.4x10^4$(cfu/ml), the count from fried fish was $7.4x10^4$, $2.1x10^4$, $1.6x10^5$(cfu/ml), the count obtained from moi-moi was $1.0x10^4$, $3.2x10^3$, $2.2x10^4$(cfu/ml), the count obtained from suya was $9.3x10^4$, $2.8x10^4$, $2.0x10^5$(cfu/ml), the count obtained from meat pie was $6.4x10^4$, $2.7x10^3$, $2.4x10^5$(cfu/ml) and the count obtained from fried plantain was $7.6x10^4$, $3.3x10^4$, $1.2x10^5$. This information is represented in the table 1 below.

3.3 Distribution of *Staphylococcus aureus* with respect to food type

The number of samples contaminated with *Staphylococcus aureus* and their percentage prevalence was indicated in which moi-moi and meat pie are the most contaminated with a percentage of 83.33% followed by suya with a percentage of 66.6%, fried yam with a percentage of 50%, fried plantain with a percentage of 33.3%, fried fish had the least contamination with a percentage of 16.6%.The information is illustrated in the Figure 1 below.

3.3 Distribution of *Staphylococcus aureus* Isolates with Respect to Location

The contamination of samples according to location were also recorded. Samples gotten from Akai-Effa and Ikot-Ishie showed the highest contamination with a percentage of 83:33% followed by samples gotten from Marian and Effio-ette with a percentage of 50.00%, Eta Agbo road and Akim market had the least contamination with a percentage of 33.33%. The following information is represented in the figures 2 below.

3.4 Antibiotic susceptibility of *Staphylococcus aureus* in ready-to-eat food

The *Staphylococcus aureus* strains isolated were further subjected to antibiotic susceptibility testing and the zones of inhibition for the sensitive strains were measured in millimeters (mm), the bacterium was highly sensitive tolevofloxacin. The resistant patterns to selected antibiotics were also recorded. One out of three (33.3%) of the isolates from fried yam was resistant to ciprofloxacin and Amoxil, two out of three (66.6%) was resistant to Norfloxacin, Eythromycin&Ampiclox, none was resistant to Gentamycin, streptomycin, rifampicin, chloramphenicol and levofloxacin. The isolates from fried fish showed no resistance to eight (8) out of the ten (10) antibiotics assayed. Resistance was however shown to Norfloxacin and Streptomycin with a frequency of 33.3% respectively.

None of the isolate from moi-moi was resistant to ciprofloxacin, Norfloxacin, Gentamycin, chloramphenicol and levofloxacin. Resistance was shown to Amoxil, streptomycin, Rifampicin, Erythromycin and Ampiclox with a percentage of 40%, 20%, 20%, 40% & 60% respectively. Two of four (50%) of the isolate from suya was resistant to ciprofloxacin and chloramphenicol,three out of four (75%) was resistant to Amoxil, Ampiclox and levofloxacin, none was resistant to Gentamycin, streptomycin and erythromycin. Isolate from meat-pie was resistant to all but ciprofloxacin andlevofloxacin. The percentage resistance was 80%, 40%, 60%, 60%, 20%, 60%, 20%, 40% to Norfloxacin, Gentamycin, Amoxil, Strepromycin, Rifampicin, Erythromyun, Chloramphenicol, Ampiclox, respectively.

One out of two (50%) of the isolate from fried plantain was resistant to ciprofloxacin, Rifampicin, erythromycin and Ampiclox, none was resistant to Norfloxacin, Gentamycin, Amoxil, streptomycin, chloramphenicol & levofloxacin. Generally, isolates' antibiotic-

resistance was highest to Norfloxacin in that ten out of the twenty (50%) of the isolates were resistant to it.This information is represented in the figure 3 below.

4. DISCUSSION AND CONCLUSION

The study revealed that street foods are mostly contaminated by *Staphylococcus aureus* and consequently unsuitable for consumption. These results are in agreement with the data of Mensah *et al.*,(2002) and Sina *et al.,* (2011),which showed that salad and macaroni dishes in Accra (Ghana)had high levels of *S. aureus* contamination (Sina *et al.,* 2011). The high occurrence of *S. aureus* in moi-moi& meat pie depicts a deplorable state of poor hygienic and sanitary practices employed in the processing & packaging of these food products (Clarence *et al.,* 2009). In addition,the high incidence of *S. aureus* in food sold in Eta-Agboroad and Akai-efa is due to the poor personal hygiene of most vendors and the state of the environment where these food vendors stay to carry out their activities.

Street vended food sample are frequently contaminated by *Staphylococcus aureus* (Ghosh *et al.,* 2007) and these could serve as potential vehicles for the transmission of resistant strains of the pathogen (Bello *et al.,* 2013) thus, this study showed that *Staphylococcus aureus* was resistant to Norfloxacin by 50% and Levofloxacin by 5%.It therefore indicates that levofloxacin can be used as drug of choice for the treatment *of S. aureus* (food) infection. The occurrence of antibiotic resistant in this study might be explained by self-medication habit of the population due to the level of poverty and the fact that these antibiotics are cheap and can be bought without prescription. This practice enhances a frequent exposures of bacteria to antibiotic leading to the development of antibiotic resistant (Sina *et al.,* 2011).The bacterium was also highly sensitive to levofloxacin, this may be attributed to the fact that these antibiotics were able to penetrate the cell wall membrane and damage the nucleic acid of the isolates (Nwachukwu and Nwaigwe, 2013).

Staphylococcus aureus is highly vulnerable to destruction by heat treatment and nearly all sanitizing agents. Therefore, the presence of this bacterium or it's enterotoxin in processed foods or on food equipment is generally an indication of poor sanitation (Barron *et al.,* 2006).

The role of food vendors in determining the microbial loads in food cannot be over emphasized as they contribute to the well-being of the general population with regards to their activities.Street food is prepared by the vendors at home or at the roadside stalls (Muinde and Kuria, 2005). Vendor's sites are mostly within five to ten meters radius of dusty roads and foot paths (Mwadime, 2001). The vending sites are self-allocated with absence of sanitary amenities (Mwangi, 2002). Foods are held in different ways before selling, fish are placed openly on the stalls and chips are held in cup boards next to the stalls while fruit salads are held in open bowls (Muinde and Kuria, 2005).

After the food is prepared, it is not reheated to high temperatures before serving (Muinde and Kuria, 2005). The stalls are poorly constructed and increase the exposure to contamination of dust and smoke on the road side (Muinde and Kuria, 2005). Street vendors use tap water supplied from the municipal council or buy from water kiosks (Mwangi; 2002; Mwadime, 2001). In other instances water is ferried from home of the food vendors because there is no portable water available in their area of operation. This water is not enough for washing and food preparation and vendors do not wash fresh foods properly (Muinde and Kuria, 2005). Mensah, 1997 noted that without formal education, the street food vendor lack knowledge on proper food handling and may play a role in transmission of food borne pathogens (Ikpoh *et al.*, 2013).

Ready-to-eat food in Calabar municipality, Nigeria were analyzed for the presence of *Staphylococcus aureus* and were found to contain notable load of the organism. The presence of *Staphylococcus aureus* in food is considered a major cause of food poisoning and a health concern. *Staphylococcus aureus* is recorded high in street vended food and this could be associated with poor sanitation as most of the foods examined were considered poorly processed.

From this study, it can be concluded that exposed and poorly handled ready-to-eat food are readily prone to infection by *Staphylococcus aureus* as well as other microbial infections. The presence of the organism has been implicated in food intoxication. In order to prevent outbreak of food poisoning, public health establishments should enforce proper handling of food and proper hygiene should be practiced by production personnel and food vendors (Nwachukwu and Nwaigwe, 2013).

Quality control unit should be established in our food industry and the hazard analysis and critical control point (HACCP) concept should be applicable to the processing and vending of ready-to-eat food products. Regular inspection of the vended foods by local authorities to ensure that their respective managements comply with standard food hygiene and safety measures at every stage in the food chain to prevent food contamination. Also, there should be regular training/retraining and health education of food handlers in all aspects of food hygiene and safety (Bello *et al.,* 2013). Special attention should be given to the preparation, storage and service of highly patronized foods such as moi-moi& meat pie as resistant strains of *Staphylococcus aureus* were high in them and all food should not be reprocessed for sale on the second day of preparation (Bello *et al.,* 2013). To prevent resistant strain of *S. aureus*, it is recommend that an infected person should consult a trained physician, be given immediate treatment and also the dosage for the prescribed drug must be completed.

There is therefore need to educate the retailers and hawkers of street-vended foods on the hazards associated with the cultivation of non-chalant attitudes to hygienic processing, proper display and proper packaging of these food products, control measures should be inculcated during sales of products and these includes displaying foods in glass cabinets, washing hands at regular intervals, disallowing customers form picking up and returning products with bare hands and/or without thorough hand washing procedures. It is also important to mention that street-vended food are best within 24 hours of their production and thus, becomes necessary that vendors prepare fresh products for consumers. There apparently will prevent or reduces the potential health risks associated with the consumption of street-vended food.

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Table 1: Bacteria count of the various food samples analyzed

Food Sample	Mean bacteria count (cfu/ml)	Minimum bacteria count (cfu/ml)	Maximum bacteria count (cfu/ml)
Fried yam	$1.0x10^4$	$3.0x10^3$	$2.4x10^4$
Fried fish	$7.4x10^4$	$2.1x10^4$	$1.6x10^5$
Moi-moi	$1.0x10^4$	$3.2x10^3$	$2.2x10^4$
Suya	$9.3x10^4$	$2.8x10^4$	$2.0x10^5$
Meat pie	$6.4x10^4$	$2.7x10^3$	$2.4x10^5$
Fried plantain	$7.6x10^4$	$3.3x10^4$	$1.2x10^5$

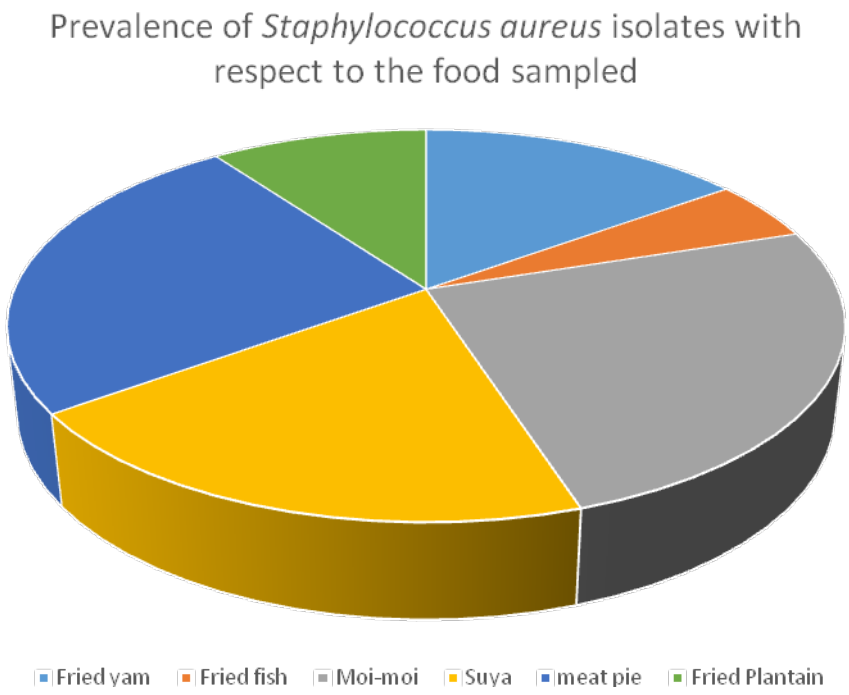

Figure 1: Prevalence of *Staphylococcus aureus* with respect to food type

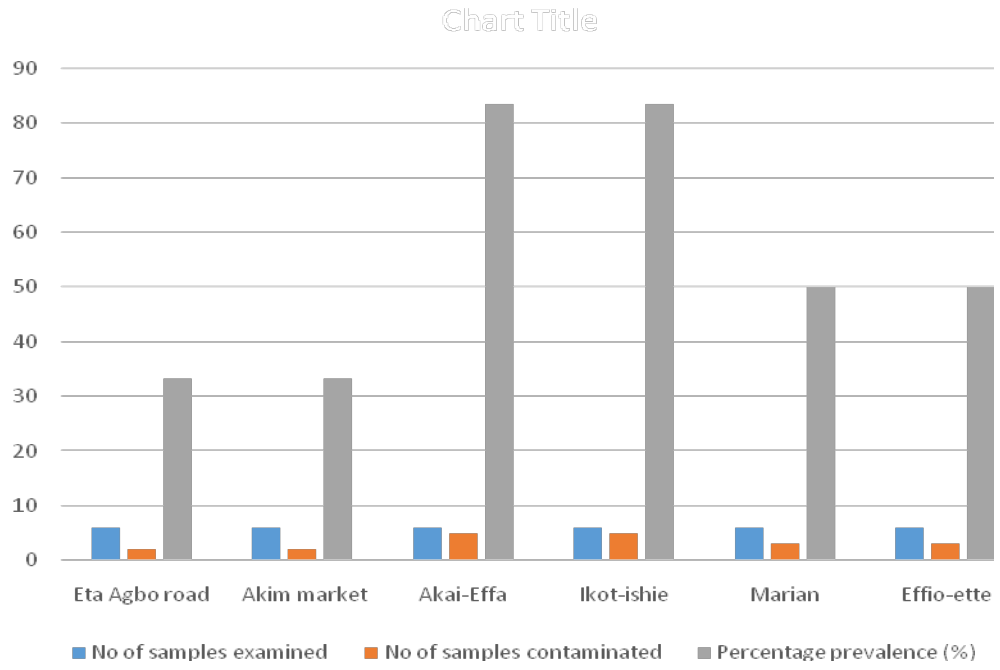

Figure 2: Percentage prevalence of *Staphylococcus aureus* isolates with respect to location

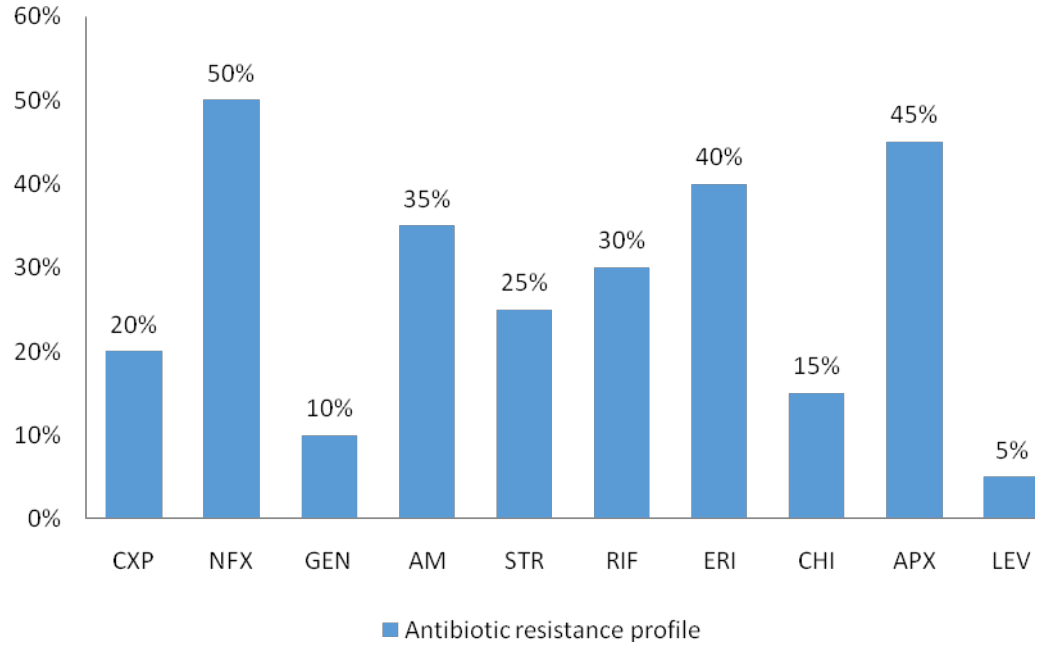

Figure 3:Antibiotic susceptibility profile of *Staphylococcusaureus*in ready-to-eat foods sampled.

KEY:

	CXP	– Ciprofloxacin
	STR	- Streptomycin
	APX	- Ampliclox
	NFX	– Norfloxacin
	RIF	- Rifampicin
	GEN	- Gentamycin
	ERY	- Erythromycin
	AMX	– Amoxil
	CHL	- Chloramphenicol
	APX	- Ampliclox
	LEV	- Levofloxacin