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Antimicrobial Susceptibility Pattern of *Staphylococcus aureus* isolated from Ready-to-eat food (moi-moi)

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ABSTRACT Street vended foods are the major source of food-borne diseases in most parts of Nigeria. In this study, 36 moi-moi samples, from six different vended sites (locations) in the Rivers State University were evaluated for the presence of *Staphylococcus aureus* and its antimicrobial susceptibility pattern using standard microbiological techniques. The samples were cultured on two media (Nutrient Agar and Mannitol Salt Agar). The bacterial colonies were counted, calculated and recorded in cfu/g. There were significant differences (p>0.05) in the total heterotrophic bacterial counts (THBC) total Staphylococcal counts (TSC) at the various locations sampled. The THBC and TSC ranged between 9.3 ± 1.41 x 10^5cfu/g (Department of Mathematics) and 64.65 ± 10.39 x10^5cfu/g (Department of Medical Laboratory Science), 4.5 ± 3.1 x 10^5 cfu/g (Department of Mathematics) and 36.0 ± 15.13 x10^5cfu/g (Department of Computer Science), respectively. In addition to isolating *Staphylococcus aureus* other four genera identified with their frequencies include, *Bacillus spp.* (21.7%), *Klebsiella* spp (13.1%), *Escherichia coli* (8.7%) and *Salmonella* spp (8.7%), with *Staphylococcus aureus* (47.8%) occurring most. Result of the antibiotic susceptibility revealed that the isolated *Staphylococcus aureus* was highly susceptible to Levofloxacin (100%), Ciprofloxacin (91%), Streptomycin (91%), Gentamycin (73%), and Rifampicin (73%). Resistance was least to Gentamycin (27.3%), Ciprofloxacin (9.1%), Streptomycin (9.1%) and Levofloxacin (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*, health care and other institutions should enforce proper processing, handling and storage of moi-moi in the Rivers State University, Port Harcourt, Nigeria.

Keywords: Susceptibility pattern, *Staphylococcus aureus*, ready-to-eat food, moi-moi

Introduction

Staphylococcus aureus, an opportunistic pathogen residing in the oral cavity, nasal cavity, the skin as a normal flora, has been reportedly found to be carried by about 30-40% of the population in addition to becoming a significant growing world-wide problem in public health systems (Grundman *et al*., 2006). It has strains that are able to resist some antimicrobial agents, hence resistant to treatment in humans (Lowy, 2003), and result in increased mortality, morbidity and increased health care cost (Cosgrove *et al*., 2003).

Because some members are normal flora of the mucous membranes and skin of humans; they cause abscess formation, suppuration, a variety of pyogenic infections and even fatal septicemia. *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 consumption has been identified as an important pathway through which humans are infected with *S. aureus*, especially the antimicrobial resistant strains present in foods, thus special attention in the preparation, processing, handling, to the consumption stage of Ready-to-Eat food (moi-moi) is necessary (Okeke *et al*., 2000). According to World Health Organization-WHO (1989), foodborne disease is the disease with the highest microbial origin and the most widespread complications in the contemporary world, which is responsible for about one-third of death world-wide. Also, food-borne illnesses arising from microbial infection or intoxication of food are a major health complications associated with the consumption of vended foods (Feglo and Sakyl 2012; Nyenje *et al*., 2012).

Food has been described as a vehicle for the transmission of microbial diseases (Nkere *et al*., 2011), especially Ready-to-Eat food. Several reasons why people eat RTE foods, include among others absence from home while travelling, at work, and may resort to buying street vended food that may have been poorly processed. This situation however, has resulted to the transfer of poor sanitary measures and handling from individuals/families to the food vendors who rarely enforce such practices (Musa and Akande, 2002).

When *Staphylococcus aureus* are allowed to grow in foods, it can produce toxins that cause illness, although cooking destroys the bacterium, but the toxin produced is heat-stable and may not be easily destroyed. The pathogenesis of this bacterium causing food-borne poisoning or infections depends on its capacity to cause intoxication, and among all the bacteria predominantly involved in this form of infection, *S. aureus* is a leading cause, which can cause complications especially Gastroenteritis resulting from the consumption of contaminated food (Loir *et al*., 2003).

Poor sanitary conditions in most of the local markets, vending sites, and in the environment where this food is produced/sold, being highly polluted and charged with pathogenic flora, has been reported to be likely the major source of contamination of food items sold by these vendors and may also encourage the growth and multiplication of this pathogenic organism (Egeonu, 2002). Also, since *S. aureus* can attach itself to equipment or other materials, in cases of poor sanitary conditions, this organism adhere to the surface of equipment used in food processing or food material itself and if not properly sterilized, the organism is transferred to the food and cause food-borne illness when consumed.

Majority of staff and students on campus don't prepare food themselves or take it along with them to the campus and this led to an increase in demand for Ready-to-eat food which gives opportunity to cafeterias and canteens to serve as the major vending sites where both staff and students purchase food daily and in most cases these foods are not adequately processed, protected from flies and usually refrigeration is unavailable.

This unhygienic condition under which food vendors operate couple with their lack of basic hygienic safety led to this present study. This study was designed to evaluate the microbial quality of a ready-to-eat food (moi-moi) sold in the Rivers

State University, Port-Harcourt, Nigeria, and investigate the occurrence of *Staphylococcus aureus* in the said sample as well as determining the antimicrobial susceptibility pattern of *Staphylococcus aureus* isolates with a view to proffering solutions for effective preparation and handling of food by the vendors.

Materials and Methods

Collection of samples

Thirty-six (36) samples of ready-to-eat food (moi-moi) wrapped in aluminium foil were randomly obtained from the 6 vending sites in the Rivers State University, Port Harcourt. The choice of the sample sites was partly due to the situating of vending sites at those locations with about 2 – 5 students visiting the sites at any time. The sites include: Department of Accounting, Department of Computer Science, Department of Mathematics, Department of Medical Laboratory Science, College of Medicine and Department of Microbiology. The samples were put into a sterile specimen container and immediately transferred in cooled packs, under aseptic conditions to the Laboratory for microbiological analysis within 30 minutes of collection.

Microbiological Analysis

One (1) gram of each moi-moi samples was homogenized in 9ml of sterile normal saline to give a dilution of 1:10. Subsequent serial dilutions were made by adding 1.0 ml to 9.0 ml fresh sterile diluents. Finally, 0.1 ml of appropriate dilutions (10^{-5} and 10^{-6}) was plated out on sterile Nutrient Agar plate (for heterotrophic bacteria) and Mannitol Salt Agar plates (for Staphylococcal counts) in triplicates, using spread plate method (Cheesebrough, 2006). All set up were incubated at 37^{o}C for 24 h. At the end of incubation period, the bacterial colonies were counted manually, recorded, and were expressed in colony unit per gram (cfu/g). Characteristic colonies were subcultured on sterile Nutrient Agar (NA) plates to obtain pure colonies. Pure isolates were identified using morphological and biochemical tests such as Gram's reaction, motility , citrate utilization , Methyl Red – Voges Proskauer (MR-VP), Oxidase, Coagulase, Catalase, Indole and sugar fermentation(including glucose, lactose, mannitol and maltose) tests (Cheesebrough, 2006; 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.

Antimicrobial Susceptibility Testing

The antimicrobeial susceptibility pattern 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 disks produced by Optun laboratory Nigeria limited included the following antibiotics with known concentrations; Amoxil (AMX)-20µg Ampiclox (APX)-20µg, Chloramphenicol (CH)-30µg, Gentamycin (CN)-10µg, Ciprofloxacin (CPX)-10µg, Erythromycin (E)-30µg, Levofloxacin (LEV)-20µg, Neobimycin (NB)-10µg, Rifampicin (RF)-20µg and Streptomycin (STR)-30µg.

The commercial antibiotics disks were introduced on 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). All tests were done in triplicates.

Data Analysis

Data obtained from the aerobic plate count and measurement of zones of inhibition of each antibiotic on isolates from each moi-moi sample were subjected to statistical package for the social sciences (SPSS) version 22. Descriptive statistics (mean, standard deviation, etc.) was applied to summarize data for tabulation and graphical representation. Analysis of variance (ANOVA) was used to test for significant difference between Departments at $p \geq 0.05$. Where differences existed, Student-Newman-Keul's (S-N-K) method of separating means was used to separate means between the various Departments at 95% probability.

Results

The results of total bacterial and Staphylococcal counts in the moi-moi samples revealed that there was a significant difference ($p \leq 0.05$) at the various investigated sites and that the samples had high levels of bacterial load as well as *Staphylococcus aureus* (Tables 1 and 2). Medical Laboratory Science recorded the highest mean total heterotrophic bacterial counts (THBC) of 64.65 ± 10.39 x10^{5}cfu/g, while the Department of Mathematics had the least with 9.3 ± 1.41 x 10^{5}cfu/g. The mean Staphylococcal counts followed the same pattern, being higher in the department of Med. Lab. Sci. (36.0 ± 15.13 x10^{5}cfu/g) and least in the department of Mathematics (4.5 ± 3.11 x10^{5} cfu/g) (Table 1).

Table 1: Variation in mean total heterotrophic bacterial and Staphylococcal counts in moi-moi samples in the different Departments

Location/Department	Total heterotrophic bacterial count (x10^{5}cfu/g)	Total Staphylococcal counts (x10^{5} cfu/g)
Accountancy	64.15±21.43^{c}	32.30±9.89^{c}
Computer Sci.	62.80±14.85^{c}	36.20±4.95^{c}
Mathematics	9.30±1.41^{a}	4.50±3.11^{a}
Med. Lab. Sci.	64.65±10.39^{c}	36.00±15.13^{c}
Medicine	39.15±30.90^{ab}	12.50±13.01^{ab}
Microbiology	34.15±22.42^{ab}	12.20±0.71^{ab}

*Mean with the same superscript along the columns is not significantly different ($p \leq 0.05$)

Table 2: ANOVA table showing the level of significance between microbiological parameters and locations

			Sum of Squares	df	Mean Square	F	Sig.
Total heterotrophic bacterial count * Locations/sites	Between Groups	(Combined)	4986.360	5	997.272	2.663	0.132
	Within Groups		2246.880	6	374.480		
	Total		7233.240	11			
Total Staphylococcal counts * Locations/sites	Between Groups	(Combined)	1991.577	5	398.315	4.501	0.047
	Within Groups		530.940	6	88.490		
	Total		2522.517	11			

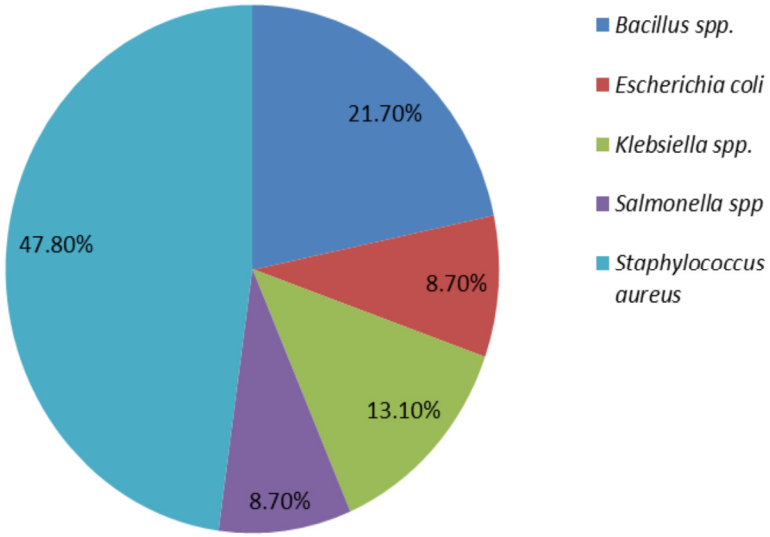

Fig. 1: Occurrence of bacterial Isolates in the moi-moi samples studied

Other bacteria identified in varying frequencies include, *Bacillus spp.* (21.70%), *Klebsiella* spp (13.10%), *Escherichia coli* (8.70%) and *Salmonella* spp (8.70%), with *Staphylococcus aureus* (47.80%) occurring most (Fig. 1).

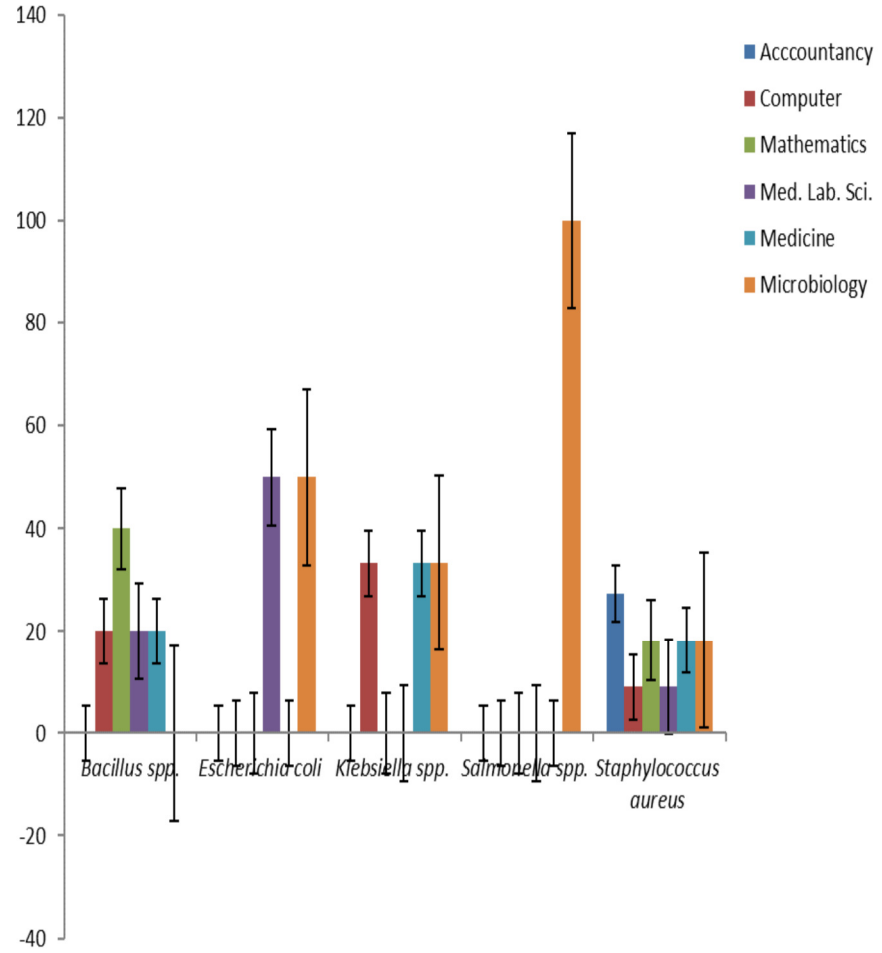

Fig. 2: Occurrence of bacterial isolates in the different vending sites

The results of frequency of bacterial isolates in all the vendings sites studied showed that *Stahpylococcus aureus* occurred at all the sites with varying frequencies while *Bacillus* spp occurred only in four departments (Fig. 2).

Result of susceptibility pattern of *Staphylococcus aureus* isolates as depicted by the zone of inhibition showed varying susceptibility pattern depending on the vending site with Levofloxacin having the highest percentage of 100%, while Norfloxacin was the least sensitive with a percentage of 0% (Tables 3 and 4). Generally, *Staphylococcus aureus* isolates from moi-moi were highly susceptible to Levofloxacin (100%), closely followed by Ciprofloxacin and Streptomycin, both with a frequency of 91%, and Gentamycin and Rifampicin (73% each), but were resistant to Norfloxacin (91%), closely followed by Ampiclox (81.8%) and then Erythromycin (72.7%), Chloramphenicol (63.6%) and Amoxil (54.6%) as shown in Table 5

Table 3: Antimicrobial susceptibility pattern of *S. aureus* isolates showing the size of zone of inhibition (milliliters)

Isolate code	Antibiotics									
	AMX (20µg)	APX (20µg)	CH (30µg)	CN (10µg)	CPX (10µg)	E (30µg)	LEV (20µg)	NB (10µg)	RD (20µg)	STR (30µg)
AC.1	14	R	R	23	R	R	22	R	17	18
AC.2	R	R	R	R	17	R	26	R	R	R
AC.3	14	R	R	25	27	R	25	R	13	10
C.1	12	R	R	16	20	R	20	R	14	17
MT.1	R	R	R	20	18	10	25	R	R	16
MT.2	R	R	12	R	13	R	20	R	17	15
ML.2	R	18	20	20	20	18	25	R	20	16
MD.1	16	15	16	22	16	R	23	R	R	11
MD.2	R	R	16	17	16	R	21	11	15	20
MB.1	13	R	R	18	20	11	27	R	24	18
MB.2	R	R	R	R	11	R	14	R	15	12

Key: R: Resistance, AMX: Amoxil, APX: Ampiclox, CH: Chloramphenicol , CN: Gentamycin, CPX: Ciprofloxacin, E: Erythromycin, LEV: Levofloxacin, NB: Norfloxacin,, RD: Rifampicin, STR: Streptomycin.

Table 4: Percentage of Drug sensitivity pattern of *S. aureus* isolated from various vending sites

Sites/department (no of *S. aureus* isolates)	Antibiotics/% drug sensitivity									
	AMX (20µg)	APX (20µg)	CH (30µg)	CN (10µg)	CPX (10µg)	E (30µg)	LEV (20µg)	NB (10µg)	RD (20µg)	STR (30µg)
Accountancy (3)	66.7	0	0	66.7	66.7	0	100	0	66.7	66.7
Computer (1)	100	0	0	100	100	0	100	0	100	100
Mathematics (2)	0	0	50	50	100	50	100	0	50	100
Med. Lab. Sci. (1)	0	100	100	100	100	100	100	0	100	100
Medicine (2)	50	50	100	100	100	0	100	50	50	100
Microbiology (2)	50	0	0	50	100	50	100	0	100	100

Key : AMX: Amoxil, APX: Ampiclox, CH: Chloramphenicol , CN: Gentamycin, CPX: Ciprofloxacin,
E: Erythromycin, LEV: Levofloxacin, NB: Norfloxacin,, RD: Rifampicin, STR: Streptomycin.

Table 5: Frequency and percentage of resistance and Sensitivity pattern exhibited by *S. aureus* isolated from moi-moi samples

Antibiotics (conc. in µg)	RESISTANCE		SENSITIVITY	
	No. of isolates	Percentage (%)	No. of isolates	Percentage (%)
AMX (20µg)	6	54.5	5	45.5
APX (20µg)	9	81.8	2	18.2
CH (30µg)	7	63.6	4	36.4
CN (10µg)	3	27.3	8	73
CPX (10µg)	1	9.1	10	91
E (30µg)	8	72.7	3	27.3
LEV (20µg)	0	0	11	100
NB (10µg)	10	91	1	9.1
RD (20µg)	3	27.3	8	73
STR (30µg)	1	9.1	10	91

Key: R: Resistance, AMX: Amoxil, APX: Ampiclox, CH: Chloramphenicol , CN: Gentamycin, CPX: Ciprofloxacin, E: Erythromycin, LEV: Levofloxacin, NB: Norfloxacin,, RD: Rifampicin, STR: Streptomycin.

Discussion

Staphylococcus aureus is known to be an important pathogen of man as it produces several virulence factors that enhance its pathogenecity (Palavecino, 2006). Some strains have been known to be resistant to antibiotics such as amoxicillin, penicillin, oxacillin, and methicillin (Al-Zoubi *et al.,* 2015). The organism has become widespread globally causing different types of infection and therefore developing resistance strains due to the indiscriminate use of antibiotics (Lowy, 2003). In this present study, it was revealed that total bacterial and Staphylococcal counts in the moi-moi samples was significantly different ($p \leq 0.05$) at the various investigated sites and that the samples had high levels of bacterial load as well as *Staphylococcus aureus*. The moi-moi sold in the Department of Medical Laboratory Science had the highest bacterial load as well as a greater population of *Staphylococcus aureus*, and according to the International Commission for Microbiological standards of food (ICMSF), these food samples are not acceptable for consumption but tolerable. This result is in agreement with the data of Mensah *et al*. (2002) and Sina *et al*. (2011). The high levels of these group of organisms in this department could probably be due to deplorable state of poor hygienic and sanitary practices employed in the processing and packaging of these food products (moi-moi) (Clarence *et al*., 2009). In addition, the high incidence of *S. aureus* in moi-moi sold in these vending sites may be due to the poor personal hygiene of most vendors and the state of the environment where these food vendors carry out their activities. The Department of Medical Laboratory Science is also highly populated with students, when compared to other departments, having many activities around the vending sites and may have introduced these organisms into the moi-moi. Other bacteria identified in varying frequencies include,

Bacillus spp. (21.7%), *Klebsiella* spp (13.1%), *Escherichia coli* (8.7%) and *Salmonella* spp (8.7%), with *Staphylococcus aureus* (47.8%) occurring most (Fig. 1). The role of food vendors especially in determining the microbial loads in food (moi-moi) cannot be over emphasized. The high incidence of *Staphylococcus aureus* (47.8%), a normal flora of human skin may have been introduced into the food during the handling process hence contributing to the well-being of the population. The results of frequency of bacterial isolates in all the vendings sites studied showed that *Staphylococcus aureus* occurred at all the sites with varying frequencies while *Bacillus* spp occurred only in four departments (Fig. 2). The presence of *Bacillus* spp. could be due its ability to form spores which withstand adverse environmental conditions. The prevalence of *Bacillus cereus* and *Staphylococcus aureus* was also reported by Isara *et al*. (2010) when they studied the prevalence and contamination of some fast food restaurants in Benin City. They queried the unhygienic practices of the vendors. On the other hand, *Salmonella* spp. did not occur in any of the sites but the department of Microbiology probably because of the proximity of the site to the toilets. Other enteric like *Klebsiella* spp and *E. coli* were not isolated from some samples from other departments. The presence of these organisms in mio-moi could constitute a health hazard.

Vended food sample are frequently contaminated by *Staphylococcus aureus* (Ghosh *et al*., 2007) and 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* isolates were highly susceptible to Levofloxacin (100%), closely followed by Ciprofloxacin and Streptomycin, both with a frequency of 91%, and Rifampicin (74%), but were resistant to Norfloxacin (91%), closely followed by Ampiclox (81.8%) and then Erythromycin (72.7%), as revealed in Table 5. This therefore indicates that Levofloxacin, Streptomycin and Ciprofloxacin can be used as drugs of choice for the treatment of *S. aureus* (food) infection. This result is similar to that of Agbo *et al.* (2016) who reported 50% sensitivity of Levofloxacin to *Staphylococcus* spp isolated from some vended foods. According to them the occurrence of antibiotic resistant of the strains as exhibited by its 5% resistant to Norfloxacin as also recorded in this study may be due to 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 tends to enhance a frequent exposure of bacteria to antibiotic leading to the development of antibiotic resistant (Sina *et al*., 2011). The bacterium being highly sensitive to Levofloxacin, 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). Furthermore, *Staphylococcus aureus* is highly vulnerable to destruction by heat treatment and nearly all sanitizing agents. Therefore, the presence of this bacterium or its enterotoxin in processed foods or on food equipment is generally an indication of poor sanitation (Barron *et al*., 2006).

Conclusion

To conclude, this study showed that moi-moi sold at some vended sites in the Rivers State University contained notable load of bacteria and significant level of *Staphylococcus aureus*. The significant level of *Staphylococcus aureus* in moi-moi is alarm-

ing and should be considered a major cause of food poisoning and a health concern. Although varying patterns of sensitivity to antibiotics tested was observed, the study also revealed that the drug of choice is Levofloxacin for the treatment of infections due to *Staphylococcus aureus* isolated from vended foods in Rivers State University

Recommendations

The significant level of bacterial load and presence of *Staphylococcus aureus* in the moi-moi samples is a major health concern and it is therefore necessary to educate the vendors of moi-moi on the hazards associated with the cultivation of non-chalant attitudes to hygienic principles. It is also important to mention that antibiotic sensitivity pattern of *Staphylococcus aureus* and strict vending policy for food sold within the University and outside is monitored. Moi-moi is best within 12 hours of their production and thus, becomes necessary that vendors prepare fresh products for consumers. These apparently will prevent or reduce the potential health risks associated with the consumption of vended moi-moi sold in Rivers State University.

Limitations of Study

The study was conducted within a short period of three (3) months hence the sample size of thirty-six (36). In addition, moi-moi samples were not always available at the designated vending sites. In future, more samples collected over a longer period could be conducted and more departments included in the study. Other limitation was the non-availabilty of constant power supply which was very frustrating as some of the experiments were repeated because of contaminants.

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