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Occurrence of antibiotic-resistant Staphylococcus aureus in some street-vended foods in Ogun State, Nigeria

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ABSTRACT

Food borne illnesses of microbial origin are a major international health problem associated with food safety and an important cause of death in developing countries. This study was carried out to investigate the occurrence of antibiotic-resistant Staphylococcus aureus in some street-vended foods in Ogun State, Southwerstern Nigeria. A total of 140 street-vended food samples which included 20 samples each of fish sausages, meat sausages, fried fish, fried meat, fried yam, moin-moin and jollof rice were purchased from vendors in three different communities (Sabo, Isale-Oko and Makun) in Sagamu, Ogun State, Nigeria. Demographic survey was carried out on the hygienic and safety attitudes cultivated by the vendors recruited for this study. Microbiological analyses were carried out on the food products to isolate typical S. aureus strains. The samples were serially diluted and dilution factors of up 10^{-6} were cultured on Mannitol salt agar medium employing the spread plate technique. The disc-diffusion method was employed to determine the antibiotic resistance patterns of the isolated S. aureus strains. Most vendors were aware of the heath risk associated with unhygienic practices. Percentage products contaminated ranged from 0%, as obtained from fried yam, to 40% obtained from fish sausages. Prevalence of S. aureus strains obtained from samples ranged from 0 (as in fried yam) to 5.20 $\pm$ 1.2 cfu ml^{-1} (as obtained from jollof rice). The isolates were subjected to antibiotic susceptibility assay employing the disc diffusion technique. Results on the resistance patterns of the isolated S. aureus strains revealed that resistance was highest to gentamycin (45.8%) and lowest to cotrimoxazole (4.2%) and erythromycin (4.2%). In conclusion, street vended food samples are frequently contaminated with S. aureus and that these could serve as potential vehicle for the transmission of resistant strains of the pathogen. Increased resistance of S. aureus to certain broad spectrum antibiotics such as gentamicin and amoxicillin should stimulate the interest of researchers.

Keywords: S. aureus, antibiotics, vended, foods, resistance, health, risk.

Discipline and Sub-discipline: Microbiology (Food Microbiology/ Public Health)

SUBJECT CLASSIFICATION: Bacteriological Quality of Food/Public Health

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1.0 INTRODUCTION

The emergence of drug resistant bacteria is a major problem in antibiotic therapy. Staphylococcus aureus has been recognized historically as a virulent and important human pathogen. Its capacity to produce human disease has not diminished with the introduction of antibiotics [1]. It is still one of the most frequently encountered single bacterial species in hospitals and continues to be a frequent cause of burns and wound sepsis [2]. Animals, human food and the inanimate environment can provide a favorable environment for the admission of S. aureus [3,4]. S. aureus as an indicator of contamination of processed foods could come from the skin, mouth, or nose of handlers [5]. The presence of staphylococci in food is an indication of postharvest contamination due to poor personnel hygiene, and/or diseased food product [6]. In Nigeria, food-borne microbes including S. aureus are a major cause of food poisoning [7]. Overseas, S. aureus food poisoning due to consumption of fish and other street-vended foods was reported [7,8,9,10]. Some species of staphylococci have developed resistance to all β-lactam antibiotics through acquisition of the mobile staphylococcal chromosomal cassette carrying the methicillin-resistant gene mecA (staphylococcal cassette chromosome mec, SCCmec). Methicillin-resistant S. aureus (MRSA) and methicillin-resistant coagulase-negative staphylococci (MR-CoNS) are acknowledged as internationally distributed zoonotic multidrug-resistant pathogens that cause nosocomial infections and community-acquired diseases in humans, and as infections in many animal species [11,12,13]. The emergence of antibiotic-resistant Staphylococcus aureus in fish has been recently noted [14]. The role of food handlers in preparation of street-vended foods is crucial in determining the hygienic status of the final product, with poor handling resulting in increased likelihood of contamination by human-borne microbes including multidrug-resistant and/or enterotoxigenic staphylococci. In Nigeria, like other developing countries, street food vending generates income to households and majorly dominated by women. Street foods are defined as ready-to-eat foods and beverages prepared and/or sold by vendors on the street from push-carts or buckets or balance poles or stalls or from shops having fewer than four permanent walls [15]. The safety of street foods is affected by several factors starting from the quality of the raw materials, to food handling and storage practices. In most cases, the flow of water from taps is not regular for hand- and dishwashing, cooking or drinking, leading the street vendors to store water under vulnerable conditions subject to contamination. Street foods are exposed to appalling environmental conditions, such as the presence of insects, rodents, domestic animals/other animals and air pollution [16]. Besides, most food vendors do not observe good food handling practices, exposing foods to dangerous conditions such as cross contamination, unsafe storage and poor time-temperature conditions [17]. There is, a general perception that street vended foods are unsafe, mainly because of the environment under which they are prepared, sold and or consumed, which exposes the food to contamination [18]. Street food vendors operate from such places as bus terminals, industrial sites, market places, school compounds or around the gates, road sides and other street corners where there are ready and numerous clienteles. Unfortunately, these locations usually do not meet all food safety requirements. Street food vending has been associated with causing food borne illnesses in the population owing to the difficulties inherent in ensuring that food is prepared and sold under hygienic conditions [19,20,21]. In recent years studies have found antibiotic resistant Staphylococcus aureus in retail cuts of pork, chicken, beef, and other meats in the United States, Europe, and Asia [22]. However, no data has yet been presented regarding antibiotic resistant Staphylococcus aureus isolated from retailed fried fish and meat, fish and meat sausages and moin-moin (a popular cowpea pudding) as presented in this study.

2.0 MATERIALS AND METHODS

2.1 Geographical Area

Sagamu (Coordinates: 6°50′N 3°39′ E) is a city and the headquarters of the Local Government Area of the same name in southwestern Nigeria located in Ogun State near the Ibu River. The Sagamu region is underlain by major deposits of limestone, which is used in the city's major industry, the production of cement. Agricultural products of the region include cocoa and kola nuts. Sagamu is the largest kola nut collecting center in the country. The kola nut industry supports several secondary industries such as basket and rope manufacturing, which are used to store the kola nuts. Sagamu is the part of the Yoruba cultural region of southwestern Nigeria. The city was founded in the mid-19th century when several small towns united for purposes of defense during the wars brought about by the fall of the Oyo Empire. Sagamu has experienced both population and economic growth since the 1950s due to its position between the cities of Ibadan and Lagos. The population in 1995 was 114,300 but 2007 estimates place it as high as 228,382. The great Olabisi Onabanjo University Teaching Hospital (OOUTH) is located in Sagamu.

2.2 Study Population and Survey

The survey to evaluate the food safety knowledge and practices of street food vendors within Sagamu was carried out between May and August, 2012. One hundred and forty street vendors operating on major streets, open market, motor parks, schools and offices, in and around Sabo, Isale-Oko and Makun, all in Sagamu, were recruited for this study. Data to build up questionnaires were partly adopted from the work of Omemu and Aderoju [23] and Bruno et al. [24], with some modifications. The survey tool contained forty-one questions categorized under nine headings. These covered socio-economic characteristics, health and personal hygiene knowledge of vendors. Informed consent was obtained from prospective respondents while confidentiality was ascertained. The survey tool was then administered to participants. More than one response per item was permitted. Results were collated as demographic profiles.

2.3 Samples Collection

The study was conducted on street vended foods from Sabo, Isale-Oko and Makun, Sagamu. The districts were chosen due to their high population and concentration of street-food vendors. The study was carried out during the period of June

to November, 2012. A total of 140 street food samples were collected in this study. The street food vendors were mostly stationary with shelter, owned stalls and prepare full meals in open, and serve on tables in stalls. Others were stationary without shelter and prepare their foods in the open air by the road side and sell at that spot. The food focused on were the commonly patronized foods such as moin-moin (cowpea pudding) prepared by placing slurries, contained in small aluminum plates or wrapped in leaves or nylon in water and boiling until the slurries set as firm gels; fried fish and meat products; fried yam; fish and meat sausages. All samples were transported to the laboratory for microbiological evaluations within 12 hrs of collections.

2.4 Samples Analyses

Ten (10) grams each of the samples was weighed and transferred into 90 mls of sterile distilled water. This was shaken thoroughly and appropriate dilutions of up to 10^{-8} were prepared for microbiological studies [25]. Using the spread plate technique, dilutions prepared were made on Mannitol Salt Agar (Oxoid Ltd, UK) and incubated for 24 – 48 h at 37^0C. Typical S. aureus colonies on MSA were transferred to nutrient agar slants and incubated at 37^0C. Coagulase positive strains were tested further in accordance with standard procedures [26]. Staphylococci isolates were inoculated onto blood agar plates and were incubated overnight at 37^0C to identify mucoid and pigmented strains. Randomly selected S. aureus isolates were subjected to certain biochemical tests such as catalase test [25], nitrate reduction in nitrate broth and coagulase activity by tube assay [27]. Further characterization included production of acid from glucose, arabinose, mannitol, mannose, lactose, sucrose and xylose [28].

2.5 Antibiotics Susceptibility Testing

The susceptibility of S. aureus isolates to various conventional antibiotics was determined using Mueller-Hinton agar (Oxoid Ltd, UK) while employing the disk diffusion method in accordance with the guidelines of the National Committee for Clinical Laboratory Standards [29]. The antibiotics used in this study included Amoxycillin (Amx), Chloramphenicol (Chl), Ciprofloxacin (Cpx), Cloxacillin (Clo), Cotrimoxazole (Cot), Erythromycin (Ery), Gentamycin (Gen), Norfloxacin (Nfx), Rifampicin (Rfp), Streptomycin (Str) and Tetracycline (Tet). The commercial antibiotic disks were introduced on to agar plates previously seeded with 18 h-broth cultures of the test organisms. The plates were incubated at 37^0C for 48 h. The inhibition zones were measured, scored as sensitive, intermediate susceptibility and resistant according to the NCCLS recommendations [29]. Staphylococcus aureus ATCC 12600 was used as a reference strain.

3.0 RESULTS AND DISCUSSION

Table 1: Demographic profiles of attitudes of food vendors in Sagamu, Ogun State

(N= 140)

Parameter	%	Parameter	%
1. Factors influencing the purchase of food to be vended		6. Methods used in cleaning utensils	
Price	90	Washing with sponge, soap and water	69
Freshness	72	Washing with soap and water	22
Volume	90	Washing with hot water and soap	9
Based on reputation of food handlers	65		
		7. Personal Hygiene	
2. Food handling practices		Long fingernails and presence of rings	58
Food cooked and stored in warmer	67	Clean wears/Aprons	65
Food cooked at intervals	81	Head cover	61
Food sold from tray with covering	94	Constant washing of hands during the operations	67
Food sold from tray with no covering	6	Prior training in the hygiene and handling of foods	41
Food handled at ground level	12	Presence of rings and other accessories	21
Food placed on counters and tables	88	Knowledge that microorganisms can contaminate foods	46
Food reheated before sale	50		
Food maintained at cooking temperature	58		
Thorough washing of food to be cooked	69	8. Operations	
		RM acquired from registered suppliers	7

3. Serving of food		RM stored appropriately	9
Food served with fork/spoon	71	RM protected from vectors during the operations	70
Food served into cup/plate	75	Continual and adequate hygiene of the equipments?	85
Food served into paper/leaves	68		
		9. Installations	
4. Left-over food management policy		Presence of rubbish	10
Throw away	15	Adequate source of water	98
Eaten at home	35	Adequate cleaning of establishment	30
Refrigerated and reheated	18	Designated waste bin for the residues generated	65
No answer/no left-over	22		
5. Source of water for hand washing and cooking			
Borehole	57		
Deep well	43		
Streams	0		

Demographic profiles of the attitudes of vendors as regards hygienic practices and other critical parameters showed that most vendors considered both price (90%) and volume (90%) when purchasing food products or raw materials to be processed for vending, as compared to freshness (72%) of the products and reputation of the food handlers (65%). This is similar to the work of Omemu and Aderoju [23] who reported 94% and 93% on the preference of vendors on volume and price, respectively. However, in this study, more vendors cooked their foods at intervals (68%) in considerably small volumes than those that cooked and stored in warmers from morning (32%). Most vendors were aware of the heath risk and unhygienic practice which was dictated by the positioning of their vended foods, as most had their foods placed on counters or tables (88%) when directly compared to those that placed their foods at ground level (12%). In summary, it was deduced that most vendors were aware of good hygienic practices but in varying degrees, except that very few acquired their raw materials from registered suppliers (7%) and stored them appropriately (9%). Borehole (57%) and deep well (43%) were the sources of the water used for the preparation of the vended foods (Table 1). These water sources are usually regarded as safe depending on the depth and hygienic practices of the users [30].

Table 2: Prevalence of S. aureus in some street-vended foods in Sagamu, Nigeria

Samples	Number assayed	Number/Percentage of product contaminated	Cfu ml^{-1} or cfu g^{-1} (log_{10})
Fish Sausages	20	8 (40%)	*3.40 $\pm$ 0.9
Meat Sausages	20	7 (35%)	*3.38 $\pm$ 0.9
Fried Fish	20	2 (10%)	*2.60 $\pm$ 0.8
Fried Meat	20	1 (5%)	*1.01 $\pm$ 0.75
Fried Yam	20	0	Nil
Moin-moin	20	3 (15%)	*4.10 $\pm$ 1.16
Jollof Rice	20	7 (35%)	*5.20 $\pm$ 1.2
Total	140	28 (20%)	

*Values show the means of triplicate determinations

Table 2 showed the prevalence of S. aureus in street-vended foods. The occurrence of S. aureus was highest in jollof rice from food preparatory centers with an average value of 5.20 $\pm$ 1.2 cfu ml^{-1} which occurred in 35% of the samples. This was followed by moin-moin whose 15% of contaminated samples turned out 4.10 $\pm$ 1.16 cfu g^{-1} of the isolate on the average. However, number of samples implicated for the presence of the organism was highest in fish sausages as it was found to occur in 40% of the samples with an average of 3.40 $\pm$ 0.9 cfu g^{-1}. Though, it was the product most contaminated in this study but not with the highest prevalence of the organism. It was interesting to see that no S. aureus strain was isolated from samples of fried yam under study. This confirmed frying as one of the reliable methods of preserving food. One of twenty (5%) samples of fried meat was implicated for the presence of S. aureus with 1.01 $\pm$ 0.75 cfu g^{-1} on the average, and this showed the least occurrence of the organism. Considering the total number of samples (140), 28 (20%) was

indicted for the presence of S. aureus, while it was clearly noted that fish sausage was the product most contaminated by S. aureus (40%), followed by meat sausages and jollof rice which both possessed percentage contamination of 35% (Table 2). The results of this study agreed with the findings of Sokari [31] and Mosupye and von Holy [32] who reported that percentage contamination of ready-to-eat foods by S. aureus ranged from 13% to 86%. However, higher prevalence and mean counts of S. aureus have been reported in other ready-to-eat food products as reported by Umoh and Odaba [21].

Table 3: Antibiotic resistance of S. aureus strains in ready-to-eat foods

Samples	No. of Isolates	Number/ Percentage resistance to antibiotics									
		Amx	Chl	Clo	Cot	Cpx	Ery	Gen	Nfx	Str	Tet
Fish and Meat Sausages	15	7 (46.7%)	0.0	4 (26.7%)	2 (13.3%)	3 (20%)	2 (13.3%)	5 (33.3%)	0.0	4 (26.7%)	4 (26.7%)
Fried Fish and Meat	3	0.0	0.0	1 (33.3%)	0.0	0.0	0.0	2 (66.7%)	0.0	1 (33.3%)	1 (33.3%)
Moin-moin	3	1 (33.3%)	1 (33.3%)	1 (33.3%)	0.0	1 (33.3%)	0.0	2 (66.7%)	1 (33.3%)	1 (33.3%)	2 (66.7%)
Jollof Rice	7	3 (42.9%)	2 (28.6%)	2 (28.6%)	0.0	1 (14.3%)	0.0	3 (42.9%)	1 (14.3%)	2 (28.6%)	1 (14.3%)
Total/ Average percentage resistance	24	9 (37.5%)	3 (12.5%)	7 (29.2%)	1 (4.2%)	4 (16.7%)	1 (4.2%)	11 (45.8%)	2 (8.3%)	7 (29.2%)	7 (29.2%)

Keys:

Amx – Amoxycilin; Chl – Chloramphenicol; Clo – Cloxacillin

Cot – Cotrimoxazole; Cpx – Ciprofloxacin; Ery – Erythromycin

Gen – Gentamycin; Nfx – Norfloxacin Str – Streptomycin

Tet – Tetracycline.

The isolated strains were further subjected to antibiotic susceptibility testing to determine their resistant patterns to selected conventional antibiotics (Table 3). Results revealed that seven out of fifteen (46.7%) of the isolates from fish and meat sausages were resistant to amoxycilin; none was resistant to chloramphenicol and norfloxacin. Five of fifteen (33.3%) of the isolates were found to be resistant to gentamycin; 26.7% were resistant to cloxacillin, streptomycin and tetracycline, 20% resistance was shown to ciprofloxacin while the percentage resistance of isolates to cotrimoxazole and erythromycin was 13.3% (Table 3). No resistance was shown by isolates from fried fish and meat to six out of the ten antibiotics assayed. Resistance was, however, shown to cloxacillin, gentamycin, streptomycin and tetracycline with percentage resistance of 33.3%, 66.6%, 33.3% and 33.3% respectively. Isolates from moin-moin and jollof rice were found to be resistant to all, but cotrimoxazole and erythromycin. Percentage resistance ranged from 14.3%, as in the cases of isolates from jollof rice against ciprofloxacin, norfloxacin and tetracycline, to 66.7% as in the cases of isolates from moin-moin to gentamycin and tetracycline. In all, it was noticeable that resistance of isolates was highest to gentamycin as indicated by the resistance of eleven out of twenty four (45.8%) of isolates to antibiotics (Table 3).

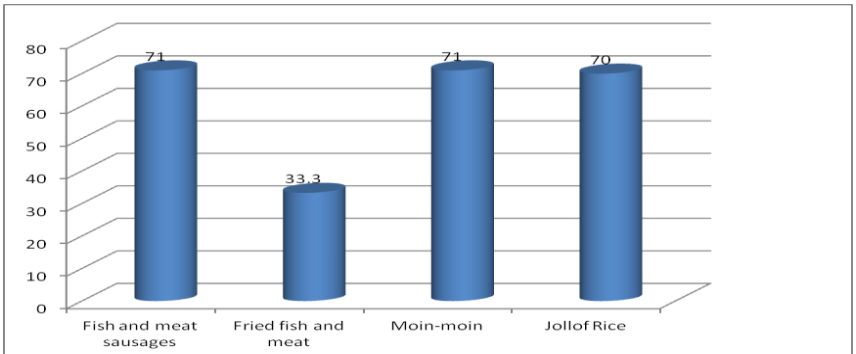

Figure 1: Percentage resistance of *S. aureus* strains of different origins to two or more antibiotics

Most S. aureus strains isolated from fish and meat sausages were found to be resistant to at least two of the antibiotics as revealed by percentage resistance of 71%. Similarly, it was observed that isolates from moin-moin also showed percentage resistance as high as 71% to two or more antibiotics. Isolates from jollof rice exhibited percentage resistance of 70% to at least two of the antibiotics while the least resistance was exhibited by isolates from fried fish and meat products as shown by percentage resistance of 33.3%.

The isolation and enumeration of S. aureus in food products is employed generally as a sanitation index [22]. The presence of these organisms in food beyond certain critical limit is interpreted as indicating that the food in question has been exposed to condition that might introduce or allow proliferation of pathogenic microorganisms [33]. S. aureus is an important food-poisoning organism because of its cosmopolitan distribution in nature. Its presence in ready-to-eat foods may be traced back to the environment as they are important normal flora of humans [34,35,36,37]. The antibiotic-resistant isolates might have originated from humans considering the fact that the level of carriage of S. aureus among humans is over 40% in Nigeria as reported by Paul et al. [38] and Lamikanra et al. [39]. It has been isolated from foods such as rice, spices, meat and dairy products [6,9,31,40]. The level of resistance to antimicrobial drugs is a reflection of the indiscrimate use of antibiotics which is becoming a common practice without legal caution in our environment [13].

4.0 CONCLUSION AND RECOMMENDATIONS

In conclusion, this study revealed a high prevalence of antibiotic resistant S. aureus in street-vended foods. The antibiotic resistance patterns of the S. aureus strains isolated here suggest some health risks as there is even the possibility of transfer of resistance to other forms of bacteria since they form commensal flora through food chain. Though, demographic data obtained showed that most vendors were aware of health risks associated with unhygienic practices, there is still need for more 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. Special attention should be given to the preparation, storage and service of highly patronized foods such as jollof rice and moinmoin as resistant strains of S. aureus were high in them, and all foods should not be reprocessed for sale on the second day of preparations. Of greater concerns are cases of acquired resistance, where initially susceptible populations of bacteria become resistant to an antibacterial agent and proliferate and spread under the selective pressure of use of that agent. There is need to educate the retailers and hawkers of ready-to-eat foods on the hazards associated with the cultivation of non-chalant attitudes to hygienic processing, display and packaging of these food products. Control measures should be inculcated during sales of products, and these include displaying foods in glass cabinets, washing hands at regular intervals, disallowing customers from picking up and returning products with bare hands and/or without thorough hand-washing procedures. It is also important to mention that street-vended foods are best within 24 hours of their productions, 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 street-vended foods.

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BIOGRAPHY

I am BELLO, Olorunjuwon Omolaja, Male, Nigerian. I possess National Diploma (ND) in Science Laboratory Technology from The Polytechnic, Ibadan, Nigeria and proceeded to study Microbiology in Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria, where I graduated with a strong Second Class (Honours) Upper Division in 2006. I was given the "award of the most intellectually stable" in the Department of Microbiology in 2005. I was retained as an academic staff because of my outstanding academic performance and assumed duty as a Graduate Assistant in 2008 shortly after my National Youth Service Corps (NYSC) programme.

I had my Master's Degree in Microbiology and graduated with a distinction in 2010 and, presently, at the terminal stage of my PhD programme. I am presently rounding off my bench–work in the Microbial Biotechnology Laboratory, Department of Biological Sciences, Faculty of Science and Technology, North-West University, Mafikeng Campus, South Africa, where I register as a visiting doctoral research student. It is also worthy of mention that I am presently being considered for promotion to Lecturer two (L2) having served for two years as a Graduate Assistant (2008 – 2010) and three years as Assistant Lecturer (July 2010 till date), and with over ten publications in reputable International Journals.

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