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Staphylococcus aureus in soup samples from restaurants/food canteens and some families in Jalingo Metropolis, North-Eastern Nigeria

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A prospective study was conducted in 2011 and 2012 to determine the occurrence and antibiotic resistant pattern of *Staphylococcus aureus* in soups prepared and sold in restaurants/food canteens and some selected families in Jalingo Metropolis, Taraba State, Nigeria. Two hundred (200) soup samples comprising of 100 from restaurants/food canteens and 100 from selected families were collected and processed. Out of the 200 soup samples, 84 (42%) were positive for *S. aureus.* The occurrence of *S. aureus* in soup samples from restaurants was significantly higher ($p < 0.05$) than that of the families (48 vs. 36%). The occurrence of *S. aureus* according to the types of soup samples examined showed that green leaf (Aleyehu) soup has the highest (65.0%), followed by sorrel (Yakuwa) (57.5%), melon (Eguisi) (40.0%), stew (36.7%), okro (25.0%) and bitter leaf soup (0.0%). The antibiotic sensitivity result of the 84 *S. aureus* revealed that all (100%) the isolates were resistant to ampicillin and penicillin, 60 (71.4%) were resistant to tetracycline, 30 (35.7%) to erythromycin, 11 (13.1%) to sulphamethoxazole/trimethoprim, 10 (11.9%) to gentamicin and 5 (6.0%) to chloramphenicol. None of the isolates was resistant to amikacin and ciprofloxacin. Unhygienic practices during processing and serving (post processing contamination) were the factors responsible for the high occurrence of *S. aureus* in the soup samples examined. The study recommends regular inspection of restaurants/food canteens and households by regulatory agencies and sanitary inspectors to ensure proper sanitary conditions before, during and after food preparation.

Key words: Antibiotic, resistant pattern, *Staphylococcus aureus,* soups.

INTRODUCTION

Foodborne pathogens remains the common cause of illness and death in less developed countries, and responsible for millions of cases of infectious gastro-intestinal diseases in developed countries costing billon of dollars in medical care. They are the major factors in the health of the society and *S. aureus* ranks the third as a factor involved in food borne diseases (Normanne, 2005). One of the major concerns of public health hazards in restaurants, hotels and various households is bacterial contamination of foods before and after prepara-

tion. Many diseases such as salmonellosis, tuberculosis, brucellosis, dysentery, shigellosis and other enteric diseases are transmitted from infected persons to susceptible people through contaminated foods and water (Hall et al., 2005; Arlington, 2007; Tauxe et al., 2010; Kirk et al., 2010; El-Kholy et al., 2014). Hence, it is of importance to monitor and determine the food safety risks along the entire food production chain. The retail outlets are the final point in the food production chain before the products reach the consumers. Monitoring microbial food safety risks at this level of food chain and ensuring safety is extremely important as the producers do not have any control on the product quality and safety once it is sold to consumers (Jawetz et al., 1991; Normanno, 2005). Foods commonly associated with staphylococcal food poisoning include protein foods such as ham, processed meats, chicken, milk, potato and creamed potatoes (USFDA, 2013). The restaurant business is lucrative in many parts of the World, as such in Nigeria more people have ventured into the business, but however, efficient regulatory agencies to monitor and assess the hygienic standards of these restaurants during or after preparation of soups are lacking or unavailable. Therefore, the patronage of these restaurants/food canteens puts the health of the populace at risk.

Staphylococcus aureus is the major cause of gastroenteritis resulting from consumption of contaminated food (Bergdol, 1989; Le Loir et al., 2003), and poisoning is due to the release of toxins in the food during its growth, causing symptoms ranging from abdominal pain to nausea, vomiting, and sometimes diarrhoea (Wieneke et al., 1993; Talaro and Talaro, 2002). *S. aureus* enterotoxins (SEs) involved in food poisoning are highly stable and resistant to neutralization by proteolytic enzymes, such as pepsin or trypsin (Bergdol, 1989; Akineden et al., 2001; Kerouanton et al., 2007). *S. aureus* is considered the most resistant of all non-spore forming pathogens, with well developed capacities to withstand high salt concentrations (7.5 - 10%), extremes in pH and high temperatures (up to 60°C for 60 min). It also remains viable after months of air-drying and resists the effects of many disinfectants and antibiotics (Talaro and Talaro, 2002).

In a study by Umoh and Odoba (1999), 6 (18.3%) of the 32 coagulase positive *S. aureus* tested produced enterotoxin A (SEA) and 50% were resistant to the common antimicrobial drugs used in the treatment of staphylococcal and wound infections. The main sources of contamination of these food are food-handlers by manual contact, coughing or sneezing since up to 50-70% of the human population are *S. aureus* carriers (Solberg et al., 2000; Vogel et al., 2001) and food contact surfaces (Montville et al., 2002; Sudheesh et al., 2013). Other sources include contamination from animal origins either by animal carriage or zoonosis (Le Loir et al., 2003; Umaru et al., 2012).

In Nigeria, several studies have confirmed the occurrence of *S. aureus* and SEs in various foods such as milk (Umoh et al., 1990; Umaru et al., 2012), street foods (Umoh and Odoba, 1999), 'Kunun-zaki' (local non-alcoholic beverage drink) (Oranusi et al., 2003), etc. However, literature is scarce on the occurrence in Taraba State, Nigeria. This study therefore, was aimed at providing information on whether *S. aureus* may be present in soups from restaurants/food canteens and some households during consumption, which may likely be due to post processing contamination. The study also provided the antibiotic resistant profiles of the isolated *S. aureus* and possible factors responsible for the contamination.

MATERIALS AND METHODS

Sample collection

This study was carried out in Jalingo Metropolis, Taraba State, Nigeria in 2011 and 2012 between the months of April and October. The restaurants/food canteens and some selected families were randomly selected within the populated areas including schools. These areas are: College of Agriculture, College of Education, Road Block, Barde Ward, GRA and Market. The participants were well educated on the reason and needs of taking and analyzing their soup samples. Each of the restaurant/food canteens as well as families within the selected areas was visited, where fresh soup samples were collected directly into sterile sample bottles prior to consumption. The soups collected comprised of 40 green leaves or spinach (Aleyehu), 40 sorrels (Yakuwa), 30 melons (Egusi), 30 stews and 40 "okra". The samples (from families) were refrigerated at 4°C while those from restaurants/food canteens were then placed on ice and transported to the laboratory where standard bacteriological examinations were conducted (Tamagnini et al., 2006).

Isolation of *Staphylococcus aureus*

All samples were inoculated onto the surface of the Baird Parker medium (Oxoid, Basingstoke, UK) supplemented with egg yolk and potassium tellurite by streaking (Ikeagwu et. al., 2008), in order to obtain colonies according to the protocol for testing of foods for *Staphylococcus aureus* (Tamagnini et al., 2006). The plates were then incubated aerobically for 24 h at 37°C, and finally observed for growth (Tamagnini et al., 2006).

Presumptive coagulase-positive *S. aureus* colonies appeared as black, grey or white and were surrounded by an opaque halo of precipitation which signifies the coagulase reaction (O' Brien et al., 2009). All the positive samples were further identified using colonial morphology, Gram' staining, and catalase, coagulase and motility tests (Ryan and Ray, 2004; Talaro and Talaro, 2002; Geidam et al., 2012).

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Table 1. Prevalence of *Staphylococcus aureus* in soup in Jalingo Metropolis, Northeastern Nigeria.

Location	No. of samples examined	No. of samples positive for *S. aureus*	Samples positive for *S. aureus (*%)
Families	100	36	36[a]
Restaurants	100	48	48[b]
Total	200	84	42

Values in the same column with different superscript differ significantly (P < 0.05).

Table 2. Isolation rate of *S. aureus* from different soup types in Jalingo Metropolis, Northeastern Nigeria.

Types of soup	No. of samples examined	No. of samples positive for *S. aureus*	Samples positive for *S. aureus* (%)
"Aleyehu"	40	26	65.0[a]
Bitter leaf	40	0	0.0
"Egusi"	30	12	40.0[b]
Okra	40	10	25.0[c]
Stew	30	11	36.7[d]
"Yakuwa"	25	23	57.5[e]
Total	200	84	42.0

Values in the same column with different superscript occurred significantly (P < 0.05).

Antimicrobial susceptibility tests

Antimicrobial resistance and susceptibility tests were carried out using the Kirby-Bauer disk diffusion method (Bauer et al., 1996), as recommended by Clinical Laboratory Standards Institute (CLSI, 2010). All the *S. aureus* isolates were grown in Brain Heart Infusion Broth (Biotech Laboratories, United Kingdom) and incubated at 37°C for 6 h when the turbidity of 0.5 McFarland standards was achieved. The isolates were then inoculated by spreading onto Muller Hinton Agar (Amershan, England) using cotton swabs, and the antimicrobial discs applied on the surface of the medium with a disc dispenser (Oxoid, Basingstoke, UK). The plates were then incubated at 37°C for 24 h. Zones of inhibition were observed, measured with a ruler and finally compared with the breakpoints provided by the CLSI (2010). The isolates were tested against a panel of nine (9) antibiotics with the following concentrations: Ampicillin (30 µg), Amikacin (30 µg), Chloramphenicol (12 µg), Ciprofloxacin (5 µg), Erythromycin (10 µg), Gentamicin (10 µg), Penicillin (10 µg), Sulphamethoxazole/trimethoprim (50 µg) and Tetracycline (30 µg). (Oxoid, England). *S. aureus* ATCC 3359 strain was used as a positive control.

Data analysis

Data obtained from the study were analyzed statistically using Statistical Package for Social Sciences (SPSS) Version 13 software. Frequencies were obtained and percentages for study variables were calculated. Chi-square at 5% level of confidence was used and compare between the variables, and significance was determined. A P value <0.05 was considered significant for all comparisons.

RESULTS

Of the 200 soup samples, 84 (42%) yielded positive culture of *S. aureus* (Table 1). The occurrence of *S. aureus* in soup samples from restaurants (48%) is significantly higher (P < 0.05) than that of the families (36 %).

Green leaf (Aleyehu) soup has the highest prevalence of 65.0%, followed by sorrel (Yakuwa) (57.5%), melon (Egusi) (40.0%), stew (36.7%), "okra" (25.0%) and bitter leaf (0.0%) (Table 2). Hence the prevalence in the four (4) soups occurred at a significant rate (P < 0.05). The antibiotic resistant profiles of the 84 *S. aureus* revealed that all (100%) the isolates were resistant to ampicillin and penicillin, 60 (71.4%) were resistant to tetracycline, 30 (35.7%) to erythromycin, 11 (13.1%) to sulphamethoxazole/trimethoprim, 10 (11.9%) to gentamicin and 5 (6.0%) to chloramphenicol (Table 3). None of the 84 isolates was resistant to amikacin and ciprofloxacin.

DISCUSION

In this study, isolation of 84 (42.0%) *S. aureus* sampled soups is of public health concern considering that soups were cooked and expected not to contain *S. aureus.* Data on the prevalence of *S. aureus* in foods in the study area is lacking as shown in the present study. However, this result is higher than the 15.0% prevalence of *S. aureus* reported by Umoh and Odoba (1999) in street foods in Zaria, Kaduna State, Nigeria and lower than the 54.0% reported by Suleiman et al. (2013) in chickens in Maiduguri, Nigeria. This could be due to the differences in the types of foods used for the study and the locations

Table 3. Antibiotic resistance profile of *S. aureus* isolated from soup served in restaurant/food canteens and some selected families in Jalingo Metropolis, Northeastern Nigeria.

Antibiotic	No. (%) resistance	No. (%) susceptibility
Amikacin	0 (0.0)	84 (100.0)
Ampicillin	84 (100.0)	0 (0.0)
Chloramphenicol	5 (6.0)	79 (94.0)
Ciprofloxacin	0 (0.0)	84 (100.0)
Erythromycin	30 (35.7)	31 (64.3)
Gentamicin	10 (11.9)	74 (88.1)
Penicillin	84 (100.0)	0 (0.0)
Sulphamethoxazole/trimethoprim	11 (13.1)	73 (86.9)
Tetracycline	60 (71..4)	24 (29.6)

of the study areas. Although, soups from restaurants/food canteens recorded the highest occurrence of the pathogen (48%) when compared with families, the occurrence in families was also significant ($P < 0.05$). The higher occurrence from this present study could be attributed to unhygienic practices during preparation and processing and also to a lesser extent, improper cooking of soup to high temperatures. Also, most of the families and restaurants depend on the commercial water vendors (popularly known as "Yan-Garuwa") for the supply of water. The "Yan-Garuwa" in Jalingo largely fetches water from streams and ponds from which water quality is compromised (This need to be verified). This could have served as means for the spread of *S. aureus* to the food environment. In less developed countries, improper sanitary conditions in the whole food production chain starting from primary production to the consumers and the occurrence of a wide range of food borne diseases create vulnerability in food safety (Arlington, 2006).

The results showed that spinach (Aleyehu) and sorrel (Yakuwa) soups recorded the higher occurrence of the pathogen (65.0 and 57.5%) than "egusi", stew and okra (25%) (40.0, 36.0 and 25%) but all occurred at a significant rate ($P < 0.05$). The reason could be that "Aleyehu" and "Yakuwa" do not undergo vigorous washing and prolonged cooking because they do not have any objectionable taste couple with the fact that they have very high content of water in them. So long period of cooking at high temperatures would make them overcooked and soft to the consumer, and hence may be unpalatable. Also "Aleyehu" and "Yakuwa" are mostly farmed around gardens (known as "lambu") where heavy contamination with human and animal faecal material exist. Hence high level of *S. aureus* was recovered from "Aleyehu" and "Yakuwa". This is not the case with the bitter-leaf soups examined in which none of the samples examined was positive for the pathogen. This could be due to active washing to remove the bitter taste prior to cooking. Hence the organisms were thus mechanically eliminated and any amount left after the washing was further subjected to the prolonged period of cooking at high temperature. This present study supports earlier studies that post processing contamination of foods by both humans and animals is possible and common (Umoh and Odoba, 1999). This may probably explain the total absence of the pathogen in the bitter leaf soup. Most of the organisms were isolated in soups obtained from populated areas like Road Block and Jalingo Main Market which because of the population, unhygienic practices such as indiscriminate dumping of refuse and faecal material is common. This served as breeding environments for flies which are known to be factor causing food contamination, coupled with the humid tropical weather of the study area. Hence, the environments where restaurants/food canteens are located and that of households may appear to be a contributing factor in food borne bacterial diseases such as staphylococcosis.

The antibiotic resistance profiles of the isolates showed that all the isolates (100.0%) were resistant to ampicillin and penicillin, while 71.4% was resistant to tetracycline. These are the antibiotics mostly used in Jalingo and environs for the treatment of bacterial infections in both humans and animals. However, very low resistance was recorded against chloramphenicol (6.0%), gentamicin (11.9%) and sulphamethoxazole/trimethoprim (13.1%). In contrast, all the isolates were susceptible to amikacin and ciprofloxacin. This may suggest that amikacin and ciprofloxacin could be the drug of choice in the treatment of *S. aureus* infection in the study area. This result is consistent with the findings of Suleiman et al. (2013) who also recorded a 100% resistance of coagulase-positive *S. aureus* against penicillin and ampicillin, respectively, in chickens in Maiduguri, Nigeria, and 100% susceptibility to ciprofloxacin.

The total resistance of the isolates to ampicillin and penicillin may suggest the high abuse of these antibiotics in the treatment of infections in the area. In contrast, the susceptibility to amikacin, ciprofloxacin, chloramphenicol, gentamicin and sulphamethoxazole/ trimethoprim may suggest that these antibiotics are less abused, and therefore the drug of choice in the treatment of staphylo-

coccal infection in the study area.

Conclusion

From the results obtained, it was concluded that soups served in restaurants/food canteens and some families in Jalingo Metropolis were highly contaminated with *S. aureus.* Contributing factors could be secondary contamination during preparation, processing and handling due to unhygienic practices.

RECOMMENDATIONS

1. Restaurants/food canteens proprietors and families should ensure adequate sanitary and hygienic measures during preparation of soups and foods meant for human consumption
2. Soups and other food items for human consumption should be properly cooked to higher temperatures in other to kill *S. aureus.*
3. Humans and animal faecal material should be disposed properly so that they do not contaminate streams and gardens (lambus) which are the water point for most of the "Yan-Garuwa" that provide water to the people in Jalingo Metropolis.
4. Water intended for cooking should be clean and free from *S. aureus*.
5. Sanitary officers or inspectors should be empowered to visit, monitor and inspect restaurants/food canteens and households to ensure all necessary sanitary procedures are strictly observed or adhere to.
6. Regulatory agencies like National Agency Food Drugs Administration and Control (NAFDAC) and Public Health Officers should also be inspecting restaurants and households for proper education on the public health implications of *S. aureus* and other bacteria.

Conflict of Interests

The authors have not declared any conflict of interests.

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