Journal of Applied Biosciences 46: 3133– 3143

ISSN 1997–5902

Characterization of *Staphylococcus aureus* isolated from street foods: Toxin profile and prevalence of antibiotic resistance

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Original Submitted In 8th September 2011. Published online at www.biosciences.elewa.org on October 28, 2011.

ABSTRACT

Objective: In this study, we characterized the toxins profile and occurrence of antibiotic resistance of *Staphylococcus aureus* (*S. aureus*) stains isolated from street foods in Cotonou (Benin).

Methodology and results: *S. aureus* was isolated on growth agar media and confirmed by Gram staining, catalase activity, and coagulation of citrated rabbit plasma. The leucotoxins and epidermolysins were identificated by radial immunoprécipitation. Enterotoxins and TSST-1 were assessed by agglutination method. The antimicrobial susceptibility was tested by diffusion method on Mueller Hinton agar. About 56.25% of food dishes analyzed were contaminated. They produce Panton and Valentine Leucocidin (13.33%) and various enterotoxins such as, the enterotoxins A (56.29%), B (37.77%), D (13.33%), the TSST (15.92%), the leucotoxin LukE/LukD (69.25%), and the Epidermolysin A (14.07%). About 15.18% of the *S. aureus* colonies were resistant to methicillin.

Conclusions and main findings: This study reveals a wide range of *S. aureus* food contamination producing various disease mediated toxins. Interestingly, we report here the presence of MRSA outside health centers or hospital environments. Our results suggest an urgent need to instruct/educate street food salesmen and the community on food hygiene to avoid food intoxication and subsequent diseases.

Keywords: *Staphylococcus aureus*, enterotoxins, leucotoxins, street foods, antibiotic resistance, Benin.

INTRODUCTION

The poverty level of community, the demographic expansion and a continuous urbanization factors in various developing countries have led to the emergence of a novel and most dominant form of restaurants known as “street foods”. Generally, street foods are readymade foods sold by salesmen or by peddlers moving from one location to the other (Canet, 1997). Due to its relatively low cost, street foods represent one of the most cost efficient forms of feeding among low paid public workers, students, craftsmen, and high school students (Chauliac et al., 1998). Unfortunately no hygienic standard is respected during handling or selling of foods.

The security of street foods depends on several factors including the quality of different utensils, the hygienic practices during handling of the foods, the conservation and distribution of the foods. Unfortunately, these hygienic factors are generally ignored in the selling process leading to diseases such as gastroenteritis and the diarrheas of microbial origins (Barro, 2000; Chauliac et al., 1998). Recently, the epidemiological data from various hospitals around the world showed a prevalence of 19% of diarrheal diseases, of which 20 to 70% were linked to lack of hygienic practices in street food handling. According to Chauliac et al. (1998), street foods are often exposed to dangerous environmental conditions such as presence of insects, flies, other animals and air pollution. In addition, food ingredients used in street foods are below standardized hygienic requirement. This situation is also aggravated by the use of low quality water, various non authorized additives/spices and poorly cleaned food packaging gadgets to mention but a few (Dawson et al., 1991). Moreover, the salesmen have no idea of good practices of food hygiene, exposing the foods to adequate conditions for all kinds of crossed contaminations (Sansonetti, 1987).

Various fatal diseases caused by street food intoxications have been lately reported (Barro et al., 2002). Interestingly, there have been ~300 to 350 more street food intoxications than generally reported (Dacosta, 1995). In reported street food epidemiology studies, *Staphylococcus aureus* is the most predominant virulent bacteria responsible for a wide range of human diseases (Lopez et al., 1993). *S. aureus* is the most predominantly virulent human staphylococcus pathogens causing a wide range of diseases (Wertheim et al., 2005). It represents the major causal agent of food intoxication through its enterotoxin products (Le Loir et al., 2003). In recent survey, bacterial infections represented more than 90% of street food contamination; of which and 75% were attributed to *S. aureus* infection alone (China, 2002). In France for instance, *S. aureus* was the second cause of collective food toxi-infections between 1996 and 2005 (Delmas et al., 2006). While the prevalence of bacterial infections and occurrence of antibiotic resistance has been well studied in animal nutrition (Le Hayes et al., 2003), there is elusive understanding of bacterial infection and antibiotic resistance profile of bacterial virulence in human nutrition (Johnston and Jaykus, 2004).

In this paper, we endeavored to isolate and study the toxicity profile as well as the occurrence pattern of antibiotic resistance of different *S. aureus* colonies isolated from street foods in different public places and streets of the city of Cotonou in Benin (West Africa). The ultimate goal was to provide valuable contribution towards establishing national hygienic norms for street foods in rural and urban communities of the city.

MATERIALS AND METHODS

Study area and typology of street food sellers: Five (5) districts namely Agla, Dantokpa, Gbegamé, Avotrou and Zogo in the city of Cotonou were concerning by the present study (Figure 1). Cotonou (6°20 N; 2°20 E) is in the southern Benin covering 79 Km^2. Food samples were collected from various public places and streets in districts cited above. A particular attention was focused on the environment and hygiene practices of the sellers. For the site of sample collections, we took into account: foods prepared by the sellers and brought to a fix located selling point (foods sold on the shoulders of streets without a proper designated selling point) and foods prepared within a restaurant like structure nearby public streets.

Food sampling: Food samples were collected based on the consumers' preference and their tendency to be easily contaminated. The samples were collected in Cotonou, the largest city of Benin, West Africa. Precaution was taken to avoid contamination during sample collection. Four (macaroni, salad, bean cooks in sauce and rice in sauce) most preferable dishes by the consumers were considered in this study. Per dish, 20 vendors (4 vendors per districts) of street food were randomly selected for the food sampling. Two (2) samples were collected per dish per vendor. That made 40 sampling per dish. A total of 160 samples were collected for the 4 dishes in the 5 districts. The collected samples were then packaged and immediately (at most 2 hours) transported under hygienic conditions at 4 °C to the laboratory for analysis. Microbial screening was immediately carried out or samples were kept at 4°C to be screened not later than 24 hours.

Isolation and identification of *S. aureus:* For the bacterial colony isolation serial dilutions of the samples were made and diluted sample (100µl) was streaked on Baird-Parker agar supplemented with egg yolk tellurite enrichment suspension (Difco, Detroit, MI, US) and incubated at 37°C for 48 hours as previously described (Speck, 1976). The physical identification characteristics of the bacterial colonies such as black, smooth, convex to uniform outline with one or two halos were recorded (Lancet and Bennett, 2001). *S. aureus* was confirmed by colonial morphology, Gram staining, catalase activity, and coagulation of citrated rabbit plasma (bioMe´rieux, Marcy l'Etoile, France) (Riegel et al., 2006).

Identification of *S. aureus* virulence factors

Identification of leucotoxins and epidermolysins by radial immunoprecipitation: The different leucotoxins (Panton-Valentine leucocidin [PVL], leucotoxins LukELukD and B (ETB) were evidenced from culture supernatants after 18 h of growth in yeast-Casamino acids-pyruvate (YCP) medium by Ouchterlony method also called radial gel immunodiffusion as previously described (Gravet et al., 1998). Specific anti-leucotoxins and anti-epidermolysin rabbit antibodies were used to detect the presence of respective toxins.

Identification of enterotoxins and TSST-1 by latex agglutination Test: The enterotoxins A (SEA), B (SEB), C (SEC), D (SED) and the Toxic Shock Syndrome Toxin (TSST-1) were assessed by a sensitive and specific semi-quantitative SET-RPLA™ (Passive Reversed Latex Agglutination) and TST-RPLA™ (Oxoid, Hampshire, England) detection kits respectively (Brett, 1998; Gravet et al., 1999). The test was performed in ELISA 96 well-microplates according to the manufacturer recommendation.

Antibiotic resistance profile of *S. aureus* colonies isolated from street foods: Antimicrobial susceptibility was tested by agar diffusion method on Mueller Hinton agar (Bio-Rad-Diagnostic Pasteur, Marnes la Coquette, France) as recommended by the National Committee for Clinical Laboratory Standards (NCCLS). Interpretation of antimicrobial susceptibility followed the recommendations of the Antibiogram Committee of the French Microbiology Society (http://www.sfm.asso.fr/). Evaluation of methicillin resistance was performed by plating the strains on buffered Mueller-Hinton (Bio-Rad-Diagnostic Pasteur, Marnes la Coquette, France) with NaCl 2% (wt/vol) at 37°C for 24 h. Fifteen antibiotics were tested: Penicillin G (PeG), Oxacillin (Ox), Vancomycin (VA), Teicoplanin (TEC), Fosfomycin (Fos), Rifampicin (RA), Fusidic acid (FA), Gentamycin (GM), Linezolid (LZD), Trimetroprim Sulfamethozolin (SXT), Ofloxacin (OXF), Pristinamycin (PT), Erythromicin (E), Tobramycin (T), and Chloramphenicol (C).

Statistic analysis: The statistical analysis was performed using Microsoft office Excel 2007 software. The T-test was used to determine the statistical significance of virulence factors via Epi Info 6 software versions 6.04 cfr January 1999.

RESULTS

Environmental characteristics of the street foods: The street food sellers generally used the sidewalk of streets together with other commercial kiosks (Figure 2). It is often easy to find the street food selling installations nearby stagnant waters (Figure 2A). In almost all street food installation, they are pills of garbage (Figure 2C) and other kiosks as depicted in Figure 2B. The foods to be sold are generally disposed on tables (Figure 2A) without any continuous re-heating system. In addition, the majority of food sellers served the customers with their hands without using sanitary disposable gloves. Having no cashier personnel, the food street sellers handled the food and the money (mostly dirty and germ carrier agents) with the same hands, increasing therefore the risk of food contamination. The dish washer systems are disposed in open air. They are notoriously dirty and the water used in cleaning and rinsing the plates are generally dirty as well (Figure 2D, E).

***S. aureus* identification and characterization from street foods:** *S. aureus* associated with the street food contamination are summarized in Table 1. Interestingly we found that macaroni and salad dishes were more contaminated than other dishes. As revealed in Table 1, the microbial contamination loads in macaroni and salad dishes were above the admitted norms. The reason of the high contamination of macaroni and salad is because is the fresh dishes. Furthermore, the prevalence of *S aureus* in the street foods was assessed (Table 2). The microbial screening showed that 56.25% of the food samples were contaminated by *S. aureus* (Table 2), and salad dishes were the most contaminated by *S. aureus* followed by macaroni dishes.

Prevalence of toxins produced by street food *S. aureus* colonies: As expected, various toxins have been identified in *S. aureus* colonies examined (Figure 3). Among the enterotoxins identified, the SEA (56.29%) and the SEB (37.77%) were predominantly produced. None of the colonies produced SEC toxin. The Panton and Valentine leucocidin (PVL), one of the most virulent factors of *S. aureus* was produced by 13.33% of all identified colonies. On the other hand, the leucotoxin Luk E/D was produced by the majority of *S. aureus* (69.25%) (Figure 4). However, none of the bacteria produced the epidermolysin B (ETB). Almost all bacterial colonies producing enterotoxin A (SEA) produced Luk E/D as well. Table 3 illustrates the distribution of toxin production according to the food sample varieties. Interestingly, the toxins follow no food variability pattern.

Resistance of *S. aureus* colonies to different antibiotics: We tested the resistance profile of the bacteria under different antibiotics. The results of the antibiogram profile are summarized in Figure 4. Our data revealed that 15.18% of *S. aureus* were resistant to methicillin. All isolated bacterial colonies are resistant to penicillin G. However, they were all sensitive to Vancomycin, Teicoplanin, ofloxacin and Linezolid. The bacterial colonies resistant to Methicillin were also resistant to Kanamycin, Gentamycin, Tobramycin and to Erythromycin. Our data showed that the bacteria were resistant to most of the antibiotics tested in this study.

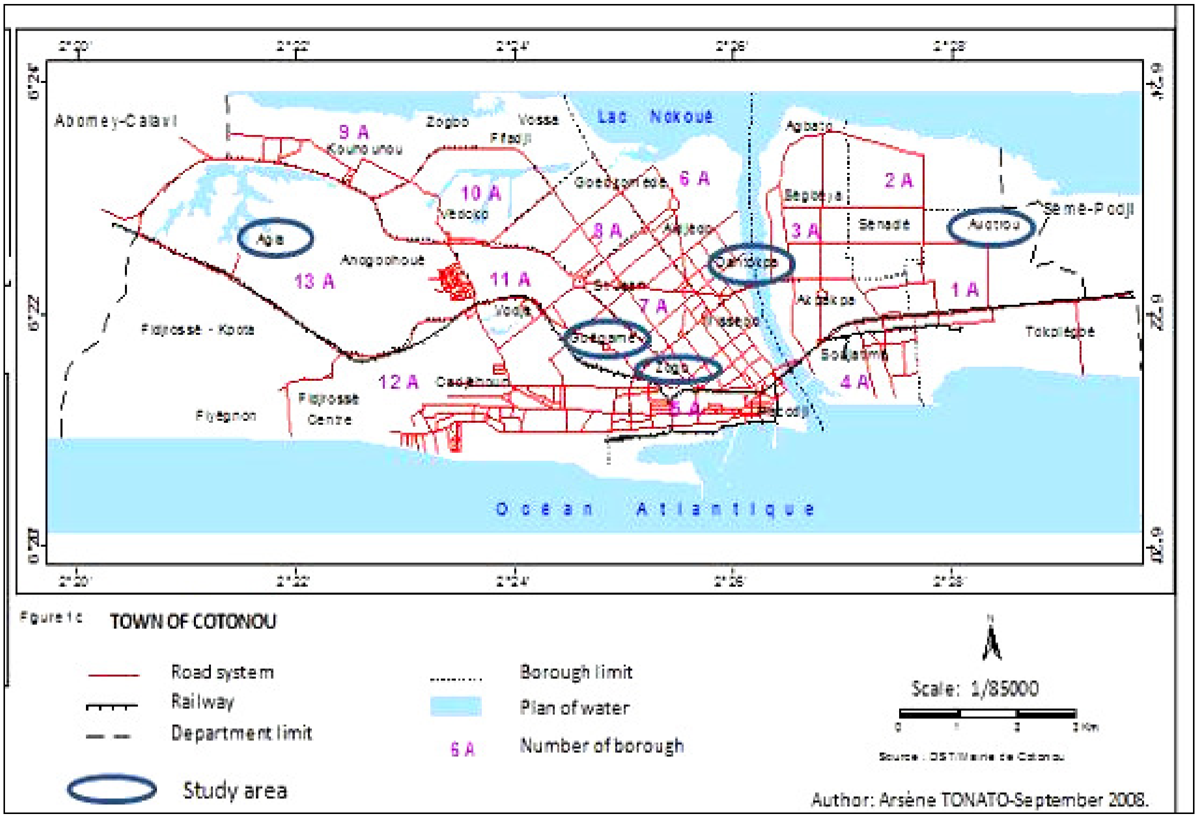

Figure 1: Map of Cotonou showing the study area

Figure 2: Characterization of the street food commercial area.
(A): General overview of the street food location showing polluted stagnating water. (B): Another selling kiosk of vulcanization near the street food. (C-D): The environment of dish washer and the dish cleaning water are depicted.

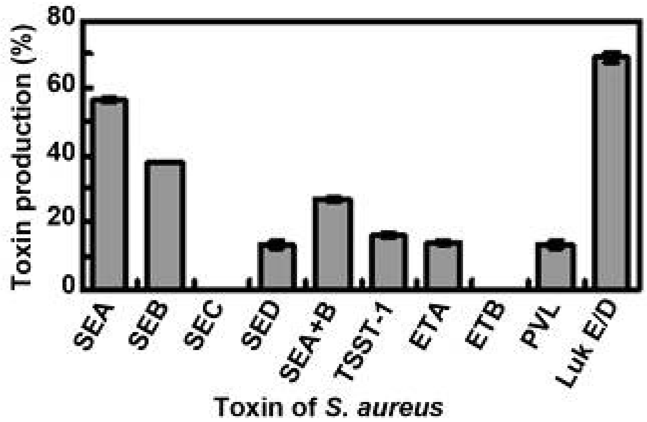

Figure 3: Toxin profile produced by *S. aureus* isolated from street foods

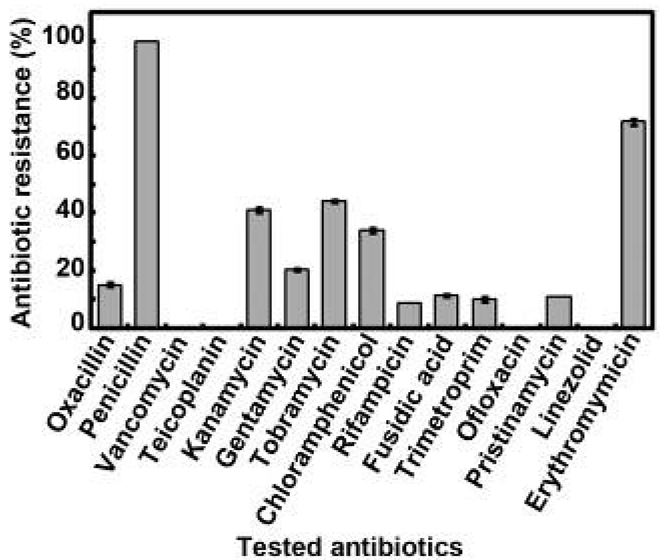

Figure 4: Antibiotic resistance profile of *S. aureus* isolated from street foods

DISCUSSION

This study revealed that street foods are mostly contaminated by *S. aureus* (Table 1), and consequently unsuitable for consumption. Recently, Mensah et al. (2002) showed that salad and macaroni dishes in Accra (Ghana) had unacceptably high levels of *S. aureus* contamination in the range of 3.7 and 4 log10 CFU/g respectively, while the bean dishes contained approximately 0.6 log10 CFU/g. Our results are in agreement with the data of Mensah et al. (2002) (Table 1, Table 2). Recently, Moushumi et al. (2007) and Barro et al. (2002) in Burkina-Faso confirmed the contamination trends of street foods in India and Burkina-Faso respectively, revealing a crucial need to educate street food sellers about basic hygienic behavior in order to keep the food save for consumption.

Table 1: Level of microbial contamination on street foods

Food variety	Food microflora*		*S. aureus**	
	Measured	Standard limit**	Measured	Standard limit**
Macaroni	6.37 ± 1.52	6.00	4.2 ± 1.68	3.0
Salad	6.42 ± 0.73	6.00	3.9 ± 1.42	3.0
Bean	5.97 ± 0.93	6.00	3.5 ± 1.01	3.0
Rice	5.61 ± 1.97	6.00	2.9 ± 0.55	3.0

* The values are measured in Log10 cfu/g of food.
**According to French Agency for Food Safety

Table 2: Prevalence of sample contaminated by *S. aureus* per type of street food

Food varieties	*S. aureus* contamination rate (%)
Macaroni	24/40 (60%)
Salad	34/40 (85%)
Bean	18/40 (45%)
Rice	14/40 (35%)
Total	90/160 (56.25%)

Table 3: Production profile of toxins in indicated street foods

Toxins	Macaroni	Salad	Bean	Rice	P-Value	Difference
SEA	58.33	58.82	44.44	57.14	0.9036	NS
SEB	50	47.05	22.22	14.28	0.2663	NS
SEC	0	0	0	0	0	-
SED	8.33	17.65	0	28.57	0.5152	NS
TSST-1	25	14.28	11.11	14.28	0.7662	NS
ETA	8.33	17.65	22.22	0	0.6616	NS
ETB	0	0	0	0	0	-
PVL	8.33	0	11.11	57.14	0.0288	S
LukE/D	75	76.47	44.44	71.47	0.3624	NS

S: Significant (P-Value <0.05); **NS**: Non Significant (P-Value > 0.05); (**-**): Not applicable

These polluted environmental conditions and the lack of hygienic practices of street food sellers easily attract flies, vectors of toxic microbial germs resulting to disease mediating vectors (Todd et al., 2007). Research evidence showed that a lack of keeping foods in appropriate temperature for long time represents one of the major factors of food toxi-infection (Roberts, 1982). Mensah et al. (2002) showed that 36% of food sellers used their hands to sell foods. This practice increases the contamination risks. Under this condition, the sellers are themselves carriers of pathogenic germs, because the bacterial enteropathogens can survive in the hands, under the fingers for more than 3 hours and be transmitted from mothers to children (Mensah, 1997). The bacterial colonies (*S. aureus*) are predominantly present on the mucous and human skin. Recent study demonstrated that 20% of collective nutritional intoxication cases resulted from food contamination or from people that have handled contaminated foods (Greig et al., 2007). Without a cashier in street food selling system, the food sellers are the cashiers (handling money) as well as the food servers. This observation has been reported by previous studies (Barro et al., 2000; Tjoa, 1977).

In this study, 56.25% of the dishes were contaminated by *S. aureus* (Table 2, Figure 3); while studies carried out in Ghana and Thailand showed 39.1% and 17.9% of dishes contaminated by *S. aureus* respectively (Fang et al., 2003; Mensah et al., 2002). Surprisingly we recorded 85% of contaminated salad dishes. This high level of contamination could not only be explained by handling the food with hands as above mentioned. We suspected that the hygienic practices related to cleaning processing, preparation, and packaging of the foods was simply ignored by food sellers. This is strengthened by the poor and unsuitable quality of water used to rinse, and clean the food serving accessories (Figure 2D, E). Our results agreed with the recent study of Moushumi et al. (2007) revealing that 86% of salad dishes were also contaminated with *S. aureus* in India.

In order to demonstrate that the contaminated foods could potentially lead to disease and seriously jeopardize human health upon consumption, we next endeavor to prove that the bacterial germs identified in the foods produced disease promoting toxins. As expected, our data revealed that the bacterial germs produced different type of toxins suggesting that the *S. aureus* are highly virulent to cause food mediating human disease upon consumption. In enterotoxins, SEA, were frequently identified in contaminated foods followed by SEB toxins. This observation has been also reported in previous studies (John et al., 2005; Tamarapu et al., 2001). These two classes of toxins were responsible for 80% of collective nutritional intoxication cases in France for instance. In the United States, the SEA is responsible for ~80% of food intoxications, while SEB is responsible for 10% of food contamination (Atanassova et al., 2001). The same observation was made in Miras Gerais in Brazil confirming that SEA and SEB were indeed the major toxins of food intoxications (Veras et al., 2007). In the city of São Paulo in Brazil, a recent study revealed that 41% of bacterial colonies produced SEA toxins, and only 7.7% of the colonies produced SEB toxins (Rall et al., 2008). In the present study, we discovered that SED toxin was produced by 13.33% of isolated bacterial colonies, which is in the range of SED value (12.8%) found by Rall et al. (2008). However, none of our bacterial colonies produced SEC toxins contrary to the findings of Rall et al. (2008) and those of Rosec et al. (1997) showing that 20.5 and 77% of colonies studied in Brazil and France produced SEC toxins respectively. This variability might be explained by differences in the origin of the colonies. In fact, Rosec et al. (1997) worked on meats demonstrated that foods/meats were contaminated with bacteria of human origins. It is therefore desirable to carry out additional bacterial biotype tests to determine/establish the origin of the bacterial germs identified in the study.

Our study showed that all colonies producing SEA produced also leucotoxin LukE/LukD. This is in agreement with our previous findings (Baba-Moussa et al., 2006) and explained the link between food intoxications and diarrheal diseases. Early studies have proved that LukE/LukD toxins produced by *S. aureus* are responsible for the outbreak of diarrheal diseases in France (Gravet et al., 1998). 80% of diarrheal diseases are associated to post-antibiotic treated *S. aureus*. The ETA toxin was produced by 14.07% of the bacterial colonies. However, none of them produced ETB toxin as previously reported (Baba-Moussa et al., 2010). Our data indicate the important role of *S. aureus* in the outbreak of diarrheas mediated by food contamination. Epidemiological links have been established between the outbreak of diarrheas and the consumption of street foods (Ericsson et al., 1980; Heinze and Yackovich, 1988; Mensah, 1997).

The antibiotic resistance profile revealed that *S. aureus* colonies were resistant to a wide range of antibiotics (figure 4). About 15% of *S. aureus* colonies were resistant to methicillin (Oxacillin), while only 4% of the same colonies found in Tunisia were resistant to methicillin (Ben Hassen, 2003). Likewise, about 3% of *S. aureus* isolated from animal derived foods (meats, milk, etc…) in different cities of Italy were resistant to methicillin (Corrente et al., 2007; Normanno et al., 2007). We found that 15% of colonies resistant to methicillin in our study are too high for the extra-hospital samples. However, an increasing presence of *S. aureus* has been reported outside hospital environment. Indeed reports of *S. aureus* resistant to methicillin (MRSA) from samples outside hospital environment have been lately more frequent (Mahoudeau, 1997). Until recently, MRSA and other antibiotics appeared rarely in samples collected outside hospitals or health care centers. Recent study of Centers for Disease Control and Prevention revealed that infections linked to MRSA, coming from samples outside the hospital environments have significantly increased (Fridkin, 2005). In addition, epidemiological screening of MRSA mediated diseases in the cities of Baltimore, Atlanta and State of Minnesota between 2001 and 2002 showed that 17% of community based colonies were resistant to antibiotics (Fridkin et al., 2005). From this community based *S. aureus*, 6% of them corresponded to invasive infections (meningitides, pneumonias, etc.) and 77% to coetaneous and skin infections. We therefore postulated that the street food sellers might also be vectors of *S. aureus* mediating street food contamination in Cotonou, Benin. It is therefore important to include an epidemiological study on the food sellers to systematically establish the sources of the contaminations and cross-link contamination. Interestingly none of the bacteria analyzed in this study was resistant to vancomycin, teicoplanin, ofloxacin and linezolid. This observation has been also confirmed by Ben Hassen (2003) indicating that the *S. aureus* identified in the street foods might not be responsible for the antibiotic resistance phenomena observed in human pathology.

In this study, all bacteria were resistant to Penicillin G against 64% of them resistant to the same antibiotic in previous study (Ben Hassen, 2003). Our data revealed that 71. 85% of bacteria were resistant to erythromycin, while none of the colonies analyzed by Ben Hassen (2003) was resistant to erythromycin. In addition, we noticed 20.37% of colonies resistant to gentamycin, 8.88% to rifampicin, 9.99% to trimetroprim-sulfamethozolin, 11.11% to pristinamycin, while none of bacterial colonies studied by Ben Hassen (2003) was resistant to the same above mentioned antibiotics. The occurrence of antibiotic resistance in this study might be explained by self-medication habit of the population in Cotonou, Benin, due to their level of poverty, and the fact that these antibiotics were sheep and can be bought without prescription. This practice enhances a frequent exposure of bacteria to antibiotics leading to a development of antibiotic resistance within the community.

CONCLUSION

This study highlights the toxicity and antibiotic resistance profiles of *S. aureus* isolated from streets foods in public places in Cotonou (Benin). This study reveals a wide range of *S. aureus* food contamination producing various disease mediated toxins. Interestingly, we report here the presence of MRSA outside health centers or hospital environments. Furthermore, the source of *S. aureus* contamination should be known in order to efficiently tackle the outbreak of bacterial toxin inducing diarrheal diseases. Due to the potential virulence of bacterial toxins and the antibiotic resistance of isolated *S. aureus*, it is evident that street foods might constitute a major problem for public health in Cotonou. However, the poverty level of the community has constrained the population to favor the street food form of alimentation. It is therefore important to educate the community and particularly the street food sellers about food quality and security.

CONFLICT OF INTEREST STATEMENT

None declared.

ACKNOWLOGEMENTS

The authors would like to acknowledge the International Foundation of Science (IFS) for financial support.

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