Original Research Article

Prevalence of *Staphylococcus aureus* isolated from some street Hot Beverages in Abidjan, Côte d'Ivoire.

ABSTRACT

Introduction: *Staphylococcus aureus* is one of the main pathogens found in street food, including hot beverages. However, information about *S. aureus* isolated from street hot beverages from coffee carts is very limited in Abidjan, Côte d'Ivoire.
Aims: We aimed to characterize phenotypically *S. aureus* isolated from street hot beverages sold in Abidjan.
Methodology:A total of 400 samples of hot beverage were collected and analyzed. The identification was made through conventional microbial and biochemical analysis. Macroscopic identification on the Baird Parker agar supplement with egg yolk tellurite. Microscopic observation through Gram staining as well as biochemical tests such as catalase; DNase and coagulase were performed. to confirm staphylococcal strains, the MALDI-TOF mass spectrometry was used. After identification, the susceptibility of the staphylococcal isolates was evaluated using disc diffusion method.
Results: Result showed that most of *Staphylococcus aureus* (18.4%)were isolated from tea.All the strains of *staphylococcus aureus* isolated from street beverages were sensitive to cefoxitin and vancomycin. All the *S. aureus* isolated from milk were resistant to Erythromycin. Although beverages are consumed hot, the presence of *S. aureus* in ready-to-drink beverage transmitted infections to consumers.
Conclusion: This drink need attention for the seller and the user to avoid some infections.

Keywords: Staphylococcus aureus,hot beverages, Susceptibility to antibiotics, food safety, Abidjan.

1. INTRODUCTION

Street foodshave easy access and generatejobsopportunities, especially for low- or uneducatedpeople[1]. The hot drink culture is highly developed in industrialized countries and constitutes an important part of street catering. The developing country use these types of drink, but severalstudies have shownthatmanufacturers of streetfoods use rawmaterials and ingredients of poormicrobiologicalqualityandnon-authorizedpersons are involved in thissector of activity[2-3]. Food safety remains a global concern despite efforts made by health and sanitation organizations [5]. Food contamination remains a real and permanent risk, especially in cities where this risk is closely linked to the consumption of ready to eat street foods [6-7]. Foodborne infections are reported worldwide, and it was estimates to be involved in more than 2 million deaths per year, mainly children [8], due solely to food and water contaminated by microorganisms. Across the world and specifically in Africa, several

countries have experienced episodes of food poisoning without scientifically proofed of food involvement. Several bacteria are involved in food poisoning [7, 9].

Staphylococcus aureus(*S. aureus*) is a ubiquitous commensal bacterium on human skins and anterior nares, but frequently causes severe infections in humans [10]. *S. aureus* has been described as one of the most common causal agents of food poisoning associated with the consumption of raw milk cheese and is also listed as one of the pathogens likely to pose the greatest risk to human health [11-13]. The presence of this pathogen in final products is related to several factors but is usually associated with the use of contaminated raw milk and endogenous starter cultures in manufacturing the cheese, as well as with asymptomatic human carriers of *S. aureus*[14-15]. Another serious public health problem associated with *S. aureus* in food is its resistance to antimicrobials because it triggers serious infections with limited therapeutic options [16-17]. Methicillin-resistant *S. aureus* (MRSA) is a leading example of bacterial resistance in the world [16] and is characterized by acquisition of the *mec*A gene, which is located on the chromosomal cassette *mec* (SCCmec) [18]. For many years, MRSA staphylococcal isolates have been recognized as pathogens unique to hospitals. However, studies have documented the spread of MRSA in foods [19]. The aim of this study was to investigate the presence of *S. aureus* in some hot beverages (coffee, tea and cocoa) and the antimicrobial resistance profile of staphylococcal isolates from those street hot beverages collected from Abidjan, Côte d'Ivoire.

2. MATERIAL AND METHODS

2.1. Study areas

The survey was carried out in five municipalities (Abobo, Adjamé, Yopougon, Cocody and Port-Bouet) of Abidjan to draw the characteristics of consumers of street Hot Beverages. Abidjan is located is located in the south of Côte d'Ivoire, and covers 57,735 ha^2.Abidjan had been divided into districts, arrondissements and delegations. The arrondissements corresponded to large groups, such as the Plateau, Treichville, or Adjamé, and their number increased from eight in 1967 to twelve in 1978 [20]. These districts are the basis of the delimitation of the perimeters of the ten autonomous municipalities which became the administrative units for the organization of the management of the city: Abobo, Adjamé, Attécoubé, Cocody, Koumassi, Le Plateau, Marcory, Port-Bouët, Treichville and Yopougon[21].

2.2. Survey

The survey was carried out in five municipalities (Abobo, Adjamé, Yopougon, Cocody and Port-Bouet) of Abidjan to draw the characteristics of consumers of street Hot Beverages.The criteria for including respondents were sellers and consumers who agreed to answer the questionnaires. Each consumer and seller were subjected to a series of questionnaires coupled with direct observations on hygiene. The questions relate to the behavior and preferences of the respondents as well as the health status linked to the consumption of coffee, tea and cocoa-based beverages at itinerant or fixed sellers and the consumption of these hot drinks at these sellers.

2.3. Sample collection

Per municipalities, three kinds of hot beverages (tea, coffee, and/or cocoa) were targeted. A total of 400 samples of hot beverage made of tea (139), coffee (138), or cocoa (123) were collected in sterile tubes (Table 1). The samples were collected hot in stomacher paper and transported immediately or within 2 hours in cooler to the laboratory for microbiological analyzes.

Table1. Distribution of samples collected according to the collected places and the kind of hot beverages

Communes	**Tea**	**Coffee**	**Cocoa**	**Total**
Abobo	28	26	23	77
Adjamé	28	26	26	80
Yopougon	27	29	27	83
Cocody	27	29	25	81
Port-Bouët	28	29	22	79
Total	138	139	123	400

2.4. Microbial Analysis

One (1) ml of each of the collected beverage samples was used for a decimal dilution. Each dilution (1 ml) was spread on Baird Parker agar (Biokar Diagnostics, France) supplemented with egg yolk tellurite [21]before its incubation at 37°C for 48 hours for presumptive identification of *Staphylococcus sp*. *S. aureus* referencing strain of *S. aureus* ATCC 25923 was used as a positive control. Macroscopic identification was made on Baird Parker agar supplemented with egg yolktellurite. Microscopic observation was performed byGram staining as well as biochemical tests such as catalase, DNase and coagulase and fermentation of mannitol.

2.5. Identification of isolates by MALDI-TOF MS

After isolation, the MALDI-TOF (Bruker Daltonics^{TM}) mass spectrometry was used to confirm the *Staphylococcus aureus*. Samples were covered with 1.5 µl of the matrix solution (Sigma, Lyon, France). After 10 min of sonication, a centrifugation (13000 g, 5min) was performed before transferring to a clean polypropylene tube. The target plate and matrix were then dried at room temperature before identification [22]. A positive control was used (*S. aureus* ATCC 25923).

2.6. Antibiotic susceptibility testing

Antibiotic susceptibilities were determined by the disc diffusion method on Mueller-Hinton agar (Oxoid, France) according standards and recommendations of the Antibiogram Committee of the French Society of Microbiology [23]. The antimicrobial tested and their concentrations on the discs of antibiotics (Bio-Rad, Marnes-LaCoquette, France) were as following: penicillin (PEN 10µg), amoxicillin (AMX 25 µg), cefoxitin (FOX 30 µg), tobramycin (TM 10 µg), gentamicin (GM 15 µg), erythromycin (ERY 15 µg), clindamycin (CMN 2 µg), levofloxacin (LVX 5 µg); norfloxacin (NOR 5 µg), ofloxacin (OFX 5 µg), vancomycin (VAN 30 µg). The reference strain *Staphylococcus aureus* ATCC 25923 was used as test control processed in the same conditions. Isolates were classified as susceptible or resistant to the drug.

2.7. Data Analysis

The data obtainedfrom the survey and observations checklists wereanalyzedusing the SPSS version 20.0 statistical software package, and thenexported to Microsoft Excel for scoring. Descriptive analyses wereused to summarize the variables of interest and determinerelationshipsbetweenthem. The resultswereexpressed as mean ± standard deviations (±SD), frequencies, and percentages. The Chi-squaredfrequency test (χ^2 test) wasused to test the relationshipsbetween the variables. Statisticallysignificantdifferenceswerebased on 95% confidence limits.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Probable origin of contaminants

The probable origin of contaminants in relation to hot beverage process are compiled in table 2. Thus, during hot beverage preparation, some contaminants occurred related to the process. Also, the highest factor risks were recorded through the treatment of products, materials or ingredients. The cooking and conservation process are also one of the determining factors linked to the beverage poisoning risks. The quality of water used to prepare and rinse recyclable cup could be a supplement contamination factor. Hot beverage contamination varied according to several factors.

Table 2: Probable risk related to beverage consumption sold by street vendors

Category		Probable contaminants level
Ingredients	Coffee, Cocoa, Tea	+
	Sugar, Lemon, Mint	+
Materials	Thermos for hot water, Spoon, Knife, Scissors	+
	Lemon squeezer	++
Others	Water used to prepare and rinse recyclable cups	++
	Disposable cups	+
	Recyclable cups	++
	Handling of products, materials or ingredients	+++
	Beverage cooking process	++
	Hygiene of vendors	++

+ Low risk, ++ Moderate risk, +++ High risk

3.1.2. Phenotypic Characterization and Species Identification

All staphylococcal isolates were confirmed as gram-positive cocci, catalase positive, and coagulase and DNase positive. Presumptive staphylococcal isolates were confirmed at the species level by proteomic analysis using MALDI TOF MS, validating identification of the species as *S. aureus*.The contamination prevalence of the sampled hot beverage was 8.5% and vary according to the kind of beverage (Table 3) and locality of collections (Table 4). According to the kind of beverage, the highest *S. aureus* contamination level was recorded with tea samples (18.4%), followed by cocoa's (8.7%) and coffee's (1.4%).

Table 3. distribution of *S. aureus* strains according to the sampled hot beverages

Beverages	Proportion of *S. aureus* % (n/N)
Tea	18.4% (26/138)
Coffee	1.4% (2/139)
Cocoa	8.7% (6/123)
Total	8.5% (34/400)

The Table 4 indicated that the most contaminated samples by *Staphylococcus aureus* were collected from Yopougon(15.7%) followed by Adjamé(13.7%). The lest contaminated commune was Cocody (1.2%).

Table 4. Distribution of *Staphylococcus aureus* in communes

Commune	*S. aureus* n (%)
Abobo	2 (2.6)
Adjamé	11 (13.7)

Yopougon	13 (15.7)
Cocody	1 (1.2)
Port-Bouët	6 (7.8)
Total	34 (8.5)

3.1.3. Antibiotic susceptibilities of *Staphylococcus aureus* isolated from street beverages

There is a variability of resistance profile of the isolated *S. aureus* strains (Figure 1). All the isolated strains from street beverages were sensitive to cefoxitin and vancomycin (Figure 1). In addition, most of *S. aureus* were sensitive to the aminoglycosides and quinolones tested.

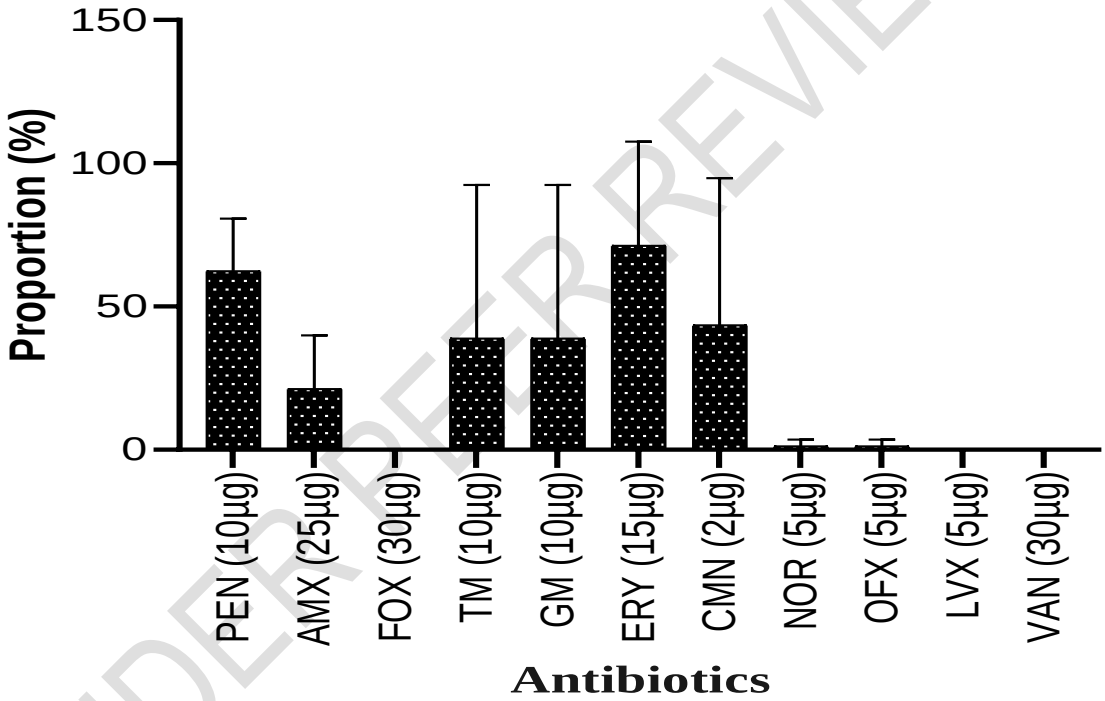

Figure 1. Global antibiotic susceptibility of *Staphylococcus aureus* isolated from street beverages

3.1.4. Distribution of *S. aureus* resistance according to the beverages

According to the kind of beverage, it is observed a variability of resistance profile (Figure 2).

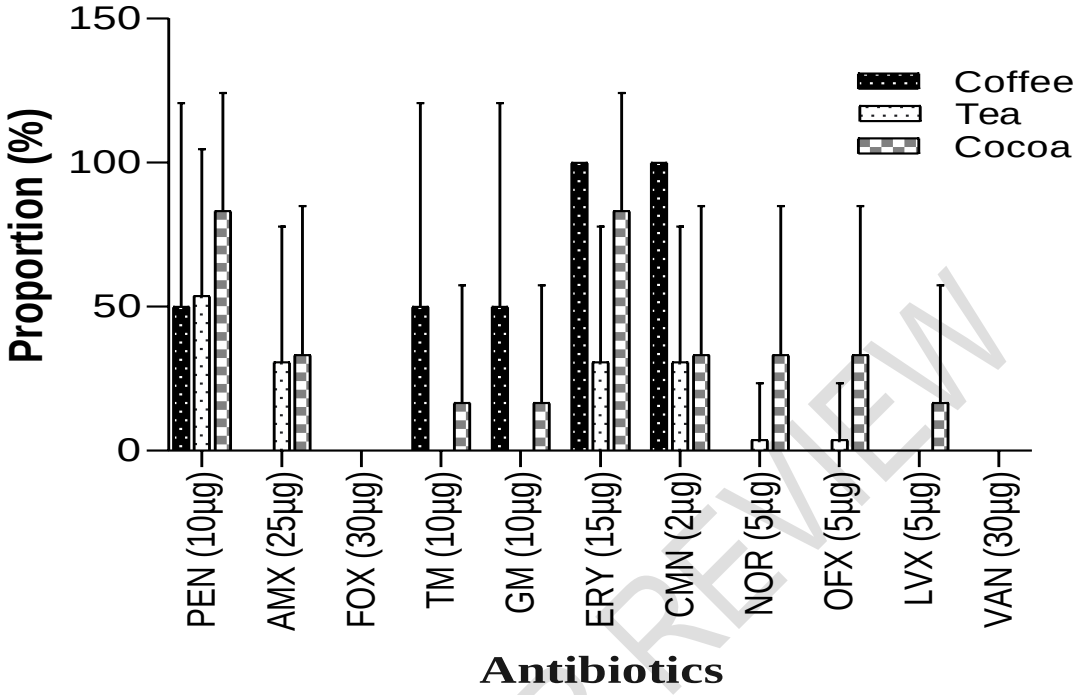

Figure 2. Distribution of isolated *S. aureus* resistance according to their origin

3.2. Discussion

For Lieberman *et al*. [24], foods and beverageswhich are prepared and sold on places like streets, market, festival areas and consumed on the run are known as streetfood. Street foodsespecially show the eating habits of people living in big citiessuch as Abidjan.Survey resultsshowedthat, majority of hot streetbeveragevendorswereforeigners (88.0%) and illiterate (71.3%). Ma *et al.*[25] reportedthatpreparing and sellingfood on the streetsprovide a constant income for millions of uneducated people. Regarding the employment, streetfoodprovides a good job opportunity and income for sellerswithsmallcapitals and especially for women[26-27]. In addition, Atobla*et al.* [28]showedthatvendors' knowledgeispoor and hadlowereducationlevels, whichisalsoreflected in theirlargelyinadequatefacilities and unhygienicbehaviorwhilesellingfood.

The strains of *S. aureus* were isolated from samples of hot drinks, on all the samples analyzed, with high frequencies in tea (67.3%), cocoa (44.6%) and coffee (34.6%). Hot drinks exposed to contamination by *S. aureus* represent a significant risk for human health, in fact this pathogen is supposed to have been eliminated by heat during treatment therefore its presence in hot drinks is generally indicative of bad hygienic conditions as highlighted [29]. These results are evidence of manual contamination. The spread of *S. aureus* by hot drink handlers is a concern and should be given careful attention in the manufacturing and sales chain.

The variation in the resistance to antibiotics of strains of *Staphylococcus aureus* isolated from hot drinks based on coffee, tea and cocoa is important because strains resistant to several antibiotics have been obtained. Resistance varies depending on the antibiotic used.

Note that all strains are sensitive to levofloxacin, norfloxacin, ofloxacin, ceftriaxone and vancomycin. These results have also been reported by Attien et al. [17]. In addition, certain antibiotics which were initially sensitive have increasingly become resistant. Variable resistance was observed depending on the origin of the strains to antibiotics such as penicillin and erythromycin, amoxicillin and gentamicin. This trend was obtained by Resch et al. [30], who showed that strains of coagulase staphylococci isolated from food had resistance to oxacillin. This is reported by Magiorakos et al. [31] who stated that today almost all strains of *S. aureus* have acquired resistance to penicillin. These results could be an indicator of a prophylactic route for the treatment of *S. aureus* infections. Penicillin was the first antibiotic discovered in 1929 by Alexander Fleming, *S. aureus* were sensitive at this time. Resistance to oxacillin indicates the presence of methicillin-resistant strains among the strains isolated. This would increase the risk of infection in the event of contamination by *S. aureus*methi- resistant which is very difficult to manage. However, additional tests are required toclarify the presence of this type of strain.

4. CONCLUSION

It is observed that street vendorspreferreddrinking coffee to boost of energy to sell. According to them, teawas the mostbeveragesconsumed by Ivoirians. The resultsfromthisstreetvendorsshowedthatmoststreetvendors are foreigners and illiterate. Thesestreetvendorsthereforeneed training in basic hygiene practice to avoidrisksssuch as food contamination or throwing plastic cups on the street. In addition, several bacteria were isolated; highlighting the potential risk incurred by compomers. The isolated bacteria are mostly multiresistant to conventional molecule antibiotics.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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