



## **Attieké street vendors: a potential source for dissemination of virulent strains of *Staphylococcus aureus* in consumers**

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### **ABSTRACT**

The *Attieké* is a typical Ivorian side dish made from cassava pulp and more than 34% of households in Abidjan consume *attieké* at least once a day with fish or meat. There is also a high consumption in street catering and before being served to customer, *attieké* is kneaded with hand by saleswomen in order to detach the granules. Our objective was to assess the presence of *S. aureus* on the hands of *Attieké* vendors and investigate for their virulence factors and antibiotics resistance profile. *S. aureus* strains were recovered from fifty-four street vendors of *Attieké* and screened by PCR to detected toxins genes (*LukS*, *EtA* and *tst*) and then tested for susceptibility against antibiotics by disc diffusion method. Of the 54 vendors, 42 (77.8%) were found to be harboring *S. aureus* in their hands. The genes *EtA*, *EtB* and *tst* were detected respectively in 2.4%, 4.8% and 2.4% *S. aureus* strains. None of strains contained *LukS* gene. All the strains were methicillin-susceptible *S. aureus* and resistant to penicillin and 61.9% of strains were multiple drug resistant. A high prevalence of antibiotics resistance and the presence of virulent *S. aureus* strains among street vendors represent a potential health hazard for consumers of *Attieké*.

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**Keywords:** *Attieké*, *S. aureus*, Staphylococcal toxins, food handler, Exfoliative toxins.

### **INTRODUCTION**

The *Attieké* is a typical Ivorian side dish made from cassava pulp (*Manihot esculenta* Crantz) that is a part of Côte d'Ivoire cuisine. It is cassava semolina fermented, partially dehydrated, steamed and agglomerated in appearance which is similar in texture to couscous (Gnagne et al., 2016; NI 484, 2013). In Abidjan, more than 34% of households consume *Attieké* at least once a day with fish or meat (Assanvo et al., 2006; Djeni et al., 2011). There is also a high

consumption of *Attieké* in Abidjan streets due to the rapid urbanization and the lower cost of this dish. Several authors have reported the high microbial contamination of *Attieké* produced and marketed in the informal sector and not always respecting the good hygiene practices and conservation. Previous studies (Kouamé et al., 2013; Yobouet et al., 2016) have revealed the presence of faecal coliforms, *Fusarium* species, *Bacillus cereus* group and *Staphylococcus aureus* in *Attieké* sold in Abidjan. The presence of these

bacteria in food occurs frequently due to inappropriate manipulation of food by carriers of this microorganism. For food poisoning outbreaks, *Staphylococcus aureus* carriers in food handlers were described as the major source of contamination by epidemiological and molecular data. *Staphylococcus aureus* is considered as a normal flora or commensal of the skin and nasal cavities of humans and various animal species (Grema et al., 2015). Previous studies have shown that 20-50% of the human populations are often colonized with *S. aureus* (Mat et al., 2014; Mat et al., 2017). Humans, food animals, food contact surfaces, and tools or environments can serve as vehicles for the transfer of *S. aureus* to foods (Chao et al., 2015). Food handlers contribute to food safety, being potential sources of bacteria that cause foodborne diseases due to the introduction of pathogens during its processing, distribution and manipulation (Rall et al., 2010). Food is a suitable growth environment for toxin-producing bacteria such as *Staphylococcus aureus*, which is able to grow and express its virulence in a wide variety of foods (Normanno et al., 2005, 2007; Adams and Moss, 2008). This species is one of the pathogens responsible for foodborne disease outbreaks worldwide (Li et al., 2015). Staphylococcal food poisoning is a major health problem (Kadariya et al., 2014) because *S. aureus* possesses an arsenal of virulence factors that contribute to evasion of host defenses. Important virulence factors implicated in the pathogenesis of *S. aureus* include staphylococcal enterotoxins (SEs), or enterotoxin-like proteins (SEL), exfoliative toxins, superantigen staphylococcal toxic shock syndrome toxin-1 (TSST-1), and Panton-Valentine Leucocidin (PVL), and other exoproteins are also responsible for particular clinical manifestations (Vancraeynest et al., 2006 ; Aung et al., 2017; vitale et al., 2018). As years pass by, *S. aureus* has developed resistance towards antibiotics, starting from penicillin, then methicillin and more recently towards vancomycin. The rise of drug-resistant virulent strains of *Staphylococcus aureus*, particularly

methicillin-resistant *S. aureus* (MRSA) is a serious problem in the treatment and control of Staphylococcal infections. Methicillin-resistant Staphylococci (MRS) cause hard-to-treat infections. The most striking situation is that MRSA strains have emerged with concomitant resistance to many commonly used antibiotics of groups, aminoglycosides, macrolides, fluoroquinolones, chloramphenicol, and tetracycline (Kaur and Chate, 2015). MRSA can be easily transmitted from person to person either by direct contact with the person who is infected, a carrier or by touching contaminated things or substances. The carriage of enterotoxigenic *S. aureus* by food handlers is an important source of staphylococcal food contamination in restaurants and fast food outlets (Colombari et al., 2007). Therefore it is important to screen for *S. aureus* carriage among food handlers in order to ascertain the epidemiology, pattern of antibiotic susceptibility and to prevent possible food contamination by them that may result in food poisoning. This will also help develop preventive measures as well as treatment possibilities (Emeakaroha et al., 2017). Food handlers may constitute a reservoir of virulent strains of *S. aureus* and may be vehicles of their transmission to food. In fact, food poisoning outbreaks associated with post-process contamination of foods with *S. aureus* are in part the responsibility of food handlers who carry enterotoxigenic staphylococci in their nares or on their skin (Castro et al., 2016). The aim of this study was to determine the prevalence of *S. aureus* in hands of Attiéké street vendors and then investigated for their virulence genes and antibiotics resistance.

## MATERIALS AND METHODS

### Study area

The study was carried out at Abidjan, a city on the southern Atlantic coast of Côte d'Ivoire, in West Africa. It is the economic capital and the country's major urban center. It is one of the areas of high production and consumption of Attiéké. The daily production of freshly Attiéké reaches over 100 tons for artisanal sector. Most of these attiéké are

processed by small scale producers, thus making quality control difficult (Djeni et al., 2011). The annual consumption of attiéké was estimated to 450000 tons (Yobouet et al., 2016).

### Sample collection and bacterial isolation

This study was conducted from October 2017 to march 2018 in two (2) sites of Yopougon a municipality of Abidjan: Adiopodoume and Lievre-rouge. Street retail *attiéké* vendors were selected based on their willingness for participate to this study. In these street restaurants, the *attiéké* dish is served with raw vegetables and fried fish on the roadside. The *attiéké* before being served to consumer is kneaded with fingers (without gloves) by the vendor to detach the granules. A total of 54 vendors were selected in this study especially at Adiopodoume (n=29) and Lievre-rouge (n=25). Samples were collected from the hands of vendors according the imprint method described by Pittet et al., (1999). The imprint of the 5 fingertips of the vendor's dominant hand on Chapman plates was taken in the sales site of *attiéké*. Fingertips were gently pressed for 5 seconds. As soon as possible (until 1 hour) after the sampling, Chapman plates were transported (+4 °C) and incubated at 37 °C for 24 hours in the laboratory. After incubation, suspect colonies (golden or yellow) showing the typical features of staphylococci were identified by standard microbiologic procedures including Gram staining, catalase activity, mannitol fermentation and the ability to coagulate rabbit plasma (Amini et al., 2012).

### Amplification of *16SrRNA*, *FemA*, *LukS*, *EtA*, *EtB* and *tst* genes

After Overnight culture of bacterial strains in Brain-Heart Infusion, cells were harvested at 12000 rpm for 3 min. Then, DNA was extracted by using Qiagen DNA isolation kit (Hilden, Germany) according to the manufacturer's instructions.

Genomic DNA was used as a template for polymerase chain reactions (PCR) amplification. According to modified methods

of multiplex PCR previously described by Kacou et al. (2011), six genes were screened in two multiplex PCR. The first multiplex PCR was performed to identify the *Staphylococcus* genus (*16S rRNA* gene), to differentiate *S. aureus* from Coagulase-negative *Staphylococci* (CNS) (*FemA* gene), and to detect Panton-Valentine leukocidin toxin (*LukS*) genes simultaneously. This first PCR was performed in a final volume reaction of 25 µL containing 9.75 µL nuclease-free water (Ambion), 5 µL PCR buffer (5X), 1.5 µL Magnesium Chloride (MgCl<sub>2</sub>) (25 mM) (Promega Corporation, Madison, USA), 0.5 µL Deoxynucleotide Triphosphates (dNTPs) (10 mM), 0.5 µL of each primer (20mM) (Table 1), 0.25 µL Go Tag®G2 Flexi DNA polymerase 5 U/µL (Promega Corporation, Madison, USA) and 5 µL of template DNA. The PCR method was performed according to the following program: initial denaturation (94 °C, 5 minutes), 35 cycles each composed of initial denaturation (94 °C, 30 seconds), primer annealing (60 °C, 1 minute) and extension (72 °C, 1 minute) and a final extension (72 °C, 5 minutes). A previously known (Kacou et al., 2011) *LukS* gene positive *S. aureus* strain was used as a control strain. The second multiplex PCR was performed to detect staphylococcal exfoliative toxin genes (*EtA* and *EtB*) and staphylococcal toxic shock syndrome 1 (*tst*) gene in the same conditions than the first multiplex. The difference was the PCR program: initial denaturation (94 °C, 5 minutes), 35 cycles each composed of initial denaturation (94 °C, 30 seconds), primer annealing (55 °C, 1 minute) and extension (72 °C, 1 minute) and a final extension (72 °C, 5minutes). All PCR amplification products were revealed on a gel Doc EZ® imager (Bio-Rad) after electrophoresis in 2% agarose gel containing Syber safe (Invitrogen).

### Antimicrobial susceptibility test

The susceptibility testing was carried out by culturing *S. aureus* strains on Mueller-Hinton agar (Bio-rad, Marne-la-coquette, France) in accordance with the performance standards for antimicrobial susceptibility

**Table 1 :** The primer sequences.

Genes	Primers	Primer sequences (5'-3')	Size of amplified products (bp)	References
<i>16S</i>	16S rRNA-F	GCAAGCGTTATCCGGATTT	597	Al-Talib et al. (2009)
<i>rRNA</i>	16S rRNA-R	CTTAATGATGGCAACTAAGC		
<i>LukS</i>	LukS-F	CAGGAGGTAATGGTTCATTT	151	
	LukS-R	ATGTCCAGACATTTTACCTAA		
<i>FemA</i>	FemA-F	CGATCCATATTTACCATATCA	450	
	FemA-R	ATCACGCTCTTCGTTTAGTT		
<i>EtA</i>	etA-F	CTAGTGCATTTGTTATTCAAGACG	119	Becker et al. (1998)
	etA-R	TGCATTGACACCATAGTACTTATTC		
<i>EtB</i>	etB-F	ACGGCTATATACATTCAATTCAATG	262	
	etB-R	AAAGTTATTCATTTAATGCACTGTCTC		
<i>tst</i>	tst-F	AAG CCC TTT GTT GCT TGC G	445	
	tst-R	ATC GAA CTT TGG CCC ATA CTT T		

testing, recommended by the Committee of antibiogram of French Society of Microbiology (CA-SFM/EUCAST, 2016). Antibiotics used for susceptibility testing included penicillin 6 µg, cefoxitin 30 µg, ciprofloxacin 5 µg, ofloxacin 5 µg, norfloxacin 5 µg, kanamycin 30 µg, gentamicin 10 µg, tobramycin 10 µg, erythromycin 15 µg, clindamycin 2 µg, minocyclin 30 µg, tigecyclin 30 µg, tetracyclin 30 µg, chloramphenicol 30 µg, fosfomicin 10µg, fusidic acid 10 µg, nitrofurantoin 100 µg and rifampicin 5 µg. All antibiotics were obtained from Bio-rad. It should be indicated that the reference strain *Staphylococcus aureus* ATCC 29213 were used as positive-control and provided by the National Reference Center for Antibiotics, Pasteur Institute of Côte d'Ivoire.

## RESULTS

### Prevalence of *S. aureus* hand carriage among attiéké vendors

All the 54 vendors of attiéké in this study were female. *S. aureus* strains isolated

were identified by Gram-positive cocci, catalase positivity, mannitol fermentation, coagulase production. However, strains that were Gram-positive cocci, catalase positive, and coagulase negative were considered as coagulase-negative Staphylococci. This phenotypic identification was confirmed by PCR results (Figure 1). Of the 54 vendors of attiéké, 48 (88.9%) had *Staphylococci spp* in their hands. Among 48 *Staphylococci spp* isolated, 42 strains (87.5%) were *Staphylococcus aureus* (*16SRNA* positive and *femA* positive) and 6 strains (12.5%) were Coagulase-negative Staphylococci (*16SRNA* positive and *femA* negative). The prevalence of *S. aureus* hand carriage in vendors of attiéké was 77.8% (42/54).

### *Staphylococci* hand colonization associated with sites of sale

The *Staphylococci* strains were isolated in the hands of vendors of Adiopodoume and both Lievre-rouge vendors. Prevalence of *S. aureus* colonization among attiéké vendors of Adiopodoume was 75.8% (22/29) and 80%

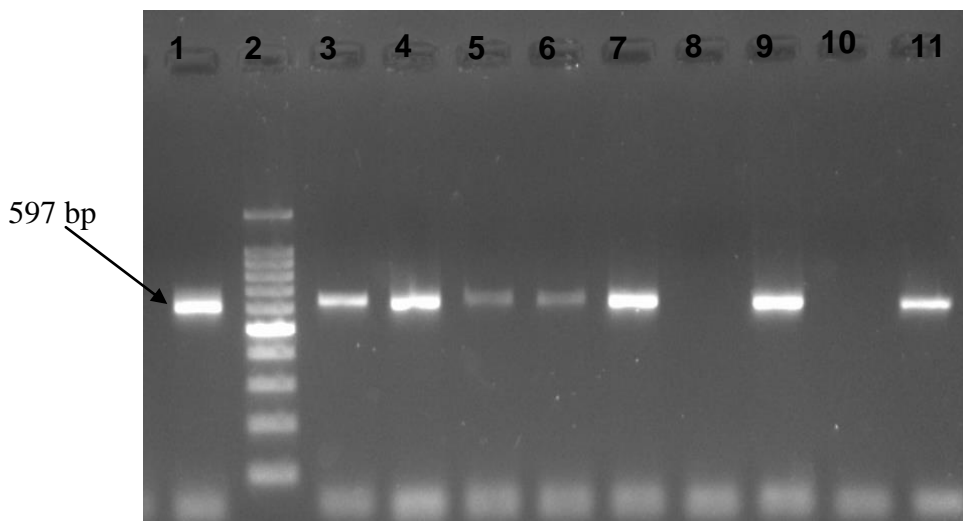
(20/25) among vendors of Lievre-rouge site (Table 2). There is no significant difference of the prevalence of *S.aureus* hand carriage between vendors of Adiopodoume and Lievre-rouge ( $P= 0,487$ ;  $P > 0, 05$ ).

#### Virulence genes detected among *S. aureus* strains

Four *S. aureus* strains (9.5%) carrying virulence genes (*EtA*, *EtB* and *tst*) (Figure 2 and 3). *EtB* was the most prevalent gene in strains recovered from hands of attiéké vendors. None Panton-Valentine Leukotoxin-encoding gene (*LukS*) was detected. In 42 *S. aureus* strains, genes encoding for Staphylococcal exfoliative toxins *EtA*, *EtB* and staphylococcal toxic shock syndrome 1 *tst* were detected in 2.4% (1/42), 4.8% (2/42) and 2.4% (1/42) of *S. aureus* strains respectively (Table 3). The majority of virulence genes was detected among hand *S. aureus* carriage by vendors of Lievre-rouge site (3/4).

#### Antibiotics susceptibility of *S.aureus* strains isolated from vendors hands

The result of the antimicrobial sensitivity pattern of 42 *S. aureus* strains from hands of attiéké vendors shows that, all strains (100%) were susceptible to ceftioxin and gentamicin, but resistant to penicillin (Table 4). The frequency of strains resistant to fosfomycin, fusidic acid, tetracyclin, minocyclin, rifampicin, chloramphenicol, clindamycin and erythromycin was 78.6%, 61.9%, 54.8%, 52.4%, 38.1%, 35.7%, 31% and 35.7% respectively. The lower rate of resistance was observed for ofloxacin (16.7%), kanamycin (14.3%), tobramycin (9.5%), ciprofloxacin (9.5%), nitrofurantoin (7.1%) and tigecyclin (4.8%). Multiple Drug Resistant (MDR) organisms are microorganisms resistant to three or more classes of microbial agents. In this study, 61.9% (26/42) of *S. aureus* strains carriage by food vendors were MDR.



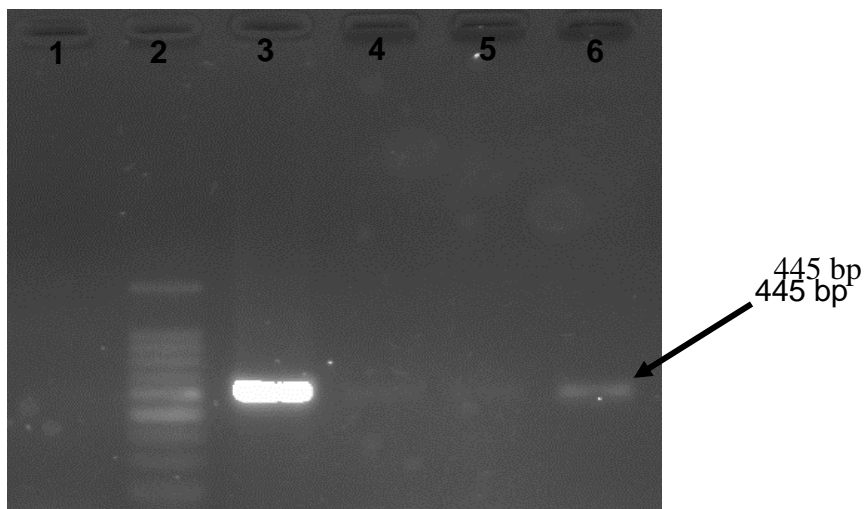
**Figure 1:** PCR amplification for detection of *16SRNA* gene among *S. aureus* strains from attiéké vendors.

Lane 1: Positive control (*16SRNA*+); Lane 2: 100 bp DNA ladder ; Lanes 3-7 and 9, 11: *S. aureus* strains positive for *16SRNA* gene.

**Table 2:** Prevalence of *Staphylococci* colonization among the attieké vendors by place of sale.

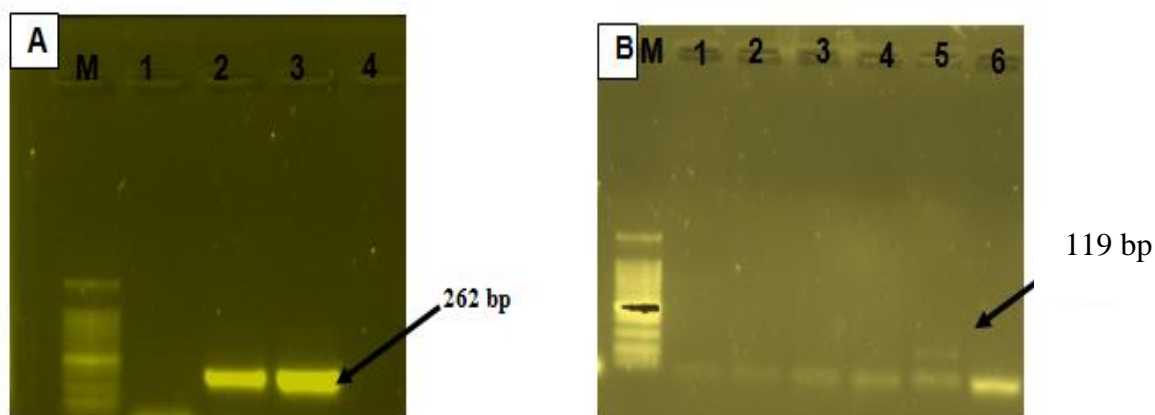
Attieké vendors by site (n=54)	Number of <i>Staphylococci</i> hands carriage	
	<i>S. aureus</i> (%)	CNS (%)
Adiopodoume (n=29)	22 (75.8%)	5 (17.2%)
Lievre-rouge (n=25)	20 (80%)	1(4%)

CNS : Coagulase-negative *Staphylococci*.



**Figure 2:** Electrophoresis result of *tst* gene.

Lane 1: Negative control (*tst*-); lane 2: 100 bp DNA marker; lane 3: Positive control (*tst*+); lanes 6: *S. aureus* strains positive for *tst* gene.



**Figure 3 (A & B):** Electrophoresis result of *EtA* and *EtB* genes.

(A): Lane M: 100 bp DNA marker; Lanes 2-3: *S. aureus* strains positive for *EtB* gene; (B): Lane M: 100 bp DNA marker; Lanes 5: *S. aureus* strain positive for *EtA* gene.

**Table 3:** Virulence genes detected among hand *S.aureus* strains.

Sites	Number of virulence genes positive <i>S. aureus</i>			
	<i>LukS</i>	<i>EtA</i>	<i>EtB</i>	<i>tst</i>
Adiopodoume Strains	0	0	1	0
Lievre-rouge Strains	0	1	1	1
Total (n=42)	0	1 (2.4%)	2 (4.8%)	1(2.4%)

**Table 4:** Antibiotics resistance of *S. aureus* strains recovered from hands of food vendors.

Antibiotics	Number (%) of resistant strains	
	Resistance (n=42)	Percentage %
Penicillin	42	100
Cefoxitin	0	0
Ciprofloxacin	4	9.5
Ofloxacin	7	16.7
Norfloxacin	3	7.1
Kanamycin	6	14.3
Gentamicin	0	0
Tobramycin	4	9.5
Erytromycin	15	35.7
Clindamycin	13	31
Minocyclin	22	52.4
Tigecyclin	2	4.8
Tetracyclin	23	54.8
Chloramphenicol	15	35.7
Fosfomycin	33	78.6
Fusidic acid	26	61.9
Nitrofurantoin	3	7.1
Rifampicin	16	38.1

## DISCUSSION

Food handler may be a vector of foodborne disease spreading, due to inadequate personal hygiene or cross contamination (Bass et al., 2006). In our study, the carriage of potential pathogens in the hands of attieké vendors was 88.9% out of which 87.5% was *S. aureus* and 12.5% was Coagulase-negative Staphylococci. In our study, the carriage of potential pathogens in

the hands of attieké vendors was 88.9% out of which 87.5% was *S. aureus* and 12.5% was Coagulase-negative Staphylococci. Our findings are in line with those of several authors who have studied the presence of *S. aureus* in the hands of food handlers. Previous study conducted by Djeni et al. (2014) showed the highest level of contamination of attieké handlers (producers) by *S.aureus* in Abidjan (60%), Dabou (73.6%) and Jacqueville

(93.3%). Various species such as *S. aureus*, *Klebsiella pneumoniae*, *Enterobacter agglomerans*, *Citrobacter youngae*, *Klebsiella oxytoca* and *Citrobacter freundii* were isolated with high loads in this study. In a food company in Portugal (Castro et al., 2016) a lower prevalence of *S. aureus* was found on the hands (11.1%; 18/162) of food handlers. However, our rate is lower than those reported from Woh et al., (2017) among food handlers in Malaysia where 63.4% of hand swabs tested were positive for *Staphylococcus aureus* (Woh et al., 2017). *Staphylococcus aureus* was the predominant bacteria isolated from fingernail specimens (46%; 63/137), among food handlers in the north of Iran (Nasrolahei et al., 2017). In addition, a previous studies (Djeni et al., 2011 ; Kouamé et al., 2013) conducted on attiéké marketed in Côte d'Ivoire reported a contamination by *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Citrobacter freundii*, *Enterobacter amnigenus*, *Citrobacter youngae*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Enterobacter agglomerans* and *Klebsiella oxytoca*. The presence of such microorganisms in attiéké samples could be due to an after steaming contamination by the contact of the product with air, the product handling and the plastic bag used and lack of hygiene practices during packaging, the use of unhygienic utensils but also by poor personal hygiene of attiéké handlers. A study carried out by Attien et al., (2014) on meat sold in Abidjan streets, showed the link between food contamination by *S. aureus* (19.79%) and handlers or the unsterile containers. Moreover, there is no significant difference between prevalence of *S. aureus* hand carriage in the 2 sites of sampling (Adiopodoume and Lievre-rouge). In the two sites, the street vendors had the same bad hygiene practices. During the sale, saleswomen use hands to serve customers. They do not wash their hands before and after serving customers and use an unhygienic cotton towel to wipe their hands. Indeed, the places of attiéké sale of the 2 sites (Adiopodoume and Lievre-rouge) are located near the roads, in an environment influenced by pollution and heavily overloaded by fumes and exhaust gases from vehicles. The sellers of the Lievre-rouge site are located near a

very busy market frequented by the population of Yopougon, with open gutter, poultry sales areas, cuts of beef and mutton and chickens. The majority of these vendors are close with other types of activity. Some attiéké sellers are installed right next to garbages and sell practically in the open air. In fact, food poisoning outbreaks associated with post-process contamination of foods with *S. aureus* are in part the responsibility of food handlers who carry enterotoxigenic staphylococci in their nares or on their skin (Lues et al., 2007). With regard to virulence genes, the prevalences of *EtA*, *EtB* and *tst* genes among *S. aureus* were low (2.4%, 4.48%, and 2.4% respectively). In the study conducted by Argudín et al. (2012), in Spain, *S. aureus* isolated from foods and food handlers contained exfoliatin genes *EtA* (6.5%), *EtB* (3.2%) and *tst* gene (25.8%) with prevalence higher than those detected in our study. *tst* genes have been previously (Castro et al., 2016) detected in *S. aureus* isolates recovered from food handlers, in higher percentage (8.7%) than in our study. The detection of *tst* gene among hand of vendors is a major hazard to costumers, because this gene was the first marker identified for Staphylococcal toxic shock syndrome (TSS), which is an acute and potentially fatal illness that is characterized by a high fever, diffuse erythematous rash, desquamation of the skin one to two weeks after onset (if not fatal before this time) and hypotension (Grumann et al., 2014). Several studies have revealed exfoliative toxin production by *S. aureus* strains isolated from foods sold in the streets. Sina et al. (2011) have detected *S. aureus* producing exfoliative A toxin (*EtA*) from food handled such as Macaroni (8.33%), salad (17.65%), bean (22.2%), sold on the streets of Cotonou in Benin. Exfoliative toxins (also known as “epidermolytic” toxins) are responsible for the clinical manifestation of staphylococcal scalded skin syndrome. It is primarily characterized by skin exfoliation, early with fever, malaise, lethargy, and poor feeding. These symptoms are followed by an erythematous rash and the formation of large, fragile, fluid-filled blisters. (Bukowski et al., 2010). The prevalence of *EtA* does not differ significantly among methicillin-resistant (MRSA) and methicillin-susceptible (MSSA)



strains. The presence of these toxins genes among isolates in the Abidjan food vendors represents a potential health hazard for consumers of Attiéké. Antimicrobial susceptibility of isolates shows that all the isolated *S. aureus* were susceptible to cefoxitin and gentamicin, but resistant to penicillin. The strains susceptible to cefoxitin were classified as those strains methicillin-susceptible. In this study, 100% of *S. aureus* strains were methicillin-susceptible *S. aureus* (MSSA). The frequency of others antibiotics resistance among *S. aureus* isolated in this study was ranging from 4.8% to 78.6% and 61.9% of strains were MDR. According to Udo et al. (2009), food handlers represent a section of the healthy population in the community, and the detection of high prevalence of antibiotic resistance in *S. aureus* isolated from them also highlights the growing problem of antibiotic resistance in the community.

### Conclusion

Street food vending has become an important public health issue and a great concern to countries of sub-Saharan Africa as Côte d'Ivoire, due to widespread food borne diseases, due to the mushrooming of wayside food vendors who lack an adequate understanding of the basic food safety. This study showed the presence of *S. aureus* carried several virulence genes and resistant to antibiotics in food handlers. The presence of these strains in the food chain poses a threat to public health, since the strains were from the healthy vendors and deserves further attention. Adherence to strict hand washing protocols before and after touching the food will definitely bring down the carriage of toxinogenic *S. aureus* in these vendors there by bringing down its infections rates in consumers.

### COMPETING INTERESTS

Authors disclose any commercial associations that might create a conflict of interest in connection with this manuscript.

### AUTHORS' CONTRIBUTIONS

SMK-S is the instigator of this work and oversaw the overall work and the manuscript writing. OK has carried out

laboratory tests and data management; FK has carried out antibiotics susceptibility tests; DCN provided guidance in laboratory analysis and manuscript editing. KSK, SN-K and AD have participated in the manuscript correction and data analysis.

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