Microbiology and Nature
Volume 1, Issue 4 pages 120-129
ISSN 2664-388X
https://doi.org/10.26167/BMVH-0739

Microbiol. Nat.

Société Ivoirienne de Microbiologie

MICROBIOLOGY AND NATURE
Journal homepage: www.microbiologyandnature.com

Occurrence of *Staphylococcus aureus* isolates harboring methicillin-resistance and enterotoxin genes in various foods from Abidjan, Côte d'Ivoire

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Received, 25th November 2020; Revised, 30th April 2021; Accepted 3rd May 2021; Published online 30th May 2021

Abstract

Staphylococcus aureus, in particular its enterotoxigenic strain, is a well-known pathogen that causes food poisoning. The objective of this study was to investigate the prevalence of *S. aureus*, according to methicillin resistance and enterotoxin profile. *S. aureus* strains were isolated from different foods. Suspected *S. aureus* colonies were used to detect both methicillin resistance and enterotoxin genes by multiplex PCR. Out of a total of 325 colonies analyzed, 92 were shown to be *Staphylococcus aureus* strains representing a prevalence of 28.3 %. Among these strains, 66 (72.8 %) predominantly expressed at least one toxin gene. Hence *mec*A gene was identified in 21 strains (31.3%) while *se*A gene was found in 59.6% of isolated strains among which 25.3 % presented association with other enterotoxin genes notably *se*B, *se*C, *se*D and *Se*E. *se*B, *se*C and *se*D genes that were less dominant and detected respectively at 25.4%, 10.4% and 13.4 %. The results showed a high prevalence of *S. aureus* and a significant presence of enterotoxigenic strains in food in Abidjan where dairy products were the most contaminated. Moreover, the prevalence of methicillin resistant *S. aureus* (MRSA) strains was high and therefore requires an improvement of the food hygienic conditions in order to reduce the risk of MRSA dissemination. Furthermore, a rational use of antibiotics in the environments related to food resources is recommended.

Key words: food, *Staphylococcus aureus*, enterotoxins, methicillin-resistant *Staphylococcus aureus*.

Résumé

Staphylococcus aureus, en particulier ses souches entérotoxigènes, est un pathogène bien connu qui provoque une intoxication alimentaire. L'objectif de cette étude était d'étudier la prévalence de S. aureus, en fonction de la résistance à la méticilline et du profil des entérotoxines. Des souches de *S. aureus* ont été isolées à partir de différents aliments. Des colonies suspectes de *S. aureus* ont été utilisées pour détecter à la fois la résistance à la méthicilline et les gènes d'entérotoxines par PCR multiplex. Sur un total de 325 colonies analysées, 92 se sont avérées être des souches de *S. aureus* représentant une prévalence de 28,3%. Parmi ces souches, 66 (72,8%) exprimaient majoritairement au moins un gène de toxine. Ainsi, le gène mecA a été identifié dans 21 souches (31,3%) tandis que le gène seA a été trouvé dans 59,6% des souches isolées parmi lesquelles 25,3% présentaient une association avec d'autres gènes d'entérotoxines, notamment *se*B, *se*C, *se*D et *Se*E. Les gènes *se*B, *se*C et *se*D étaient moins dominants et détectés respectivement à 25,4%, 10,4% et 13,4%. Les résultats ont montré une forte prévalence de *S. aureus* et une présence significative de souches entérotoxigènes dans les aliments à Abidjan où les produits laitiers étaient les plus contaminés. De plus, la prévalence des souches de *S. aureus* résistantes à la méthicilline (MRSA) était élevée et nécessite donc une amélioration des conditions d'hygiène alimentaire afin de réduire le risque de dissémination des MRSA. De plus, l'utilisation rationnelle des antibiotiques dans les environnements liés aux ressources alimentaires est recommandée.

Mots-clés: .*Staphylococcus aureus*, entérotoxines, résistance à la methicilline

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Introduction

Staphylococcus aureus is recognized worldwide as a major pathogen that causes various infections (Lina et al., 1999 ; Tong et al., 2015). *S. aureus* is also an important foodborne pathogen because of its ability to produce a wide range of extracellular toxin proteins and virulence factors that contribute to its pathogenicity (Zecconi et al., 2013 ; Kong et al., 2016 ; Fisher et al., 2018). *S. aureus* food poisoning (SFP) has a rapid onset (2–8 h), that includes nausea, violent vomiting, abdominal cramping, with or without diarrhea (Murray, 2005 ; Argudin et al., 2010 ; Fisher et al., 2018). The disease is usually self-limiting and typically resolved within 24–48h after onset. Occasionally it can be severe enough to warrant hospitalization, particularly when infants, elderly or debilitated people are concerned (Murray 2005). *S. aureus* is able to produce one or more staphylococcal enterotoxins (SEs) which are part of the main virulence factors of the pathogen. The repertoire of *S. aureus* SEs/SEIs comprised 22 members, excluding molecular variants: (i) the classical SEA, SEB, SEC, SED and SEE involved in SFP outbreaks, and classified in distinct serological types and (ii) the new types of SEs (SEG, SEH, SEI, SER, SES, SET) and SEIs (SEIJ, SEIK, SEIL, SEIM, SEIN, SEIO, SEIP, SEIQ, SEIU, SEIU2, and SEIV). TSST-1, the toxic shock staphylococcal toxin, initially designated as SEF, lacks emetic activity (Thomas et al., 2007 ; Larking et al., 2009 ; Agurdin et al., 2010 ; Hennekine et al. 2012, Ono et al., 2015). Members of these SEs play an essential role in food poisoning outbreaks and other infections that are septic-related (Fisher et al., 2018). Staphylococcal enterotoxins are heat stable and, therefore, are able to thrive and maintain their activity in food previously contaminated with the pathogen. Two distinct activities of SEs, i.e., enterotoxicity and superantigenicity are described (Balaban et al., 2000 ; Kong et al. 2016 ; Argudin et al., 2010). After ingestion, SEs stimulate the vagus nerve in the abdominal viscera, which transmits the signal to the vomiting center in the brain (Addis et al., 2015; Fisher et al., 2018). In addition, SEs are able to penetrate the gut lining and activate local and systemic immune responses. Release of inflammatory mediators (including histamine, leukotrienes, and neuroenteric peptide substance P) causes vomiting (Addis et al., 2015; Fisher et al., 2018). Local immune system activation could also be responsible for the gastrointestinal damage associated with SE ingestion. Inflammatory changes are observed in several regions of the gastrointestinal tract, but the most severe lesions appear in the stomach and the upper part of the small intestine (Fisher et al., 2018). The diarrhea sometimes associated with SEs intoxication may be due to the inhibition of water and electrolyte reabsorption in the small intestine (Argudin et al., 2010 ; Kong et al. 2016). *se* genes are located on accessory genetic elements, including plasmids, prophages, *S. aureus* pathogenicity islands (SaPIs), genomic island vSa, or next to the staphylococcal cassette chromosome (SCC) elements (Argudin et al., 2010). Most of these are mobile genetic elements and their spread among *S. aureus* isolates can modify their ability to cause disease and contribute to the evolution of this important pathogen (Omoe et al., 2003 ; Fueyou et al., 2005 ; Novick et al., 2007 ; Baba et al., 2008 ; Goerke et al. 2009). In addition to enterotoxins, *S. aureus* is also characterized by the ability to acquire a variety of mechanisms of resistance to antimicrobial agents. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important multidrug-resistant pathogens around the world (Stapleton et al., 2002 ; Taubes 2008, Lakhundi et al., 2018). MRSA is generated when methicillin-susceptible *S. aureus* (MSSA) exogenously acquires a methicillin resistance gene *mec*A, carried by a mobile genetic element, staphylococcal cassette chromosome *mec* (SCCmec) (Ito et al., 1999 ; Lakhundiet al., 2018). The gene encodes a 78-kDa Penicillin-binding protein (PBP2a) with a lower affinity to methicillin and other beta-lactam antibiotics (Tsubakishita et al., 2010, Lakhundi 2018). In addition to mecA, other chromosomal factors such as femA are also associated with

the expression of methicillin resistance (De Lencastre et al., 1999). The *fem*A gene, which encodes a protein precursor involved in peptidoglycan biosynthesis. *fem*A and ribosomal mRNA are used as a molecular marker for the identification of *S. aureus* (Jukes et al., 2010). *S. aureus* is a common commensal of the skin and mucosal membranes of humans, with estimates of 20–30% for persistent and 60% for intermittent colonization (Kluytmans et al., 2005). Thus, food handlers carrying enterotoxin-producing *S. aureus* in their noses or on their hands are regarded as the main source of food contamination, via manual contact or through respiratory secretions. Due to lack of good competition with indigenous microbiota in raw foods, *S. aureus* contamination is mainly associated with improper handling of cooked or processed foods, followed by storage under conditions which allow growth of *S. aureus* and production of enterotoxin(s). These contaminated foods could be a source of maintenance and subsequent spread of MRSA and virulent strains. Also, in the last decade, investigation of MRSA in food and food-producing animals has received considerable attention (Wendlandt et al., 2013 ; Sergelidis et al., 2017). In Côte d'Ivoire, the main objective concerning studies of food products, was usually to analyze the presence of *S. aureus* and to count CFU (Colony Forming Units) in order to determine the rate of food contamination (Sina et al., 2011 ; Zinzendorf et al., 2009), as well as antibiotic resistance evaluation (Attien et al., 2013). This study focused on the detection of the genes involved both in methicillin resistance and enterotoxin production to show the health risk associated with food consumption in Abidjan

Materials and Methods

Bacterial isolates

S. aureus strains were isolated and characterized from food products in the National Public Health Laboratory, (Abidjan, Côte d'Ivoire). The phenotypic identification focused on cocci Gram+, catalase+, desoxyribonuclease (DNase)+ and coagulase+, the morphology of bacteria from Gram staining, the presence of catalase and an agglutination to Slidex Staph Plus test (BioMerieux ®) which simultaneously detects the clumping factor, protein A and *S. aureus* capsular antigens. All strains were maintained in Tryptic Soy Broth (TSB) supplemented with 25% glycerol and stored at −80°C.

Bacterial DNA extraction

To prepare DNA extracts, *S. aureus* strains were cultured on blood agar (5% from sheep blood) up to 24 hours at 37°C. Bacterial DNA was extracted by the thermal shock method (Kacou-N'douba et al., 2011). Briefly, a loop of typical colonies was removed and resuspended in 400 µl of sterile distilled water in a 1.5ml Eppendorf tube. The suspension was frozen at -20°C for 20 minutes and then the frozen suspension was incubated for 20 minutes in a heating block preheated at 100°C. After centrifugation at 13,000 rpm for 10 minutes, the supernatant containing bacterial DNA was recovered and stored at -20°C.

Specific detection of *S. aureus*

In order to specifically detect *S. aureus*, a multiplex PCR was performed using *S. aureus* specific primers to amplify *mec*A, *fem*A and 16SrRNA genes (Al-Talib et al. 2009). Primer sequences are described in Table 1. A multiplex PCR was performed to detect the presence of the three genes (16S rRNA, *fem*A and *mec*A) as follows: a reaction was carried out in a final volume of 50 µl containing 10 mM Tris-HCl (pH 8.9), 50 mM of KCl, 3 mM of MgCl 2, 200 µM of each desoxynucleotide triphosphate (dNTPs), 0.6 pmol of primer 16S rRNA, 0.8 pmol of primer *fem*A, 1 pmol of primer *mec*A. The PCR amplification was carried out in a thermocycler GeneAmp Perkin Elmer 9700 (Applied Biosystems, Courtaboeuf, France) as described by Al-Talib et al. (2009). An initial denaturation of DNA at 94°C for 5 minutes was followed by another 30 cycles at 94°C/30, hybridization 60°C/1min and elongation 72° C/1min. The amplification was completed by a final elongation at 72°C/5min. After PCRs, amplicons were separated on 1.5% agarose gel in 1× Tris-borate-EDTA (TBE) at 100 V for 90 min and viewed under Gel Doc XR UV transilluminator (Bio-Rad Laboratories, Hercules, CA). The gel images were analyzed with the QuantityOne software (Bio-Rad Laboratories) after ethidium bromide (0.5 µg/mL) staining.

Detection of genes encoding enterotoxins

Enterotoxin genes *se*A, *se*B, *se*C, and *se*D, were detected using the same multiplex PCR protocol described above except that the cycling conditions included an initiation step at 93°C/15 mn; 35 cycles of denaturation at 92° C/40s, 45–55°C/60s and 72°C/90 s; a final extension at 72°C/7 mn (Johnson et al. 1991) was added.

Table 1: Primers used for multiplex PCR

Genes	Primer	Sequence 5' – 3'	Size (pb)	Reference
16SrRNA	16SrRNA-F	GCAAGCGTTATCCGGATTT	597	Al-Talib et al., 2009
	16SrRNA-R	CTTAATGATGGCAACTAAGC		
*fem*A	femA--F	CGATCCATATTTACCATATCA	450	
	femA-R	ATCACGCTCTTCGTTTAGTT		
*mec*A	mecA-F	ACGAGTAGATGCTCAATATAA	293	
	mecA-R	CTTAGTTCTTTAGCGATTGC		
*se*A	seA-F	TTGGAAACGGTTAAAACGAA	120	Johnson et al., 1991
	seA-R	GAACCTTCCCATCAAAAACA		
*se*B	seB-F	TCGCATCAAACTGACAAACG	478	
	seB-R	GCAGGTACTCTATAAGTGCC		
*se*C	seC-F	GACATAAAAGCTAGGAATTT	257	
	seC-R	AAATCGGATTAACATTATCC		
*se*D	seD-F	CTAGTTTGGTAATATCTCCT	317	
	seD-R	TAATGCTATATCTTATAGGG		
*se*E	seE-F	TAGATAAAGTTAAAACAAGC	170	
	seE-R	TAACTTACCGTGGACCCTTC		

After PCRs, amplicons were separated and analyzed as previously described.

Results and discussion

This is the first report on the prevalence of MRSA harboring enterotoxin genes in diverse food in Abidjan, Côte d'Ivoire. Out of 325 food samples analyzed, a total of 101 isolates of *S. aureus* were obtained using phenotypic methods and ninety two strains (92) were effectively identified as MRSA based on specific detection of genes *mec*A, *fem*A and 16S rRNA (Al-Talib et al. 2009). The majority of strains 44/92 (65.7%) were isolated from dairy products which were the most contaminated (40.20 %) (Table 2).

Table 2. Prevalence (%) of *Staphylococcus aureus* from different foods

	isolates of *S. aureus*	
Food	**N**	**Prévalence* (%)**
dairy products (149)	60	40.2
cooked meals (55)	8	14.5
fruit juice (44)	11	25.0
raw foods (31)	7	22.5
hamburger (29)	3	10.3
drinking water (17)	3	17.6
Total (325)	92	

It is evident that MRSA can be occasionally present in foods, posing a potential public health risk. So, it is important to identify the origin of food-related MRSA and to evaluate the potential pathogenicity of these MRSA isolates. To date, lots of studies have reported the isolation with variable frequencies of MRSA from derived foods, both raw and ready to eat (Wendlandt et al. 2013; Kraushaar et al. 2014; Lozano et al. 2016, Dekhordi et al., 2019). Compared with other countries' studies, the prevalence of MRSA from food in Abidjan, Côte d'Ivoire can be considered high (Hao et al., 2015, Wang et al., 2017, Wu et al. 2019). However, such variation may be attributed to a number of factors, such as the sample size, sampling site, types of samples or isolation methods.

S. aureus is also an important foodborne pathogen because of its ability to produce a wide range of extracellular toxin proteins and virulence factors that contribute to its pathogenicity (Zecconi et al., 2013 ; Kong et al., 2016; Fisher et al., 2018). In this study, we also investigated most of *S. aureus* enterotoxin genes. A total of 31,3% MRSA strains were recorded as positive for genes encoding these toxins. These results are contrary to other studies with low frequencies (0,21% - 6,8%) of enterotoxigenic

MRSA (Fri et al., 2020 ; Wang et al., 2017 ; Wu et al., 2019 ; Kim et al., 2015 ; Ge et al., 2017). Moreover, it was shown that 67/92 (72.8%) of the *S. aureus* strains expressed at least one toxin gene. Indeed, the *se*A gene was found in 59.6% of the isolated strains of which 25.3 % harbored other enterotoxin genes notably *se*B, *se*C, *se*D and *se*C. According to the food type, the *se*A gene was the most detected in dairy products, fruit juice, raw foods and hamburger with respectively a rate of 43.5%, 30.7%, 37.5% and 40%. Moreover, the association of *se*A + *se*B was most frequently found in ready-made meals, 26.5%. In the case of dairy products, *se*D gene was detected with a rate of 34.8% followed by *se*B, 21.7% (Table 3). Genes *se*A (38.5%) and *se*C (14.2%) were dominant in MRSA strains while *se*A and *se*D were abundant in MSSA strains, respectively 17.5% and 10.7%. The *se*E was identified in one case, associated with *se*A and *se*D (1.5%) in a MRSA strain (Fig 1).

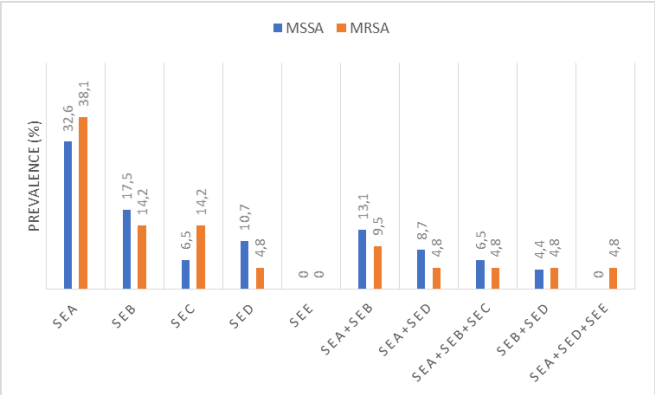

Figure 1. Distribution of *staphylococcus* enterotoxin genes profiles according to methicillin resistance

Prevalence (%) is the ratio between the numbers of S. aureus isolates and the the number of food samples*

The rate of prevalence was 28.3%. Such ratio of *S. aureus* prevalence was reported in previous studies (Chaalal et al., 2018; Govender et al., 2019) and can be considered high with regards to that reported in other studies. Difference in geographical location, seasonal variety, sample kind or the sensitivity of detection methods may impact this prevalence and explain this typical variation between countries (Wang et al. 2017, Wu et al. 2019). The presence of *S. aureus* in food samples can also be influenced by environmental conditions (Jhalta et al., 2014; Marcia et al., 2020) notably food handlers through coughing and sneezing; storage of food at high temperature; and/or some processed foods, which constitute a good culture medium for bacteria (Isara et al., 2010 ; European Food Safety Authority, European Centre for Disease Prevention and Control & European Medicines Agency 2009 ; Ho et al., 2014). The majority of strains 44/92 (65.7%) were isolated from dairy products showing that dairy products were the most contaminated 40.2% of case (Table 2). This could be due to conditions of dairy food processing that constitute a good culture medium for bacteria (Schmid et al.,2009; Isara et al., 2010; European Food Safety Authority, European Centre for Disease Prevention and Control & European Medicines Agency 2009; Ho et al., 2014). *S. aureus* strains carrying genes have the potential to produce SEs toxin in foods under favorable conditions for growth and SE production. In this study 67 strains out of 92 (72.8%) carried the genes. In Egypt, SE genes were identified in 20.7% of raw goat milk samples and 11.1% of meat samples (Kalifa et al. 2016). In Nigeria, 269 strains out of 552 (48%) isolated from ready to eat food were enterotoxigenic (Sokari et al. 1991). The *se*A gene was found in 40 (59.6%) of strains and was the most frequently carried type. Several studies have investigated the distribution of enterotoxins genes in *S. aureus* from foods and staphylococcus food poisoning (SFP) outbreaks in european an asian countries. The *se*A gene is the most common cause of staphylococcal food poisoning outbreaks in european an asian contries (Kérouanton et al. 2007, Chiang et al. 2008, Cha et al. 2006, Asao et al. 2003, Kitanoto et al. 2009, Vitale et al. 2015). In Africa, these genes have also been found in isolates from food samples. In Nigeria, enterotoxigenic strains of *S. aureus* isolated from ready to eat food, enterotoxin A being the most commonly found toxin (Sokari et al. 1991).

In Egypt, *se*A genes from strains isolated from goat's food and human contact was the most prevalent type detected in 8 (50%) out of the enterotoxigenic isolates followed by *se*B, *se*C, *se*D and *se*A+*se*C genes (Khalifa et al., 2015). From bovine milk produced in central Ethiopia, at least one type of *S. aureus* enterotoxin gene (*se*E) was carried in 73 (66.9%) of the isolates. The most frequently encountered gene was *se*A (40; 36.7%) followed by *se*B (19; 17.4%), seE (18; 16.5%), seC (12; 11.01%), and seD (7; 6.4%) (Seyoun et al., 2016). In Africa, although these foods were not associated with food poisoning, the presence of *se* genes, indicates the risk associated with their consumption. In agreement with the predominance of *se*A gene, the toxin SEA, either alone or together with other SEs/SEls, is the enterotoxin most commonly reported in foods, and is also considered as the main cause of staphylococcal food poisoning (SFP), probably due to its extraordinarily high resistance to proteolytic enzymes (Balaban et al. 2000). As these foods were not associated with infection, the detection of toxins in the foods was therefore not indicated. The high rate of enterotoxinic *S. aureus* from food could cause SPF and presents public health risk to the consumers in Abidjan. SPF should be prevented and controlled by proper cooking and preparing of food as well as storing, adequate refrigeration of food, improve personal hygiene, adequate cooking and heating processing. The control method or measures also includes, education of those who prepare the food at home and other food handlers, prohibiting individuals with absences or other skin lesions from handling food. It is also recommended to put food in cold place in order to prevent bacterial multiplication and toxin building (WHO, 2018). A total of 29/92 (31,5%) MRSA strains were recorded as positive for toxin genes showing the evidence that MRSA are present in foods in Abidjan. This poses a potential public health risk. These results are contrary to other studies with low frequencies (0,21% - 6,8%) of enterotoxigenic MRSA (Fri et al., 2020 ; Wang et al., 2017 ; Wu et al., 2019 ; Kim et al., 2015 ; Ge et al., 2017). Our results are in line with the higher frequencies found in MRSA elsewhere (Holeckova et al., 2002 ; Rhee et al., 2010 ; Aafatahery et al. 2016) Reports showed higher MRSA isolation rate than our estimate, even up to 70% (Asiimwe et al., 2017 ; Riva et al., 2017). In addition, except the fact that the *se*E gene-expressing strain was isolated from a MRSA strain, all other toxins were identified in MRSA and MSSA strains. These results emphazized the fact that there is no evidence for a positive correlation between antibiotic resistance and enterotoxigenicity or for different behaviour among MRSA and MSSA concerning enterotoxin production. Therefore, if food production and storage conditions are favourable for enterotoxin production, MRSA strains carrying enterotoxin genes should be capable of producing enterotoxins, similar to any other enterotoxigenic *S. aureus* (Sergelidis et al. 2017).

Conclusion

This study revealed a high prevalence of *S. aureus* and *se* genes. These results show a real risk of staphylococcus food poisoning in foods consumed in Abidjan. Moreover, the high presence of MRSA strains isolated from food may promote the dissemination of the pathogen in the community. Thus, there is a need for continuous and better control of sources of food contamination and the spread of antimicrobial -resistant bacteria since the pathogenic potential of these strains cannot be ignored.

Acknowledgements

This study was conducted with the technical support of National Laboratory of Public Health

Conflict of Interest

No conflict of interests is declared

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