Prevalence and antibiotic resistance of *Staphylococcus* strains isolated from meat products sold in Abidjan streets (Ivory Coast)

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Our study aimed to carry out the prevalence and antibiotic resistance of the coagulase positive and negative *Staphylococcus* isolated from meat product sold in streets in Abidjan (Ivory Coast). Two hundred and forty (240) samples from three kind of meat product (beef, pork and chickens) were collected in four popular communes (Abobo, Adjamé, Treichville and Yopougon) of Abidjan. These samples were composed of 80 samples of each kind of meat. After seeding on appropriate medium, suspected *Staphylococcus* strains were preliminary identified using API Staph protocol. The exact bacteria identity was confirmed by MALDI-TOF mass spectrometry. The *Staphylococcus* strains susceptibility to 18 antibiotics was determined using the disc diffusion method on Mueller Hinton medium. Out of the 240 tested samples, 96 *Staphylococcus* strains were isolated and identified. The coagulase positive specie isolated was *Staphylococcus aureus* with 19/96 (19.79%). Among the 77 coagulase negative strains, *S. sciuri* (32/77) was the most isolated followed by *S. simulans* (15/77). The highest resistance level was observed with erythromycin (100% for coagulase positive and 69.5% for coagulase negative). None resistance was observed with imipenem. The observed resistance to antibiotics of *Staphylococcus* strains suggests that the streets meat products sold at Abidjan are not appropriate and can be able to present a public health danger for the consumer.

Key words: *Staphylococcus*, coagulases, MALDI-TOF-MS, antibiotics, meat products, Ivory coast.

INTRODUCTION

Food safety became a worldwide concern (Ackah et al., 2011). In spite of the efforts made by World health organization, food contaminations remained a serious problem because those affections closely related to world

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mortality rates (WHO, 2002). Food poisoning is frequent in developing countries and is a permanent risk in developed countries (Akhtar et al., 2012). The problem is amplified by public feeding habit with developing of the street vended foods. Street foods can be defined as foods and beverages prepared and/or sold by vendors in streets and other public places for immediate consumption or consumption at a later time without further processing or preparation (Tambeckar et al., 2008). Apart of providing a source of income for the vendors, street foods are said to provide a source of readily available, inexpensive, nutritional meals (Ghosh et al., 2007). This kind of food is appreciated not only for its unique flavors and convenience, but also for maintaining the nutritional status of the population. They also assure food security for low-income urban population and livelihood for a significant proportion of the population in many developing countries (Tambeckar et al., 2008), including Ivory Coast. In spite of these potential benefits, street foods have been reported to be contaminated with pathogens and have also been implicated in food-borne disease (Nyenje et al., 2012).

Among the most appreciated street foods, meat products occupied a very good position. However the poor sanitary practices during preparation and sale, multiple routes of entry, and high ambient temperatures especially in tropical environments have been described as the major factors responsible for facilitating the access and multiplication of bacterial contaminants in meat products (Mankee et al., 2004). Meat product vending is prevalent in Ivory Coast, with a large number of people from all ages and income groups consuming a variety of meats. However, the unhygienic conditions under which meat products vendors operate and lack of basic food safety training of the street vendors makes these kinds of meats more vulnerable to bacterial contaminants (Mensah et al., 2002; Barro et al., 2006).

Salmonella, Staphylococcus, Coliformes and Clostridium are the mostly identified bacteria in case of food poisoning. The ingestion of those bacteria or their toxins can cause metabolic problems to the consumer (Baba-Moussa et al., 2010). Apart from their ability to induce such problem, bacteria are source of health care taking because of the upsurge of strains resistances to antibiotics (Chambers and DeLeo, 2009). Indeed, antimicrobial resistance is an important public health concern worldwide. The development of resistance both in human and animal bacterial pathogens has been associated with the extensive therapeutic use of antimicrobials or with their administration as growth promoters in food animal production (Barber et al., 2003).

Lately, Ivory Coast faces serious political crises with their high migratory movements that increase the food poisoning risk. In order to contribute effectively to the food poisoning caused by the Staphylococcus risk prevention, the aim of our work was to evaluate the microbiological quality of three meat products (pork, beef and chicken) sold in Abidjan streets (Ivory Coast).

MATERIAL AND METHODS

Sample collection

From February to September 2010, three kinds of meat (beef, pork and chickens) were collected in four most popular commune of Abidjan (Abobo, Yopougon, Adjame and Treichville). At each commune, four places were selected regarding their high diurnal and nocturnal people frequentation. Meat products samples were collected from street vendors as braise meat. One sample of each kind of meat (beef, pork and chickens) was collected five times (one per month) at each site. For the whole study, 240 samples with 80 samples of each kind of meat product were collected. The samples were collected in sterile Stomacher papers and then carried to the laboratory in icebox at < 4°C.

Microbiological analysis

Once at the laboratory, 10 g of each sample were homogenized in 90 ml of sterile bacteriological peptone (Oxoid, Hampshire, England) and then incubated at 37°C for 1 to 3 h (Akoachere et al., 2009). To perform the isolation of Staphylococcus strains, 0.1 ml of serial decimal dilutions were plated in duplicate on Baird-Parker Agar (Biobad, South Africa) medium (Baird-Parker, 1990) with 50 ml egg-yolk tellurite emulsion (Merck, Darmstadt, Germany) and incubated at 37°C for 48 h.

Staphylococcus strains identification

Standard microbiological methods for microorganism’s identification were used (Akoachere et al., 2009). Then, suspected Staphylococcus colony was subcultured on Mueller-Hinton agar (bioMérieux, Marcy l’Etoile, France) and identified by subsequent Gram staining, catalase test and Slide Staph Plus (bioMérieux, Marcy l’Etoile, France) and the coagulase test with the rabbit plasma (Cheesbrough, 2004). Finally, the strains were analyzed by API Staph (bioMérieux, Marcy l’Etoile, France).

Staphylococcus species identification by MALDI-TOF-MS

MALDI-TOF- MS was used to confirm the identification of Staphylococcus species identified by Api-Staph. Staphylococcus strains were grown on Mueller-Hinton agar (bioMérieux, Marcy l’Etoile, France) and incubated 24 h at 37°C. An isolated colony was harvested in 100 µl of sterile water; 1 µl of this mixture was deposited on a target plate (Bruker Daltonics, Bremen, Germany) in three replicates and allowed to dry at room temperature. One micro-liter of absolute ethanol was then added in each well. After the mixture had dried, 1 µl of matrix solution DHB (2.5-dihydroxybenzoic acid at 50 mg/ml, 30% acetanilide at 0.1% trifluoroacetic acid) was added. Samples were then processed in the MALDI-TOF-MS spectrometer (Autoflex; Bruker Daltonics, Germany) with flex control software (Bruker Daltonics, Germany). Positive ions were extracted with an accelerating voltage of 20 Hz in linear mode. Each spectrum was the sum of the ions obtained from 200 laser shots performed in five different regions of the same well. The spectra have been analyzed in an m/z range of 1,000 to 11,000. The analysis was performed with the flex analysis software and calibrated with protein calibration standard T (Protein I; Bruker Daltonics, Germany). The profiles were analyzed and compared (Rujoi et al., 2004; Rohner et al., 2005).

Antibiotic susceptibility

Antimicrobial susceptibility was determined by the disc diffusion
method of Kirby-Bauer on agar Mueller-Hinton (bioMérieux, Marcy l'Etoile, France) as recommended by the Antibiogram Committee of the French Microbiology Society (CASFM, 2012). After 24 h at 37°C, inhibition zone was measured. For susceptibility to oxacillin, inoculum of $10^8$ CFU/ml was prepared and the plate was incubated at 37°C for 24 h on Mueller-Hinton agar + 2% NaCl.

The tested antibiotics (Bio-Rad, Marne la Coquette, France) were: Pristinamycin; Erythromycin; Lincomycin; Oxacillin; Amoxicillin; Ceftriaxone; Gentamicin; Tobramycin; Sisomicin; Oxytetracycline; Tetracycline; Trimethoprim/sulfamides; Cefotaxime; Ofloxacin; Pefloxacin; Vancomycin; Rifampicin; Imipenem.

Statistical analysis
Microsoft Excel Spreadsheet has been used for data processing. For comparison tests of positive isolates in various meat kinds meat product, the Student T test, and the Fisher's test were used for lower number series (GraphPad Prism 5). $P<0.05$ was considered statistically significant.

RESULTS
Bacterial identification
Out of the 240 meat sample, 96 strains of *Staphylococcus* were isolated. The identification tools used in this study revealed about 20% (19/96) of coagulase positive and 80% (77/96) of coagulase negative strains. A total of 11 species were identified at different proportion after MALDI-TOF assay (Figure 1). Only *S. aureus* was identified among coagulase positive strains while ten (*S. sciuri, S. simulans, S. xylosus, S. cohnii, S. lentus, S. haemolyticus, S. saprophyticus, S. capitis, S. succinus, and S. equorum*) were identified among the 77 coagulase negative strains (Figure 1). Strains were variably isolated from the three kind of meat product. Some species (*S. capitis, S. succinus and S. equorum*) were isolated only from chickens' meats while *S. sciuri, S. aureus, S. simulans* and *S. xylosus* were isolated from the three kind of meat (Figure 2). At last, it was observed a variability of species identification regarding the sample collection commune (Figure 3).

Taking into consideration the sample collection area, variation of strains distribution was observed. The data show that all the four communes have their meat products samples contaminated by *Staphylococcus* strains (Figure 4). At Abobo 57.5% of collected meat samples were infected by *Staphylococcus* followed by Adjame (47.5%), Yopougon (32.5%) and Treichville (22.5%). A variability of samples contamination was observed according to the collection site (Figure 4). Thus, it was observed that the samples collected on sites closed to the market (C) and bus stations (D) were the most contaminated (Figure 2) in comparison to sites closed to habitation (A) and public places (B).

There was a variability of contamination according to...
the kind of meat product (Figure 5). Thus, chickens meat product were the most contaminated (60%) followed by beef (35%) and pork (27.5%). These contamination proportions also vary according to the collection commune.
Figure 4. Contamination frequency of meat product samples according to their collection site by communes. Collected site: A: closed to habitation; B: closed to public places; C: closed to the market; D: closed to the bus stations. ABOBO: Abobo; ADJM: Adjame; YOP: Yopougon; TREICH: Treichville.

Figure 5. Contamination frequency of different kind meat product according to their collection site by communes. ABOBO: Abobo; ADJM: Adjame; YOP: Yopougon; TREICH: Treichville.

(Figure 5).

Antibiotic susceptibility

All the coagulase positive (S. aureus) and negatives strains isolated in our study were resistant at different proportion to the tested antibiotics (Figure 6). However, for S. aureus strains, we noticed 100% of resistance to Erythromycin followed by oxytetracycline (84.0%), ceftri-axone (79.0%). With coagulase negative, the highest
resistance levels were observed using erythromycin (69.5%), oxytetracycline (63.9%), lincomycin and ceftriaxone (56.2%) (Figure 6). The isolated S. aureus strains resistance level to oxacillin was higher (58.0%) than those observed with coagulase negative (38.3%).

**DISCUSSION**

We observed in this study 19.7% of coagulase positive and 80.3% of coagulase negative *Staphylococcus* (Figure 1). The presence of the *Staphylococcus* strains on meat (Olsson et al., 2003; Shale et al., 2005) and sausage (Cocolin et al., 2004; Rantsiou et al., 2005a, b) was proven in various proportions (10 to 72 %). In our study, 11 species were identified using MALDI-TOF-MS. *Staphylococcus* strains were identified as well as coagulase positive and coagulase negatives'. Concerning the coagulase positive’s, we founded *S. aureus* in all kind of meat products with highest prevalence in beef (63.16%, 12/19). Our proportion seems less than the 88% obtained by Fang et al. (2003) in the beef meat sandwiches collected in Thailand. Likewise, in Cameroon, M'bawala et al. (2010) showed a high *S. aureus* contamination level of samples coming from Kilishi, a beer of dried beef. Regarding previous studies, we can see those beef meat products are an appropriate field for contamination by *S. aureus* in spite of the fact that some species like *S. sciuri, S. lentus*, and *S. xylosus* are involved in beef meat contamination during slaughtering (Shale et al., 2005). Then, the identification in our study of the mentioned above *Staphylococcus* species can be explained by slaughtering contamination. In fact, beef consumed part is generally separated of the animal skin which constitutes a filter of microorganisms. After removing the skin of beef, the consumed parts become exposed to manual contamination during the transformation process.

The *Staphylococcus* species according to the kind of, meat products shows that chicken are the most contaminated regarding the number and in diversity of *Staphylococcus* species (Figure 5). *S. sciuri* was the most dominant species. Our results are similar to the high percentage of coagulase negatives obtained in chicken meat by previous studies (Kawano et al., 1996; Ifesan et al., 2009). In the chicken meat products samples, 9 of the 11 species were isolated (only *S. haemolyticus* and *S. saprophyticus* were not identified). These *Staphylococcus* Strains that contaminate chicken meats are those observed in the environment. This observation can be ex-
explained by the fact that the contamination occurs in dirty poultry farms during the slaughtering in the Abidjan's markets. The pork was contaminated by seven \textit{Staphylococcus} species with the predominance of \textit{S. simulans} (27.27%) and \textit{S. aureus} (22.72%). Early in 1979, Narucka (1979) drew the international opinion attention concerning the hazard related to the contamination of pork by \textit{S. aureus}. The presence of \textit{S. aureus} in pork meats products was shown by Olsen et al. (2000) and Ifesan et al. (2009) but the proportions obtained were lower than ours. This may be due to the cooking mode and sellers' hygiene. Many other work on the pork proved the contamination by \textit{S. aureus} with significant variable proportions (Bakr et al., 2004; Gundogan et al., 2005; Rantsiou et al., 2005a; Lindblad et al., 2006). The presence of the other species, like \textit{S. xylosus} (48%) and \textit{S. saprophyticus} (1%), was also notified in fresh sausages (Cordero and Zumalacarregui, 2000; Cocolin et al., 2004; Rantsiou et al., 2005b). Those proportions are different from ours. Likewise, in Italian sausages, Rebecchi et al. (1998), isolated \textit{S. sciuri} (30%), \textit{S. xylosus} (25%) and \textit{S. saprophyticus} (20%). These results appear higher than ours for \textit{S. sciuri} (12.5%) and \textit{S. xylosus} (33.33%). The great staphylococcal flora diversity observed in pork can be explained by the farms unhealthy environment and the sale places. In fact, Labadie (1999) meant that the carcasses initial contamination is generally stronger in the pork, and the cooking of carcasses with, the skin constitutes a supplement risk of contamination.

Abobo was the most contaminate commune (57.5%) followed by Adjame (47.5%), Yopougon (32.5%) and Treichville (22.5%). These can be comparing with the variation observed from one district to another studying traditional beer in Cameroon (M'Bawala et al., 2010). Regarding the site of samples collection, we notice that that samples collected close to the markets (C) and bus stations (D) were the most contaminated (Figure 4) in comparison to those collected close to habitation (A) and public places (B). This notice can be explained by a great people's influx (travelers, customers and traders), the atmospheric pollution (the proximity with artisans, smoked, dirt and dust), and the bad hygienic statue of sellers. In addition, we can note that the hawkers use, at the same time, their (dirty) hand to serve food and receive and/or give money (Barro et al., 2002; Sina et al., 2011). The meats are usually packed in old sheets of papers (news papers, cements papers) and served in dirty plates.

The three kinds of meat products of Abobo remains the most contaminates. The population density can explain that, because this commune is one of the biggest in term of populations in Ivory Coast. We can remark that \textit{Staphylococcus} grow up more in area with human high density (Conly and Johnston, 2003; Simor et al., 2005). A strong contamination by \textit{Staphylococcus} strains were reported among prisoners, nurseries children, soldiers, the itinerants and young athletics (football, fencing, fight) (Adem et al., 2005). The resistance of coagulase positive \textit{Staphylococcus} to antibiotics was variable (Figure 6).

The presence of resistant \textit{S. aureus} strains in food is a big health risk (Van den Broek, 2003). In the same way, Barton et al. (2003) revealed the presence of Methicillin resistant \textit{Staphylococcus} strains in all kinds of animals and Ann and Julian (2007) raise the alarm concerning the increase of the resistance towards other antibiotics like fluoroquinolones. The coagulase negative \textit{Staphylococcus} strains are often use as ferment and/or as flavoring in the manufacturing of meat products (Rodriguez et al., 1996; Even et al., 2010). Although the coagulase negative \textit{Staphylococci} are part of the normal flora of human skin (Roth and James, 1988), they are increasingly recognized as agents of clinically significant infection of the bloodstream and other sites. Risk factors for coagulase negative \textit{Staphylococcus} infection include foreign bodies (such as indwelling prosthetic devices or intravascular catheters) and immune compromised.

All the \textit{S. aureus} strains are resistant to Erythromycin (100%) followed by Oxy-tetracycline (84%), ceftriaxone (79%), Amoxicillin (68%), oxacillin (58%), lincomycin (42%) and Vancomycin (37%). Our results are different from those obtained by Ben Hassen et al. (2003) for oxacillin (4%) and tetracycline (36%). The major part of \textit{S. aureus} (75%) isolated from beef meats are resistant to Oxy-tetracycline and amoxicillin when ¼ are resistant to oxacillin.

Some \textit{Staphylococcus} coagulase negative (\textit{S. sciuri} and \textit{S. simulans}) isolated from pork and chicken display high resistant level (from 44 to 100%) to erythromycin, lincomycin, oxacillin and oxytetracycline. The similar results were obtained by Resch et al. (2008). Quinolons (pefloxacin and ofloxacine) were efficient against all the tested strains when only few (<05 %) were resistant to Aminosides (Sisomicin). It was also observed a resistance to fluoroquinolones with some coagulase negative strains (\textit{S. sciuri} and \textit{S. simulans}). The resistance to fluoroquinolones was already reported with \textit{S. aureus} resistant to methicillin (SARM) strains (Ann and Julian, 2007). The appearance of resistance strains among coagulase negative's bacteria becomes dangerous for human being in case of invasion. This wide spray of resistance can be explained by the fact that medicines (antibiotics) are available on the markets through illegal distribution circuit and without any medical prescriptions. All these contribute to increase the antibiotics (particularly those cheap and/or easy of supply) resistance level in clinical and community \textit{Staphylococcus} strains.

CONCLUSION

These findings of microorganisms demonstrate that ready to eat meat sold in the streets of Abidjan constitutes a likely potential hazard to human health. The isolated microorganisms display resistance to antibiotics.

We must pay a particular attention before buying and eating street meat product because, the presence of \textit{Staphylococcus aureus} in meat products sold in Abidjan, regarding it ability to produce toxins may be a dangerous
for consumers.

REFERENCES


