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Phenotypic Characterization and Antibiotic Resistance of Strains of *Staphyloccocus aureus* Isolated from Food Sold in the Streets of N'Djamena, Chad

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A B S T R A C T

The preparation and sale of street food constitutes a socio-cultural and economic fact in all countries of the world. However, the hygienic failures observed during this activity constitute a source of contamination of these foods. The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) noted at the beginning of the 1960s is now experiencing rapid spread across the world. The objective of this study was to determine the phenotypic resistance characteristics of strains of *Staphylococcus aureus* isolated from three types of food sold on public roads in the city of N'Djamena in Chad. A total of 115 food samples were collected in the city of N'Djamena between April 2015 and May 2017. The research and characterization of *Staphylococcus aureus* were carried out following ISO standardized methods. The overall prevalence of *Staphylococcus aureus* was 62.49%. The evaluation of the antibiogram showed that all *Staphylococcus aureus* isolates were sensitive to Ciprofloxacin, Rifampicin, Ofloxacin, Teicoplanin and Fosfomycin. The isolates showed the highest resistance to Penicillin G (100%), Oxacillin (100%) and Erythromycin (60%). This resistance is the consequence of inadequate prescriptions and especially self-medication. The use of antibiotics on Chadian territory must be subject to strict control.

Introduction

According to the Codex Alimentarius Commission (FAO/WHO), the term street food refers to food ready to be consumed, prepared and/or sold by vendors or hawkers, particularly in streets and other public places (Codex, 1999). Street food vending is one of the most visible features of developing countries where it is a popular informal activity (Cross, 2000; NASVI, 2001; Maruyama *et al.,* 2010; Milgram, 2011; Maneepong *et*

al., 2013). Foods commonly contaminated with *Staphylococcus aureus* (*S. aureus*) include milk, cream, pastries, butter, ham, cheeses, sausages, canned meats, salads, cooked meals and sandwich fillings (Le Loir *et al.,* 2003). Over the past decades, there has been a significant increase in the number of cases of *S. aureus* infections worldwide. For example, in the United States, in 2003 alone, *S. aureus* was responsible for 400,000 hospitalizations with 19,000 deaths (Boucher, 2008). *S. aureus* have been implicated in diseases such as dermatitis, pneumonia, sepsis, osteomyelitis, and meningitis in humans and pigs, as well as bovine mastitis in cattle and bumble bee mastitis in poultry (Quinn *et al.,* 2000).

The emergence of methicillin-resistant *S. aureus* (MRSA) was noted in the early 1960s, first in Europe and was followed by rapid spread throughout the world (Jevans, 1961). Methicillin-resistant *S. aureus* or oxacillin-resistant *S. aureus* (MRSA) are strains that are resistant to methicillin group antibiotics. A study carried out on dairy products in Egypt highlighted a strong presence of MRSA (Kamal *et al.,* 2013). Strains of *S. aureus* isolated from broilers and layers in Kaduna State, Nigeria, revealed high resistance against tetracycline (76.8%), ciprofloxacin (60.4%), oxacillin (36.6%) and cotrimoxazole (26.6%) (Onaolapo *et al.,* 2017).

The objective of this study was to determine the phenotypic characteristics of antibiotic resistance of *S. aureus* strains isolated from some foods sold on public roads in the city of N'Djamena in Chad.

Materials and Methods

Period, Setting and Location of Study

This study was carried out between April 2015 and May 2017 in Chad and more precisely in N'Djamena, the capital of the Republic of Chad and also the largest city in the country. The city of N'Djamena covers an area of approximately 15,000 hectares and is divided into 10 districts with a total of 64 neighborhoods.

This is a descriptive and prospective study which covered 8 of the 10 districts in the city of N'Djamena. Districts No. 1, 2, 3, 4, 5, 6, 7 and 9 were retained. Districts No. 8 and 10 were abandoned for security reasons.

In the eight districts selected, the specially targeted sites were markets, primary schools, high schools, industrial zones, stations (taxi and travel), avenues and heavy roads.

Sampling

The samples under study mainly include ready-made meals. Around 115 samples were taken. The random sampling concerned the minced beef sandwich (n= 42), the "ball" with okra sauce (n= 42) and the fried fish (n= 31).

Samples of a quantity of 500 g were collected in sterile bags or bottles, transported to the laboratory at 4°C and then analyzed in the hours that followed.

Isolation and identification of *Staphyloccocus aureus*

For the search for *S. aureus*, the technique of spreading on the surface of 0.1 ml of the corresponding dilution on complete Baird Parker medium made in duplicate was used.

Then the Petri dishes were incubated at 37°C for 24 hours according to Standard V08-057-1. For identification, we marked the characteristic colonies on the bottom of the plates (black or gray, shiny and convex surrounded by a clear halo) and reincubated the same plates at 37°C for 24 hours. The first confirmation phase consisted of taking 3 characteristic colonies and submitting them to the catalase test. When the test is negative, this certifies the absence of *S. aureus*. Otherwise, a colony was taken and cultured in Brain HeartBroth at 37°C for 20 to 24 hours. The second confirmation phase consisted of the search for coagulasse, that is to say, 0.1 ml of Brain HeartBroth culture from a colony was added to 0.3 ml of Rabbit Plasma. When the coagulase is negative, this certifies the absence of *S. aureus*, otherwise, the result is positive.

Antimicrobial Susceptibility Testing

To carry out the antibiogram, the agar diffusion method was used (Bauer *et al.,* 1966). Thus, the *S. aureus* strains previously isolated from food samples were subcultured on Mueller Hinton agar (Liofilchem, Italy) then incubated at 37°C. After incubation for 18 to 24 hours, a few bacterial colonies were suspended in physiological water. The suspension obtained was calibrated to the MacFarland 0.5 standard (~108 CFU/ml) using a densimat.

The antibiotics tested were those commonly used for treatments in human medicine and veterinary medicine in Chad. Thus, the following antibiotics were tested on the strains of *S. aureus*: Penicillins (Penicillin G (10 µ unit), Oxacillin (5 µg)); Macrolide (Erythromycin (15µg)); Lincosamine (Lincomycin (15µg)); Fluoroquinolones (Ofloxacin (5µg), Ciprofloxacin(5µg)); Cephalosporin (Cefoxitin (30µg)); Glycopeptide (Teicoplanin (30µg)); Fosfomicin (Fosfomycin (200 µg)) and Rifampicin (Rifampycin (30 µg)).

At the end of the incubation, circular sterile culture zones were observed around certain antibiotic disks. The diameter of these zones or inhibition diameter is proportional to the antibacterial activity of the antibiotic. Thus, these inhibition diameters were measured using a caliper. The results were interpreted in accordance with the recommendations of CA-SFM/EUCAST (2016).

Data Processing Statistical Analyzes of Data

The data collected was entered into Excel and analyzed using Statistical Package for the Social Sciences (SPSS) and Excel software. Differences were considered significant when $p < 0.05$.

Results and Discussion

Description of the dishes taken into account by the study

Prevalence of *Staphyloccocus aureus* in food sold on public roads in N'Djamena

Resistance phenotype of *Staphyloccocus aureus* isolated from food sold on the public roads of N'Djamena

The sensitivity situation of *S. aureus* to the different antibiotics tested is reported in Table 3. The 30 strains are mainly isolated from gumbo sauce "ball", fried fish and sandwiches. The test revealed a very high range of antibiotics to which *S. aureus* showed significant sensitivities.

All *S. aureus* isolates were susceptible to Ciprofloxacin, Rifampicin, Ofloxacin, Teicoplanin and Fosfomycin. The isolates showed the highest resistance to Penicillin G (100%), Oxacillin (100%) and Erythromycin (60%). Of the 30 strains of *S. aureus* isolated, 10 antibiotics were tested to determine the prevalence of resistant strains. The different strains showed total resistance (100%) to penicillins (Penicillin G and Oxacillin). The 100% rate obtained for resistance to Penicillin G is identical to that of work carried out on a range of street foods in Benin and Ethiopia (Sina *et al.,* 2011; Beyene, 2016).

The 100% resistance rate to Oxacillin obtained as part of this study is a clear increase compared to other work carried out on food samples, in Tunisia, Italy and Benin where resistance rates of strains of *S. aureus* were very low at 3%, 4% and 15% respectively (Ben Hassen, 2003; Corrente *et al.,* 2007; Normanno *et al.,* 2007; Sina *et al.,* 2011). This growth in resistance of *S. aureus* strains could be justified by the fact that these antibiotics are widely used whether in veterinary medicine or in human medicine.

Average resistance was also observed against the group of Macrolides and Lincosamides (50% for Lincomycin and 60% for Erythromycin). *S. aureus* strains isolated from food and environmental samples also showed rates of resistance to Erythromycin very close to the rate obtained in the present study (Sina *et al.,* 2011; Olufemi *et al.,* 2017). We note remarkable activities of the group of Fluoroquinolones (100% sensitivity for Ofloxacin and Ciprofloxacin). Some studies have also reported such high sensitivities of *S. aureus* strains to fluoroquinolones (Sina *et al.,* 2011; Olufemi *et al.,* 2017). The rate of 100% sensitivity to Rifampicin obtained as part of our study corroborates the results of Sina and colleagues (2011), however it is in contradiction with the conclusions of Olufemi and colleagues (2017) who found resistance of the strains of *S. aureus* to Rifampicin by more than 80%.

The significant resistance of our strains to certain antibiotics is due to several factors, mainly their overuse. However, no strain showed resistance to glycopeptides (teicoplanin). These three types of widely consumed foods sold on the streets of the city of N'Djamena show fairly high prevalences of *S. aureus*.

Although street food represents a crucial socio-economic and nutritional necessity, the existence of *S. aureus* in these foods highly prized by consumers indicates the lack of good practices among sellers during preparation and sale. It was revealed that strains of *S. aureus* showed strong resistance to the antibiotics tested. It was revealed that strains of *S. aureus* showed strong resistance to the antibiotics tested. Monitoring these resistant strains is a key element in preventing and controlling their spread.

Table.1 Description of the types of food analyzed

N°	Type of food	Description of the food
Cereal-based foods		
01	"Ball" with okra sauce	Rice flour dough prepared and eaten with okra meat sauce (beef or mutton).
02	Minced Beef Sandwich	Bread loaded with minced meat cooked in a sauce with tomato, potato, onions, mayonnaise, aroma of oil and salt
Fish based foods		
03	Fried fish	Fried fish

Table.2 Prevalence of *S. aureus* in cooked meals sold on the public roads of N'Djamena

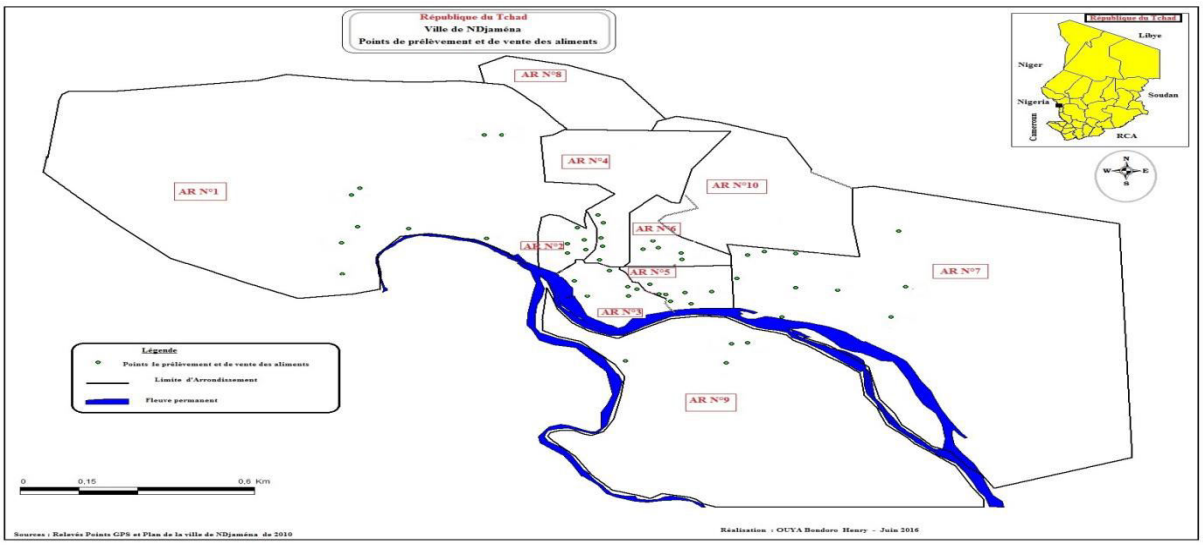
Types de dishes	Frequency (%)
Minced Beef Sandwich (n= 42)	32 (76,19)
"Ball" with okra sauce (n= 42)	35 (83,33)
Fried fish(n= 31)	24 (77,42)
Total (n= 115)	**91 (62,43)**

Table.3 Resistance phenotype of S. aureus strains**.**

Antibiotics	N = 30	
	S (n/%)	R + I (n/%)
Penicillins		
Penicillin G	00 (0%)	30 (100%)
Oxacillin	00 (0%)	30 (100%)
Fluoroquinolones		
Ofloxacin	30 (100%)	0 (0%)
Ciprofloxacin	30 (100%)	0 (0%)
Macrolide, Lincosamine and Cephalosporin		
Erythromycin	12 (40%)	18 (60%)
Lincomycin	15 (50%)	15 (50%)
Cefoxitin	20 (66.67%)	10 (33.33%)
Glycopeptide, Fosfomicin and Rifampicin		
Teicoplanin	30 (100%)	0 (0%)
Fosfomycin	30 (100%)	0 (0%)
Rifampycin	30 (100%)	0 (0%)

S= sensitive; R + I = resistant plus intermediate; N= number of strains

Figure.1 Sampling Sites



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Author Contributions

MayoréAtéba Djibrine: Investigation, formal analysis, writing—original draft. A. T. Kuan: Validation, methodology, writing—reviewing. Bodering Alain:—Formal analysis, writing—review and editing. Abdelsalam Tidjani: Investigation, writing—reviewing. Nicolas Barro: Resources, investigation writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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