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Antimicrobial resistance of *Staphylococcus aureus* and *Pseudomonas* spp. isolated from coated skewers sold in Ouagadougou, Burkina Faso

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ABSTRACT

Coated skewers are very popular in Ouagadougou. This work aims to assess the microbial quality ndaof coated skewers sold in the streets of Ouagadougou and their isolated *Staphylococcus aureus* *Pseudomonas* spp antimicrobial resistance. A total of 30 coated skewers samples were collected from various processors for microbial analysis using standard methods. The antimicrobial resistance test was performed using the agar plate diffusion method. The microbial load varied from 6.0×10^4-1.7×10^8 cfu/g (aerobic mesophilic bacteria (AMB)), 1.5×10-2.7×10^7 cfu/g (coliform), 0.0 to 6.1×10^1 (*S. aureus*) and 10-25 cfu/g (*Pseudomonas* spp.). Globally, 70% (21/30), 30% (09/30), 40% (12/30) and 6.67% (02/30) of the analyzed samples were unacceptable based on the load of AMB, coliform, *S. aureus* and *Pseudomonas* spp., respectively. Four coated skewers were contaminated by *Pseudomonas* spp. About susceptibility to antimicrobial agents, 91.67% of *S. aureus* strains were resistant to ceftazidime and aztreonam, while a low resistance rate was observed for the others antibiotics. *Pseudomonas* spp strains, were resistant to cotrimoxazol (75%) but sensitive to the other antibiotics. These results highlight poor hygienic conditions of coated skewers preparation in Ouagadougou. These practices contribute to their microbial contamination. Isolated bacteria showed a different level of resistance to the tested antibiotics. There is a need of good manufacturing practices to improve the hygienic quality of coated skewers.

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1. Introduction

The increase of population in developing countries has imposed new food habits especially street food.

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According to Food and Agriculture Organization (FAO), street foods sector takes particular dimension in African urban centers. There rapid urbanization and economic difficulties have encouraged increase in the number of street food sellers (1).

Over the past twenty years, cases of collective food poisoning associated with the consumption of street foods have increased in developed countries, involving pathogens such us *Salmonella*, pathogenic *E. coli* and *S. aureus* (2,3). Illnesses associated with the consumption of contaminated street foods are common in many parts of developing countries, but are underestimated due to the lack of reliable survey and surveillance data (4,5). In these countries, the main risk factors for contamination are generally poor hygiene and conservation practices during meat processing (6).

In Burkina Faso, coated skewers are one of the most popular street foods with low-cost and accessible. The poor sanitary practices during cooking process and sale increase the risk of microbial contamination (6). In those same places, people use antibiotics without any medical consultation or service to deal with diseases associated to street foods. It then result an increase in multidrug resistant bacteria in developing countries (7). In addition, the uncontrolled use of antibiotics in breeding contribute to increase the spreading of antibiotic-resistant bacteria (8,9). Regarding all the above, there is very little data to our knowledge concerning food poisoning of meat products in Burkina Faso.

This study, aims to assess the microbiological quality and the antibiotic resistance of S. aureus and Pseudomonas spp. strains in the coated skewers sold in Ouagadougou, Burkina Faso. Specifically, it is about to (i) assess good hygiene practices, (ii) analyze the microbiological quality of coated skewers, and (iii) test the resistance of S. aureus and Pseudomonas spp. strains to some currently used antibiotics by the populations.

2.Materials and Methods

2.1. Survey

The survey was carried out from March to August 2020, in Ouagadougou, Burkina Faso among coated skewers sellers. It's focused on the socio-demographic characteristics (age and school level) and good hygiene and manufacturing practices criteria using Ishikawa methods of 5M (Raw materials, materials, environments, labor and methods).

2.2. Sample collection

The city was divided into 3 crowns. According to the number of markets per crown, 4 sites were selected in zone 1, 3 sites in zone 2 and 3 sites in zone 3. A total of 30 coated skewers samples were collected from 10 sellers (3 samples per seller) in 10 sites. Samples concern three successive production during 3 weeks. Samples were collected in stomacher sachet, introduced in a cooler containing ice and transported to laboratory and kept at 4°C for analyses within 24 h.

2.3. Microbiological analysis

The preparation of stock solution was carried out according to standard (10) by introducing 10 g coated skewer meat into a flask containing 90 ml of sterile diluent (Liofilchem, Teramo, Italy). A homogeneous solution was obtained after homogenization with a Stomacher (11). This solution was used to make a serial dilutions until the appropriate dilution.

2.4. Enumeration of aerobic mesophilic bacteria

These microorganisms were enumerated on plate count agar (PCA) (HIMEDIA REF M091-500G) medium according to standard (12). Briefly, 1 ml of each dilution was plated on PCA and incubated at 30°C for 72 ± 3 h (12). The colonies enumeration was carried out on two successive dilution dishes (less than 300 colonies and more than 4 colonies on a dish) according to standard (12).

2.5. Enumeration of Coliforms

Seeding was carried out in a double layer on violet red bile lactose agar and incubated at 37°C for 24 h according to standard (13). Bacterial colonies were enumerated according to the formula described below. According to AFSSA standard, the threshold of detection of this germ was 10^2 cfu/g (14).

2.6. Enumeration of *Staphylococcus aureus*

About 0.1 ml of the suspension was streaked on to Baird Parker agar supplemented with egg yolk with potassium tellurite and carefully spread using a spreader (15). Incubation was carried out at 37°C for 24–48 h. After incubation, all the dishes where there was growth of small black, shiny colonies surrounded by a transparent halo and an opaque border of 2–5 mm in diameter were retained for enumeration. The enumeration was carried out according to the formula described above. The threshold of detection of this germ was 10^3 cfu/g according to AFSSA (14). After enumeration, 5 characteristic colonies were randomly chosen from each dish and inoculated into sterilized tubes containing 5 ml of brain heart broth and incubated at 37°C for 24 h. After the incubation, 0.5 ml of the medium was added with 0.5 ml of oxalated rabbit plasma. This new mixture is incubated at 37°C for 24 h for observation of the solidification of the plasma in the tubes (15).

2.7. Enumeration of *Pseudomonas* spp.

For *Pseudomonas* enumeration, Cetrimide Agar was used (16). Thus, 0.1 ml of the initial suspension or the appropriate dilution is spread-plated on the surface of the agar in Petri dishes. Incubation was done at 42°C for 24 h to observe blue-green colonies considered as suspected colonies (16). The count of these colonies was scored at less than 150 colonies per dish. Five representative colonies were randomly selected from all types of colonies of the selected dishes and submitted to confirmation test using Moistened filter paper with oxidase reagent. Selected colony was scraped using a loop in platinum or plastic and placed on a moistened filter paper. Positive and negative controls were used for the confirmation of results. Colonies which showed positive oxidase reaction were presumptively considered as *Pseudomonas* spp.

2.8. Expression of microbiological results

The number of colonies on each dish was counted after incubation period. The dishes of two successive dilutions containing between 4 and 300 colonies (AMB) and 4 and 150 colonies (Coliforms, *S. aureus* and *Pseudomonas* spp.) were selected and the number of microorganisms N was calculated according to the following formula:

$$N = \frac{\Sigma c}{V(n1 + 0.1\ n2)d}(\mathrm{CFU/g})$$

With Σc = the total number of colonies counted on all the retained dishes of two successive dilutions and of which at least one dish contains 10 colonies; V = volume of inoculum applied to each dish in milliliters; n1 = dish number retained at the first dilution; n2 = dish number retained, at the second dilution; 0.1 = dilution factor; d = dilution ratio, corresponding to the first dilution retained.

All the results were interpreted according to the standard AFSSA criteria (14).

2.9. Antimicrobial resistance test

All isolates were also tested for susceptibility to different antimicrobial agents using the disk diffusion method on Mueller Hinton II agar (Bio-Rad France), according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (17). *E. coli* ATCC 25922 and ATCC 35218 were used as a control. The antimicrobial disks (Himedia, India) used were amoxyclav (30 µg), aztreonam (30 µg), ceftazidim (30 µg), tobramycin (10 µg), amikacin (30 µg), erythromycin (15 µg), norfloxacin (10 µg), vancomycin (30 µg), chloramphenicol (30 µg), cotrimoxazol (25 µg) for *S. aureus* and amoxyclav (30 µg), aztreonam (30 µg), ceftazidim (30 µg), ceftriaxon (30 µg), tobramycin (10 µg), amikacin (30 µg), norfloxacin (10 µg), chloramphenicol (30 µg), cotrimoxazol (25 µg), colistin (50 µg) for *Pseudomonas* spp.

2.10. Statistical analysis

Excel software made it possible to classify and enter the results. Then the data were analyzed using XLSTAT Pro 2014 version software. Shapiro test was used for the normality of the data. Kruskal-Wallis test was used for the multiples comparisons because data were not normally distributed. The Principal Component Analysis test was performed to analyze correlations between contaminating bacteria and to see samples less contaminated or more contaminated by the germs researched.

3. Results

3.1. Socio-demographic characteristics of processors/ sellers and hygiene practices during processing

Survey showed that 72.41% of processors are aged between 18 and 35 years, 44.83% were unschooled, 34.48% had a primary level and 3.45% had university level (Fig. 1). According to origin of the meat, 80% of the meat came from slaughterhouses, 10% from large market and the other 10% from *Zabre daaga* market. About, 90% of meat is generally transported on motorcycles and 10% on tricycles. During transport, the meat is either not protected or sometimes covered by paper or bags which are reused several times. Sixty percent (60%) of production sites are located next to gutters and along the tracks. Forty percent (40%), are located at the edge of tracks, 20% are tiled, 60% are in simple beaten earth and 20% are cemented.

After production, 80% of processors expose their coated skewers on open-air and 20% kept down their products in boxes (Fig. 2). In addition, 70% of the coated meat is sold with cement paper and 30% with aluminum foil.

Table 1. The average loads of different microorganisms analyzed

	Microbes			
Processors	AMB	Coliformes	*S. aureus*	*Pseudomonas*
P01	6.0×10^{4} a	3.9×10^{2} a	0.0	1.0×10^{1} a
P02	4.4×10^{6} a	2.1×10^{2} a	8.2×10^{1} a	1.0×10^{1} a
P03	1.2×10^{8} a	5.1×10^{2} a	6.1×10^{3} a	1.0×10^{1} a
P04	6.8×10^{6} a	2.7×10^{3} a	3.0×10^{4} a	5.8×10^{1} a
P05	2.9×10^{6} a	6.3×10^{2} a	1.9×10^{3} a	2.3×10^{1} a
P06	2.5×10^{7} a	3.7×10^{3} a	3.5×10^{3} a	1.0×10^{1} a
P07	3.7×10^{6} a	1.5×10^{1} a	1.0×10^{2} a	1.0×10^{1} a
P08	1.3×10^{7} a	1.1×10^{6} a	1.3×10^{2} a	1.0×10^{1} a
P09	1.7×10^{8}a	1.3×10^{2} a	3.6×10^{3} a	1.0×10^{1} a
P10	5.8×10^{7} a	2.7×10^{7} a	5.8×10^{3} a	1.0×10^{1} a
p-value	0.167	0.261	0.706	0.505

Indication: in the same column, the data assigned the same letter [a] are not significantly different at the 5% threshold according to Kruskal-Wallis test.
Legend: AMB: Aerobic Mesophilic Bacteria

3.2. Microbiological analysis

The results of the microbiological analysis showed that the coated skewers samples were loaded with AMB, coliforms, *S. aureus* and *Pseudomonas* spp. in different proportions. The average loads of AMB varied between 6.0×10^{4} and 1.7×10^{8} cfu/g with an average charge of 4.04×10^{7} cfu/g. The multiple comparison analyses using Kruskal-Wallis test indicated in table 1 showed that the microbiological analysis does not vary between producers (p-values > 0.05).

The results showed that each microorganism load is clearly greater than the fixed threshold of 5%. There is no a correlation between the microbial load production sites.

Base on microbial standard, 70% of coated skewers analyzed are unsatisfactory according to their load in AMB, 40% according to *S. aureus*, 30% according to coliforms and 6.67% for *Pseudomonas* spp. Among the 30 samples analyzed, 73.33% are unacceptable. Results of this study show that, contamination were more observed with sellers aged 18 to 35 years, sellers unschooled followed primary level. Also, most sellers aged 18 to 35 years were unschooled.

Table 2. Antimicrobial resistance testing of *S. aureus* and *Pseudomonas* spp.

Antibiotics	*S. aureus* (%)		*Pseudomonas* spp. (%)	
	Sensitive	Resistant	Sensitive	Resistant
Amoxyclav	50	50	0.00	100
Aztreonam	8.33	91.67	25	75
Ceftazidime	8.33	91.67	25	75
Ceftriaxone	-	-	50	50
Tobramycin	100	0.00	100	0.00
Amikacin	83.33	16.67	100	0.00
Erythromycin	58.33	41.67	-	-
Norfloxacin	91.67	8.33	50	50
Vancomycin	83.33	16.67	-	-
Chloramphenicol	91.67	8.33	50	50
Cotrimoxazol	75	25	25	75
Colistin	-	-	100	0.00

Legend: - = this antibiotic was not tested

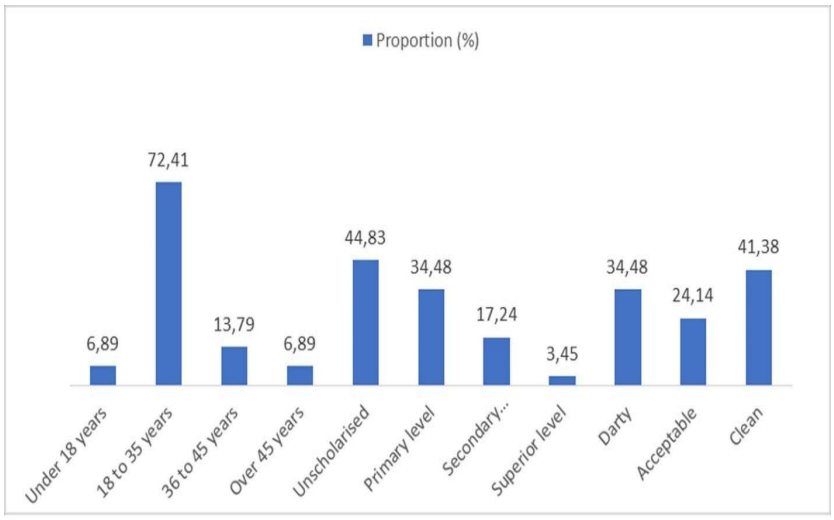

Figure 1. The socio-demographic data of coated skewers sellers

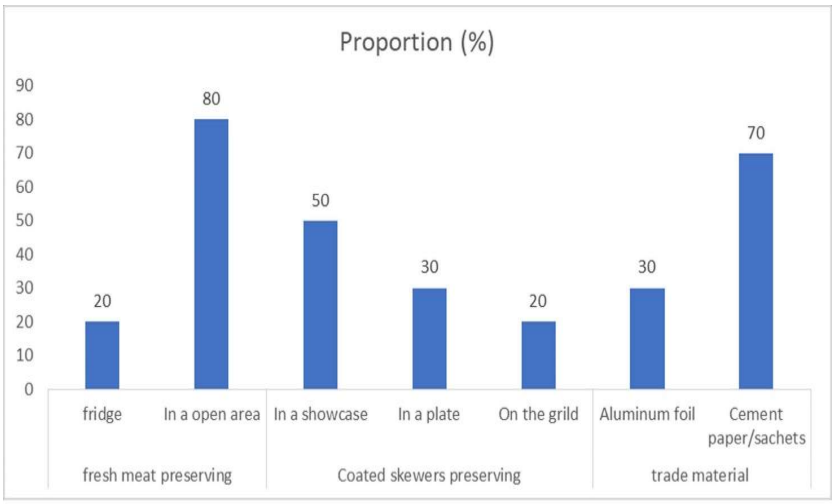

Figure 2. Methods of coated skewers conservation after production

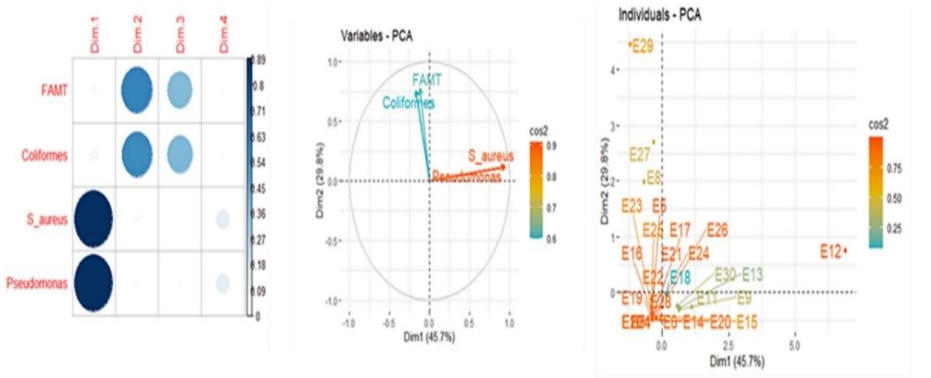

Figure 3.a: PCA of bacteria in coated skewers

Figure 3.b: correlation circle

Figure 3.c: individuals graph in PCA

Figure 3. Principal component analysis of bacteria isolated in coated skewers in Ouagadougou

3.3. Principal component analysis

Fig. 3.a, 3.b and 3.c are about correlation between enumerated microorganisms. These figures show 45.7% of variance of first dimension (Dim1) and 29.8% which for the second dimension (Dim2) with a total of 75.5% of variance. This value is an evidence of a good representation of results. Based on Fig. 3.a and 3.b, the samples which were on or near to Dim1 axis are more contaminated with *S. aureus* and *Pseudomonas* spp.and those near axis 2 were more contaminated with AMB and coliforms. According to Fig. 3.b, sample E12 was the most contaminated with S. aureus and Pseudomonas spp. and E29 was more contaminated with AMB and coliform. However, E18 was less contaminated. Similarly, E9, E11, E13 and E30 were weakly contaminated by S. aureus and Pseudomonas spp. when E8 and E28 were less contaminated with

AMB and coliforms.

3.4. Antimicrobial resistance testing of *S. aueus* and *Pseudomonas* spp. isolated from coated skewers

A total of 12 suspected strains of *S. aureus* and 4 of *Pseudomonas* spp. were isolated from coated skewers. All the isolates were screened for susceptibility to different antibiotics discs. Results are shown in Table II. Concerning *S. aureus*, a high rate of resistance was notified to aztreonam and ceftazidime (91.67%), amoxyclav (50%) and erythromycin (41.67%). For *Pseudomonas* spp., resistance was notified with amoxyclav (100%), ceftazidime, aztreonam, cotrimoxazol (75% each one), ceftriaxone, norfloxacin and chloramphenicol (50% each one).

4.Discussion

Increase in urban demographic in West Africa has favored development of a dynamic artisanal and inexpensive food sector which provides processors with a substantial income. Coated skewers are among these products. The conditions and practices of processing and distribution of coated skewers increase their risk of contamination and lead to a serious public health matters. The ignorance of street sellers on food-borne illnesses is a risk factor (18-20). The microbial results showed that most of the coated skewers was contaminated. These coated skewers were charged in AMB with 4×10^7 cfu /g as an average. Several recent studies have reported similar results for meat products sold as street food in Burkina Faso and others developing countries (6, 21).

In our study contamination were more observed with sellers aged 18 to 35 years, sellers unscholarised followed primary level. According to the survey results, most of seller's aged 18 to 35 years were unschooled. These results could be justified by ignorance and lack of training in good hygiene and manufacturing practices among unschooled sellers and those at the primary level. Coated skewers were also mostly charged in coliforms and *S. aureus*. *S. aureus* is indicator a contamination by hand or linked to personal hygiene of processors. Coliforms and *S. aureus* in food are indicators of direct fecal contamination, of the sales environment or of the handler himself because *S. aureus* are naturally localized in the skin, mucous membranes, hands and nasal passages (22-24). According to survey, there are poor hygiene practices in slaughtering, transporting, processing, preserving and marketing meat. The high level of contaminations of the skewers by microorganisms could be then due to a lack of

hygiene when processing meat to skewers in polluted environments. About, 60% of sellers are located at edge of tracks and gutters which contain solid waste and stagnant water. In addition, the use of some ingredients for coating the meat and water of questionable quality could contaminated skewers. Peanut cakes and spices dried in open air (dust), mills and mortars used for grinding may be source of contamination. Moreover, there is a lack of hygiene throughout the production chain. So, the high concentration of these total coliforms found in the meat could come from fecal contamination or a poor hygienic condition and the use of water of questionable quality since the sellers do not have a tap next to them from their points of sale corners (19). Coated skewers are also charged in *Pseudomonas* spp. And, heat would probably be one it's limiting factors that would have destroyed the maximum of this germ since it is sensitive to heat. Then this low contamination could be justified by cross-contamination due to the absence of the system of the forward movement on all the sites of studies. Barro et al. and Gedik, et al. showed that money handling constitutes another risk factor of street foods contamination. Money can get contaminated and then play a key role in transmission of microorganisms during the selling (25,26). These streets contaminated foods consumption could be a public health risk. In fact, *P. aeruginosa* secretes a wide range of extracellular toxins, including exotoxin A and enterotoxins. Other substances such as hydrocyanic acid, proteolytic enzymes, biofilms and haemolytic substances may also contribute to the pathogenicity of this species.

The combination of toxins and hazardous substances is a key factor in the high virulence of *P. aeruginosa* in different hosts Footnote (27,28).

According to the statistical analysis, *S. aureus* and *Pseudomonas* spp. contribute strongly to Dim1. These correlated germs strongly contaminate coated meat. Because these germs are generally found in the environment (water, soil, air) and animals bodies. This why these germs evolve together. Dim 2 shows that coliforms are strongly linked to AMB. AMB is a set of microorganisms growing at 30°C. They group together spoilage germs and pathogens. Coliforms then strongly influence AMB.

Street food are often contaminated with pathogenic bacteria which are responsible for infectious diseases (29,30). Up to day, studies carried out in Burkina Faso on resistance mechanisms to antibiotics have more focused on clinical strains, whereas food is mostly part of disease sources. This study is a first in Burkina Faso. Many studies around the world show that bacterial strains are increasingly developing resistance to antibiotics. The use of antibiotics in breeding mainly spread out resistant strains throughout meat and meat products. A total of 12 *S. aureus* and 4 *Pseudomonas* spp. were isolated and carried out for antimicrobial resistance tests with different antibiotics discs. The majority of *S. aureus* and *Pseudomonas* spp. isolates from coated skewers were resistant to amoxyclav, aztreonam, ceftazidime and norfloxacin and sensitives to other antibiotics. This is similar to other studies that observed a high sensitivity of *S. aureus* (23,31,32).

Nowadays, antibiotics are mostly used to increase animals' growth. There are used to treat some animal illnesses. In developing countries, the lack of financial and the ignorance due to the low level of education of the breeders make them use antibiotics without any veterinary assistance (33,34). Veterinary antibiotics are now sold cheaper in the public square by ambulant sellers who have no idea of antibiotic retention principles and expiry date. In addition, poverty is one of the factors associated with emergence of antibiotic resistance in developing countries. Indeed, the poor economic conditions associate the malnutrition, inaccessibility to safe drinking water and hygienic conditions increase the risk of acquiring infections and transmitting resistant bacteria.

5.Conclusion

This study carried out on the coated skewers showed high levels of contamination with pathogenic bacteria in general and *S. aureus* and *Pseudomonas* spp. in particular, with associated risk for consumers' health. It's highlight that coated skewers in Ouagadougou are prepared in poor hygienic conditions which contribute its bacterial contamination. Antimicrobial resistance, showed that isolated *S. aureus* and *Pseudomonas* spp. are resistant to the main b-lactam antibiotic family. As a solution, quality management during coated skewers processing, preservation and sale need to be increased to ensure the quality of finished products. Microbial and antimicrobial resistance plans must be implemented as tools for infection prevention.

Conflict of interests

The authors declare that they have no competing interests

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