Journal of Agriculture and Food Research 2 (2020) 100063

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.journals.elsevier.com/journal-of-agriculture-and-food-research/

Toxin production and antibiotic resistance of *Staphylococcus aureus* strains isolated from market gardening products and their irrigation water in cotonou, Benin

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ARTICLE INFO

Keywords:
Market gardening products
Irrigation waters
Staphylococcus aureus
Toxins
Antibiotics
Benin

ABSTRACT

Consumption of market gardening product has displayed several cases of food poisoning because they are being consumed raw often. This study aims to establish antibiotics resistance and toxins production profiles of *Staphylococcus aureus* strain isolated from market gardening products and irrigation water in Cotonou. In this regard, 4 kind of market gardening products (Lettuce, Carrot, Nightshade, and Cabbage) and 22 samples of irrigation water (pool, well and drilling water) were collected from four market gardeners and were analyzed. The *S. aureus* strains were identified using microbial method and classical biochemical tests. The antimicrobial susceptibility of 17 antibiotics was performed by the agar disk diffusion method. The toxins production was detected by the radial gel immunodiffusion method. Out of the 112 samples analyzed, 69.81% were contaminated by *S. aureus*. Market gardening products were contaminated by *S. aureus* at the rate of 82.44% and the irrigation water was at 17.56%. Among these products, nightshade was the most contaminated (32.79%), followed by lettuce (27.86%), cabbage (21.31%) and carrot (18.04%). Based on the type of water samples used, it appears that 53.85% of the well water contained *S. aureus*. Globally, the highest resistance proportion has been observed with lincomycin (62.06%) and none of the strains were resistant to ciprofloxacin, whereas about 20.69% of the strains were resistant to the methicillin (MRSA). ETA and LPV toxins were the most produced (32.69%) followed by ETB (28.85%), and LukE-D (5.77%). From the toxin-producing strains of *S. aureus*, 46.43% were MRSA. High contamination rate of toxins producer and resistant *S. aureus* strains isolated were recorded on market gardening products. It is important to promote monitoring and sensitizing farmers in aim to reduce the contamination rate of market gardening products.

1. Introduction

In Africa, the rapid growth of the population in the urban areas has made supply a major problem and a stake in food security policies said [1]. Thus, Benin, as one of the sub-Saharan African countries, has in recent years experience a rise in urban and peri-urban agriculture because of the high population growth leading to an increase in food needs [1]. Ba and Cantoreggi [2], states that Peri-urban agriculture is characterized by market gardening and is often an informal activity.

In the development of market gardening schemes, some major constraints are being faced, such as land pressure (inadequate land space), resulting in overexploitation of land which causes huge production losses. To reduce these occurrences, market gardeners use mineral, organic fertilizers and chemical pesticides. In the absence of an adequate control mechanism, the use of pesticides rarely meets health and environmental standards [3]. Besides, the lacks financial means for the supply of drinking water and synthetic fertilizers, makes market gardeners use wastewater for irrigation [4].

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https://doi.org/10.1016/j.jafr.2020.100063
Received 25 May 2020; Received in revised form 23 July 2020; Accepted 23 July 2020

The harms faced, as a result of consumption of market gardening products among which food poisoning and infections are a public health problem. This occurs due to the frequency, severity and has increased the Foodborne illness 300 to 350 times more than indicated numbers reported cases [5]. They result from the consumption of food contaminated by a harmful microorganism or a pathogen producing toxins [6–8]. Among the microorganisms involved in these digestive manifestations, *S. aureus* occupies an important space, due to the production of a wide range of virulence factors [9]. *S. aureus* is the most pathogenic microbial species among staphylococci and causes several diseases [10]. It is also the main cause of food poisoning due to the production of enterotoxins by species contaminating food products [11]. More than 90% of foodborne infections whose determinism is known are due to bacteria. Among these, *S. aureus* accounts for 75% of reported cases. Thus, for the treatment of infections on bacterial origin, the abusive are often uncontrolled use of antibiotics by self-medication has revealed a phenomenon of resistance in most bacteria. Worldwide, the spread of resistant pathogenic microorganisms is of great concern [12]. Indeed, many cases of multidrug-resistant bacteria are reported in Benin [13–15], Côte d'Ivoire [16], Burkina-Faso [17,18].

However, *S. aureus* infections are facilitated by the expression of several virulence factors, which include several groups of toxins such as Panton and Valentine's Leucocidin (LPV), toxic shock syndrome toxin, adhesins, hemolysins, exfoliative toxins (ETA and ETB), and staphylococcal enterotoxins. Ingestion of *S. aureus* in high doses in foodstuffs contaminated with staphylococcal toxins can produce food poisoning [19].

Given the numerous cases of *S. aureus* food poisoning, and the emergence of *S. aureus* resistant strains, it is necessary to investigate the food microbial quality to prevent health risks related to the consumption of market gardening products by the Beninese population. This study aims to look for the antibiotic resistance profile and toxins produced by *S. aureus* strains isolated from sprinkling water and market garden products sold in Cotonou.

2. Material and methods

2.1. Samples sites and collections

For this study, samples of market garden products and water were collected from four (Fidjrossè, Houéyiho, Akpapka, and Cadjehoun) truck farming sites in Cotonou (Fig. 1). The four sites were selected after a prior investigation. The main objective of the prior investigation was to sift all the truck farming sites in Cotonou focusing on i) the market garden products and ii) the sprinkling water used. The selected site were those on which not only the four-targeted garden products were product but

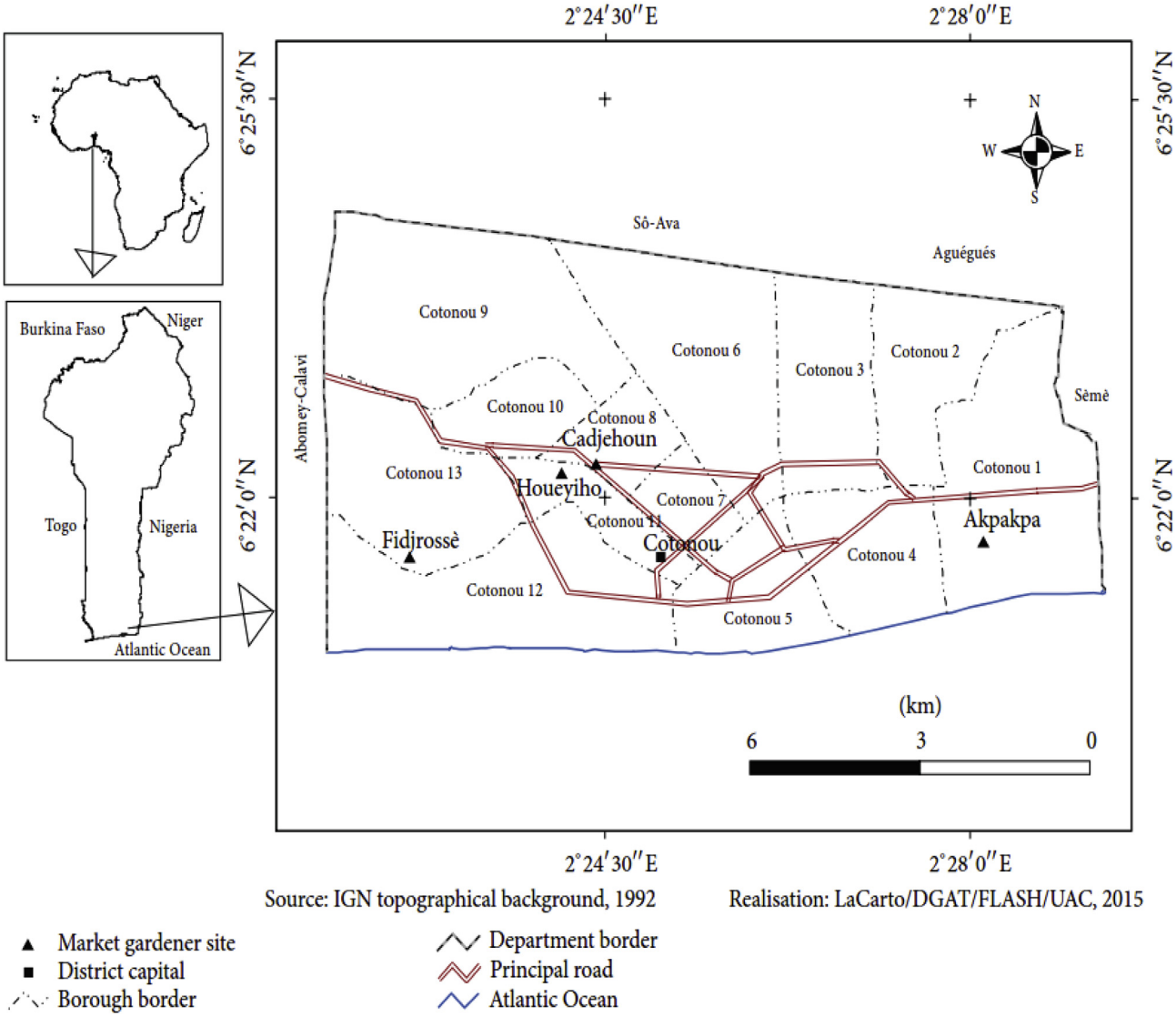


Fig. 1. Map showing the samples collection sites.

also the three (wells, pools, and drilling) sprinkling water used.

2.1.1. Market garden products samples

Per the site, three gardeners were randomly selected among those who grow the four-targeted products (lettuce, cabbage, nightshades and carrot) and two round of samples for each product were collected per gardener. The samples were collected in the dry season (January–February) and in the rainy season (October–November). Per season, 88 samples (22 lettuces, 22 cabbages, 22 nightshades, and 22 carrots) were collected aseptically and carried to laboratory in icebox (4 °C).

2.1.2. Irrigation water samples

From these four sites, three kinds of irrigation water (wells, pools, and drilling) were collected. The water samples were only collected in the dry season (January–February). Water samples were collected following a standard technique [20]. Briefly, before taking samples, the exterior of the containers was cleaned with 70% ethanol. After that, the targeted sprinkler water was mixed before collecting the sample from the surface. Per the farm site, the three gardeners were randomly selected among those who grow the three targeted irrigation water samples and two samples of each kind of water were collected per gardener. Then, 24 irrigation water samples (8 wells, 8 pools, and 8 drilling sites) were collected aseptically and then carried to the laboratory in the icebox (4 °C).

2.2. Microbiological analysis

2.2.1. Market garden products samples

At the laboratory, 10 g of each sample was homogenized in 90 ml of sterile distilled water and then was incubated at 37 °C for about 2 h. For the isolation of *Staphylococcus* strains, 0.1 ml of serial decimal dilutions were incubated at 37 °C for 48 h on Baird-Parker Agar medium [21,22].

2.2.2. Irrigation water

The samples of irrigation waters, once carried at the laboratory, were filtered ($\theta = 0.45\,\mu m$) and the filter was incubated at 37 °C for 24 h on Plate Count Agar.

2.3. Isolation and identification of S. aureus strains

Standard microbiological methods for microorganism's identification were used [23]. Then, *S. aureus* identification was based on Gram staining, morphology, catalase positivity, agglutination in the Pastorex Staph Plus test (Bio-Rad, Marnes la Coquette, France) and free coagulase production with lyophilized rabbit plasma [24]. Finally, the API Staph (bioMérieux, Marcy l'Etoile, France) Gallery staining test was used to confirm the *S. aureus* species [25].

2.4. Susceptibility of S. aureus strains to antibiotics

The susceptibility of the identified *Staphylococcus aureus* to seventeen (17) conventional antibiotics was performed using the French Society of Microbiology recommendations [26]. The tested antibiotics were: penicillin G (P 10 µg), amoxycillin (AML10 µg), ciprofloxacin (Cip 5 µg), gentamycin (Gen 10UI), tobramycin (TM 10 µg), fosfomycin (Fos 50 µg), fusidic acid (FA 10 µg), kanamycin (K 30 µg), erythromycin (E 15 µg), lincomycin (L 10 µg), amikacin (AN 30 µg), vancomycin (VA 30 µg), pristinamycin (PT 15 µg), linezolid (LZD 30 µg), trimethoprim/sulfamethoxazole (Sxt 23,75 µg), teicoplanin (TEC 30 µg) and oxacillin (OX 1 µg). Oxacillin resistance was determined by the growth inhibition diameter on 5% (w/v) NaCl hyper-saline agar after 24 h of incubation at 37 °C.

2.5. Phenotypic detection of S. aureus toxins production

The production of Panton and Valentine Leucotoxin (PVL), leukotoxin (LukE-LukD) and epidermolysin (ETA, ETB) were phenotypically detected by radial gel immunodiffusion [27]. The toxins mentioned were evidenced from culture supernatants after 18 h of growth in Yeast Casamino-acid Pyruvate (YCP) medium [28] in 0.6% (wt/vol) agarose with component-specific rabbit polyclonal and affinity-purified antibodies [27].

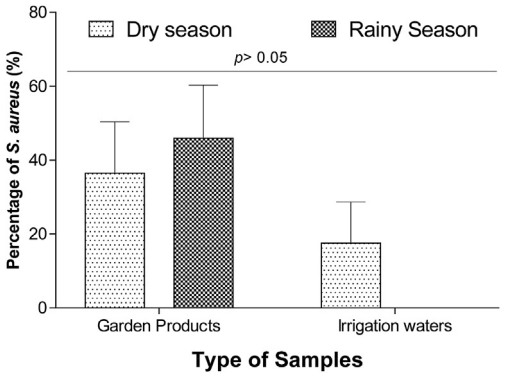


Fig. 2. Global contamination rate of market garden products and irrigation water by *S. aureus* according to the season.*; $p < 0.05$.

2.6. Data analyzes

The raw data was collected on benchtop sheets and the Microsoft Office Excel 2013 spreadsheet was used for graphing. The statistical texts were produced using the Epi Info 7.2.3.1 and GraphPad Prism 7. The significance level was $p < 0.05$.

3. Results

3.1. Distribution of S. aureus strains

Globally, 69.1% of the 112 samples were contaminated *Staphylococcus aureus* strains. Market gardening products were contaminated at a rate of 82.44% and the irrigation water was contaminated at 17.56%. It was observed that the *S. aureus* strains had contaminated the market garden products more in the rainy season (45.95%) than those in the dry season (36.49%) (Fig. 2). However, there was no statistically significant difference in the contamination rate of the different types of samples ($p > 0.05$).

3.2. Distribution of S. aureus strains according to the type of market garden produce and the season

Among the market gardening products, the nightshade samples were the most contaminated (32.79%), the least contaminated were carrot (18.04%). According to the season, in the dry season, the lettuce samples were the most contaminated (16.39%) and the least contaminated were carrot (4.92%). For the rainy season, samples of nightshade were the most contaminated (21.32%) followed by carrots (13.12%). The least contaminated were the cabbage samples with 9.84% (Fig. 3). The contamination rate statistically vary from a type of samples to another and from a season to another ($p < 0.05$).

3.3. Distribution of S. aureus strains according to the type of irrigation water

The proportion of contamination in irrigation water is variable depending on the type of water (Fig. 4). The well water samples were the most contaminated (53.85%) followed by pool water (30.77%) and finally drilling water (15.38%). The proportions of contamination according to the type of irrigation water are not statistically different ($p > 0.05$).

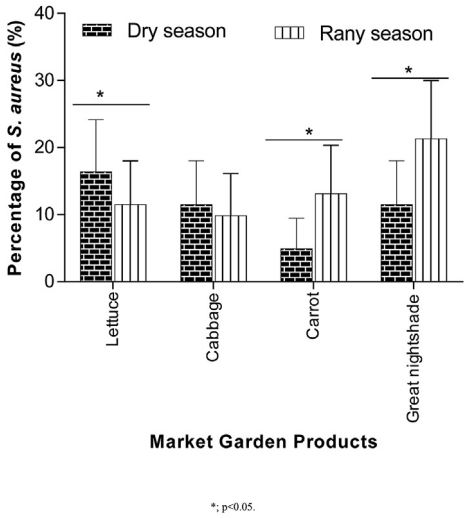


Fig. 3. Contamination rate of *S. aureus* strains according to the type of market garden product and the season.

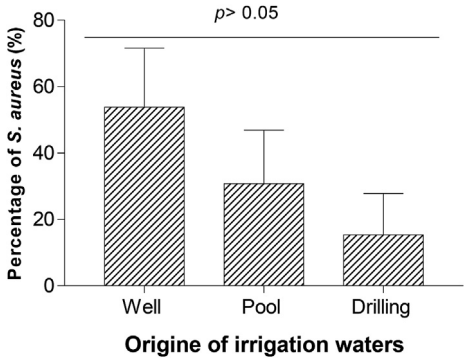


Fig. 4. Contamination rate of *S. aureus* strains isolated from irrigation water.**P.G**: Penicillin G, **Amo**: Amoxycilin, **Cip**: Ciprofloxacin, **Gent**: Gentamycin, **Tob**: Tobramycin, **Fos**: Fosfomycin, **A.F**: Fusidic acid, **Kan**: Kanamycin, **Ery**: Erythromycin, **Lin**: Lincomycin, **Ani**: Amikacin, **Van**: Vancomycin, **Pri**: Pristinamycin, **Lin**: Linezolide, **Trim**: trimethoprim/sulfamethoxazole, **Tei**: Teicoplanin and **Oxa**: Oxacillin.*; $p < 0.05$.

Considering all the samples, it was observed that the market gardening of Fidjossè was the most contaminated (31.08%) and the least contaminated was the one of Cadjehoun (14.86%). In the rainy season, the market gardening products samples from the Akpakpa site were the most contaminated with the *S. aureus* strains (24.59%) and the least contaminated were those from the Cadjehoun (3.28%.). On the other hand, in the dry season, the samples from the Houéyiho were the most contaminated (18.04%) and the least contaminated is that of Akpakpa (6.56%) (Table 1). The difference in proportion between the different sites is statistically significant ($p < 0.05$). Taking into account the irrigation water, the market gardening sites of Fidjrossè, Houéyiho and Cadjehoun were contaminated by the strains of *S. aureus* (30.77%) each and then Akpakpa (7.69%) (Table 1). The difference in proportion between the different sites is statistically significant ($p < 0.05$).

3.4. Susceptibility to antibiotics of isolated strains of S. aureus

The susceptibility of the isolated *S. aureus* strains to the 17 antibiotics tested was variable ($p < 0.05$) (Fig. 5). We observed a high proportion of *S. aureus* strains resistant to lincomycin (62.06%) followed by pristinamycin (55.17%). The resistance to penicillin G was 48.27%. The most active antibiotic was ciprofloxacin, which had inhibitory activity on all the strains of *S. aureus* tested. A rate of 20.69% of the strains of *S. aureus* was resistant to Oxacillin.

Table 1
Distribution rate of isolated *S. aureus* strains according to the sampling sites and the kind of samples.

Sample collection site	Market Garden Products		Irrigation Waters	Total	P-value
	Dry season	Rainy season			
Fidjrossè	9.46%	16.22%	5.40%	**31.08%**	***p***
Houéyiho	14.86%	6.77%	5.40%	**27.03%**	**<0.05**
Akpakpa	5.40%	20.27%	1.35%	**27.02%**	
Cadjehoun	6.77%	2.70%	5.40%	**14.87%**	
Total	**36.49%**	**45.96%**	**17.55%**	**100.00%**	

3.5. Prevalence of toxins produced by S. aureus isolated

The four types of toxins evaluated (Table 2) during our study were produced in varying proportions. Among the isolated *S. aureus* strains, 70.27% produced toxins. Thus, it was observed that the leukotoxin of Panton and Valentine (PVL) and ETA were the most produced (32.69%) followed by ETB (28.85%) and leukotoxin LukE-D (5.77%) (Table 2). This difference in toxin production is highly significant ($p < 0.0005$).

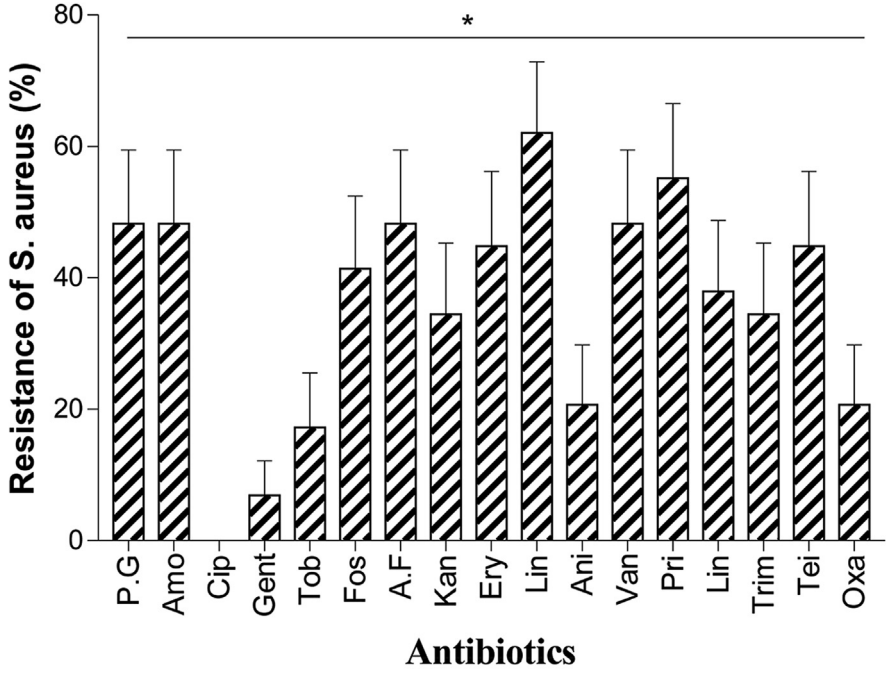
Among the toxin-producing *S. aureus* strains, 46.15% were resistant to Methicillin ETA, followed by LukS-F were the most produced toxins by Methicillin-Resistant *Staphylococcus aureus* (MRSA). Whereas, the Methicillin sensitive *Staphylococcus aureus* (MSSA) strains produced ETB followed by LukS-F. The least produced toxin was LukE-D for both MRSA (8.33%) and MSSA (3.57%) (Table 2).

4. Discussion

We observed that 69.81% of the samples were contaminated by *S. aureus*. This overall rate observed varies according to the type of water (irrigation water and market garden products). Thus, it was found that 17.56% of irrigation water and 82.44% of market gardening products were contaminated with *S. aureus*. Thus, the high contamination of market gardening product observed, is consistent with the observations obtained by other authors around the world in foods [28–31]. The potential origin of the high proportions of contamination of the two types of samples (irrigation water and market garden products) by *S. aureus* may be due to the poor hygienic quality of irrigation water. Since Staphylococci are ubiquitous germs, it is very easy to witness manual contamination [32].

In our study, we found out that the Market Gardeners carry out the various watering operations with bare hands and in an unsanitary environment. In view of the above, it is recommended that market garden products must, therefore, be treated with great care before consumption to reduce microbial density. Also, good hygiene practices: by washing market gardening products appropriately is highly recommended. Otherwise, when these hygienic factors are ignored, they lead to diseases such as gastroenteritis and diarrhea of microbial origin [33,34].

Among the market gardening products, carrot was the least contaminated (4.92%) in the dry season by the strains of *S. aureus* compared to the three leafy vegetables (Fig. 3). Note that this difference is statistically significant ($p < 0.05$). This can be explained by the fact that leafy vegetables are the most exposed to water, unlike the carrot which is an underground vegetable. During the rainy season, the cabbage samples were the least contaminated by *S. aureus* (Fig. 3). This observation could find its explanation in the morphology of the cabbage. Indeed, cabbage has leaves that close up forming a sort of crown. This crown would constitute a favorable environment that can prevent the development of bacteria such as *S. aureus*. Also, the outer leaves are currently washed out by rainwater. In addition, the variation in the contamination rate from



P.G: Penicillin G, **Amo**: Amoxycilin, **Cip**: Ciprofloxacin, **Gent**: Gentamycin, **Tob**: Tobramycin, **Fos**: Fosfomycin, **A.F**: Fusidic acid, **Kan**: Kanamycin, **Ery**: Erythromycin, **Lin**: Lincomycin, **Ani**: Amikacin, **Van**: Vancomycin, **Pri**: Pristinamycin, **Lin**: Linezolide, **Trim**: trimethoprim / sulfamethoxazole, **Tei**: Teicoplanin and **Oxa**: Oxacillin.*; p<0.05.

Fig. 5. Susceptibility of the isolated *S. aureus* strains to the seventeen tested antibiotics.

Table 2
Variation of toxins production by *S. aureus* isolated from irrigation water and market garden products.

Toxins	Methicillin Resistant *S. aureus*	Methicillin sensitive *S. aureus*	Total	P-value
Luk S/F	15.38%	17.31%	32.69%	***p* <0.0005**
Luk E/D	3.85%	1.92%	5.77%	
ETA	7.69%	25.00%	32.69%	
ETB	19.24%	9.61%	28.85%	
Total	**46.16%**	**53.84%**	**100.00%**	

Luk S/F: Leucotoxin S and F, Luk E/D: Leucotoxin E and D, ETA: Epidermolysin A, ETB: Epidermolysin B.

season to season can be the result of poor environmental quality. Indeed, during the dry season, there is generally an accumulation of dirt such as dust, waste. They are suitable compost for the development of potentially pathogenic microorganisms. Unlike the rainy season, rainwater cleans most places with dirt.

Irrigation water is often considered as a source of contamination for market garden products [35]. Compared to the other irrigation water, it was found that well water was the most contaminated with *S. aureus* (Fig. 4) in comparison with drilling and pool water. This fact is not surprising, in Cotonou, the groundwater is close to the surface of the ground and generally high permeability ensures the infiltration of rainwater and wastewater [36]. The agricultural technique practiced by most market gardeners also plays an important role in the contamination of irrigation water. Indeed, Market Gardeners use a large number of poultry drops (sometimes fresh) as fertilizer for soil fertilization. This farming practice would encourage permanent fecal contamination of irrigation water from shallow, uncovered wells [37]. Such sprinkling water can thus propagate in cultures many pathogenic microorganisms or even Norwalk and hepatitis A viruses [38,39]. Pollution of irrigation water could also be due to the unsanitary conditions of cultivation areas, located not far from household waste dumps and gutters. This is in agreement with the observations made by Matthys et al. [40] during their work on the social network of market gardeners in Abidjan.

The presence of a germ in a portion of food alone is not enough to say that it is responsible for a disease. It must be shown that this germ has toxinogenic equipment. Toxin research revealed that PVL and ETA were the most produced (Table 2). The PVL production obtained in our study is far from the 15% obtained in Benin by Baba-Moussa et al. [41] in all kinds of samples. Baba-Moussa et al. [42] showed that 21.50% of clinical *S. aureus* isolates produced PVL. This toxin, in clinical practice, is associated with skin diseases such as boils, abscesses [43–46]. Thus, the production of PVL in a higher proportion by food strains must moreover challenge us, especially about its virulence. This presence may be due to the portage by market gardeners, which would facilitate transmission. Sina et al. [47] obtained a rate of 14.07% of ETA production by street foods *S. aureus* strains. This rate is low at 32.69% of ETA obtained in our study. This different proportion maybe because of our strains from market gardening products that are not cooked. Production of the toxin LukE/D (5.77%) is observed in the strains of *S. aureus* (Table 2). This rate shows that the consumption of raw vegetable products can endanger humans because studies showed in 1998 in France the leukotoxin LukE-D plays an important role in the occurrence of diarrheal infections [27].

The antibiogram performed on the various strains of *S. aureus* against the 17 antibiotics revealed very varied resistance (Fig. 5). Markhous et al. [31] have observed similar resistances for strains of *S. aureus* from market garden products in Tchad. The presence of multi-resistant *S. aureus* in market garden products can be explained by the fact that they come from market gardeners who have already practiced

self-medication by treating infections caused by their working tools (cutting -cup, knives, watering cans, etc.). *S. aureus* being ubiquitous, as soon as they plunge their feet or their hands carrying these various cuts in the irrigation water, the microorganisms resistant to antibiotics are transmitted. Also, several studies have shown that the multi-resistance strains come more from the community environment [13,15,48]. In our study, no strain of *S. aureus* isolated from market garden products had resistance to Ciprofloxacin. However, we observe a high rate of resistance to Lincomycin (62.06%) and Pristinamycin (55.17%). This resistance rate to pristinamycin belonging to the lincosamide family is abnormal because it is an antibiotic recommended for the treatment of staphylococcal infections [49]. About 21% of the *S. aureus* strains were resistant to methicillin. Studies in Oklahoma have shown about 1.8% were resistant to methicillin [50]. Another study carried out in Tunisia by Chairat et al. [19] revealed that 2.5% of the strains were resistant to methicillin. The rate of 20.69% found in our study is high for food origin strains. Nevertheless, today several studies show that *S. aureus* widens its pathogenicity territory by developing resistance to several antimicrobial agents [51]. The presence of multi-resistant *S. aureus* in our samples can be explained by the fact that they come from market gardeners who have already practiced self-medication by treating infections caused by their working tools.

The presence of toxin-producing MRSA was observed, 41.67% and 33.33% of MRSA produced ETB and LukS-F, respectively. The presence of MRSA in these foods could constitute a supplementary risk for the consumer, not only by the zoonotic potential (presence of toxinogenic strains, of enterotoxin strains) but also by the pathogenic potential in certain circumstances especially in immunocompromised subjects, the elderly and children [52]. The risks of contamination of vegetables would, therefore, exist in Benin. Indeed, these vegetables can carry many infectious diseases [53].

5. Conclusion

Foodborne illness due to their recurrence remains a major concern of the authorities responsible for public health. *S. aureus* was present in both irrigation water and market gardening products. Likewise, all *S. aureus* isolated strains from market gardening products and their irrigation water produced toxins, which remains a very important virulence factor. Thus, this study brings new knowledge on the microbiological quality of market gardening products as well as their sprinkling water. Therefore, this study will contribute to better management of food security.

Declaration of competing interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This work was supported by Grant E/4332-2F of International foundation of science (IFS).

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