

Article

Prevalence, Enterotoxigenic Potential and Antimicrobial Resistance of *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolated from Algerian Ready to Eat Foods

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Abstract: *Staphylococcus aureus* causes a foodborne intoxication due to the production of enterotoxins and shows antimicrobial resistance, as in the case of methicillin-resistant strains (MRSA). Herein, we analyzed 207 ready-to-eat foods collected in Algeria, reporting a *S. aureus* prevalence of 23.2% (48/207) and respective loads of coagulase positive staphylococci (CPS) ranging from 1.00 ± 0.5 to 5.11 ± 0.24 Log CFU/g. The 48 *S. aureus* isolates were widely characterized by staphylococcal enterotoxin gene (SEg)-typing and 16S-23S rDNA intergenic spacer region (ISR)-PCR, as well as by detecting *tst* and *mecA* genes, genetic determinants of toxic shock syndrome toxin-1 and methicillin resistance, respectively. We found that the *S. aureus* isolates belonged to seven different SEg-types harboring the following combinations of genes: (1) *seIW, seIX*; (2) *egc (seG, seI, seM, seN, seO), seIW, seIX*; (3) *seA, seH, seK, seQ, seIW, seIX*; (4) *seB, seIW, seIX*; (5) *seD, seIJ, seR, seIW, seIX*; (6) *seH, seIW, seIX, seIY*; and (7) *seA, egc, seIW, seIX*, while among these, 2.1% and 4.2% were *tst*- and *mecA*- (staphylococcal chromosomal cassette *mec*-type IV) positive, respectively. Selected strains belonging to the 12 detected ISR-types were resistant towards antimicrobials including benzylpenicillin, ofloxacin, erythromycin, lincomycin, tetracyclin, kanamycin, oxacillin, and cefoxitin; 8.3% (1/12) were confirmed as MRSA and 16.7% (2/12) were multidrug resistant. The present study shows the heterogeneity of the *S. aureus* population in Algerian ready-to-eat foods as for their toxigenic potential and antimicrobial resistance, shedding the light on the quality and safety related to the consume of ready-to-eat foods in Algeria.



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Keywords: *Staphylococcus aureus*; staphylococcal enterotoxin; enterotoxin gene cluster (*egc*); staphylococcal food poisoning; staphylococcal chromosomal cassette *mec* (*SCCmec*); methicillin resistant *Staphylococcus aureus* (MRSA); antibiotic resistance; toxic shock syndrome toxin-1; 16S-23S rDNA intergenic spacer region PCR (ISR-PCR); ready-to-eat foods

Key Contribution: The consumption of Algerian ready-to-eat foods may represent a risk for the consumer's health due to the presence of enterotoxigenic and antimicrobial-resistant *S. aureus* strains. To our knowledge, we performed the widest genetic characterization conducted so far in Algeria to investigate the enterotoxigenic potential of foodborne *S. aureus* isolates.

1. Introduction

Food-associated health problems, caused by food contamination or malnutrition, have a worldwide impact on public health and economy [1]. Particularly, foodborne diseases (FBDs) represent a major issue for public health, and there is a growing number of outbreaks

and sporadic cases of disease associated with various type of foods [2–4]. Furthermore, FBDs have a significant economic impact on both the industry and the public health system in terms of loss of productivity, cost of treatment, and food safety governance [5]. On top of that, FBDs are responsible for nearly 600 million illnesses and 420,000 deaths each year [6].

Staphylococcus aureus is one of the major pathogens of humans. It causes various diseases including staphylococcal food poisoning (SFP), toxic shock syndrome and other systemic diseases, bacteremia, pneumonia, and skin and soft tissue infections, to cite a few [7,8]. Its pathogenicity is promoted via several virulence factors, such as staphylococcal enterotoxins (SEs), toxic shock syndrome toxin-1, haemolysins, and fibronectin-binding proteins [9]. This microorganism is not only considered as the most pathogenic of staphylococci but, as reported by Havelaar et al. [10] and Fusco et al. [11], it is also acknowledged among the top 10 causes of bacterial FBDs, therefore playing a major role in terms of food safety [12].

Staphylococcal enterotoxins (SEs), produced by enterotoxigenic *S. aureus* strains, are a super family of pyrogenic exotoxins that share structural and functional similarities, causing vomiting, diarrhea, and cramps upon ingestion. To date, at least 28 SEs and staphylococcal enterotoxin-like toxins (SElts) have been identified [13]. SE and SElts are globular, single polypeptides with molecular weights ranging from 22 to 29 kDa. They can be encoded in prophages, plasmids, or chromosomal pathogenicity islands. The location of the *se* and *sel* genes on mobile genetic elements presents an additional risk factor in *S. aureus* food intoxication, due to possible horizontal gene transfer [14].

Foods that have been frequently incriminated in SFP include meat and meat products, poultry and egg products, milk and dairy products, salads, bakery products, particularly cream-filled pastries and cakes, and sandwich fillings [15]. However, according to le Loir et al. [16], foods implicated with SFP vary from country to country, particularly due to variation in consumption and food habits. In particular, food contamination with *S. aureus* and its SEs is mainly due to its ability to enter the food chain through contaminated raw material, inappropriate handling of processed food, and failure to maintain the cold chain [17].

Moreover, *S. aureus* is a well-known bacterium that develops antibiotic resistance [18] due to its ability to acquire a variety of resistance mechanisms towards antimicrobial agents [19], such as the resistance to methicillin. The latter mainly depends on the acquisition of the staphylococcal chromosomal cassette *mec* (SCC*mec*) harboring the *mecA* gene that encodes for penicillin-binding protein 2a (PBP2a) [20] that has low affinity for β -lactam antibiotics. In recent years, methicillin-resistant *S. aureus* (MRSA) strains have been recovered from several animal-derived foods such as poultry, pork, and beef meats, suggesting that foods may serve as a reservoir and source of MRSA [21]. Apart from direct transmission between humans and animals, the latter being considered natural reservoirs of this organism, transmission of MRSA might occur via exposure to, or ingestion of, contaminated foods [22,23]. Therefore, *S. aureus* and MRSA are considered a significant public health concern given their ability to contaminate foods and to colonize and infect both humans and animals [23,24].

Various ready-to-eat products are becoming increasingly popular in developing countries, particularly in the metropolitan areas. The prevalence and characteristics of enterotoxigenic *S. aureus* in ready-to-eat food products has been studied in different parts of the world [25–27]; however, as highlighted by Lozano et al. [28], studies on foodborne *S. aureus* in Africa are limited and, to date, mainly restricted to few countries, such as South Africa, which in general is reported to contribute to the most part of the African investigations, followed by Egypt and Nigeria [29]. Moreover, the available studies are focused primarily on the assessment of the prevalence of *S. aureus* and corresponding loads in various foods [28], while a further characterization is not systematically addressed. Thus, the information on enterotoxigenic *S. aureus* and MRSA from food in the African continent is, to date, fragmented and still incomplete. In order to overcome these limits,

the present study aims to investigate the prevalence, the enterotoxigenic potential, and the antimicrobial resistance of *S. aureus* in ready-to-eat foods sampled in Algeria, Africa.

2. Results

2.1. Prevalence of *Staphylococcus aureus* in Algerian Ready to Eat Foods

In this study, *S. aureus* was found in 23.2% (48/207) of ready-to-eat foods sampled in Algeria. High prevalence was found in meat/fish-based foods (38.2%, 21/55), followed by vegetable-based foods (22.2%, 16/72), cereals (17.6%, 3/17), pastries (16.3%, 7/43), and various foods (milk- and egg-based foods) (5.0%, 1/20) (Table 1).

Table 1. Prevalence of *S. aureus* in ready-to-eat foods sampled in Algeria and related counts of coagulase positive staphylococci.

Food Category	Number of Samples	<i>S. aureus</i> Positive Samples (% of Positive Samples)	CPS Count ^a	CPS Count Range ^b
Meat/fish-based foods	55	21 (38.2%)	3.48 ± 0.86	1.40 ± 0.12–4.49 ± 0.26
Vegetable-based foods	72	16 (22.2%)	3.19 ± 1.03	1.00 ± 0.5–4.43 ± 0.15
Pastries	43	7 (16.3%)	3.57 ± 0.80	2.84 ± 0.11–5.11 ± 0.24
Cereals	17	3 (17.6%)	2.40 ± 1.10	2.08 ± 0.16–4.13 ± 0.14
Various foods (milk- and egg-based foods)	20	1 (5.0%)	1.70 ± 0.10	–
Total	207	48 (23.2%)	3.38 ± 0.94	1.00 ± 0.5–5.11 ± 0.24

^a Coagulase positive staphylococci count expressed as Log CFU/g ± standard deviation in *S. aureus* positive samples. The count is expressed as median ± standard deviation for the categories “meat/fish-based foods”, “vegetable-based foods”, “pastries”, and “cereals” where more than one *S. aureus* positive sample was found, and “total”. ^b Minimum and maximum counts of coagulase positive staphylococci found in *S. aureus* positive samples, expressed as Log CFU/g ± standard deviation.

The coagulase positive staphylococci (CPS) counts in the *S. aureus* positive samples showed a median of 3.38 ± 0.94 Log CFU (colony forming unit)/g, ranging between 1.00 ± 0.5 and 5.11 ± 0.24 Log CFU/g as minimum and maximum counts, respectively. Pastries showed the highest CPS counts (between 2.84 ± 0.11 and 5.11 ± 0.24 Log CFU/g; median: 3.57 ± 0.80 Log CFU/g), while various foods (milk- and egg-based foods) showed the lowest (1.70 ± 0.10 Log CFU/g) (Table 1).

2.2. 16S-23S rDNA Intergenic Spacer Region (ISR)-PCR, Staphylococcal Enterotoxin Gene (SEg)-Typing and Presence of *tst* and *mecA* Genes

The 48 *S. aureus* isolated from the ready-to-eat foods sampled in Algeria are listed in Table 2.

Table 2. *S. aureus* isolated from Algerian ready-to-eat foods and corresponding genotypes, compared to *S. aureus* type strain DSM 20231.

Isolate	Origin	ISR-Type	SEg-Type ^a	<i>tst</i>	<i>mecA</i> (SCC <i>mec</i> Type)
SA01	Potato in sauce ^b	I	1	-	-
SA06	Chicken ^c	I	1	-	-
SA11	Couscous with meat ^c	I	1	-	-
SA13	Rice ^d	I	1	-	-
SA23	Minced meat ^c	I	1	-	-
SA33	Mashed potatoes ^b	I	1	-	-
SA41	Salad ^b	I	1	-	-
SA43	Rice ^d	I	1	-	-
SA44	Salad ^b	I	1	-	-
SA50	Salad ^b	I	1	-	-
SA53	Minced meat ^c	I	1	-	-
SA54	Beet salad ^b	I	1	-	-

Table 2. Cont.

Isolate	Origin	ISR-Type	SEg-Type ^a	tst	mecA (SCCmec Type)
SA58	Meat ^c	I	1	-	-
SA73	Salad ^b	I	1	-	-
SA78	Lentil soup ^b	I	1	-	-
SA83	Beans ^b	I	1	-	-
SA84	Chicken ^c	I	1	-	-
SA86	Sausages ^c	I	1	-	-
SA87	Pastry ^e	I	1	-	-
SA07	Meat ^c	IV	1	-	-
SA22	Salad ^b	IV	1	-	-
SA38	Spaghetti with tomato sauce ^b	IV	1	-	-
SA39	Vegetable and meat sauce ^c	IV	1	-	-
SA49	Potato ^b	IV	1	-	-
SA10	Meat ^c	VI	1	-	-
SA02	Meat ^c	II	2	-	-
SA30	Meat ^c	II	2	-	-
SA08	Meat ^c	V	2	-	-
SA19	Pastry ^e	IX	2	-	-
SA24	Lentil soup ^b	IX	2	-	-
SA34	Meat ^c	IX	2	-	-
SA37	Meat ^c	IX	2	-	-
SA82	Sautéed beef with potato ^c	XII	2	+	-
SA03	Salad ^b	III	3	-	-
SA04	Meat ^c	III	3	-	-
SA09	Pastry ^e	III	3	-	-
SA29	Meat ^c	III	3	-	-
SA31	Salad ^b	III	3	-	-
SA72	Pastry ^e	III	3	-	-
SA05	Pastry ^e	VII	4	-	-
SA18	Pizza ^d	VII	4	-	-
SA55	Pastry ^e	VII	4	-	-
SA56	Pastry ^e	VII	4	-	-
SA59	Russian salad ^b	VII	4	-	-
SA20	Meat ^c	VIII	5	-	-
SA17	Turkey pieces ^c	X	6	-	+ (SCCmec IV)
SA46	Braised beef ^c	X	6	-	+ (SCCmec IV)
SA51	Fermented milk ^f	XI	7	-	-
DSM 20231 ^T	Human pleural fluid	XIII	1	-	-

^a SEg-type: 1 (*seW*, *seX*); 2 [*egc* (*seG*, *seI*, *seM*, *seN*, *seO*), *seW*, *seX*]; 3 (*seA*, *seH*, *seK*, *seQ*, *seW*, *seX*); 4 (*seB*, *seW*, *seX*); 5 (*seD*, *seJ*, *seR*, *seW*, *seX*); 6 (*seH*, *seW*, *seX*, *seY*); 7 [*seA*, *egc* (*seG*, *seI*, *seM*, *seN*, *seO*), *seW*, *seX*]; ^b vegetable-based foods; ^c meat/fish-based foods; ^d cereals;

^e pastries; ^f various foods (milk- and egg-based foods).

The 16S-23S rDNA intergenic spacer region (ISR)-PCR allowed us to identify 12 different ISR-types, as shown in Figure 1.

Thirty-nine point six percent of the *S. aureus* (19/48) belonged to ISR-type I; 12.5% (6/48) to ISR-type III; 10.4% (5/48) to ISR-type IV and VII, respectively; 8.3% (4/48) to ISR-type IX; and 4.2% (2/48) to ISR-type II and X, respectively, while 2.1%, of the *S. aureus* isolates belonged to ISR-types V, VI, VIII, XI, and XII, which were represented by one isolate each (1/48, respectively) (Table 2).

The staphylococcal enterotoxin gene (SEg)-typing allowed us to detect seven different SEg-types (Table 2). The most prevalent was SEg-type 1 (*seW*, *seX*) detected in 52.1% (25/48) of the *S. aureus* that belonged to three different ISR-types (I, IV, and VI). It was followed by SEg-type 2 [*egc* (*seG*, *seI*, *seM*, *seN*, *seO*), *seW*, *seX*] (16.7%) (8/48) observed in *S. aureus* belonging to ISR-types II, V, IX, and XII, SEg-type 3 (*seA*, *seH*, *seK*, *seQ*, *seW*, *seX*) (12.5%) (6/48) in *S. aureus* belonging to ISR-types III, SEg-type 4 (*seB*, *seW*, *seX*) (10.4%) (5/48) in *S. aureus* belonging to ISR-type VII, and SEg-type 6 (*seH*, *seW*, *seX*, *seY*) (4.2%) (2/48) in *S. aureus* belonging to ISR-type X. The least prevalent were SEg-types 5 (*seD*, *seJ*,

seR, selW, selX) and 7 [*seA, egc (seG, seI, seM, seN, seO), selW, selX*] (2.1%) (1/48), which were detected in *S. aureus* belonging to ISR-type VIII and XI, respectively (Table 2).

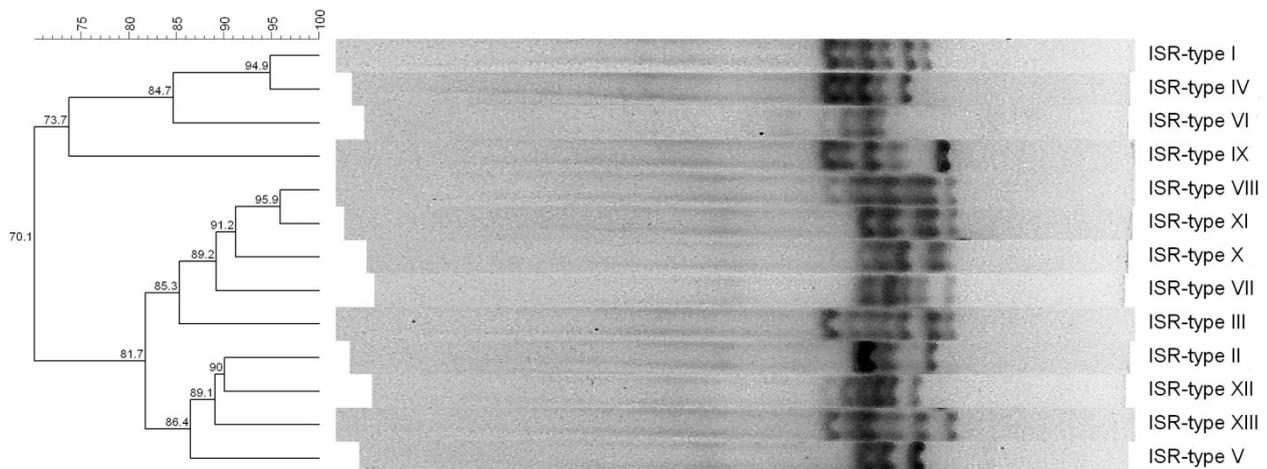


Figure 1. UPGMA (unweighted pair group method with arithmetic mean) dendrogram of the different ISR-types of the *S. aureus* isolated from Algerian ready-to-eat foods (ISR-types I to XII), compared to *S. aureus* type strain DSM 20231 (ISR-type XIII).

One out of the 48 *S. aureus* isolates (2.1%) that belonged to ISR-type XII was positive for the *tst* gene, and two *S. aureus* (4.2%) belonging to ISR-type X were positive for the *mecA* gene (Table 2).

It is noteworthy that *S. aureus* isolates belonging to the same ISR-type harbored the same genes as detected by PCRs (Table 2).

In regard to the prevalence of the *se* and *sel* genes, *selW* and *selX* were detected in all the isolates (48/48), followed by the enterotoxin gene cluster (*egc*) and its related genes (*seG, seI, seM, seN, seO*) (18.8%, 9/48), *seH* (16.7%, 8/48), *seA* (14.6%, 7/48), *seK* and *seQ* (12.5%, 6/48 respectively), *seB* (10.4%, 5/48), *selY* (4.2%, 2/48), and *seD, selJ, and seR* (2.1%, 1/48, respectively).

Lastly, the distribution of the *S. aureus* in relation to their genotypes and the corresponding sources of isolation is displayed in Table 3.

Table 3. Distribution of *S. aureus* isolated from Algerian ready-to-eat foods based on their genotypes.

ISR-Type	SEg-Type ^a	<i>tst</i>	<i>mecA</i> (SCC <i>mec</i> Type)	Origin and Number of <i>S. aureus</i> Isolates (% Out of the Total for Each Genotype)					Total (% of Total Isolates)
				Meat/Fish-Based Foods	Vegetable-Based Foods	Pastries	Cereals	Various Foods (Milk- and Egg-Based Foods)	
I	1	-	-	7 (36.8%)	9 (47.4%)	1 (5.3%)	2 (10.5%)		19 (39.6%)
IV	1	-	-	2 (40.0%)	3 (60.0%)				5 (10.4%)
VI	1	-	-	1 (100%)					1 (2.1%)
II	2	-	-	2 (100%)					2 (4.2%)
V	2	-	-	1 (100%)					1 (2.1%)
IX	2	-	-	2 (50.0%)	1 (25.0%)	1 (25.0%)			4 (8.3%)
XII	2	+	-	1 (100%)					1 (2.1%)
III	3	-	-	2 (33.3%)	2 (33.3%)	2 (33.3%)			6 (12.5%)
VII	4	-	-		1 (20.0%)	3 (60.0%)	1 (20.0%)		5 (10.4%)
VIII	5	-	-	1 (100%)					1 (2.1%)
X	6	-	+ (SCC <i>mec</i> IV)	2 (100%)					2 (4.2%)
XI	7	-	-					1 (100%)	1 (2.1%)
Total (% of total isolates)				21 (43.8%)	16 (33.3%)	7 (14.6%)	3 (6.2%)	1 (2.1%)	48 (100%)

^a SEg-type: 1 (*selW, selX*); 2 [*egc (seG, seI, seM, seN, seO), selW, selX*]; 3 (*seA, seH, seK, seQ, selW, selX*); 4 (*seB, selW, selX*); 5 (*seD, selJ, seR, selW, selX*); 6 (*seH, selW, selX, selY*); 7 [*seA, egc (seG, seI, seM, seN, seO), selW, selX*].

Table 4. Cont.

Class	Antimicrobials	<i>S. aureus</i> Strains												
		SA01	SA02	SA04	SA07	SA08	SA10	SA18	SA20	SA24	SA46	SA51	SA82	DSM 20231 ^T
Glycopeptides	TEI	S	S	S	S	S	S	S	S	S	S	S	S	S
	VAN	S	S	S	S	S	S	S	S	S	S	S	S	S
Tetracyclines	TE	S	R	S	S	S	S	R	S	S	S	S	S	S
Fosfomycins	FOS	S	S	S	S	S	S	S	S	S	S	S	S	S
Nitrofurans	NIT	S	S	S	S	S	S	S	S	S	S	S	S	S
Steroidal	FA	S	S	I	S	S	S	S	S	S	S	S	S	S
Ansamycins	RIF	S	S	S	S	S	S	S	S	S	S	S	S	S
Folate pathway inhibitors	COT	S	S	S	S	S	S	S	S	S	S	S	S	S
	Resistance profile ^a	P	OF, ERY, L, TE	P	P	P	P	P, KA, TE	P	P	P, OXA, FOX, KA	- ^b	P	- ^b

P: Benzylpenicillin, OXA: Oxacillin, FOX: Cefoxitin, GEN: Gentamicin, KA: Kanamycin, TOB: Tobramycin, OF: Ofloxacin, ERY: Erythromycin, L: Lincomycin, CLI: Clindamycin, PRI: Pristinamycin, LZ: Linezolid, TEL: Teicoplanin, VAN: Vancomycin, TE: Tetracyclin, FOS: Fosfomycin, NIT: Nitrofurantoin, FA: Fusidic acid, RIF: Rifampicin, COT: Co-trimoxazole; R: resistant; S: susceptible; I: intermediate; ^a antimicrobials to which the tested *S. aureus* strains are R; ^b sensitive to all tested antimicrobials.

3. Discussion

In the present study, we found *S. aureus* in Algerian ready-to-eat foods with a prevalence of 23.2%. Other studies analyzed the presence of this microorganism in ready-to-eat foods in African countries, but only fragmented and incomplete information about the enterotoxigenic *S. aureus* and MRSA isolated from African ready-to-eat foods is available so far. Similar to our findings, Chaalal et al. [31] reported an overall *S. aureus* prevalence of 23.8% in pastries and cooked dishes sampled from supermarkets and university cities in Western Algeria, and Titouche et al. [32], in the same country of the aforementioned study, found a prevalence of 14.46% in pastries collected from several market points (in Tizi Ouzou area), while no *S. aureus* contamination was found by these authors in the sampled sandwiches [32]. A prevalence of 33.26% was reported in ready-to-eat meat products collected in all the provinces of South Africa [33], while in Egypt (in Benha city), a prevalence of 50.8% was reported for ready-to-eat meat products sampled from restaurants and street vendors [34], and in Nigeria (in Port Harcourt Metropolis), 100% of the analyzed street ready-to-eat meals were reported to be contaminated by *S. aureus* [35].

As shown by these studies, the prevalence of *S. aureus* contamination in ready-to-eat foods may greatly vary and, as highlighted by some authors [32,36], such differences are related to several factors that include the source (e.g., street-vendors or shops) and type of samples (e.g., animal- or non-animal-derived foods), the sample size, the accuracy of the identification method (based on cultivation characteristics, biochemical tests, or molecular biology techniques), the manufacturing procedures (e.g., involving bactericidal temperatures or not), and the overall hygienic measures that are implemented during the preparation and handling of the foods. In particular, *S. aureus*, being a commensal bacterium present on the skin, the nose, and mucous membranes of animals and humans [28], may contaminate food especially when poor hygienic practices and conditions exist. It is noteworthy that, beyond the contamination that may originate from animals at the primary production stage, food handlers are recognized as the main source of food contamination with *S. aureus* [31,32,37], and Sezer et al. [38] found that the 79% of the food handlers employed in a catering establishment were carriers of *S. aureus*. Nevertheless, it should be taken into account that contamination may also happen and spread by cross-contamination among foods and/or surfaces in the environment surrounding the manufacturing and storage of the ready-to-eat foods, since *S. aureus* may survive on inanimate surfaces for prolonged times [39], especially in cases of improper or ineffective sanitizing procedures [40].

When contamination occurs, *S. aureus* may replicate, and its load may increase in food. *S. aureus* is mostly a coagulase positive bacterium, and according to the Interministerial Decree of the Algerian Republic Official Journal No 39 (published on 2 July 2017) establishing

the microbiological criteria for food products, the limits of CPS in ready-to-eat foods are set to values equal to 10^2 CFU/g (“m” value), below which the product quality is considered satisfactory, and 10^3 CFU/g (“M” value), above which the product quality is considered unacceptable. In the ready-to-eat products sampled in this study, we found CPS loads, above the “M” value, in four of the five analyzed food categories (meat/fish-based foods, vegetable-based foods, pastries, and cereals), in which the highest CPS counts reached values higher than 4 and 5 Log CFU/g. Additionally, according to the Decree, samples in which CPS counts are superior or equal to 10^5 CFU/g are considered as toxic. Therefore, these findings shed light on the quality and safety of the Algerian retail ready-to-eat foods.

Some other studies assessed CPS loads, even reported as *S. aureus* counts, in ready-to-eat foods in the African continent. Although Mahami et al. [41] found low contamination of cooked and smoked sausages, reporting *S. aureus* loads ranging from 1.85 to 2.15 Log CFU/g in samples purchased from a factory in Ghana (in Accra city) and less than 1 Log CFU/g in samples from a shopping mall, Oguttu et al. [42], analyzing ready-to-eat chicken sold in informal markets in South Africa (in Tshwane Metropolitan City), reported the presence of unsatisfactory quality samples with loads of *S. aureus* greater than 3 Log CFU/g, and an overall mean of 3.6 Log CFU/g. Similarly, Shiningeni et al. [43] found ready-to-eat beef and chicken meats of unsatisfactory quality, with loads of *S. aureus* equal or greater than 3 Log CFU/g, that were purchased from street vendors in Namibia (Windhoek city). These authors reported that, in relation to the vending sites, the mean counts in the ready-to-eat meat samples ranged from 0 to 3.46 Log CFU/g, and the highest load was 5.12 Log CFU/g [43].

Since the issue regarding *S. aureus* and corresponding loads in foods is related to the possible contamination of food by staphylococcal enterotoxins, that in very round figures may occur at hazardous levels if *S. aureus* load reaches around 5 Log CFU/g [40], a great importance was given in this study to the investigation of the enterotoxigenic potential of the *S. aureus* we isolated, and to the best of our knowledge this is the first study in Algeria in which the presence of a total of 27 *se* and *sel* genes was sought in foodborne *S. aureus*.

We found that the 48 *S. aureus* belonged to seven different SEg-types harboring the following combination of *se* and *sel* genes: (1) *selW*, *selX*; (2) *egc* (*seG*, *seI*, *seM*, *seN*, *seO*), *selW*, *selX*; (3) *seA*, *seH*, *seK*, *seQ*, *selW*, *selX*; (4) *seB*, *selW*, *selX*; (5) *seD*, *selJ*, *seR*, *selW*, *selX*; (6) *seH*, *selW*, *selX*, *selY*; and (7) *seA*, *egc* (*seG*, *seI*, *seM*, *seN*, *seO*), *selW*, *selX*. Previous studies detecting a high number of *se* and *sel* genes (17 to 27 genes) found 6 different genotypes in 53 *S. aureus* from raw milk [13], 11 different genotypes in 50 *S. aureus* from raw minced meat and sausages [44], and 120 enterotoxin gene patterns in a group comprising 568 *S. aureus* from humans, animals, foods, and the environment [45]. Our study corroborates these findings and indicates the heterogeneity of the enterotoxigenic potential that can be encountered in *S. aureus* isolates from ready-to-eat foods in Algeria.

In our study, we found that 100% (48/48) of the analyzed *S. aureus* harbored *selW* and *selX*. These are located in the chromosome and are reported as highly prevalent genes; indeed, they were detected in more than 92.0% and in more than 79.0% of the *S. aureus* analyzed in previous studies, respectively [46,47]. The recent identification of this and other new *se* and *sel* genes indicates that the pathogenic potential of *S. aureus* may be greater than previously thought [48]. Moreover, based on the findings of Aung et al. [46], it was suggested that *selW*, and to a greater extent *selX*, may play a universal role in the virulence of *S. aureus* [46].

The enterotoxin gene cluster (*egc*) was found in the 18.8% (9/48) of the *S. aureus* herein analyzed. This cluster, located in a variable genomic island (*vSaβ*) inserted in the chromosome [37], has been similarly reported with a prevalence of 16.2% [49] and of 26.4% [13] in *S. aureus* from various sources, including food. In our study, the presence of the *egc* was confirmed by the detection, in the same strains, of the *egc* encoded genes *seG*, *seI*, *seM*, *seN*, and *seO*. Various types of *egc* exist in *S. aureus* (*egc1* to *egc5*) and, considering our findings, the strains herein analyzed may harbor *egc1* [50] or the newly described *egc5* [13]. It should be specified that these two *egc* types, beyond harboring the enterotoxin genes we

detected (*seG*, *seI*, *seM*, *seN*, *seO*), might also include two pseudogenes (*ψent1-ψent2*) (*egc1*) or *selU2* (*egc5*) [13,50].

Since *selU2* results from a single adenine deletion in the *ψent1-ψent2* region (being just a 1 nucleotide frameshift of the *ψent1-ψent2* sequence that deletes the stop codon of pseudogene *ψent1*) [51], the design of primers able to specifically detect *ψent1-ψent2* or *selU2* is not achievable, making impracticable their easy and affordable detection by a PCR screening such as the one we performed.

The *seH* gene was detected in the *S. aureus* herein analyzed with a prevalence of 16.7% (8/48). This gene, located on a presumptive transposon [52], was reported with lower prevalence in some previous studies, being in the range of 4.05–4.8% in *S. aureus* from food and other sources [32,45], while a higher prevalence, more similar to our findings, and ranging between 24.6 and 28.0%, was reported by other authors analyzing *S. aureus* from raw milk, raw meat, and ready-to-eat foods [13,44,53].

The *egc*-encoded enterotoxins are reported with increasingly probability to have a role in staphylococcal foodborne poisoning (SFP) [54,55], and the *seH* encoded enterotoxin (SEH) is the first non-classical SE that has been reported to have caused SFP outbreaks [56,57], highlighting therefore the relevance of the detection of these genes in foodborne *S. aureus*.

When the classical *se* genes are considered, in our study we found *seA*, *seB*, and *seD* with a prevalence of 14.6% (7/48), 10.4% (5/48), and 2.1% (1/48), respectively. The presence of these genes, whose encoded enterotoxins are the most frequently reported causes of SFP outbreaks [58], is frequently investigated, and in African countries prevalences ranging between 6.7–90.0%, 0.0–18.3%, and 0.0–8.0% for each of them have been reported in *S. aureus* isolated from food products [32,59,60].

In our investigation, we also detected *selJ*, *seK*, *seQ*, *seR*, and *selY*. In general, scarce knowledge is available on the prevalence of these new *se* and *sel* genes in foodborne *S. aureus*. This study helps to improve this paucity of data, representing one of the few African reports available to date. Interestingly, we observed that some of the above-mentioned genes were found in association in the *S. aureus* herein analyzed, in particular, *selJ* and *seR* along with *seD*, and *seK* and *seQ* along with *seA*. The first association is carried on plasmids (pIB485-like), and the second is carried on prophages [37], and both of these associations are being found in *S. aureus* isolates from SFP outbreaks [61–64]. Considering that the emetic properties of the encoded SEs (SEK, SEQ, and SER) have been demonstrated [65,66], these new *se* genes may also have a role in the genesis of staphylococcal intoxication that to date is still not fully understood.

tst and *mecA* genes were found at low prevalence in the *S. aureus* analyzed in our study (2.1% (1/48) and 4.2% (2/48), respectively), and they were not in association in the same strains. These genes, being the genetic determinants of toxic shock syndrome toxin-1 (*tst*), which causes a range of systemic diseases, and methicillin resistance (*mecA*), which confers a general resistance to β -lactam antibiotics in MRSA, represent important virulence and antimicrobial resistance attributes that can worsen clinical conditions when *S. aureus* infections occur.

In general, a low prevalence of *tst* gene in foodborne *S. aureus* has also been reported in previous studies conducted in various parts of the world including African countries [31,67–69]. Although the association of *tst* and *mecA* genes has been found in *S. aureus* isolated especially from clinical samples [70,71], our findings resemble those of Chaalal et al. [31] that recently analyzed *S. aureus* from food products in Algeria detecting a low prevalence of *tst* gene (3.2%) that was found only in *mecA*-negative isolates.

Nevertheless, the presence of *mecA* has been reported in foodborne *S. aureus* isolates in Algeria, with a prevalence of 3.03 [72], 4.81 [32], and 16.9% [31], and, additionally, in the aforementioned studies, an overall low prevalence of MRSA in the analyzed foods has been reported, which is consistent with our findings [31,32,72]. The massive and inappropriate use of antimicrobials in veterinary and human medicine is considered the main cause for the emergence of antimicrobial-resistant strains [73]; however, the prevalence of MRSA in

food is described to still be low and reported in the range of 1.6–6.4% in investigations also conducted in other countries [74].

Transmission of MRSA occurs between animals and humans but, notably, food contamination may serve as a vehicle to increase the dissemination of MRSA [73]. The community-acquired MRSA (CA-MRSA) but also the livestock-acquired MRSA (LA-MRSA) frequently carry the SCC mec -type IV [75,76], and they principally spread in human community settings and livestock populations. The detection of the SCC mec -type IV in our *S. aureus* isolates tentatively suggests their origin and, consistently with our findings, it was also the prevalent SCC mec -type found in the MRSA isolated from food in previous studies [31,77–79].

Interestingly, the ability of MRSA strains to act as a foodborne pathogens has been reported [80], and the first foodborne gastrointestinal illness outbreak caused by an enterotoxigenic MRSA strain has been described in 2002 [81]. However, to date, the actual MRSA involvement in SFP (cases or outbreaks) is not precisely elucidated, and a general underestimation is suggested [80]. Our *mecA*-SCC mec -type IV positive isolates harbored *seH*, *seIW*, *seIX*, and *seIY* genes (SEg-type 6) suggesting their potential role as SFP agents, especially considering that the *seH* encoded enterotoxin (SEH) has been already reported to have caused SFP outbreaks [56,57], as mentioned above. Additionally, the association of *seH* and *mecA* gene is known and has been previously described [52]. Moreover, our results also corroborate those of previous studies that detected *se* and *sel* genes in MRSA isolated from food products [32,77,79,82].

To further characterize our *S. aureus* isolates, we employed ISR-PCR, previously used also by other authors as a practical tool for *S. aureus* genotyping [13,83]. Interestingly, unlike results previously reported by Chieffi et al. [13], we observed that ISR-PCR had a discriminatory power higher than SEg-typing since ISR-PCR enabled us to detect 12 ISR-types despite the seven SEg-types. On the other hand, we observed that *S. aureus* belonging to the same ISR-type harbored the same genes as detected by PCRs, corroborating the aforementioned study that reported that strains belonging to the same ISR-type showed the same SEg-type.

Therefore, ISR-PCR allowed us to select 12 representative genetically diverse *S. aureus* strains that, when tested for antimicrobial resistance, showed four patterns of resistance.

In particular, the antimicrobial susceptibility test allowed us to confirm one selected strain (1/12, 8.3%) as MRSA that is in agreement with the detection in the same strain of the *mecA* gene. Moreover, two selected strains (2/12, 16.7%) were classified as multidrug-resistant, being resistant to three (i.e., benzylpenicillin, kanamycin, and tetracycline) and four (i.e., ofloxacin, erythromycin, lincomycin, and tetracycline) antimicrobials belonging to different classes.

Multidrug-resistant *S. aureus* isolated from food products were detected also in previous studies conducted in Africa and in other parts of the world, whose reported prevalence ranged between 15.38–33.3% [31,32,84] and 10.4–57.5% [85,86], respectively. Such findings represent a threat to public health since the assortment of effective antimicrobials to treat *S. aureus* infections is reduced. Moreover, as for MRSA, these multidrug strains also harbored *se* and *sel* genes belonging to SEg-type 2 [*egc* (*seG*, *seI*, *seM*, *seN*, *seO*), *seIW*, *seIX*] and SEg-type 4 (*seB*, *seIW*, *seIX*), being therefore enterotoxigenic strains with the potential to cause SFP. Interestingly, the presence in food products of multidrug-resistant *S. aureus* strains carrying *se* and *sel* genes has also been reported by other authors [87].

The resistance to penicillin is reported with high prevalence; indeed, from 60% to more than 90% of foodborne *S. aureus* isolates showed penicillin resistance in many studies [31,32,85,86,88], which is consistent with the high prevalence of resistance observed also in our investigation (10/12, 83.3%).

We found that few isolates were resistant to tetracycline and kanamycin (2/12, 16.7%) and to ofloxacin, erythromycin, and lincomycin (1/12, 8.3%). Resistance to these antimicrobials has been reported also in previous studies with various prevalences of 17.31–54.1% [32,85,86,89], 10.2–32.6% [31,89], 0.0–12.4% [31,32], 5.77–52.1% [31,32,84–86,89], and 17.6% [31], respectively.

Some antimicrobials have an important role in the clinical practice, and among these, vancomycin is the drug of choice to treat serious infections caused by MRSA [90]. All our isolates were vancomycin-susceptible, which is consistent with other investigations that are reporting the absence of resistance to vancomycin in foodborne *S. aureus* [31,84,86,89].

4. Conclusions

The information on foodborne *S. aureus* in Africa is, to date, fragmented and still incomplete, calling for studies that can cover this lack of data from most of its countries. Herein, the detailed molecular characterization, in particular aimed to investigate the actual enterotoxigenic potential of the analyzed *S. aureus* isolates, along with the findings of MRSA and multidrug-resistant strains, highlight the pathogenicity as well as the heterogeneity of *S. aureus* population in Algerian ready-to-eat foods. Additionally, the finding of generally high CPS loads in the analyzed ready-to-eat foods, with respect to the microbiological criteria established by the Algerian legislation, draws attention to the quality and safety of such foods in Algeria.

Therefore, *S. aureus* contamination represents a current risk for consumers' health, and ready-to-eat foods seem to still be far from the relevant Algerian standard, but we should point out that better hygienic practices during the manufacture of ready-to-eat foods may be an affordable strategy to successfully address these current issues.

5. Materials and Methods

5.1. Sampling

A total of 207 samples of ready-to-eat foods were randomly collected from hotels, restaurants, fast foods, and pizzerias in Algiers, capital of Algeria, during 2018 and 2019. Those samples included 55 meat-based foods, 72 vegetable-based foods, 43 pastries, 17 cereals, and 20 various foods (milk- and egg-based foods). The samples were transported on ice to the laboratory and analyzed immediately.

5.2. Isolation of Presumptive *Staphylococcus aureus*

The analysis was carried out using 25 g of homogenized food in 225 mL of pre-enrichment diluent tryptone-salt broth (Oxoid, Dardilly, France) using a Stomacher-type homogenizer. Further decimal dilutions were carried up to 10^{-5} . Thereafter, the corresponding dilutions were plate-counted in accordance with the standard reference culture method recommended by the International Organization for Standardization [91] for the enumeration of coagulase-positive staphylococci, using Baird Parker with egg yolk emulsion (BPEY) incubated at 37 °C for 24–48 h. From each food sample processed, one presumptive *Staphylococcus aureus* colony was subcultured on BPEY (Oxoid, Dardilly, France) and purified by repeated streaking. The pure cultures were stored at –80 °C in brain heart infusion broth (BHI; Conda Pronadisa, Madrid, Spain), amended with 0.6% yeast extract (Biolife Italiana, Milano, Italy) added with 20% glycerol.

5.3. DNA Extraction

The presumptive *S. aureus* pure cultures were cultivated in BHI broth (Oxoid, Dardilly, France) amended with 0.6% yeast extract (Biolife Italiana, Milano, Italy) and incubated at 37 °C for 24 h. Five hundred microliters of each broth culture were centrifuged at 12,000 rpm for 90 s, and the resulting pellets were washed with 1 mL of sterilized distilled water. DNA was extracted with InstaGene Matrix (Bio-Rad, Hercules, CA, USA) following the manufacturer's instructions. Three to five microliters of the resulting DNA solutions were used for the conventional polymerase chain reaction (PCR) protocols, while two microliters were used for the real time PCR protocols, as described below.

5.4. Identification of *Staphylococcus aureus*

The identification of *Staphylococcus aureus* isolates was carried out by a species-specific simplex polymerase chain reaction (PCR) targeting the *nuc* gene [92]. Briefly, each reaction

mixture contained 3 µL of the extracted DNA, 0.4 µM of each primer, 2.5 mM of MgCl₂ (Promega, Madison, WI, USA), 0.1 mM of each deoxynucleotide triphosphates (Promega, Madison, WI, USA), 0.8 U of GoTaq G2 hot start polymerase (Promega, Madison, WI, USA), 1X reaction buffer (Promega, Madison, WI, USA), and nuclease-free water to a final volume of 25 µL. Thermocycling conditions were the following: 94 °C for 2 min; 37 cycles of 94 °C for 1 min, 55 °C for 30 s, 72 °C for 1 min. 30 s.; final extension of 72 °C for 5 min. Amplicons were separated by electrophoresis in TAE buffer at 100V on agarose gel (1.5% w/v). *S. aureus* DSM20231^T was used as positive control.

5.5. Staphylococcal Enterotoxin Gene (SEg-) Typing and Real Time PCR of the Enterotoxin Gene Cluster (*egc*)

Conventional and real time PCR assays targeting the classical (*seA* to *seE*) and the newly described (*seG* to *selZ*, *sel27* and *sel28*) *se* and *sel* genes were carried out following the protocols described by Chieffi et al. [13], while the presence of the *egc* was assessed using the SYBR Green real-time PCR protocol described by Fusco et al. [11]. Amplicons obtained by conventional PCR were separated on agarose gel by electrophoresis in TAE buffer at 100V. *S. aureus* strains used as controls in the PCR assays are reported in Table S1 [13,50,93–97].

5.6. 16S-23S rDNA Intergenic Spacer Region PCR (ISR-PCR)

16S-23S rDNA intergenic spacer region PCR (ISR-PCR) was carried out using 5 µL of each *S. aureus* DNA following the protocol described by Chieffi et al. [13]. DNA of *S. aureus* DSM20231^T was also included in the PCR reaction as control reference strain. The resulting patterns were visualized as described by Fusco et al. [98], on agarose gel (1.7% w/v) electrophoresized in TAE buffer at 60 V for 6.5 h. Two patterns were considered different ISR-types if one or more DNA bands differed in size. The relevant analysis was carried out to construct an UPGMA (unweighted pair group method with arithmetic mean) dendrogram using the Bionumerics software version 5.1 (AppliedMaths, Sint Martens Latem, Belgium) [99], setting the Pearson correlation as a fingerprint similarity coefficient and choosing 1.0% position tolerance and 0.0% optimization as position tolerance settings.

5.7. Detection of *tst* and *mecA* Genes and Characterization of the Staphylococcal Chromosomal Cassette *mec* (SCC*mec*)

Five µL of *S. aureus* DNA were employed in the multiplex PCR protocol described by Oliveira and de Lencastre [30] for the detection of the *mecA* gene and the characterization of the SCC*mec* as well. The resulting amplicons were separated, loading 2 µL of the reaction mixture on agarose gel (2% w/v) and performing electrophoresis in TAE buffer at 100 V for 1 h 50 min. The *tst* gene was detected as described by Johnson et al. [100] with minor modifications in the reaction mixture that was prepared as follows: 3 µL of the extracted DNA, 0.4 µM of each primer, 2.5 mM of MgCl₂ (Promega, Madison, WI, USA), 0.25 mM of each deoxynucleotide triphosphate (Promega, Madison, WI, USA), 1 U of GoTaq G2 hot start polymerase (Promega, Madison, WI, USA), 1X reaction buffer (Promega, Madison, WI, USA), and nuclease-free water to a final volume of 25 µL. Amplicons were separated by electrophoresis in TAE buffer at 100 V on agarose gel (1.3% w/v). *S. aureus* strains used as controls in the PCR assays are reported in Table S1 [13,50,93–97].

5.8. Antimicrobial Susceptibility Testing

Twelve *S. aureus* strains belonging to the different ISR-types were selected for testing (Table 4).

The pure cultures of each strain were cultured in Brain Heart Infusion (BHI) agar (Conda Pronadisa, Madrid, Spain) and incubated at 37 °C for 24 h. Suspensions of 0.5 McFarland were prepared from these cultures.

Antimicrobial susceptibility was performed by Vitek 2 (bioMérieux Inc., Durham, NC, USA) using AST-P631 cards. The cards were inoculated with the prepared suspensions and loaded into the Vitek 2 automated reader. Results were interpreted by the Vitek 2 Advanced Expert System software according to the reference criteria of CLSI and EU-

CAST [101,102]. *S. aureus* DSM 20231^T was used as control. Strains resistant to cefoxitin and oxacillin were considered MRSA, and strains resistant to ≥ 3 antimicrobials of different classes were considered multidrug-resistant. The following antimicrobial agents were included: benzylpenicillin, oxacillin, cefoxitin, gentamicin, kanamycin, tobramycin, ofloxacin, erythromycin, lincomycin, clindamycin, pristinamycin, linezolid, teicoplanin, vancomycin, tetracycline, fosfomycin, nitrofurantoin, fusidic acid, rifampicin, and co-trimoxazole.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/toxins13120835/s1>, Table S1: *S. aureus* strains used as controls in conventional and real time PCR assays in the present study.

Author Contributions: V.F. conceived the work. V.F., O.A.M., and D.C. interpreted the data and wrote the manuscript. O.A.M. performed sampling, enumeration, and isolation of *S. aureus* and antimicrobial susceptibility testing. O.A.M. and D.C. carried out the PCR-based identification, staphylococcal enterotoxin gene typing, real-time PCR of the *egc*, ISR typing, detection of *mecA* and *tst* genes, and characterization of SCC_{mec} of *S. aureus* isolates. A.H., S.A.B. and F.F. performed writing—review and editing. All the authors contributed to the revision of the manuscript. All authors have read and agreed to the published version of the manuscript.

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