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Prevalence and Antibiotic Susceptibility Profile of *Staphylococcus aureus* in Commercialized Food in Oran, Algeria

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HIGHLIGHTS:

- Totally, 31.14% (109/350) of food samples were contaminated with *Staphylococcus aureus*.
- Prevalence of *S. aureus* in pastry products was significantly high with a contamination rate of 43.59%.
- The lowest contamination rate (18.18%) was registering at prepared meals.
- Among *S. aureus* isolates, 21.1% presented methicillin-resistant strains.
- Appropriate measures should be implemented to decrease the contamination level of food products.

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Acronyms and abbreviations
MDR=Multidrug Resistant
MRSA=Methicillin-Resistant *Staphylococcus aureus*

ABSTRACT

Background: With regard to health-threatening infections, *Staphylococcus aureus* is the leading cause of polymorphic infections varying from a banal tegumentary infection to numerous lethal illnesses. Furthermore, it is the third commonest bacterial cause of food-borne infections worldwide.This study aimed to investigate contamination, prevalence, and antibiotic resistance of *S. aureus* isolated from several widely marketed food products (raw and processed) in the region of Oran, Algeria.
Methods: A total of 350 food samples including prepared meals (n=110), dairy products (n=42), pastry (n=78), meat and its derivatives (n=97), and other commercially available foods (egg products, sweets, and sauces) (n=23) have been randomly purchased from diverse sale outlets and screened for *S. aureus* strains during the period from July 2021 to September 2022. The isolation and identication of *S. aureus* bacteria were preformed using conventional culture and biochemical tests such as catalase, coagulase, and DNase tests. Furthermore, the strains were screened for their resistance to five different antimicrobial drugs using the agar diffusion method.
Results: The overall prevalence of *S. aureus* determined among the collecteded samples was 31.14% (109/350), where, pastry products harbored the highest contamination rate (43.59%), and prepared meals, the lowest contamination rate (18.18%). The strains presented a high level of resistance (58.71-39.44%) for gentamycin and oxacilin, respectively. Moreover, the lowest level of resistance was observed against erythromycin 16.51%, and about 83% of strains presented multidrug resistance.
Conclusion: The significative prevalence and the high level of multidrug resistant of *S. aureus* highlights the seriousness to improve food contamination prevention programs and underlines that good hygiene practices at sale outlets has a major impact on the sanitary quality of commercialized food products.

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Introduction

Food is human's fuel, however; it can be a vector of pathogens and source of disease. Food poisoning is one of the chief hazardous worries worldwide, and most of the health organisations are trying to reduce them year by year. But they occur despite the monitoring and prevention measures taken especially in countries with a high economic standard of living (Bhalla et al., 2019).

Food-borne disease outbreaks have been associated with various food-borne microorganisms due to the consumption of contaminated food and water (Morshdy et al., 2018) leading to a serious impact on public health and economy. Among the bacteria mostly involved in these diseases, *Staphylococcus aureus* which is well-known as a versatile pathogen capable of infecting both humans and animals (Haag et al., 2019).

As the main cause of several infections occuring in both communities and healthcare facilities (Yang et al., 2016), food remains the main source of staphylococcal infections (Liang et al., 2023). This is because multiple types of food are suitable for the growth of this bacterium, such as raw high-protein animal foods like milk, dairy products (Gebremedhin et al., 2022), egg products (Pondit et al., 2018), meat and meat products (Nass et al., 2019), vegetables (Wu et al., 2018b), bakery products, particularly pastries and cream-filled cakes. Sandwich fillings, have been frequently blamed for outbreaks of Staphylococcal food poisoning (Hennekinne et al., 2012).

S. aureus is a ubiquitous Gram-positive cocci, facultative anaerobic, non-motile, and non-spore forming bacterium, with a powerful amalgamation of toxin-mediated virulence, invasive capability, and antibiotics resistance, making it the main pathogen of *Staphylococcus* genus, among the 62 species and 30 subspecies attributed (Kayili and Sanlibaba, 2020).

S. aureus is a highly adaptable organism; it can adapt to various environmental conditions and rapidly develop resistance to numerous antibacterial agents (Wu et al., 2018a). Antibiotic resistance is paves the way for *S. aureus* as a human pathogen, the flexibility of its genome allows it to adapt to all hostile conditions, including the acquisition of antibiotic resistance genes and the development of regulatory mechanisms to adapt to increasing antibiotic concentrations (Dumitrescu et al., 2010).

Antibiotics are the most current and successful weapon in the fight against bacterial infections. However, utilization of new antibiotics to fight this pathogen has frequently been followed by the appearance of Multidrug Resistant (MDR) strains (Willis et al., 2022), and consequently, *S. aureus* infections become more difficult to treat, posing significant new challenges to community health.

There are alarming figures on the real spread of drug resistance worldwide. According to the review on antimicrobial resistance, at least 700,000 people die each year from drug resistance from diseases such as bacterial infections (Mancuso et al., 2021). Today, food-borne strains of *S. aureus* are known to exhibit resistance to a wide variety of antibiotics (Safarpoor Dehkordi et al., 2017). Methicillin-Resistant *S. aureus* (MRSA) is a strain of *S. aureus* that demonstates resistance to beta-lactam antibiotics, including penicillins, cephalosporins, and methicillin, as well as more common antibiotics such as oxacillin and amoxicillin (Gurung et al., 2020). Unfortunately, in recent years, some strains of MRSA have become resistant to virtually all antibiotics. This depletion of therapeutic resources is the reason why they become a serious public health concern (Aslam et al., 2018).

Staphylococcal collective food toxi-infections are caused by the ingestion of preformed staphylococcal enterotoxins. The incriminated species is mainly *S. aureus*. Thus the diagnosis of a collective food toxi-infection with staphylococci is confirmed when at least one of the following parameters is verified: i) detection of staphylococcal enterotoxins in the food matrix. ii) isolation of the same *S. aureus* strain from the faeces of the patients and the food matrix. iii) *S. aureus* amount in the suspected food is greater than 105 Colony Forming Units (CFU)/g (Hennekinne, 2009).

Data on the spread of *S. aureus* in food items have been poorly documented in Algeria. The extensive use of antimicrobial drugs has contributed to the emerging of MDR strains, making their eradication much harder. One of the goals of this study is to assess the risk of *S. aureus* food-borne infection to which the consumer is exposed when consuming multiple high consumption food products. To achieve this goal, the authors aim to estimate the prevalence and investigate the resistance profile of *S. aureus* isolates collected from food products in Wilaya of Oran, Algeria.

Materials and methods

Sample collection

Three hundred fifty food samples including prepared meals (110), dairy products (42), pastry (78), meat and meat products (97), and other commercially available foods (egg products, sweets, and sauces) (23) have been purchased from diverse randomly selected sale outlets (supermarkets, butchers, retail stores, restaurants, fast food, and dairies) and tested for the presence of *S. aureus*. The sampling of this study was carried out between July 2021 and September 2022 in Oran, Algeria.

In addition, the samples were obtained in total accordance with their customer service without revealing any information to the store of origin. To prevent them

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from any external contamination, all samples were kept in ice box under cold state (+/-4 °C) and aseptic conditions to be transfer to the laboratory within 4 h after sampling until the analysis was conducted the same day, all food samples exhibited normal organoleptic characteristics, including odor, color, and consolidation.

Samples processing and isolation of S. aureus

The studied samples were tested by traditional approaches applying the method used in the hygiene laboratory of the Wilaya of Oran, Algeria. Therefore, 25 g of each samples were suspended into a sterile stomacher bag with 225 ml of physiological saline (Biolyse, Algeria). After homogenisation in a stomacher (Seward Medical, London, United Kingdom) for 1 min. Through streaking, a loopful of the broth culture was seeded onto mannitol salt agar (Realab, Tizzi Ouzzou, Algeria) and incubated under aerobic conditions at 37 °C for 24 to 48 h. The plates representing golden-yellow, smooth, convex, round, with clean edges colonies surrounded by a large yellow halo around growth were suspected as *S. aureus* and were recultured on mannitol salt agar plates to obtain pure cultures for further confirmation stage.

Identification of S. aureus

Following isolation, microscopic examination using the standard method of Gram staining was proformed as pre-identification step for the characteristic colonies.

Subsequently, biochemical tests were carry out such as catalase, oxidase activity, and mannitol fermentation. Catalase and mannitol positive strains were further identified using immunological tests: tube coagulase and deoxyribonuclease production (DNase) tests.

Antimicrobial susceptibility testing

Relying on the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2022), Kirby Bauer disk diffusion method on mueller hinton agar (Realab, Tizzi Ouzzou, Algeria) was applied to investigate the antimicrobial resistance. In addition, the different molecular structures and mechanisms of action were considered when selecting the antibiotics. Therefore, five different antibiotics (Bio-Rad, France) from different classes were used, namly: cefoxitin (30 µg), oxacilin (1 µg), gentamycin (10 µg), erythromycin (15 µg), and tetramycin (30 µg).

Results

Prevalence of S. aureus

The culture results of the samples are presented in Table 1. Out of all samples examined, only 39 samples did not show any positive bacterial growth, and diffrent strains were identified as other species of the genus *staphylococcus* especially coagulase negative *Staphylococcus.*

Table 2 represents the prevalence of *S. aureus* in various food samples analyzed in the laboratory of hygiene of the Wilaya of Oran, the estimated global prevalence of *S. aureus* found in the 350 samples collected from diffrent sale-outlets and screend for *S. aureus* contamination.

Antibiotic susceptibility testing

The resistance profiles of *S. aureus* isolated against the five antibiotics tested are presented in Figure 1. All *S. aureus* strains were resistant to at least one antimicrobial and 82.92% of stains were determined as MDR showing resistance to three or more antibiotics from different classes.

Twenty three of 109 *S. aureus* were confirmed as MRSA by cefoxitin disc diffusion test. The number of resistant stains for gentamycin was (64 of 109), (18 of 109) for erythromycin, (43of 109) for oxacilin, and (35 of 109) for tetramycin.

Table 1: Frequency of *Staphylococcus aureus* isolated of food samples

Number of samples	Positive cultures	Number of strains of *Staphylococcus*	Number of Strains of *Staphylococcus aureus*
350	311	153	109
100%	88.85%	49.35%	31.14%

Table 2: Prevalence of *Staphylococcus aureus* in food samples

Type of samples	Number of positive samples	Number of negative samples	% of samples positive for *Staphylococcus aureus*
Prepared meals	20	90	18.18
Dairy products	12	30	28.57
Pastry	34	44	43.59
Meat and meat products	36	61	37.11
Others	7	23	30.41

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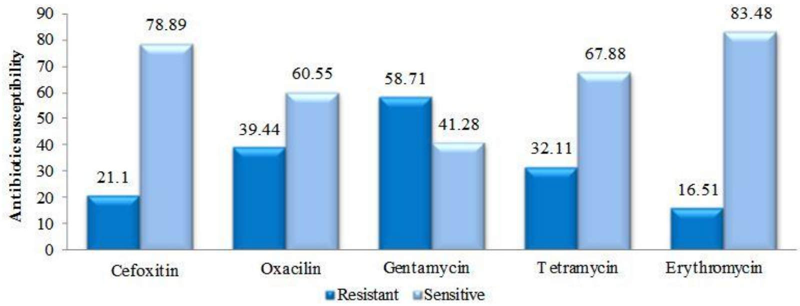

Figure 1: Antibiotic susceptibility pattern of *Staphylococcus aureus* isolated from food samples

Discussion

The current study demonstrate a notable positive result for *S. aureus* contamination in diffrent matrices of marketed food, with an overall of 31.14% (109/350) of the evaluated samples, this outcome confirms that food provides a favourable growth environment for *S. aureus*. Previosly Chaalal et al. (2018), found a similar result with an overall *S. aureus* prevalence of 30.9% out of 495 raw and ready to eat food samples in western Algeria. In the same country, the prevalence obtained in this study was higher than that earlier observed by Titouche et al. (2020), with an occurrence of 17% of *S. aureus* out of 300 food samples collected from diffrentl market points in Tizi Ouzou area, and 23.2% out of 207 Algerian ready to eat food samples found by Mekhloufi et al. (2021). However the data on *S. aureus* from food in Algeria are scarce, thus we oped for other authors world wide reports of diffrent recurrence of *S. aureus*. A higher prevalence was noted by Ballah et al. (2022) in Bangladesh, and El Bayomi et al. (2016) in Egypt with a rate of 35.95% out of 420 of different food sources and 37.5% out of 110 food samples, respectively. Other studies have shown increased prevalence of *S. aureus* regarding several food products, 42% in dairy products in Egypt and 62.7% in raw milk in Saudi Arabia (Alghizzi et al., 2021), 50% in Malaysia (Seow et al., 2021), 15.42% in Iran (Mesbah et al., 2021)**,** and 12.5% in China (Yang et al., 2016) for cooked food, 21.23% in Turkey (Şanlıbaba, 2022) and 15.7% in European Union (EU) (Rodríguez-Lázaro et al., 2017) for meat and meat products, 38.1 and 34.64% for cream pastries samples in Iran **(**Arabestani et al., 2022; Rezaei et al., 2018).

Compered to our results, these distinguished variance could be related to several factors, mainly the type, the size, and the source of samples, (eg animal or vegetable derived food, package, or bulk products), the cooking methods (broiling, grilling, roasting, baking, or frying), the utensils used, storage of samples, the geographical location, and the general hygiene measures applied in food preparation and handling. In addition, being a commensal microorganism of the skin, nose, and mucous membranes of both humans and animals, *S. aureus* could contaminate food, particularly when inadequate hygiene practices and conditions exist (Mekhloufi et al., 2021). As well these outcomes suggest that the incidence of *S. aureus* in food of vegetable origin is very minor than other types of foods (Wu et al., 2018b). Staphylococcal food poisoning commonly occurs in foods that necessitate human manipulations during processing and that are kept at ambient temperature for long durations before being consumed, as stated in our study pastry and confectionery products harbored the highest contamination rate of 43.59%, this significant level of contamination could be due to the suitable environement that provide confectionery product for *S. aureus* growth and the microbial contamination of raw ingredients like cream and utensils used in the preparation of pastries. Furthermore, meat and meat products exhibited a high prevalence of *S. aureus* (37.11%), the contamination of animal muscle reserve for consumption is a direct result of the slaughter and processing of animal carcasses (Raftari et al., 2009)**,** likewise dairy products represented 28.57% of positive samples for *S. aureus*, dairy animals commonly excrete *S. aureus* in milk as it is the most prevalent contagious agent responsible for clinical and subclinical infections in lactating cows (Benhamed and Kihal, 2013)**,** being a good substrate for the growth of *S. aureus*, milk can easily lead to food poisoning in humans.

Whereas prepared meals harbored the lowest contamination rate of 18.18%, due to its vulnerability to destruction by processing operations such as heating and disinfecting agents, the presence of *S. aureus* in processed/cooked food is usually indicative of poor hygiene (Bianchi et al., 2022).

The present study found that 100% of *S. aureus* exhibited resistance to at least one of the antibiotics tested, the most commun restantence was against gentamycin (58.71%), this result do not corroborate with those reported by several authors that *S. aureus* strains show significant sensitivity to this antibiotic indicating an average resistance between 11.1% (Chaalal et al., 2018), 31.76% (Kayili and Sanlibaba, 2020), and 36.45% (Şanlıbaba, 2022)**,** 21.1% of strains were

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determined to be MRSA according to cefoxitin antibiogram test. The presence of MRSA in food is also well reported in diffrent studies with the following rates: 21.5% in Algeria (Chaalal et al., 2018), 9.69% in Iran (Safarpoor Dehkordi et al., 2017), 26% in Bangladesh (Islam et al., 2019). Moreover, 32.7, 39.2, and 16.51% of our strains were resistant to tetracyclin, oxacilin, and erythromycin, respectively. The development of antibiotic resistance in *S. aureus* is a complex process influenced by a various factors and the resistance rates reported in our finding compared with previous studies indicate variation that may be related to a number of attributes, including geographical area, origin and source of samples, testing practices, manipulation of samples, and especially inappropriate antibiotic administration. Therefore, consumers need to be conscious that food, widely consumed in Algeria, presents potential risks and can cause food-borne diseases if handled inappropriately. Therefore it is important to provide more information on hygiene and preparation practices to the population in general and, more urgently, to groups at risk (Wu et al., 2018a).

Conclusion

To date, the data on food-borne *S. aureus* in Algeria are poorly documented and still lacking, which requires studies that can cover this lack of information.

Herein, we conducted this study to estimate the risk facing the consumer when consuming different foodstuffs. The present data has shown that marketed food is considered as public health hazard in Algeria, indicating a significant prevalence and extent of antibiotic resistance of *s. aureus* in the examined samples, reflecting that the bacterium has certainly not revealed all its secrets.

Thus, improving effective sanitation and hygiene procedures and the respect of the microbiologic requirements established by the Algerian legislationis are crucials measures to effectively manage staphylococcal food contamination.

Author contributions

N.F.C. conducted the experimental work, analyzed data, wrote the manuscript, and contributed to desining the study; N.B. designed the study and analyzed the data; I.B. contribution to experimental work (sampling); A.B. analyzed the data and revision of the paper .All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that there is no conflict of interest.

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