


Research Article

Prevalence and Characterization of Antibiotic-Resistant *Staphylococcus aureus* Recovered from Pasteurized Cheese Commercialized in Panama City Markets

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Foodborne bacteria, with a high degree of antibiotic resistance, play an important role in the morbidity and mortality of gastrointestinal diseases worldwide. Among 250 disease-causing bacteria, *Staphylococcus aureus* is one of the major causes of food poisoning, and its resistance to multiple antimicrobials remains of crucial concern. Cheese is often contaminated when proper sanitary procedures are not followed during its production and marketing. This work aimed to evaluate the microbiological quality of pasteurized white cheese commercialized in Panama City. Cheese from five different brands sold in local supermarkets were selected to determine the presence of *S. aureus* as well as its antibiotic resistance profile. The results showed significant contamination of *S. aureus* with a geometric median sample of 10^4 – 10^7 CFU/g. Four out of five (4/5) cheese brands analyzed presented risk of food poisoning by exceeding the allowed range of consumption with a geometric median sample of $1,8 \times 10^6$ – $1,4 \times 10^7$ CFU/g. Fourteen different resistance phenotypes were found. Fifty-five percent (55%) of the analyzed strains were resistant to erythromycin. The data confirm a relatively high prevalence and high levels of *S. aureus*, most likely originated during handling in Panama City retail markets. Further studies are needed to reduce bacterial contamination and to decrease the risk of food poisoning in the consumption of pasteurized cheese.

1. Introduction

Cheese, characterized by its snow-white color, crumbly texture, and a lightly salted flavor is an essential ingredient of Panamanian cuisine. Cheese is rich in nutrients, including vitamins, proteins, lactose, fats, minerals, and water. Therefore, cheese favors the growth and survival of bacterial enteric pathogens such as *Listeria monocytogenes* (*L. monocytogenes*), *Salmonella*, Shiga-toxin producing *Escherichia coli* (STEC), and *Staphylococcus aureus*

(*S. aureus*) [1–4]. Hand manipulation of processed cheese along with its high moisture content and barely acidic pH are factors that contribute to the presence and growth of foodborne pathogens [5, 6]. In Panama, cheese is either sold to wholesalers or directly to end consumers, usually in small markets and/or through door-to-door services, using no labels and undergoing minimum food-safety precautions in its transportation and storage [5, 7, 8]. The lack of hazard analysis and critical control points (HACCP) or good manufacturing practices (GMP) and good hygiene practices

(GHP) at the processing plant, along with hand manipulation by its personnel, increase the risk of cheese contamination [9, 10].

S. aureus is an important foodborne pathogen that causes a wide spectrum of infections in humans, from mild skin infections, bacteremia, systemic disease, or osteomyelitis to more life-threatening infections, such as toxic shock syndrome and staphylococcal food poisoning (SFP) [11, 12]. *S. aureus* is one of the main causative agents of SFP associated with cheese consumption [13]. In the United States of America (USA) and according to the last report available from 2017, the annual incidence of foodborne outbreaks was 841 resulting in 14,481 illnesses, including 827 hospitalizations and 20 deaths, with 2 of them reported as SFP outbreaks [14]. An estimated 77 million people in the Americas suffer an episode of foodborne illness each year, where children under the age of 5 represent 40%, with 125,000 deaths a year [15]. For example, in Ecuador, a total of 19,487 cases were reported in 2019, 12,203 of which were caused by bacteria, even though the specific causative agents were not indicated except for *Salmonella* and *Shigella* outbreaks [16]. In Panama, 277,286 illnesses associated with foodborne pathogens were reported in 2019 [17]. However, no updated data about the microorganisms responsible of these outbreaks is available since 2002, when twenty-eight percent (28%) of the investigated foodborne outbreaks were related to SFP, while *E. coli* was responsible for sixty-two percent (62%) [18].

Antibiotic-resistant *S. aureus* has been isolated from milk and cheese in many parts of the world [19–23]. To exacerbate the food-safety breach of *S. aureus* in cheese, some strains are able to elude antibiotic treatments. These strains, very often referred to as antibiotic resistant strains, have shown multiple antibiotic resistance patterns [21, 24]. This resistance to various antibiotic groups can be mediated by the bacterial chromosome, plasmids, and transposons or gene cassettes that are incorporated into integrons [25, 26], thus making the gene transfer among bacterial strains an easier process. Antibiotic resistance among bacteria is a major public health issue as a result of their persistent circulation in the environment and its consequent contamination of cheese [27]. Besides monitoring the antibiotic resistance of *S. aureus* in cheese, it is of great importance to prevent the spread of multidrug-resistant strains, which may have multiple undesirable consequences. Therefore, the aim of this study was to evaluate *S. aureus* concentration levels and its antibiotic resistance pattern, present in locally produced and commercialized pasteurized cheese in Panama City.

2. Materials and Methods

2.1. Study Area and Sample Collection. A total of five brands of pasteurized fresh white cheese sold in Panama City were analyzed. Five samples of each pasteurized fresh white cheese brand were collected from different independent local supermarkets, where all these cheeses are wrapped and kept under refrigeration at 4°C. Sample collections were done in five field visits in a row and were placed in sterile bags,

numbered, and brought in the cold chain to the Laboratory of Experimental and Applied Microbiology (LAMEXA) of the University of Panama and stored at 4°C until processing. The analysis was carried within 24 h upon collection.

2.2. Isolation and Identification of *S. aureus*. *S. aureus* was isolated and counted using the method described in the bacteriological analytical methods [28]. Briefly, 25 g of each sample was weighed aseptically into sterile stomacher bags (Seward Medical Stomacher Bags® Seward, Germany) containing 225 mL of sterilized 0.1% (w/v) peptone water (Difco, Mexico City, MEX) and homogenized for 2 min, followed by serial 10-fold dilutions until 1×10^{-6} .

Bacteriological analysis was performed by plating 0.1 mL of each dilution into triplicates on Baird–Parker agar (BP, Oxoid, Basingstoke, Hampshire, UK), supplemented with egg yolk tellurite emulsion (Oxoid, Basingstoke, Hampshire, UK). All the analyses were conducted under aseptic conditions. The plated cultures were then incubated at 37°C for 48 h. Colonies that exhibited the characteristics of *S. aureus* morphology (circular black colonies with an opaque zone within an outer clear zone) were considered for further confirmation.

2.3. Coagulase Test and Enumeration of *S. aureus*. All presumptive *S. aureus* isolates were subjected to the coagulase test as described by Koneman et al. [29]. The number of *S. aureus* was reported as CFU/g of a tested sample, taking into account the total colonies counted (TC), the number of coagulase-confirmed colonies (CC), the selected presumptive colonies (SC), the dilution (*D*), and inoculated volume (*V*) onto BP agar. The formula employed is

$$\frac{\text{CFU}}{g} = (\text{TC} \times \text{CC}) \div ((\text{SC}) \times (D) \times (V)). \quad (1)$$

2.4. Assessment of the Microbiological Quality of Pasteurized Cheese. According to guidelines set by the Laboratory Methods in Food Microbiology (LMFM) [30], the microbiological quality of pasteurized cheese can be grouped into two different categories based on their bacterial counts of *S. aureus*: (1) acceptable and (2) unacceptable/potentially hazardous. A cheese sample with *S. aureus* counts, greater or equal to 1×10^3 CFU/g, is considered unhealthy for human consumption; therefore, it fits into category (2).

2.5. Antibiotic Susceptibility Testing. Coagulase-confirmed *S. aureus* colonies were tested for susceptibility to different antibiotics, using the disk-agar diffusion method in accordance to the Clinical and Laboratory Standards Institute [31]. Inoculums from each *S. aureus* isolate were grown aerobically in 5 mL of Müller–Hinton broth (Bioxon, Mexico City, MEX) and were incubated at 37°C to reach a turbidity equal to a MacFarland 0.5 standard. Müller–Hinton agar plates were surface inoculated with each *S. aureus* culture using sterile cotton swabs, and antibiotic impregnated disks (BD Diagnostics, Mexico City,

MEX) were placed on the surface of the inoculated agar plates. A reference strain of *S. aureus* (ATCC 6538) was used as a control. Antibiotics tested were erythromycin (E) (15 µg), tetracycline (TE) (30 µg), gentamicin (GEM) (10 µg), vancomycin (VA) (30 µg), oxacillin (OX) (1 µg), chloramphenicol (CHL) (30 µg), clindamycin (CC) (2 µg), ofloxacin (OFX) (5 µg), and rifampicin (RA) (5 µg). Plates were incubated at 37°C for 18–24 h, and inhibition zone sizes were measured. Isolates were classified as resistant, intermediate, or susceptible to a particular antibiotic following the Clinical and Laboratory Standards Institute criteria [31]. Details of the antimicrobials used in this study are given in Table 1.

2.6. Statistical Analysis. Statistical differences were determined by performing a variance analysis with a significant level of 0.05%. The software SPSS Statistic 22 [32] was used for the analysis, and a *p* value ≤0.01 was considered statistically significant.

3. Results

3.1. Bacteriological Counts and Microbiological Quality of Pasteurized Cheese. A total of 25 pasteurized cheese samples from five different brands (five samples per brand) were screened for the presence of *S. aureus* (Figure 1). All the suspected *S. aureus* colonies were subjected to the coagulase test for confirmation. Coagulase-positive colonies were counted, and the levels of contamination of each brand were determined, giving the following results: the five samples analyzed from brand 1 were positive for the presence of *S. aureus* with a mean value of 6×10^3 CFU/g; two positive samples were detected in brand 2 (mean value of 2×10^4 CFU/g); four positive samples in brand 3 (mean value of 1.6×10^4 CFU/g); and 1 positive sample in brand 5 (mean value of 3.6×10^4 CFU/g). Thus, according to the Laboratory Methods in Food Microbiology, these four brands of pasteurized cheese are considered unsatisfactory for human consumption. On the other hand, brand 4 did not show any contamination by *S. aureus*, and therefore, its consumption is safe.

3.2. Antibiotic Susceptibility of *S. aureus* Isolates. Nine antimicrobial agents of veterinary and human health relevance, from different antibiotic classes, were tested. A total of one hundred *S. aureus* isolates were analyzed for antimicrobial susceptibility. Forty-nine of them were resistant to at least one of the antibiotics tested. A summary of the percentage of *S. aureus* strains, resistant to these antibiotics, is provided in Table 1. From all the examined brands, we were able to detect *S. aureus* isolates showing susceptibility to some of the following six antibiotics: GEM, VA, OX, OFX, TE, and RA (Table 1). By contrast, fifty-five percent (55%) of the *S. aureus* isolates tested showed no susceptibility to E. An intermediate resistance to CHL and CC was observed in fifty-one percent (51%) and thirty-seven (37%) of the isolates, respectively. However, a significant resistance to CC was observed in thirty-five (35%) of the *S. aureus* isolates (Table 1). Although it is not one of the highest levels of

resistance encountered in the study, the result is still startling, considering CC as one of the most common antibiotics used to treat nonlife-threatening human infections with *S. aureus*.

Susceptibility to two or more of the nine antibiotics tested was observed in forty-two percent (42%) of the *S. aureus* isolates (21 out of 49). A total of fourteen distinct antibiotic multiresistance profiles were detected (Table 2). Pattern A showed 4 phenotype resistance profiles to two antibiotics (A1–A4) and was expressed by seven isolates (14%): A1 with four isolates showing resistance to E-TE; pattern B1–B6 showed resistance to three antibiotics (20%, 9/49) including the resistance profile to CC; pattern C1, C2, and D1 exhibited resistance to four antibiotics (4%, 2/49) and to five antibiotics (2%, 1/49), respectively. One out of the forty-nine *S. aureus* isolates showed resistance to eight out of the nine antibiotics being tested. The results showed a multidrug resistance profile to more than two agents from different antibiotic classes in twenty *S. aureus* isolates out of forty-nine representing forty-two percent (42%). Multidrug resistance is frequently found in strains of human origin, whereas in veterinary medicine, it is occasionally reported [33, 34]. The brands with the highest antibiotic resistance profiles were 2 (8 antibiotic resistances) and 5 (6 resistances), followed by 1 and 3 with 5 and 2 antibiotic resistances, respectively.

4. Discussion

S. aureus is a foodborne pathogen that has been linked to various types of foodborne outbreaks related to the consumption of cheese, which have caused a significant impact on health, economic, and trade issues. Regarding to the analyzed five pasteurized cheese brands, four of them were not suitable for human consumption due to the high levels of bacteria found [35], according to guidelines set by the Laboratory Methods in Food Microbiology (LMFM) [30]. A cheese sample with *S. aureus* counts, greater or equal to 1×10^3 CFU/g, is considered unhealthy for human consumption, concluding that only brand 4 fits the criteria. The coagulase test is a tool to discriminate *Staphylococcus* pathogenic species [36]. In the present study, the total prevalence of coagulase-positive staphylococci was eighty percent (80%). The rate of contamination is higher than that observed in the survey conducted by Normano et al. [27], in which the total prevalence of coagulase-positive staphylococci in dairy products was only 17.3%. Nusrat et al. [37] showed that forty-seven percent (47%) of cheese samples harbored *S. aureus*. The presence of coagulase-positive *Staphylococcus* in the cheese analyzed in this study, elaborated with pasteurized milk, may be an indicative of sanitary deficiencies in the storage and retailing of the product [27]. On the other hand, it has been demonstrated that *S. aureus* may enter via contaminated raw milk due to subclinical mastitis in cows, and due to improper food handling, it may promote its colonization throughout the processing plant and consequently contaminate the final product [9, 38, 39]. The robustness of *S. aureus* in food increases the risk of staphylococcal enterotoxins production because the bacteria

TABLE 1: Antibiotic resistance profiles of *S. aureus* isolates from five pasteurized cheese brands in Panama.

Class of antibiotic	Antibiotic	Abbreviation	Inhibition zone (mm) ^a			Antibiotic profile				
			R ^b	I	S	1	2	3	5	T (%)
Macrolides	Erythromycin	E (15) ^c	≤12	13–17	≥18	4-12-3 ^d	0-2-0	5-3-0	18-2-0	55-39-6
Tetracyclines	Tetracycline	TE (30)	≤14	15–20	≥21	1-8-10	0-1-1	6-1-1	5-4-11	24-29-47
Aminoglycosides	Gentamicin	GEN (10)	≤13	14–22	≥23	0-0-19	0-0-2	1-3-4	0-2-18	2-10-88
Glycopeptides	Vancomycin	VA (30)	≤12	13-14	≥15	0-0-19	0-0-2	0-0-8	1-1-18	2-2-96
Penicillinase-stable penicillin	Oxacillin	OX (1)	≤12	13–15	≥16	0-0-19	1-1-0	4-0-4	5-1-14	20-4-76
Phenicols	Chloramphenicol	CHL (30)	≤10	11-12	≥13	1-13-5	0-0-2	1-6-1	0-6-14	4-51-45
Lincosamide	Clindamycin	CC (2)	≤16	17–19	≥20	3-12-4	1-1-0	7-1-0	6-4-10	36-36-28
Fluoroquinolones	Ofloxacin	OFX (5)	≤14	15–18	≥19	0-0-19	0-1-1	2-2-4	1-5-14	6-16-78
Asamycins	Rifampicin	RA (5)	≤9	10-11	≥12	1-2-16	0-0-2	3-2-3	0-3-17	6-14-80

E, erythromycin; TE, tetracycline; GEM, gentamicin; VA, vancomycin; OX, oxacillin; CHL, chloramphenicol; CC, clindamycin; OFX, ofloxacin; RA, rifampicin. ^aThe concentrations used inhibition zone measurements according to the Clinical and Laboratory Standards Institute [31]. ^bAbbreviation: R, resistant; I, intermediate; S, susceptible; T, total. ^cThe antibiotic disc concentration in µg is shown in parenthesis. ^dNumber of resistant, intermediate, and susceptible isolated strains.

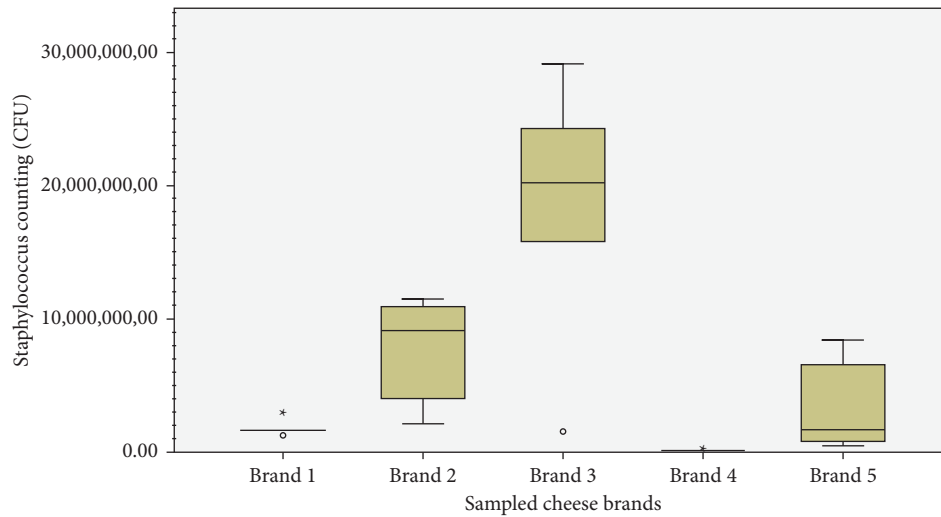


FIGURE 1: *S. aureus* CFU/g of pasteurized cheese for each brand analyzed. Boxes (in gray color) and error bars (in black) represent the median and standard deviation according to the Kruskal–Wallis statistical test.

TABLE 2: Antibiotic resistance profiles of *S. aureus* isolates from pasteurized cheese.

Antibiotic resistance phenotype	Pattern	Isolates (%)			
		1	2	3	5
E-TE	A1	1 (2)			3 (6)
CC-E	A2	1 (2)			
CC-TE	A3			1 (2)	
CC-OFX	A4		1 (2)		
CC-E-RA	B1	1 (2)			
OX-CHL-RA	B2		1 (2)		
OX-CC-TE	B3		1 (2)		1 (2)
CC-E-TE	B4		3 (6)		
OX-CC-E	B5	2 (4)			
CC-E-OFX	B6				1 (2)
VA-OX-CC-E	C1				1 (2)
OX-CC-E-TE	C2				1 (2)
OX-CC-E-TE-RA	D1		1 (2)		
GEM-OX-CC-E-OFX-TE-RA	E1		1 (2)		

E, erythromycin; TE, tetracycline; GEM, gentamicin; VA, vancomycin; OX, oxacillin; CHL, chloramphenicol; CC, clindamycin; OFX, ofloxacin; RA, rifampicin. ^aThe total number of isolates and its corresponding percentage (in parenthesis) are given.

can only be eliminated through a thermal process, but the toxins may remain and cause SFP [40, 41]. The diagnostic criterion for SFP is based upon the detection and recovery of 1×10^5 CFU/g of *S. aureus* [11, 42]. Coagulase-positive colonies counted were 6×10^3 CFU/g for brand 1 and 3.6×10^4 CFU/g for brand 5 (Figure 1), and thus, both meet the diagnostic criterion for SFP that is based upon the detection and recovery of less than 1×10^5 CFU/g of *S. aureus*. However, since values are high, especially for brand 5, it would be worthy to analyze the presence of *S. aureus* toxins that would represent a risk for public health. The lack of critical control points in the production process of cheese may also contribute to the survival and outgrowth of the pathogen [10], although we cannot exclude that handling conditions at the supermarket level might have also an impact. Thus, these results indicate an urgent need for implementation of food hygiene standards in the food industry.

On the other hand, the use and inappropriate prescriptions of antibiotics in the public, private, and agricultural sectors are considered the main factors of the increase in bacterial antimicrobial resistance [43, 44]. High levels of

antibiotic resistance were found in this study. A high percentage of resistance to erythromycin was observed, and this may be attributed to its frequent use in the treatment of human infections. In fact, both nasal and hand contamination of food from food handlers with multiresistant and virulent *S. aureus* has been reported. For example, antibiotic resistant and enterotoxin gene-positive *S. aureus* have been isolated from nasal swabs and hand fingerprints of food handlers in central Iran, Hong Kong, Portugal, or Malaysia [45–48].

Intermediate resistance of *S. aureus* to clindamycin and chloramphenicol can be due to their use in veterinary medicine. Rivera-Salazar et al. [21] reported a high frequency of *S. aureus* antibiotic resistance (56% of the isolates) from pasteurized and unpasteurized cheese samples from Venezuela. Penicillin (44%), oxacillin (20%), tetracycline (12%), erythromycin (8%), amikacin (8%), kanamycin (4%), ciprofloxacin (4%), and clindamycin (4%) are in agreement with our results. On the other hand, Nusrat et al. [37] found higher levels of *S. aureus* resistance to methicillin (58.8%) and oxacillin (100%) from cheese samples in Bangladesh. The results of the present study demonstrated that *S. aureus* isolates from pasteurized cheese in Panamanian markets were resistant to antibiotics from the macrolides class. These results are in agreement with Montoya's [49], which showed *S. aureus* resistance to macrolides such as erythromycin and clindamycin from the lincosamide class. Multidrug resistance was observed in forty-two percent (42%) of the *S. aureus* isolates, including all the antibiotic classes used in the present study (Table 2), as reported by Rivera-Salazar et al. [21]. Whether the differences in the antibiotic resistance profile observed in our work compared with those reported by Nusrat et al. [37] are due to different cheese characteristics (such humidity or acidity), manufacturing, distribution, or handling cannot be addressed, since authors do not describe these data. It would be interesting to study if the antibiotic resistance profiles of *S. aureus* isolates from food handlers as a source of dissemination would explain these differences between regions.

5. Conclusions

The occurrence of *S. aureus* in pasteurized cheese sold in Panama City's retail markets possesses a risk for consumers of these food items. The low microbiological quality of pasteurized cheese in Panama City markets suggests that the bacterium is likely to be found in other Panamanian markets with similar or even poorer sanitary conditions, justifying worker-vendor training and further research on the persistence of the pathogen, as well as the application of effective corrective actions at the market level. Consequently, continuous surveillance of *S. aureus* in Panamanian retail markets will help identify pathogenic sources and promote the development of better practices for processing and selling food. It should also improve the legislative framework that governs the presence of pathogens in food. Lastly, these data confirm that good hygienic practices and temperature control are critical for *S. aureus* control and prevention in food at retail markets.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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