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Detection of methicillin-resistant coagulase-negative staphylococci and *PVL*/*mecA* genes in cefoxitin-susceptible *Staphylococcus aureus* (t044/ST80) from unpasteurized milk sold in stores in Djelfa, Algeria

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ABSTRACT

This study was designed to determine antimicrobial resistance phenotypes and genotypes and virulence factors in *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) in unpasteurized milk sold in Djelfa, Algeria. Eighty-two unpasteurized cow milk samples were randomly obtained from 82 retail stores in Djelfa and tested to detect staphylococci. Species were identified by biochemical tests and MALDI-TOF. Antimicrobial resistance phenotypes and genotypes were determined by disk diffusion test, PCR, and sequencing. The *Staph. aureus* isolates were subjected to *spa* typing, multilocus sequence typing, and detection of virulence genes and the *scn* gene by PCR and sequencing. Forty-five (54.9%) milk samples were contaminated by staphylococci and 45 isolates were recovered: 10 *Staph. aureus* (12.2% of total samples) and 35 CNS (42.7%). Resistance to penicillin (*blaZ*), tetracycline (*tetL*/*tetK*), and erythromycin (*ermB*/*msrA*/*ermC*) were the most common phenotypes (genotypes). Three CNS were methicillin-resistant and all were *mecA*-positive. The *Staph. aureus* isolates were ascribed to the following lineages [*spa* type/sequence type/associated clonal complex (number of isolates)]: t267/ST479/CC479 (n = 6), t1510/ST5651/CC45 (n = 1), t359/ST97/CC97/ (n = 1), t346/ST15/CC15 (n = 1), and t044/ST80 (n = 1). The *mecA* gene was detected in the cefoxitin-susceptible t044/ST80 isolate and co-harbored the *lukF*/*lukS*-PV and *scn* genes. The detection of *mecA*-*PVL*-positive *Staph. aureus*, methicillin-resistant CNS, and multidrug-resistant staphylococcal species indicates a potentially serious health issue and reveals that unpasteurized milk sold in Djelfa city could be a potential vehicle for pathogenic and antimicrobial-resistant staphylococci.

Key words: *Staphylococcus aureus*, Panton-Valentine leukocidin (PVL), ST80, methicillin-resistant staphylococci

INTRODUCTION

Staphylococci species are usually considered part of the normal microbiota of humans and animals but they can also act as opportunistic pathogens involved in food-borne diseases and other clinical manifestations. Based on their ability to coagulate rabbit plasma, species of this genus are divided into 2 subgroups: coagulase-positive staphylococci (**CPS**) and coagulase-negative staphylococci (CNS). Animal-derived foods, such as dairy products, are often found to harbor CPS, mainly *Staphylococcus aureus* (Mama et al., 2019b; Titouche et al., 2019). The presence of *Staph. aureus* in milk and milk products is a public health concern, because this pathogen can produce several virulence factors that play an important role in cellular invasion, bacterial growth, and reduction of immune system cells (Jarraud et al., 2002; Lozano et al., 2011). These virulence factors are represented by cell surface components and exoproteins such as enterotoxins, exfoliatins, toxic shock syndrome toxin (encoded by the *tst* gene), and Panton-Valentine leukocidin (**PVL**, encoded by the *lukF*/*lukS*-*PV* genes) (Holmes et al., 2005). Panton-Valentine leukocidin is a pore-forming toxin that destroys polymorphonuclear cells. It is composed of 2 separate protein units, LukS-PV and LukF-PV, which behave

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synergistically to produce the toxic effect (Shrestha, 2013). It is also associated with necrotizing pneumonia (Haider and Wright, 2013). In addition, *Staph. aureus* can cause food poisoning due to consumption of food containing one or more preformed enterotoxins. Six staphylococcal enterotoxins (SEA, SEB, SEP, SEC, SED, and SEE) are known to be prevalent (Argudín et al., 2010; Titouche et al., 2020). Staphylococcal enterotoxins are heat stable and resistant to inactivation by gastrointestinal proteases (Balaban and Rassoly, 2000).

The CNS species have long been reported as contaminants. However, the role of this group of bacteria has been revised and they are currently recognized as important causative agents of bacteremia in animals and nosocomial catheter-related bloodstream infections in humans (Schulin and Voss, 2001; Piessens et al., 2011). In dairy cows, they constitute the most prevalent pathogens causing subclinical mastitis, a disease affecting milk production (Piessens et al., 2011). Nonetheless, the risk associated with staphylococci is not limited to their pathogenic properties; it includes their capacity to acquire mobile genetic elements encoding antimicrobial resistance genes via horizontal gene transfer (Gómez et al., 2017). It has been suggested that CNS isolated from cattle tend to be more resistant to antimicrobial agents than *Staph. aureus*, and they often develop multidrug resistance (Klibi et al., 2018a). Methicillin resistance, which was initially described in *Staph. aureus* (MRSA) and later in CNS (**MR-CNS**), has become an emerging concern in both veterinary and human medicine and in clinical and nonclinical settings. It is conferred primarily by expression of the *mecA* gene, located within the staphylococcal cassette chromosome (SCC*mec*; Martínez-Meléndez et al., 2015). Methicillin-resistant *Staph. aureus* are defined as *Staph. aureus* carrying the *mecA* gene (or unusually *mecB* or *mecC*) or phenotypically showing resistance to oxacillin or cefoxitin. However, oxacillin-susceptible *mecA*-positive *Staph. aureus*, also known as cefoxitin-susceptible MRSA (**CS-MRSA**), have been increasingly reported not only among clinical isolates but also in animals and food of animal origin (Andrade-Figueiredo and Leal-Balbino, 2016).

In Algeria, 3.5 billion liters of raw milk are produced per year. However, only 18% of this milk is integrated in the industry sector; the rest is sold traditionally to consumers in stores with poor hygienic conditions, either directly (unpasteurized milk) or transformed (skim milk, traditional cheeses, and other products), according to traditional, manual processes (Chenouf et al., 2016; ONIL, 2019). These products are highly appreciated by both rural and urban populations, despite the risk of carrying food-borne pathogens (Chenouf et al., 2016). In this context, few reports have dealt with the genetic characterization of *Staph. aureus* isolates in Algerian raw milk (Chaalal et al., 2018; Matallah et al., 2019; Titouche et al., 2019), with no attention given to CNS strains. Accordingly, the present study was designed to estimate the prevalence and the diversity of staphylococci species (CPS and CNS) recovered from unpasteurized milk sold in retail stores of Djelfa city, Algeria, and to investigate their antibiotic resistance mechanisms and virulence traits.

MATERIALS AND METHODS

Milk Sampling and Isolation of Staphylococcus Species

Eighty-two unpasteurized cow milk samples were obtained from small milk stores in Djelfa city during the period from 2016 to 2019. Djelfa is a steppic zone located 300 km south of Algiers and it covers 1.36% of Algeria's total surface area. Cattle breeding is less practiced in the region compared with that of small ruminants (sheep and goats). In terms of milk production, the region is classified among the fourth group (out of 5), producing between 50 and 100 million liters of milk annually (ONIL, 2019). In each store, cow milk is collected from several farms (at least 2 farms) and sold on the same day. Upon arrival at the store, milk is kept in plastic containers at room temperature for sale (one large container per store), and other milk products, such as raw cheeses, are kept in refrigerated counters. From each store and container, 100 mL of raw milk was collected once in a small sterile container and immediately transported in a 4°C cooler to the laboratory (one sample per store). Sample processing was carried out according to ISO 6888-3 (ISO, 2003), by enrichment of 1 mL of milk in Giolitti-Cantoni broth (Microbiotech, Sétif, Algeria), supplemented with 1% potassium tellurite and incubated at 37°C for 24 h. Afterward, each positive enriched culture (recognized by black coloration due to the reduction of tellurite to metallic tellurium) was inoculated on mannitol salt agar plates (Microbiotech) and incubated at 37°C for 24 to 48 h. Colonies with presumptive *Staphylococcus* morphology were selected (one isolate/positive sample) and identified using Gram staining, catalase test, coagulase test, and the MALDI-TOF MS technique (Biotyper, Bruker Corp., Billerica, MA).

Genomic DNA Extraction

One colony of *Staphylococcus* grown on brain heart infusion agar (Scharlau, Barcelona, Spain) was suspended

in 45 μL of Milli-Q water (Millipore, Burlington, MA) with 5 μL of lysostaphin (1 mg/mL), and incubated in a water bath at 37°C for 10 min. Then, the following reagents were added: 45 μL of Milli-Q water, 5 μL of proteinase K solution (2 mg/mL), and 150 μL of Tris HCl (0.1 *M*, pH 8). After 2 incubation steps, at 60°C for 10 min and 100°C for 5 min, the final suspension was centrifuged at 17,000 × *g* for 3 min at room temperature and the supernatant was collected for molecular analysis (Mama et al., 2019a).

Antimicrobial Susceptibility and Resistance Genes

Susceptibility against 11 antimicrobial agents was assessed by the disk diffusion method on Mueller-Hinton agar (Microbiotech) for all isolates (CLSI, 2018). The antimicrobials under study were as follows (μg/disk, unless otherwise noted): penicillin (10 IU), cefoxitin (30), erythromycin (15), clindamycin (2), enrofloxacin (5), kanamycin (30), gentamicin (10), trimethoprim-sulfamethoxazole (1.25/23.75), chloramphenicol (30), and tetracycline (30) (Oxoid, Basingstoke, UK). In addition, streptomycin activity (10 μg/disk) was tested (CASFM, 2018). In parallel, the D-test, involving the placement of an erythromycin disk in proximity to a clindamycin disk, was applied to detect eventual inducible clindamycin resistance (Gómez et al., 2017).

Based on the obtained antimicrobial resistance phenotypes, resistance genes were studied using conventional PCR methods (Gómez et al., 2017): penicillin (*blaZ*), tetracycline (*tetK*, *tetL*, and *tetM*), aminoglycosides [*ant*(6)-Ia, *ant*(4′)-Ia, *aac*(6′)-Ie–*aph*(2″)-Ia,], macrolides-lincosamides (*ermA*, *ermB*, *ermC*, *ermT*, *msrA*, *msrB*, *lnuA*), and trimethoprim (*dfrG*). Regardless of the methicillin-resistant phenotype, PCR and sequencing were performed in all staphylococci isolates to detect the *mecA* gene.

Virulence Genes and Molecular Typing in Staph. aureus Isolates

Staphylococcus aureus isolates were subjected to PCR and sequencing for the detection of genes encoding PVL (*lukF/lukS*-PV), enterotoxins (*sea*, *seb*, and *sep*), exfoliative toxins (*eta* and *etb*), and toxic shock syndrome toxin (*tst*). Additionally, the *scn* gene, a marker of the human immune evasion cluster (IEC), was assessed following previously described procedures (Argudín et al., 2010; Mama et al., 2019a; Titouche et al., 2019).

All recovered *Staph. aureus* isolates were typed by sequencing the repeat region of the *Staphylococcus* protein A gene (*spa*). The *spa* types were then determined with the Ridom Staph Type software, by detection and assignment of *spa* repeats (http://spaserver.ridom.de/). Targeted PCR for the detection of the clonal complex (**CC**)398 clone (Stegger et al., 2011), and multilocus sequence typing (MLST) of the 7 housekeeping loci (*arcC*, *aroE*, *glpF*, *gmK*, *pta*, *tpi*, and *yqiL*) were performed in all *Staph. aureus* isolates (Mama et al., 2019a).

RESULTS

Milk Contamination and Recovery of Staphylococci Isolates

Of the 82 unpasteurized milk samples analyzed, 45 (54.9%) were contaminated by either CPS or CNS species and consequently, 45 staphylococci isolates were recovered (one isolate per positive sample). Ten (12.2%) milk samples contained CPS isolates, all of which were identified as *Staph. aureus*. Regarding CNS, 35 isolates were identified with the following distribution: *Staph. sciuri* (n = 18), *Staph. lentus* (n = 14), *Staph. hominis* (n = 1), *Staph. chromogenes* (n = 1), and *Staph. cohnii* (n = 1).

Among the 45 contaminated milk samples, 27 (60%) came from mixed milk of 3 farms and 18 (40%) from mixed milk of 2 farms. Moreover, in all contamination cases, milk was transported from the farm to the store in mini trucks without refrigeration over 2 to 3 h (data not shown).

Molecular Typing, Antimicrobial Resistance, and Virulence in Staph. aureus Isolates

As shown in Table 1, 5 *spa* types were identified among *Staph. aureus* isolates: t267, t1510, t359, t346, and t044. They were ascribed to the following lineages (no. of isolates): ST479 (6), ST5651/CC45 (1), ST97/CC97/ (1), ST15/CC15 (1), as well as the pandemic clone ST80 (1).

Focusing on antimicrobial susceptibility testing, the highest resistance in *Staph. aureus* isolates was found toward penicillin (7 isolates, 70%), whereas all isolates showed susceptibility to cefoxitin, gentamicin, chloramphenicol, and trimethoprim-sulfamethoxazole. Three *Staph. aureus* isolates were resistant to at least one antimicrobial agent (enrofloxacin, penicillin, or kanamycin) and 2 were susceptible to all antimicrobials tested. A multidrug-resistant phenotype, defined as resistance to ≥3 drug classes, was found in 5 *Staph. aureus* isolates (Table 1).

Genotypically, resistance to penicillin occurred predominantly via production of a β-lactamase encoded by the *blaZ* gene, in 4 out of 7 resistant *Staph. aureus* isolates,

Table 1. Molecular typing, antimicrobial resistance, and virulence genes in the 10 *Staphylococcus aureus* isolates recovered from unpasteurized milk samples

Isolate code	Molecular typing[1]		Antimicrobial resistance phenotype[2]	Antimicrobial resistance genotype	Virulence genes
	spa type	ST/CC			
X1320	t267	ST479	PEN-ERY-CLI*-TET	*blaZ*, *ermB*, *ermC*, *msrA*, *tetL*	ND[3]
X1321	t267	ST479	ENR	NT[4]	ND
X1322	t1510	ST5651/CC45	PEN	*blaZ*	ND
X1323	t267	ST479	Susceptible	NT	ND
X1324	t267	ST479	PEN-KAN-TET	*blaZ*, *tetL*, *tetK*	ND
X1325	t359	ST97/CC97	PEN-ERY-CLI-TET	*ermB*, *msrA*, *tetL*, *tetK*	ND
X1326	t267	ST479	PEN-ERY-CLI-TET	*blaZ*, *ermB*, *msrA*, *tetL*	ND
X1327	t267	ST479	PEN-KAN	ND	ND
X1922	t044	ST80	Susceptible	*mecA*	*lukF/lukS*-PV, *scn*
X2026	t346	ST15/CC15	PEN-ERY-CLI*-TET	*msrA*, *tetK*	ND

[1]*spa* type = *Staph. aureus* protein A type; ST/CC = sequence type/clonal complex.

[2]PEN = penicillin, ERY = erythromycin, CLI = clindamycin, ENR = enrofloxacin, KAN = kanamycin, TET = tetracycline; *inducible resistance.

[3]None of the tested genes were detected.

[4]Not tested.

whereas tetracycline resistance was mediated by *tetL* or *tetK* genes. The 4 erythromycin–clindamycin-resistant isolates carried the *msrA* or *ermB*/*C* genes. Interestingly, *mecA* was detected in the cefoxitin-susceptible t044/ST80 isolate (CS-MRSA) and co-harbored the gene encoding PVL (*lukF/lukS*-PV). All *Staph. aureus* isolates were negative for the *sea*, *seb*, *sep*, *eta*, *etb*, *tst*, and *scn* genes, except for the t044/ST80 isolate, which carried *scn* (Table 1).

Antimicrobial Resistance Phenotypes and Genotypes in CNS Isolates

The antimicrobial resistance phenotypes and genotypes detected among the CNS isolates are summarized in Table 2. Of the 35 CNS isolates, 23 (65.7%) were susceptible to all antimicrobials studied, whereas the remaining 12 were resistant to at least one antimicrobial (34.3%). Among the resistant isolates, 4 were multidrug-resistant (2 *Staph. sciuri*, 1 *Staph. hominis*, and 1 *Staph. cohnii*). High rates of antibiotic resistance were demonstrated against tetracycline (9 isolates, 25.7%) and erythromycin (8 isolates, 22.8%), with *tetL*/*tetK* and *ermB*/*msrA*/*ermC* being the most frequent genotypes, respectively. Resistance to penicillin (*blaZ*) and enrofloxacin were also observed in 3 CNS isolates (8.6%), whereas only one *Staph. lentus* isolate expressed resistance to trimethoprim-sulfamethoxazole mediated by the *dfrG* gene. Importantly, 3 CNS isolates (*Staph. hominis*, *Staph. sciuri*, and *Staph. cohnii*) showed methicillin resistance (MR-CNS), and all harbored *mecA* (3.6% of total samples). The *Staph. hominis* isolate displayed a multidrug resistance genotype with the following genes: *blaZ*, *mecA*, *tetK*, *msrA*, *lnuA*, *ant*(6)-Ia, *ant*(4′)-Ia, and *aac*(6′)-Ie-*aph*(2″)-Ia.

DISCUSSION

In Algerian society, the consumption of unpasteurized milk and its derivatives constitutes an ancient habit associated with livestock farming. These traditions may constitute a potential health hazard. Generally, the prevalence of *Staph. aureus* strains from animal-derived food is well documented in Algeria; however, there is a lack of data on CNS. Overall, more than half of milk samples were contaminated by staphylococcal species, with a relatively high incidence of CNS compared with *Staph. aureus* (42.7% and 12.2% in analyzed samples, respectively), with *Staph. sciuri* being the most frequently isolated species. It is important to note that only one colony was selected per sample because morphologically only one type of colony was found in each mannitol salt agar plate inoculated; however, the possibility of missing cases of mixed contamination cannot be excluded. Moreover, both the presence of *Staph. aureus* and the diversity of CNS (and their antimicrobial resistance profiles) might have been underrepresented with the methods used here. Similar observations were reported in Belgium and Austria, where 90 and 94% of bulk milk samples, respectively, contained CNS (Kümmel et al., 2016; De Visscher et al., 2017). Carriage rates of *Staph. aureus* isolates in Algerian raw milk ranged from 19.9 to 34.8% (Chaalal et al., 2018; Matallah et al., 2019; Titouche et al., 2019), whereas much higher frequencies of 49.4 and 50% were reported in neighboring North African countries (Bendahou et al., 2009; Ben Said et al., 2016).

Contamination of raw milk with staphylococcal species may have numerous origins, but is mostly related to poor hygiene practices, from upstream (management of the herd) to downstream (storage of the final prod-

uct), through milking, handling, and transportation processes (Chenouf et al., 2016). Nasal carriage by cows and nasal or hand carriage by farm workers are also important sources of *Staph. aureus* and CNS (Akkou et al., 2018; Roberts et al., 2018). Moreover, the presence of staphylococci species in milk can originate from the udder itself, in the case of intramammary infection, or from the teat canal and udder skin of healthy cows. Emergence of CNS as a major cause of mastitis in dairy cows has been reported not only in Algeria (Saidi et al., 2019; Zaatout et al., 2019), but also in other countries (Klibi et al., 2018b).

Genetic diversity was observed among the *Staph. aureus* isolates. Most of our *Staph. aureus* isolates belonged to *spa* type t267, which was previously reported in Algeria in raw milk from healthy cows (Titouche et al., 2019), milk from cows with mastitis (Zaatout et al., 2019), and in nasal swabs of farmers (Akkou et al., 2018). Our findings suggest that this *spa* type might be circulating within Algerian dairy environments. Similarly, t267 was predominant in *Staph. aureus* from cattle herds in Tunisia (Klibi et al., 2018a). In our study, all CC479 isolates were concomitant with *spa* type t267. In previous surveys, CC479 was considered a ruminant-associated clone, mainly found in severe bovine mastitis cases (Boss et al., 2016; Hoekstra et al., 2019). Furthermore, one *Staph. aureus* isolate was t359/CC97; this lineage has been detected in dairy cattle from South Africa (Schmidt et al., 2017), Italy (Feltrin et al., 2015), and China (Zhang et al., 2018). It has also been reported as an uncommon cause of infections in small ruminants, pigs, and humans (Spoor et al., 2013).

A study conducted on 220 *Staph. aureus* isolates of the CC97 clone collected from different sources (bovine, human, porcine, and caprine origins) in 18 countries indicated that emergent clones of human epidemic community-associated MRSA resulted from livestock-to-human host jumps, as is the case for bovine *Staph. aureus* CC97 (Spoor et al., 2013). These results suggest the importance of dairy cattle as a source of zoonotic bacterial pathogens, with eventual spread within human populations. Three other lineages were also detected in the current findings: CC45, CC15, and ST80. They are most often found to be associated with human MRSA isolates and have been reported from patients with atopic dermatitis in Canada (Yeung et al., 2011), and with endovascular infections in Spain (Pérez-Montarelo et al., 2017).

Recently, the emergence of MRSA-CC15 in Kuwait hospitals has been reported (Udo et al., 2020). The European pandemic clone ST80 has already been detected among MRSA in Algerian hospitals, and was responsible for more than one-third of community and nosocomial infections in Algiers in the 2000s decade (35.7 and 35.8%, respectively; Ramdani-Bouguessa et al., 2006; Antri et al., 2011; Alioua et al., 2014). The first detection of the community-associated MRSA CC80 clone in Europe goes back to 1997 in Denmark, as mentioned by Stegger et al. (2014), who believed that this lineage evolved from a methicillin-susceptible *Staph. aureus* ancestor that might have originated from sub-Saharan Africa.

In the present work, only the ST80 isolate harbored the *scn* gene, as well as the *lukF/lukS*-PV genes, which

Table 2. Antimicrobial resistance phenotype/genotype in the 35 CNS isolates

Species and no. of isolates	Antimicrobial resistance phenotype[1]	Antimicrobial resistance genotype
Staphylococcus scuiri		
12	Susceptible	NT[2]
1	ERY-TET	*tetK*, *tetL*, *ermB*
1	ERY-ENR-TET	*tetK*, *tetL*, *ermC*, *msrA*, *ermB*
1	ERY	*ermB*
1	PEN-FOX	*mecA*
1	PEN-TET	*tetK*
1	ERY-ENR-TET	*tetK*, *tetL*, *msrA*, *ermB*
Staphylococcus lentus		
10	Susceptible	NT
1	ERY-SXT	*ermB*, *dfrG*
1	TET	*tetK*
2	ERY-TET	*tetK*, *tetL*, *ermB*, *ermC*
Staphylococcus hominis		
1	PEN-FOX-ERY-CLI-KAN-GEN-STR-SXT-TET	*blaZ*, *mecA*, *tetK*, *msrA*, *lnuA*, *ant*(6)-Ia, *ant*(4′)-Ia, *aac*(6′)-Ie-*aph*(2″)-Ia
Staphylococcus cohnii		
1	FOX-ENR-TET	*mecA*, *tetK*
Staphylococcus chromogenes		
1	Susceptible	NT

[1]PEN = penicillin, FOX = cefoxitin, ERY = erythromycin, CLI = clindamycin, ENR = enrofloxacin, KAN = kanamycin, GEN = gentamycin, STR = streptomycin, SXT = trimethoprim-sulfamethoxazole, TET = tetracycline.

[2]None of the antimicrobial resistance genes tested in susceptible isolates.

is considered a frequent virulence factor in this lineage. Interestingly, the presence of *scn* in this isolate suggests its potential human origin. In Algeria, few studies have described detection of PVL-containing *Staph. aureus* isolates in the cattle environment associated with mastitis cases or in food samples (Benhamed and Kihal, 2013; Chaalal et al., 2018).

The present study demonstrated the absence of the *eta*, *etb*, *tst*, *sea*, *seb*, and *sep* genes among the *Staph. aureus* isolates. In fact, toxic shock syndrome and exfoliative toxins are more frequently detected among clinical isolates. In the case of food samples, staphylococcal enterotoxins are especially relevant because of their potential implication in food poisoning. In this sense, staphylococcal enterotoxins were frequently detected in raw milk collected in Northern Algeria (Titouche et al., 2019).

The clonal lineage CC398 was not detected among our *Staph. aureus* isolates. This result could be explained by the fact that this lineage is mainly isolated from pigs (Lozano et al., 2009).

A multidrug-resistant phenotype was detected in 9 of 45 staphylococci (20%), and a variety of resistance genes were also detected. These results indicate a serious public health issue, considering that these microorganisms may be transferred to humans via milk consumption, as well as the danger of therapeutic failures. In agreement with several previous reports (Matallah et al., 2019; Titouche et al., 2019), the highest level of resistance was noted toward penicillin, tetracycline, and erythromycin, with *blaZ, tetK*, and *ermB* being the most common corresponding genotypes, respectively. The β-lactam class is known to be extensively used in Algerian dairy cattle against staphylococcal mastitis, which remains the major reason for use of antibiotics in bovines (Saidi et al., 2019). Tetracycline has a bacteriostatic effect and is commonly used and recommended in cattle for the treatment of septicemia, respiratory, digestive, genitourinary, and interdigital infections (Barour et al., 2019). Likewise, erythromycin, being the second macrolide intended for use in animals, has long been prescribed for acute mastitis caused by gram-positive bacteria in lactating dairy cows because of its good distribution in the mammary gland (Bajwa et al., 2007). The long-term use of these antibiotics, considering that they belong to older drug families prescribed in bovines, either for therapeutic or prophylactic reasons, can largely explain the high resistance rates because of selection pressure. From the animal and public health perspectives, *Staph. aureus* and CNS are of major concern, especially where animal-derived foods and humans intersect, mainly through the food chain. These results provide insight into antibio-resistance in Algerian cattle farms that may be contributing to therapeutic failures in cattle farming, the emergence of resistance by gene transfer between species, and the presence of antibiotic residues in milk. This study highlights the need to reduce and optimize the use of antibiotics in livestock animals.

The screening for methicillin resistance, performed via cefoxitin disk diffusion test, revealed 3 MR-CNS, all of which carried *mecA*. These results highlight the role that CNS may play as a potential donor of *mecA* gene to other staphylococcal species, mainly those with greater pathogenic properties, such as *Staph. aureus*. Currently, MR-CNS are receiving significant attention in human and animal health and have been described in cattle, animal handlers, and their environment (Venugopal et al., 2019), in companion animals and their owners (Gómez-Sanz et al., 2019), and also in clinical isolates from hospitals (Kitti et al., 2018). In Algeria, data on MR-CNS are lacking. Therefore, complementary studies with larger sample sizes (from cattle and cattle workers) are required to better investigate the dissemination and epidemiology of MR-CNS in cattle sector.

Phenotypically, no methicillin resistance was detected among our *Staph. aureus* isolates by antibiogram. However, the t044/ST80 isolate was found to carry the *mecA* gene (CS-MRSA). Detection of *mecA* is not performed routinely in Algerian microbiology laboratories; therefore, misclassification may occur. Detection of emerging MRSA variants, such as CS-MRSA, is increasingly described. This discrepancy between phenotype and genotype could be explained by an eventual default in *mecA* expression. Methicillin-susceptible *mecA*-containing *Staph. aureus* isolates were also reported in university and general hospitals in Brazil (Andrade-Figueiredo and Leal-Balbino, 2016) and Japan (Hososaka et al., 2007), and in processed food from Europe (Quijada et al., 2019). To the best of our knowledge, the current paper is the first report of CS-MRSA in Algeria. Further in-depth characterization of the CS-MRSA isolate detected in this study is warranted, to characterize its methicillin resistance expression. The significance of this study lies in its extensive character, describing the prevalence and genetic characterization of both CPS and CNS from raw milk sold in Djelfa city. This study could also be regarded as a food safety survey given that its findings are intended to be communicated and applied across the Algerian dairy chain.

CONCLUSIONS

Detection of *mecA-PVL*-positive *Staph. aureus*, methicillin-resistant CNS, and multidrug-resistant staphylococcal species indicates a potentially serious health issue and reveals that unpasteurized milk

destined for human consumption in Djelfa city could be a vector of pathogenic and antimicrobial-resistant staphylococci. These outcomes require particular attention from all actors in the Algerian dairy industry, but primarily food safety scientists and veterinarians prescribing antibiotics. The use of heat treatment and the application of good hygiene and manufacturing practices can minimize the risk of milk contamination. Greater controls in production and consumption of unpasteurized milk should be implemented.

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