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PCR detection of staphylococcal enterotoxin genes and exfoliative toxin genes in methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains from raw human breast milk

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SUMMARY

Background & aims: Human milk is known to be the best food for infants, as it contains all of the nutrients they need and also helps to protect them against infection. *Staphylococcus aureus* is one of the most common clinical and foodborne pathogens. It produces a variety of extracellular protein toxins, including enterotoxins, exfoliative toxin (ET), haemolysins and Panton-Valentine leukocidin (PVL). This study was carried out to evaluate the Xpert MRSA/SA nasal (Cepheid) PCR assay for the detection of Methicillin-Resistant *S. aureus* (MRSA) and Methicillin-susceptible *S. aureus* (MSSA) isolates, and to analyse the frequency of genes encoding the classical antigenic staphylococcal enterotoxins (SE) and exfoliative toxins (ET) in *S. aureus* strains isolated from raw human breast milk (HBM).

Methods: A total of 72 milk samples were collected from mothers who had delivered in the hospital between 16 February 2014 and 24 April 2016. Samples were cultured and bacterial colonies were identified phenotypically by standard bacteriological methods. All staphylococci strains isolated by routine tests were examined with MALDI-TOF-MS. Then PCR Xpert MRSA/SA nasal was performed on the closed GeneXpert® random access platform (Cepheid), then the house-PCR to detect SE genes and ET genes.

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Results: *S. aureus* was identified in 86.2% (62/72) of the 72 *Staphylococcus* spp isolated from raw milk culture. PCR results showed that 30 of the 62 *S. aureus* strains (48.3%) harboured genes coding for toxins. *sea* was the most prevalent virulence gene (24.1%), followed by *see* (12.9%) and *eta* (12.9%) genes.
Conclusions: Xpert MRSA/SA nasal complete assay allows rapid and accurate identification of MRSA and MSSA. This assay is very easy to perform and is useful for the diagnosis of milk contamination by *S. aureus* in human milk banks. These results suggest the potential infant health threat related to *S. aureus* contamination of HBM. Efforts are therefore required to improve safety standards to prevent staphylococcal food poisoning in these infants.

1. Introduction

HBM is considered to be the best food for infants, as it contains all of the nutrients they need and also helps to protect them against infection. Healthcare professionals generally agree that properly collected and stored HBM is appropriate for healthy full-term and preterm babies. However, for many reasons, breast-feeding may be impossible or inadvisable.

Milk is a good substrate for *Staphylococcus aureus* growth and enterotoxin production [1]. The presence of *S. aureus* in HBM samples can be explained by secondary contamination from the skin, breasts and nasal cavity of milk donors and healthcare professionals, or alternatively, unsatisfactory conditions of the utensils used [2]. Since its first description, MRSA has become a major public health issue because of worldwide spread of several clones. The specific genetic mechanism of its resistance has been identified as a mobile genetic element (Staphylococcal Cassette Chromosome mec – *SCCmec*) integrated into the *S. aureus* chromosome, within which the *mecA* gene encodes a specific methicillin-resistant transpeptidase (penicillin-binding protein 2a-PBP2a) [3]. A new divergent *mecA* homologue (*mecC* or *mecA* LGA251) was described in a novel *SCCmec* named type XI [4] in *S. aureus* or coagulase-negative staphylococci (CoNS).

S. aureus produce a variety of extracellular protein toxins, including enterotoxins, toxic shock syndrome toxin 1 (TSST-1), ET, haemolysins, coagulase and Panton-Valentine leukocidin (PVL). *S. aureus* is one of the most commonly isolated pathogenic bacteria and is responsible for many nosocomial infections, besides being the main causative agent of food poisoning by virtue of its wide range of enterotoxins. According to serological classification, seven classical antigenic SEs have been identified as SEA, SEB, SEC1, SEC2, SEC3, SED, SEE and SEH [5–7]. Munson et al. [8] identified and characterized *seg* and *sei*, while Zhang et al. [9] identified the gene *sej*. Several other toxins, called enterotoxins SEK, SEL, SEM, SEN, SEO, SEP, SEQ, SER and SEU, have been described, and their genes have been sequenced [1]. Toxic shock syndrome in humans and animals is caused by the presence of *S. aureus* isolates producing TSST-1 [10]. The *tsst* gene is chromosomal. Enterotoxins, as well as TSST-1, belong to a family of superantigens [11]. Some strains of *S. aureus* producing one or both of two distinct ET, A (ETA) or B (ETB), have been associated with a series of impetiginous staphylococcal diseases referred to as staphylococcal scalded-skin syndrome [12]. PVL produced by less than 5% of *S. aureus* strains is a pore-forming toxin encoded by the lukS-PV and lukF-PV genes [13].

In the light of the above findings, the present study was designed to: (i) evaluate the ability of the Xpert MRSA/SA real-time PCR assay (Cepheid, Sunnyvale, CA) to detect MRSA and MSSA isolates in a collection of staphylococci strains isolated from HBM; (ii) analyse the frequency of genes encoding SEA, SEB, SEC, SED, SEE, ETA and ETB in *S. aureus* strains isolated from raw HBM.

2. Materials and methods

2.1. Human milk bank and sample collections

We conducted a retrospective study over a 26-month period from 16 February 2014 to 24 April 2016. Seventy-two HBM samples were collected under aseptic conditions and transported frozen to the Amiens University Hospital milk bank (France), in which milk is voluntarily donated by mothers who have delivered in the hospital. To ensure optimum quality of milk donations, the donors were instructed in the appropriate methods for sanitary collection, handling, storage and transportation of the breast milk according to the French Ministry of Health recommendations [14]. In the milk bank, the milk was processed according to a detailed protocol defined by Ministry of Health recommendations [14].

The study was conducted according to the Code of Ethics of the World Medical Association (Declaration of Helsinki) and all procedures were approved by the Picardie Human Research Ethics Committee.

2.2. Bacteriological screening and Staphylococci counts

Seventy-two fresh-frozen HBM samples were transported on dry ice from the milk bank to the hygiene laboratory. One milk tube, corresponding to approximately 2 mL of raw milk, of each sample was taken. Each sample was screened for contamination by normal skin flora as follows: 10 μL of sample were seeded on sheep blood Columbia agar (5%) and Chapman agar (Bio-Rad, France). After incubation at 37 °C for 48 h, colonies presenting morphological characteristics of staphylococci were counted. Sheep blood agar was used for counting of normal skin flora and Chapman agar was used for counting of *S. aureus* colonies. Milk acceptable for dispensing raw must contain $\leq 10^6$ CFU/mL of skin flora and $<10^4$ CFU/mL of *S. aureus*.

2.3. Identification of S. aureus

Strains positive for mannitol, catalase, Gram staining and bound coagulase (Pastorex Staph Plus-Bio-Rad) were classified as *S. aureus* and stored at −80 °C in sterile Mueller-Hinton (M. H) medium enriched by horse serum, then seeded in conservation tubes.

2.4. MALDI-TOF-MS

All staphylococci strains isolated by routine tests were examined by Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) (Brucker Daltonics, Bremen, Germany) according to the previously described procedure [15]. Absolute ethanol was used for sample preparation and 1 μL of DHB matrix solution (2,5-dihydroxybenzoic acid, 50 mg/mL, 30% acetonitrile, 0.1% trifluoroacetic acid) was added. Flex control 3.0 software and Biotyper 2.0 database (Brucker Daltonics) were used to calculate and process the analytical data, as previously described [16]. Identification was performed in duplicate and the identification score cut-off was applied to each measurement, according to the manufacturer's instructions.

2.5. Methicillin agar screen

Susceptibility to oxacillin was determined by the disk diffusion method on M.H agar at 37 °C for 24 h on disks loaded with 30 μg of cefoxitin and 6 μg of benzyl penicillin, according to the recommendations of the *Comité de l'Antibiogramme de la Société Française de Microbiologie* (CA-SFM) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines V1.0 February 2013.

2.6. Xpert MRSA/SA nasal PCR assay

This test was performed on the closed GeneXpert® random access platform (Cepheid), allowing autonomous, fully integrated and automated molecular analysis: the extraction, amplification, and detection of amplified products steps are performed successively in the same cartridge. This method uses automated real-time PCR technology to detect proprietary sequences of genes that encode

S. aureus protein A, the methicillin resistance element (*mecA*) and the Staphylococcal Cassette Chromosome (*SCCmec*). The Xpert MRSA/SA nasal assay was performed according to the manufacturer's instructions on the GeneXpert® system (Cepheid).

Briefly, bacterial suspension 0.5 McFarland was diluted to 1:100 and then vortexed for 10 s. This dilution (100 μL) was transferred to the elution reagent vial, and then vortexed for 10 s. The entire sample was then dispensed into the S chamber of the cartridge, followed by reagent 1 into port 1, and reagent 2 into port 2, and the cartridge was inserted into the GeneXpert® apparatus and the assay was started. The results were available within 71 min. The following results were considered: spa^+ $mecA^-$ $SCCmec^-$ (MSSA), spa^+ $mecA^+$ $SCCmec^+$ (MRSA). Although not recommended by the manufacturer, in the case of MRSA-/SA-, the result was extrapolated to indicate methicillin-resistant coagulase-negative staphylococci (MRCoNS) when *mecA* was positive or methicillin-susceptible coagulase-negative staphylococci when *mecA* was negative (MSCoNS). The Cepheid assay used in this study has not been validated for the identification of MRSA and MSSA isolated from HBM.

2.7. DNA isolation and PCR conditions

Total DNA was extracted by using the NucliSENS easy MAG extractor apparatus (BioMérieux, France) according to the manufacturer's instructions. Oligonucleotides ranging from 120 to 200 bp were selected from the published DNA sequences of the *S. aureus* genes [17] (Table 1). PCR was performed with a final volume of 50 μL according to the Master Mixes of components from the QIAGEN Top Taq master mix kit. Each reaction contained 25 μL of Master Mix, 1 μL of each primer, 2 μL of DNA as template, 5 μL of CoralLoad and 16 μL of RNase-free water. The amplification conditions were 94 °C for 5 min followed by 35 cycles of amplification (denaturation at 94 °C for 2 min, annealing at 57 °C for 2 min, and an extension at 72 °C for 1 min), and a final extension at 72 °C for 7 min. All PCR amplifications were carried out in a Veriti Thermal Cycler (Applied Bio System, France).

3. Results

Over a 26-month of study period, 72 staphylococci strains isolated from raw milk culture were identified as *S. aureus* by agglutination test (Pastorex Staph Plus) at concentrations ranging between

Table 1
Nucleotide sequences, gene locations, and predicted sizes of amplified products for the staphylococcal toxin-specific oligonucleotide primers used in this study.

Gene[a]	Primer	Oligonucleotide sequence (5′→3′)	Location within the gene	Size of amplified product (bp)
sea	SEA-1	TTGGAAACGGTTAAAACGAA	490–509	120
	SEA-2	GAACCTTCCCATCAAAAACA	591–610	
seb	SEB-1	TCGCATCAAACTGACAAACG	634–653	478
	SEB-2	GCAGGTACTCTATAAGTGCC	1091–1110	
sec	SEC-1	GACATAAAAGCTAGGAATTT	676–695	257
	SEC-2	AAATCGGATTAACATTATCC	913–932	
sed	SED-1	CTAGTTTGGTAATATCTCCT	354–373	317
	SED-2	TAATGCTATATCTTATAGGG	632–671	
see	SEE-1	TAGATAAAGTTAAAACAAGC	491–510	170
	SEE-2	TAACTTACCGTGGACCCTTC	640–659	
eta	ETA-1	CTAGTGCATTTGTTATTCAA	374–393	119
	ETA-2	TGCATTGACACCATAGTACT	473–492	
etb	ETB-1	ACGGCTATATACATTCAATT	51–70	200
	ETB-2	TCCATCGATAATATACCTAA	231–250	

[a] Nucleotide sequence and locations derived from published nucleotide sequences [1].

Table 2
Phenotypic and genotypic characteristics of *S. aureus* and *S. lugdunensis* strains isolated from human milk before pasteurization.

Agglutination test	Identification of strains	Phenotypic characters		Genotypic characters						Total
Pastorex Staph Plus	MALDI-TOF-MS	Oxacillin	Cefoxitin	SPA^+ Mec^- SCC^-	SPA^+ Mec^+ SCC^+	SPA^+ Mec^+ SCC^-	SPA^+ Mec^- SCC^+	SPA^- Mec^- SCC^-	SPA^- Mec^+ SCC^+	
+	*S. aureus*	R	R	−	+	−	−	−	−	3
+	*S. aureus*	R	R	−	−	+	−	−	−	10
+	*S. aureus*	S	S	−	−	−	+	−	−	1
+	*S. aureus*	S	S	+	−	−	−	−	−	48
										62
+	*S. lugdunensis*	S	S	−	−	−	−	+	−	7
+	*S. lugdunensis*	R	R	−	−	−	−	−	+	1
+	*S. lugdunensis*	S	S	+	−	−	−	+	−	1
+	*S. lugdunensis*	S	S	−	−	+	−	−	−	1
										10
TOTAL										72

11.2% (8172) of cases were discordant. Eight of these discordant results were Pastorex Staph Plus positive results and Xpert negative results.

10^3 and 10^6 CFU/mL. All 72 staphylococci isolates were examined by MALDI-TOF-MS. Pastorex Staph Plus correctly identified 62 of these isolates, which were confirmed to be *S. aureus* (86.2%) and ten (13.8%) isolates, which were identified as *Staphylococcus lugdunensis*. Two of the ten *S. lugdunensis* isolates were *spa* gene positive and eight were *spa* gene-negative by GeneXpert® assay. All 62 *S. aureus* identified were *spa* gene positive. Overall, Pastorex Staph Plus results were concordant with Xpert results in 88.8% (64/72) of cases versus 11.2% (8/72) of discordant cases. Eight of these discordant results were Pastorex Staph Plus-positive results and Xpert-negative results (Table 2).

Eight (80%) of the 10 *S. lugdunensis* isolates were phenotypically methicillin-susceptible coagulase-negative staphylococci (MsCoNS) and genotypically *spa*$^-$ *mec*$^-$ *SCCmec*$^-$ and were interpreted by GeneXpert® as $MRSA^-/SA^-$; one was phenotypically MSCoNS and genotypically *spa*$^+$ *mec*$^+$ *SCCmec*$^-$ and was interpreted by GeneXpert® as $MRSA^-/SA^-$ and another was phenotypically methicillin-resistant CoNS (MRCoNS) and genotypically *spa*$^-$ *mec*$^+$ *SCCmec*$^+$ and was interpreted by GeneXpert® as $MRSA^-/SA^-$. Among the 62 *S. aureus* strains identified, 48 (77.4%) were phenotypically methicillin-susceptible *S. aureus* (MSSA) and genotypically *spa*$^+$ *mec*$^-$*SCCmec*$^-$ and were interpreted by GeneXpert® as $MRSA^-/SA^+$ (MSSA); ten (16.2%) were phenotypically methicillin-resistant *S. aureus* (MRSA) and genotypically *spa*$^+$ *mec*$^+$ *SCCmec*$^-$ and were interpreted by GeneXpert® as MSSA, three (4.8%) were phenotypically MRSA and genotypically *spa*$^+$ *mec*$^+$ *SCCmec*$^+$ and were interpreted by GeneXpert® as $MRSA^+/SA^+$, and one strain (1.6%) was phenotypically MSSA and genotypically *spa*$^+$ *mec*$^-$ *SCCmec*$^+$ and was interpreted by GeneXpert® as MSSA (Table 2). Of 62 *S. aureus* strains, 44 (70.9%) were positive for *se* or *et* genes, among which 35 (79.5%) strains carried SE genes and 9 (20%) carried ET genes. Of the genes that code for SEA-SEE, *sea* was the most frequent, it was found in 15 (24,1%), isolates, followed by *see* in 8 (12.9%) (Table 3). Of the genes that code for ETA-ETB, *eta* were the most frequent (12.9%) (Table 3).

Table 3
The percentages of *S. aureus* strains positive for staphylococcal enterotoxins (SE) and exfoliative toxins (ET) genes.

Gene	Number of strains tested	Number of positive strains	(%)
sea	62	15	(24.1)
see	62	8	(12.9)
eta	62	8	(12.9)
seb	62	6	(9.6)
sec	62	4	(6.4)
sed	62	2	(3.2)
etb	62	1	(1.6)

Among the *S. aureus* strains isolated from breast milk, 24.1% harboured *sea* genes and 12.9% harboured *see* and *eta* genes. These genes appeared to be more prevalent.

Table 4
Toxin gene profile of *S. aureus* isolated from human breast milk.

Toxin gene profiles	Number of *S. aureus* strains positive for *se and et genes*
a	1
b	3
c	2
d	1
e	11
eta	1
a + *eta*	5
a + *b* + *d* + *eta*	1
a + *b* + *eta*	1
b + *e*	1
c + *e*	2
e + *etb*	1

Sea(*a*), *seb*(*b*), *sec*(*c*), *sed*(*d*), *see*(*e*) (staphylococcal enterotoxin genes), *eta*, *etb* (exfoliative genes).

Table 4 shows 12 different genotype toxin profiles: 6 genotypes contained a single toxin gene (*a*, *b*, *c*, *d*, *e* and *eta*); 4 genotypes contained each two toxin genes (*b* + *e*, *c* + *e*, *a* + *eta*, and *e* + *etb*); 1 genotype contained three toxin genes (*a* + *b* + *eta*) and 1 genotype contained four toxin genes (*a* + *b* + *d* + *eta*).

4. Discussion

Although French legislation does not define acceptable limits for the amount of *S. aureus* in HBM, the quantity of toxins produced by toxigenic *S. aureus* are known to achieve levels that are sufficient to induce symptoms of food-borne disease for *S. aureus* concentrations $\geq 10^5$ CFU/mL, as was the case for 7 (11.2%) of the 62 raw HBM samples. Milk and milk products have frequently been implicated in staphylococcal food poisoning and contaminated raw milk is often involved. Human handlers, milking equipment, and the environment are possible sources of bulk milk contamination. Several researchers have reported different results for bacterial counts in contaminated dairy products expressed in CFU/mL [18–20]. Variations in *S. aureus* counts in milk may depend on the sanitary precautions observed during the milk processing chain. In the present study, we used the routine bacteriological procedure for isolation of *S. aureus* from HBM. In view of the findings and comparing the routine technique with Xpert MRSA/SA results, the Xpert MRSA/SA nasal test for amplification of *spa*, *mecA* and *SCCmec* genes can be considered to allow rapid diagnosis and confirmation of *S. aureus* isolates. The present study showed that 10 (13.8%) of the 72 staphylococci identified had a positive result on the Pastorex Staph Plus test, two of which were positive for *spa* gene on GeneXpert® PCR MRSA/SA and were considered to be false-positive results. These 10 staphylococci isolates were identified by MALDI-TOF-MS as *S. lugdunensis*. Previous publications have also reported false-positive results on latex agglutination tests, mainly for *Staphylococcus intermedius*, *S. lugdunensis*, *Staphylococcus schleiferi*, *Staphylococcus hycus*, and *Staphylococcus haemolyticus* [21,22], indicating that latex agglutination tests must be performed with caution. *S. lugdunensis* produces a bound coagulase (clumping factor) (which is used in the rapid slide agglutination test), a property it shares with *S. aureus*, but unlike *S. aureus*, it does not produce a free coagulase, and can therefore give positive results on the latex agglutination test. *S. haemolyticus* has been reported in other studies to give false-positive results, probably due to the production of type 5 and 8 capsular polysaccharides [21,22]. The presence of both surface antigens can easily lead to misidentification as *S. aureus*. Among the 72 staphylococcus isolates detected, 62 (86.2%) were *spa* gene positive and were confirmed as *S. aureus*, two (2.7%) were *spa* gene positive and were identified as *S. lugdunensis*. The remaining eight (11.1%) isolates without *spa* gene were identified as *S. lugdunensis*. The two *S. lugdunensis* isolates harbouring a *spa* gene were positive on the Pastorex Staph Plus test and were considered to be false-positive results. Ninety percent of protein A is found in the cell wall and the remaining 10% is free in the cytoplasm of bacteria. The Xpert MRSA/SA assay is the molecular MRSA surveillance method most commonly used in our laboratory. The use of

GeneXpert® to characterize staphylococci and their resistance to methicillin has been well documented [23,24]. The mechanism mediating methicillin resistance in *S. aureus* can be easily detected by using either *mecA* or the *SCCmec*/*orfX* junction as a marker [25,26]. GeneXpert® detects only a single staphylococcal target, corresponding to the junction at which *SCCmec*, the genetic element that contains the *mecA* gene and which confers resistance to methicillin, integrates the *S. aureus* chromosome. The integration site of the cassette into the staphylococcal genome is located in *OrfX* that is unique to *S. aureus* with its internal *mecA* gene that inserts into the *OrfX* and, in so doing, splits *orfX* into two parts. This target is called the *SCCmec*/*OrfX* junction region. This region is usually highly conserved [27,28]. Molecular characterization studies of this region identified eight *SCCmec* types (I to VIII) [29,30], as well as a large number of nontypeable and new *SCCmec* types. The *mecA* gene encodes PBP2a, which is involved in peptiglycan synthesis and confers resistance to all β-lactam antibiotics due to low-affinity binding, thereby overcoming the inhibition of native PBP conferred by these antibiotics.

A good agreement between the phenotypic method and Xpert assay was observed in 62 (86.2%) of the 72 staphylococci species isolated from HBM cultures with pure growth, while a discordance between the phenotypic method and Xpert assay was observed in 13.8% (10/72) of cases (Table 5). These strains were phenotypically methicillin-resistant and cefoxitin-resistant and genotypically *spa*$^+$ *mec*$^+$ *SCCmecA*- and were misinterpreted as MSSA by Xpert. These strains have been considered to be false-negative for MRSA. False-negatives for MRSA have been reported in isolates with atypical or novel *SCCmec* types as a result of polymorphisms. The *SCCmec*/*orfX* PCR target fails to amplify. These *spa*$^+$ *mecA*$^+$ *SCCmec*- are interpreted as MSSA by the Xpert® system [4] and these strains are called “empty cassette” variants because they have *SCCmec* lose just the *mecA* portion of the cassette through excision, while the basic structure of the element, the *mecA* gene, remains intact and the *mecA* portion of the cassette is therefore “empty”, at least in terms of its ability to confer methicillin resistance [31]. Various incorrectly identified mechanisms of MRSA isolates have been described [32]: missing *mecA* sequences (*mecA* excision, empty cassettes), prevalence of a *SCCmec*-like cassette in a CoN isolate, acquisition of a new variant *mecA* homologue (*mecC* or *mecA* LGA251) [33,34] that was recently described in a novel *SCCmec* named type XI [35]. This newly identified protein has <63% aa identity with PBP2a encoded by *mecA*. This new *mecA* homologue has been detected in bacteria from dairy cattle in England and humans in England, Scotland, Denmark [33] and France [4]. Finally, Donnio et al. [36] reported loss of *mecA* due to deletion within a type IV cassette. The *in vitro* selection for resistance

Table 5
Discordance between phenotypic and genotypic results of 11 *S. aureus* strains analysed.

Phenotypic result	Genotypic results			Interpretation
	spa$^+$ C_T *value*	*mec*$^+$ C_T *value*	*SCCmec*$^-$ C_T *value*	
Methicillin-resistant Cefoxitin-resistant	23.5	38.5	0	MRSA$^-$/SA$^+$ = MSSA
	23.6	36	0	
	23.7	37.7	0	
	24	38.7	0	
	24.4	39	0	
	24.5	39.1	0	
	24.7	36.7	0	
	25.1	36.7	0	
	25.6	36.2	0	
	32.5	37.1	0	
	spa$^+$ C_T	*mec*$^-$ C_T	*SCCmec*$^+$ C_T	
Methicillin-susceptible Cefoxitin-susceptible	24	0	26.5	MRSA$^-$/SA$^+$ = MSSA

Amplification data obtained with Xpert MRSA/SA assay for 10 MRSA phenotypes and 1 MSSA phenotype. The 10 MRSA phenotypes had MRSA$^-$/SA$^+$ genotypes with C_T values between 23.5 and 32.5 for *spa*$^+$, >35 for *mec*$^+$ and C_T value = 0 for *SCCmec*$^-$. The MSSA phenotype had a MRSA$^+$/SA$^+$ genotype with C_T values = 24.0 for *spa*$^+$, 26.5 for *SCCmec*$^+$ and 0 for *mec*+.
C_T: score cut-off.

to vancomycin has also been shown, in three different MRSA isolates carrying a type II *SCCmec*, to coincide with conversion of MRSA to MSSA [37]. Other studies have reported transposition/recombination between *ccrC1* allele 8 and *ccrC1* allele 10 [31] and site-specific chromosomal excision of *SCCmec* elements from the genome or partial deletion of *mec* gene complexes involving *IS431* elements [37] in the mechanism for MRSA-to-MSSA conversion.

In this study, a collection of 62 *S. aureus* strains isolated from HBM were investigated for the presence of classical SE genes and ET genes by PCR. *S. aureus* can produce a wide variety of virulence factors. Of the 62 *S. aureus* isolates investigated, 44 (70.9%) were found to harbour one or more SE or ET genes. Among which 79.5% strains carried SE genes and 20% carried ET genes. This rate (70.9%) is higher to the result reported by Giannatale et al. [38], and lower than the rates reported by Xie et al. [39] (90.7%) and similar to the result reported by Wang et al. [40] (68.1%). *sea* was the most prevalent enterotoxin gene detected in this study (24.1%), similar to the result reported by Mehrotra et al. [10] (19.6%) versus 41% for Rall et al. [1] and 44.4% for Xie et al. [39]. The *eta* gene rate observed in this study was 12.9%, compared to 2.4% and 0.6% for Hayakawa et al. [41], 0.9% for Xie et al. [39], and 0.8% for Wang et al. [40].

These discordant results can be attributed to improvements in the handling and sanitary procedures during milk collection and processing. Although the level of *S. aureus* in milk was considered to be not sufficient to cause disease, the presence of toxin genes can be considered to be a potential risk for infants. Several conditions, such as delayed processing, inadequate refrigeration, poor personal hygiene and post-process contamination are associated with the growth of *S. aureus* strains harbouring *se* and *et* genes.

In conclusion, conventional laboratory tests for the detection of MRSA and MSSA in human milk banks require long incubation times and do not allow rapid decision-making for selection of the most appropriate milk for infants. Most routine laboratory detection of staphylococci isolates in human milk banks is based on latex agglutination tests. This test may take as long as 48 h. MALDI-TOF-MS identification is necessary due to the false-positive results of agglutination tests, which can take at least 50–72 h for the results to reach the milk bank. The Xpert MRSA/SA assay, performed directly on fresh-frozen milk, is an alternative for routine diagnosis. It allows rapid (50 min) and accurate identification of MRSA and MSSA isolates and is suitable for testing on human milk banks. The results of this study provide important preliminary data about the prevalence of *S. aureus* strains harbouring genes coding for SE and ET in human milk.

Statement of authorships and author agreement

All authors listed on the title page participated in meetings and follow-up discussions that culminated in preparation of this manuscript. M. Biendo and A. Leke were involved in the conception and design of the study, the collection of data and drafted the manuscript and the authors participated in editing and final revisions. All authors have read and approved the final manuscript.

Submission declaration

Submission of this article implies that the work described has not been published previously and it is not under consideration for publication elsewhere.

Conflict of interest

All authors, recruited from the international clinical nutrition support community, have no conflicts of interest to report. No funding support or compensation was received by the participants.

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