

Occurrence of Methicillin-Resistant *Staphylococcus aureus* in Cheese Produced in German Farm-Dairies

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ABSTRACT

The aim of this study was to determine if raw milk cheese or cheese to be made out of heated milk, manufactured in small German cheese dairies might be a reservoir of contaminations with methicillin-resistant *Staphylococcus aureus* (MRSA). Due to predominantly manual handling during cheese making, recontaminations with *Staphylococcus* (*S.*) *aureus* or MRSA cannot be excluded. Commercial cheese products ($n = 72$) available in the region of Hanover (Lower Saxony) and dairy products offered via internet were analyzed with regard to the occurrence of *S. aureus* and MRSA. Thereof, two cheese samples, manufactured from pasteurized milk and two samples, produced from raw milk, were *S. aureus* positive with CFU/g between 1.0×10^1 and 7.0×10^1 . MRSA was not detected. All analysed cheese samples could be considered safe for consuming.

Keywords: Cheese Products; *Staphylococcus aureus*; MRSA; Small-Scale Processing Plant

1. Introduction

Staphylococcus (*S.*) *aureus* is a Gram-positive, coagulase and katalase positive coccus producing several of virulence factors as well as toxins [1]. *S. aureus* is regularly detected in bulk milk samples as a major contagious pathogen causing intramammary infections in dairy cattle [2-4]. Pasteurization of milk leads to kill this micro-organism but due to the ability to produce heat-stable enterotoxins, *S. aureus* is a common cause of food poisoning [5]. Milk and milk products, such as cheese provide a good source of *S. aureus* contaminations. Therefore, European regulations require a concentration of *S. aureus* of $<1.0 \times 10^3$ CFU/g in final cheese products manufactured from heated milk and for cheese products from raw milk, regulations require a limit value of $<1.0 \times 10^5$ CFU/g [6]. Various studies indicated the presence of *S. aureus* in artisan cheese products manufactured in small-scale farm dairies [7,8]. Furthermore, André *et al.* [9] could isolate *S. aureus* from cheese manufacturers in a dairy processing plant. Approximately 75% (3/4) of the investigated persons were colonized with *S. aureus*. The strains were recovered from both hand and nose samples.

With the introduction of methicillin for antibiotic treatment of *S. aureus* infections in humans, 60 years ago, strains became resistant to this therapeutic agent. Today,

methicillin-resistant *Staphylococcus aureus* (MRSA) is an important agent causing both hospital-acquired (haMRSA) and community-acquired (caMRSA) infections [10-12]. The methicillin resistance of *S. aureus* is caused by a specific penicillin-binding protein (PBP 2a) which is encoded by the *mecA* gene and has a low affinity to β -lactam antibiotics [13]. Due to limited options of treatment, MRSA infections turn out to be a high risk to human health because strains exhibit resistances to β -lactam antibiotics and other groups of antimicrobials.

First Voss *et al.* [14] reported on a new MRSA clone in humans called ST398 and associated with pig farming with a high potential of transmission to humans in contact with infected animals [15-17]. From that time on, the clone has also been detected in other livestock such as cattle and poultry [18-20]. Comprehensive data about the prevalence of ST398 are available in pigs. Various studies have been done on all production levels with detections of high incidence of this clone [21-24]. MRSA, predominantly ST398, could also be detected in raw meat products of different species, e.g. beef, pork, chicken and turkey [21].

MRSA ST398 has also been identified in dairy cow udders. Vicca *et al.* [20] reported of a prevalence of MRSA positive animals between 0 and approximately 14.0% of lactating dairy cows on herd-level. Overall, samples were taken from five dairies, which were proven

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positive for MRSA in previous studies. Isolates could be identified as ST398 *spa* t011, which also could be detected in pigs, poultry and in humans with close contact to infected animals [18,22-24]. MRSA ST398 *spa* t011 has also been isolated in three dairies in Southwest Germany. The prevalence on herd-level of this agent ranged between 5.1% and 16.7% [19]. In addition to meat products, MRSA contaminations were also recovered in dairy foodstuff e.g. bovine milk, mozzarella and pecorino cheeses [25].

Thus, it can be assumed that raw milk for cheese making could be a reservoir for MRSA contamination in final food products. In addition, MRSA-colonized staff could be considered as a source of contaminations in cheese. Particularly, due to predominantly manual operations during cheese making in farms with small-scale production, recontamination of final products by colonized staff could be a health risk for consumers. At present, reliable data remain unavailable.

The aim of the present study was therefore to determine if raw milk artisan cheese or cheese to be made out of pasteurized milk, manufactured in small German cheese dairies might be reservoir of contaminations with MRSA.

2. Materials

In a period of five month all available commercial cheese products were collected. Each product was included once in the study. Cause of low number of small-scale farm dairies in the region of Hanover, additional, all small-scale German processing plants which offered their cheese

products by shipment via internet were examined. Numbers of cheese samples of same dairies varied between one and seven, depending on assortment of the retailers or manufacturers. A total of 72 cheese samples were obtained. 36 samples were purchased from dairy counters or refrigerated displays in grocery stores or farmer's markets. Another 36 samples were obtained directly from manufacturers via internet. Cheese samples originated from small-scale German processing plants in Lower Saxony, North Rhine-Westphalia, Baden-Württemberg, Hesse, Bavaria and Schleswig-Holstein. In total, eight cream cheeses, five soft cheeses, eight semi-hard cheeses, one hard- and one processed cheeses, produced from pasteurized milk (Table 1) and nine soft cheeses, twenty-eight semi-hard- and twelve hard cheeses, made from raw milk, were evaluated (Table 2).

3. Methods

For quantitative analyses of *S. aureus* and MRSA, 25 g of each sample were homogenized in 225 mL buffered-peptone water (Roth, Germany) in a stomacher (AES Chemunex) for 60 s. Decimal dilutions were plated onto Baird-Parker agar (Merck) with egg yolk tellurite emulsion (Oxoid, Germany) for detection of *S. aureus* (ISO 6888-1:1999). Inoculated agar plates were incubated at 37°C for 48 h.

Quantitative detection of MRSA was carried out according to §64 LFGB L 00.100 (MPN technique). Threefold analysis of 1 mL of each decimal dilution was done by inoculating in 9 mL Mueller Hinton broth (Oxoid, Germany) with 6% NaCl, incubated at 37°C for

Table 1. Bacteria counts of *S. aureus* and MRSA recovered in cheese samples manufactured from pasteurized milk.

Type of cheese	Samples examined (<i>n</i>)	Samples positive for <i>S. aureus</i> (<i>n</i>)	CFU/g of <i>S. aureus</i>	Samples positive for MRSA (<i>n</i>)	NPM/g of MRSA
Cream cheese	8	0	<1.0 × 10 ¹	0	<0.3 × 10 ¹
Soft cheese	5	1	1.0 × 10 ¹	0	<0.3 × 10 ¹
Semi-hard cheese	8	1	2.5 × 10 ¹	0	<0.3 × 10 ¹
Hard cheese	1	0	<1.0 × 10 ¹	0	<0.3 × 10 ¹
Processed cheese	1	0	<1.0 × 10 ¹	0	<0.3 × 10 ¹
Total	23	2		0	

Table 2. Bacteria counts of *S. aureus* and MRSA recovered in cheese samples manufactured from raw milk.

Type of cheese	Samples examined (<i>n</i>)	Samples positive for <i>S. aureus</i> (<i>n</i>)	CFU/g of <i>S. aureus</i>	Samples positive for MRSA (<i>n</i>)	NPM/g of MRSA
Cream cheese	0	0	<1.0 × 10 ¹	0	<0.3 × 10 ¹
Soft cheese	9	0	<1.0 × 10 ¹	0	<0.3 × 10 ¹
Semi-hard cheese	28	1	7.0 × 10 ¹	0	<0.3 × 10 ¹
Hard cheese	12	1	3.0 × 10 ¹	0	<0.3 × 10 ¹
Total	49	2		0	

48 h. Afterwards, samples were streaked in duplicate on Brilliance MRSA 2 agar (Oxoid, Germany) and incubated at 37°C for 24 h. Blue colonies were considered as presumptive MRSA. For further analyses, colonies growing on Baird-Parker agar as well as the presumptive MRSA on Brilliance MRSA 2 agar were selected and subcultured on aesculin-blood agar (Oxoid, Germany) at 37°C for 24 h. Gram-positive, catalase-positive and clumping factor A-positive (DiaMondiaL Staph Plus Kit, Sekisui Virotech, Germany) cocci were considered as *S. aureus* and MRSA, respectively. A *mecA*-positive *S. aureus* strain (ATCC 33591) was used as a positive control on Brilliance MRSA 2 agar. Regarding molecular biology, PCR analysis as recommended by Saiful *et al.* [26] was chosen to detect the *mecA* gene and thereby confirms the presence of MRSA. A sample was considered MRSA-positive if at least one MRSA colony could be detected. MRSA counts were calculated in npm/g. Bacterial counts of *S. aureus* on Baird-Paker agar were calculated using the geometric means. For the analysis, agar plates with one up to 300 colonies were used.

4. Results

A total of 5.5% (4/72) cheese samples were positive for *S. aureus*. Thereof, two cheeses were manufactured from pasteurized milk and two samples were produced from raw milk. All samples originated from different small-scale dairies. One soft- and one semi-hard cheese sample produced from heated milk were contaminated with *S. aureus* at bacteria counts from 1.0×10^1 CFU/g and 2.5×10^1 CFU/g, respectively (**Table 1**). *S. aureus* could be detected in one semi-hard raw milk cheese at a level of 7.0×10^1 CFU/g, while one hard raw milk cheese contained 3.0×10^1 CFU/g (**Table 2**). Due to overgrowing of inoculated Baird-Parker agars with unwanted micro-organism (e.g. yeasts, streptococci, and *Bacillus* spp.), dilutions of 10^{-1} were used to calculate the bacterial counts of *S. aureus*.

Growth after incubation of samples in Mueller Hinton broth and streaking on Brilliance MRSA 2 agar occurred in 63 samples, which then showed blue colonies on the selective agar. After further testing, those colonies were identified as yeasts, streptococci, *Bacillus* spp. and Gram-negative rods. MRSA was not detectable in any of the 72 cheese samples.

Since no presumptive MRSA strains were encountered, molecular analyses were not conducted.

5. Discussion

All cheese samples examined in the current study were considered of no consequence for human health due to *S. aureus*. Detectable bacteria counts of *S. aureus* in cheese samples were below the standards established by the

European Union for coagulase-positive staphylococci in cheese made from pasteurized milk or made from raw milk [6]. MRSA was also not detectable.

However, improper pasteurization or inadequate hygiene standards during manufacturing process are prone to cause contamination of cheese products with *S. aureus*. De Luca *et al.* [27] reported 16.3% (22/135) cheese samples (produced from pasteurized milk, purchased from grocery stores) positive for *S. aureus*, bacterial counts of *S. aureus* ranging from 1.0×10^1 to 3.15×10^5 CFU/g. Bulk milk appears to be a reservoir of *S. aureus* contamination in cheese products. Microbiological testing on a dairy processing plant manufacturing raw milk cheese indicated that *S. aureus* occurs both in bulk milk samples and cheese products after manufacture [7]. Based on molecular typing method, the authors inferred that bacteria were spread with milk to the equipment and the environment during milking and cheese production.

Bulk milk might be also considered a reservoir for MRSA contaminations in cheese or other dairy products since MRSA occur in dairy cattle [19,20]. Contamination of dairy products during manufacturing on small-scale farms is not entirely inconceivable, especially in raw milk cheeses. Deficient pasteurization or inadequate hygiene standards as well as colonized manufacturers could cause contamination with MRSA and/or non-MRSA *S. aureus* in final food products. Jones *et al.* [28] isolated caMRSA from pork barbecue as the origin of a food-borne illness. Those strains originated from a sales assistant in the supermarket, where the meat was purchased. Recontamination of food has been also causing an outbreak of MRSA in a hospital in the Netherlands [29]. Information assessment of our results cannot be accomplished because of lack of data about the appearance of MRSA in cheese products as well as information of required infection application rate of MRSA. Further investigations are needed.

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