Influence of milk, milk fractions and milk proteins on the growth and viability of mastitis-causing Staphylococcus aureus strain

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\section*{ABSTRACT}

The aim of this study was to investigate the impact of milk and milk fractions (cell-reduced, skim and whey) obtained from different cows on the growth rate of mastitis-causing Staphylococcus aureus strain at low inoculum density, simulating the early phase of intramammary infection. The association of the selected milk proteins, including \(\alpha\)-lactalbumin, \(\beta\)-lactalbumin, lactoferrin, bovine serum albumin, \(\gamma\)-globulin and casein with the bacterial growth was also analysed. Twelve Polish Holstein-Friesian cows having no history of mastitis during the previous and current lactation were selected for this study. The S. aureus strain used in this study was isolated from a cow with clinical mastitis and was characterised by confirmed ability to spread among cows within a herd. Linear regression coefficients were calculated for associations between milk constituents and bacterial counts in whole milk as dependent variables. The comparison of bacterial growth between whole milk, cell reduced, skim and whey fractions was determined by a one-way analysis of variance (ANOVA). The results of the present study showed that the growth of mastitis-causing S. aureus was less stimulated by whole milk samples and their individual fractions in comparison to the nutrient microbiological medium. The strongest inhibition of bacterial growth was observed for whey fraction. Lactoferrin was the only protein causing a slight decrease in the growth of S. aureus. It was concluded that, depending on its growth medium and antimicrobial properties, milk may be among the factors of key importance for the incidence of this disease among individual cows.

\section*{Introduction}

\textit{Staphylococcus aureus} is a major cause of bovine mastitis (Smith et al. 2005; Haveri et al. 2008). The prevalence of cows infected with \textit{S. aureus} ranges from 3.4 to 8.2\% (Pitkälä et al. 2004; Østerås et al. 2006; Tenhagen et al. 2006). Most dairy cows are probably exposed to \textit{S. aureus}, as the organism is a frequent resident of the skin and mucous epithelia of dairy cows and other mammals, including humans, and is commonly found in the barn environment (Roberson et al. 1994). The virulence of \textit{S. aureus} strains is mostly due to its ability to produce a large array of virulence factors and form sessile biofilm structures displaying elevated tolerance to various antimicrobials but also to survive in different environments (Iwatsuki et al. 2006; Junka et al. 2014). It was demonstrated that \textit{S. aureus} is able to persist in mammary glands, teat canals and teat lesions of infected cows (Peterssson-Wolfe et al. 2010). Infections with \textit{S. aureus} are often spread during milking, when microbiologically contaminated milk from an infected gland comes in contact with an uninfected gland and the bacteria enter the teat canal (Roberson et al. 1994). The ability of mastitis pathogens to survive and multiply in mammary secretions was described as an important virulence factor for intramammary infections (Fang et al. 1998; Lammers et al. 2000; Kornalijnslijper et al. 2003). It was shown that milk components may play an important role in the regulation of the early steps (the period between infusion of bacteria and the influx of polymorphonuclear lymphocytes, PMN) in the pathogenesis of bovine mastitis caused by different microorganisms, including \textit{S. aureus} (Lammers et al. 2000). According to Sutra and Poutrel (1994), after a few hours of intramammary infusion of less than a hundred \textit{S. aureus} cells, significant numbers of these bacteria can be detected in milk. This indicates that \textit{S. aureus} is able to...
multiply in the milk environment of the bovine mammary gland (Fang et al. 1998; Lammers et al. 2000). Kornalijnslijper et al. (2003) demonstrated that the number of bacteria in milk depends on the balance between their growth rate and elimination rate by the host defence mechanisms. Bovine milk contains components that provide nutritive elements, immunological protection and biologically active substances to both neonates and adults (Séverin & Wenshui 2005). The major antimicrobial proteins of milk include lysozyme, lactoferrin, lactoperoxidase and immunoglobulins. The antibacterial properties were also reported for α-lactalbumin, β-lactoglobulin and caseins (Lahov & Regelson 1996; Clare & Swaisgood 2000; Pellegrini et al. 2003; Silva & Malcata 2005). However, bovine milk composition varies depending on the breed (Holstein, Holstein-Friesian, Jersey) and also the animal’s feed and the stage of lactation (Constantin & Csatlos 2010). Therefore, milk from individual cows could differ in its qualitative and quantitative composition, and thereby in its growth medium properties for microorganisms. This assumption was already confirmed in the studies aimed at investigating the growth of mastitis-inducing Escherichia coli in the milk collected from different cows (Kornalijnslijper et al. 2003). However, no similar studies concerning S. aureus, i.e. a bacterium regarded as one of the most common and most pathogenic mastitis-causing microorganism have been performed before. Thus, the aim of this study was to investigate the impact of milk and milk fractions obtained from different cows on the growth rate of mastitis-causing S. aureus strain at low inoculum density, simulating the early phase of intramammary infection. The association of selected milk proteins with the bacterial growth was also analysed.

**Materials and methods**

**Animals and milk sample collection**

Twelve Polish Holstein-Friesian cows from 30 to 120 days of second to third lactation, having no history of mastitis during the previous and current lactation and somatic cell counts (SCC) below 200,000 cells/mL were selected for this study. The diagnoses of clinical mastitis were provided by the local veterinarian. Mastitis was classified as clinical if any systemic symptoms or local signs or alterations in milk appearance were detected. California mastitis test (CMT) was also used as the indicator of the inflammation. The dairy herd consisted of about 300 cows. The animals were kept in a free stall barn and fed in a total mixed ration (TMR) system. The cows were milked twice daily. Milk samples were collected from the left front udder quarters of individual cows before the morning milking after a thorough cleaning, washing with warm water, disinfecting with Trionet (DeLaval International AB, Tumba, Sweden) and wiping dry with a paper towel around the udder. The first three squirts of milk were discarded and approximately 100 mL of foremilk was aseptically collected in a sterile container. Samples were kept under refrigeration until arrival at laboratory facilities.

**Analysis of milk samples**

All milk samples were subjected to bacteriological analysis in order to confirm that they did not contain major mastitis-causing pathogens such as Staphylococcus aureus, Streptococcus uberis, Streptococcus dysgalactiae, or coliforms. Briefly, a 50-μL aliquot of each sample was spread onto Columbia agar base with 5% sheep blood (Grasso, Starogard Gdański, Poland) and incubated at 37°C. Bacterial colonies were identified after 24 and 48 h according to accepted standards (Hogan et al. 1999).

Milk fat, proteins and lactose were determined using MilkoScan FT 120 (Foss Electric, Hillerød, Denmark), while somatic cells were determined with Somacount 150 (Bentley Instruments, Inc., Chaska, MN). For the differentiation of somatic cells (PMN, macrophages and lymphocytes), microscopic slides were prepared and stained using Newman’s Stain Solution (Sigma-Aldrich, St. Louis, MO) according to the manufacturer’s instruction. Briefly, 0.01 mL of each milk sample was spread over a glass slide. Then, the smear was rapidly dried at 50°C, cooled to room temperature and submerged into the stain for 2 min. In the next step, the slides were dried thoroughly and rinsed in water at 4°C. The prepared films were examined under an oil-immersion objective of the microscope (Axioskop 2 plus microscope, Carl Zeiss, Jena, Germany, magnification 1000 ×) and the number of cells per mL of milk was calculated.

**Milk fraction preparation**

From each whole milk sample, the following fractions were prepared: a cell-reduced fraction, a cell- and fat-free fraction (skim), cell- and fat-free and protein-reduced fraction (whey). The procedure of milk fraction preparation was performed according to Kornalijnslijper et al. (2003), with a slight modification. In order to prepare cell-reduced fractions, 15 mL of whole milk was centrifuged for 10 min at 3000 g, at 4°C (Eppendorf, Hamburg, Germany). The fat layer and
supernatant were transferred to a new tube and homogenised using vortex and then the procedure was repeated. As a result, 10 mL of cell-reduced fraction was obtained. Skim milk samples were prepared by removing the fat layer after centrifugation for 10 min at 3000 g, at 4 °C and collecting the obtained supernatant into a new tube, without pellet. The procedure was repeated twice, resulting in 10 mL of the skim fraction. To prepare the whey fraction, 20 mL of the prepared skim milk sample was subsequently subjected to acidification at pH 4.5 with 5 N HCl. Casein precipitates were removed by centrifugation at 3000 g, at 4 °C for 15 min and the pH of the obtained whey fraction was adjusted to the original value (pH = 6.6–6.9) by the addition of 5 N NaOH. Whole milk and milk fractions were sterilised through a 0.45 µm filter (Millipore, Eschborn, Germany) and kept in the fridge until the start of the experiment.

**Milk proteins**

In order to prepare milk protein suspensions, selected pure proteins were diluted in phosphate buffered saline (PBS, Sigma-Aldrich, Deisenhofen, Germany), pH 7.2 or Brain Heart Infusion (BHI, Oxoid, Basingstoke, UK) to obtain concentration in the range corresponding to the range originally occurring in cow milk (Table 1). All proteins used were purchased from Sigma-Aldrich (Deisenhofen, Germany).

**Microorganism preparation**

The *S. aureus* strain chosen for this study was isolated from a cow with clinical mastitis. The strain was the dominant bacterium causing mastitis in the herd, which has already been reported in other studies (Fijałkowski et al. 2014). The strain was stored at −80 °C on bacterial preservation beads (Viabank, BioMaxima, Lublin, Poland). For the experiment, a single bacterial preservation bead was streaked onto a Columbia agar plate with 5% sheep blood and grown for 24 h at 37 °C. A loop of bacteria was removed from the plate and used to inoculate a flask containing 100 mL of BHI broth and grown for a further 12 h at 37 °C while shaking (180 rpm). The bacteria (10 mL) were collected by centrifugation at 3000 g for 15 min, washed with sterile PBS and re-suspended in 10 mL of PBS. The absorbance at 600 nm of a 100 µL aliquot in a microtitre plate well was used to adjust the absorbance of the total suspension to 0.05 using sterile PBS corresponding to approximately $1 \times 10^8$ CFU/mL (prior to the experiment the number of *S. aureus* cells in a suspension of OD 0.05 at 600 nm was counted by performing quantitative plating on BHI agar). The obtained bacterial suspension was next diluted in PBS (first 1:1000 and again 1:1000) to obtain a concentration of approximately 100 CFU/mL. The final cell concentration was confirmed using Petroff–Hausser bacteria cell counting chamber (Hausser Scientific Co., Horsham, PA).

**Bacterial growth and viability assays**

**Milk and milk fractions**

Bacterial growth in whole milk samples and milk fractions was determined by quantitative plating in BHI agar. One hundred microlitres of the prepared bacterial suspension (approximately 100 CFU/mL) was added to 5 mL of whole milk and to 5 mL of each milk fraction and was then incubated at 37 °C for 6 h with shaking at 180 rpm. Five mL of inoculated BHI was used as a control. After incubation, six serial dilutions (10 fold) were made in PBS for each sample. One hundred µL of each dilution was pipetted onto a sterile Petri dish, and sterile liquid BHI agar was poured into each dish. The plates were allowed to cool and set for 1 h, and were then inverted and incubated at 37 °C for 24 h, followed by colony counting.

**Pure milk proteins**

Bacterial viability in the presence of pure milk proteins was determined using the Alamar Blue cell viability assay. One millilitre of each concentration of milk proteins in PBS (Table 1) was dispensed into a test vial, and was then inoculated with 100 µL of bacterial suspensions ($1 \times 10^8$ CFU/mL), vortexed and incubated for 6 h at room temperature (20 °C). One mL of inoculated PBS was used as a control. After incubation, 200 µL of the suspension was transferred into wells on a black 96 well microtitre plate (Becton Dickinson Co., Naperville, IL). Twenty microlitres of Alamar Blue (Thermo Fisher Scientific Inc., Rockford, IL) was then added to each well. The plates were sealed and incubated for 15 min at 37 °C. The fluorescence signal was measured using microplate fluorescence reader

| Protein         | Concentration, g/L |
|-----------------|--------------------|
| α-lactalbumin   | 0.5 1 1.5 3        |
| β-lactalbumin   | 1 3 4 8            |
| lactoferrin     | 0.03 0.06 0.12 0.24|
| BSA*            | 0.05 0.1 0.4 0.8   |
| γ-globulin      | 0.4 0.8 1.4 2.3    |
| casein          | 15 20 30 35        |

* Bovine serum albumine.
the value of the dependent variable (\(y_i\)). The following linear regression model was used:

\[ \text{log SCC} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \epsilon_i, \quad i = 1, \ldots, 12, \]

where: \(y_i\) – the value of the dependent variable ("simple"), \(x_{1p}, x_{2p}, x_{3p}\) – the values of independent variables, \(\beta_0, \beta_1, \beta_2, \beta_3\) – regression coefficients, \(\epsilon_i\) – random component.

The comparison of bacterial growth between whole milk, cell reduced, skim and whey fractions was determined by a one-way analysis of variance (ANOVA) and followed by Tukey-Kramer test for post-hoc comparisons. Each experiment was conducted in technical triplicates and repeated twice. In order to conduct a statistical analysis, each of the resulting six data points (three technical replicates from each of the two biological replicates) generated per assay was treated as an individual data point. The differences were considered statistically significant when the \(p\)-value was less than .05. The statistical analyses were conducted using Statistica®10 PL (StatSoft Inc., Krakow, Poland).

### Results

The whole milk constituents are presented in Table 2. As can be seen, more considerable variations were found in percentages of PMN, lymphocytes, macrophages and fat, whereas protein and lactose percentages showed the lowest variation. As expected, the procedure of milk fraction preparation resulted in decreased SCC for cell-reduced fractions, decreased SCC and fat for skim fractions, and decreased SCC, fat and protein percentages for whey fractions, respectively, while lactose percentages were not affected (Table 3).

The influence of the whole milk and the milk fraction on the growth of \(S.\ aureus\) after 6 h of incubation is presented in Table 4. The growth of \(S.\ aureus\) was less stimulated by whole milk samples and their individual fractions in comparison to BHI (control). In the case of the skim fraction, bacterial growth was inhibited by nearly 50% compared with whole milk and by 96% compared with BHI (control). The incubation of \(S.\ aureus\) in whey resulted in the growth inhibition of

#### Table 2. Milk constituents of single quarter milk samples collected from Polish Holstein-Friesian cows located on a single farm.

| Sample nr | Fat, % | Protein, % | Lactose, % | SCC, \(\times 1000/\text{mL}\) | PMN, % | MAC, % | LYM, % |
|-----------|--------|------------|------------|----------------|--------|--------|--------|
| 1         | 4.05   | 2.75       | 4.88       | 20             | 25     | 40     | 35     |
| 2         | 4.86   | 3.45       | 4.80       | 19             | 33     | 30     | 37     |
| 3         | 2.87   | 2.90       | 4.69       | 19             | 27     | 42     | 31     |
| 4         | 4.87   | 3.43       | 4.86       | 77             | 34     | 37     | 29     |
| 5         | 5.25   | 3.63       | 4.90       | 28             | 28     | 44     | 28     |
| 6         | 4.96   | 3.34       | 4.81       | 44             | 32     | 47     | 21     |
| 7         | 4.50   | 3.83       | 5.10       | 85             | 30     | 41     | 29     |
| 8         | 3.66   | 3.42       | 4.95       | 103            | 35     | 55     | 10     |
| 9         | 4.34   | 4.02       | 4.67       | 114            | 61     | 25     | 14     |
| 10        | 3.04   | 2.95       | 4.86       | 52             | 24     | 51     | 25     |
| 11        | 4.41   | 3.40       | 4.77       | 110            | 33     | 40     | 27     |
| 12        | 5.20   | 3.95       | 4.72       | 68             | 11     | 49     | 40     |
| Average   | 4.33   | 3.42       | 4.83       | 61.58          | 31.08  | 41.75  | 27.17  |
| CV, %     | 18.31  | 11.82      | 2.48       | 58.99          | 20.35  | 32.47  |

PMN: polymorphonuclear leukocytes; MAC: macrophages; LYM: lymphocytes.

#### Table 3. The SCC and percentage of fat, protein and lactose in milk and milk fraction (\(n = 12\)).

|          | Whole milk | Cell-reduced | Skim | Whey |
|----------|------------|--------------|------|------|
| SCC, \(\times 1000/\text{mL}\) | 61.6 ± 36.3 | 3 ± 0.6 | 0.4 ± 0.1 | ± 0.0 |
| CV, %    | 58.9       | 14.0         | 19.4 | 0    |
| Protein, % | 4.3 ± 0.8  | 4.2 ± 0.8    | 0.1 ± 0.0 | 0.0 ± 0.0 |
| CV, %    | 18.3       | 21.9         | 8.6  | 0    |
| Lactose, % | 3.4 ± 0.4  | 3.4 ± 0.4    | 3.3 ± 0.2 | 0.9 ± 0.1 |
| CV, %    | 11.8       | 14.2         | 13.1 | 14.5 |
| Fat, %   | 4.8 ± 0.1  | 4.8 ± 0.1    | 4.8 ± 0.1 | 4.8 ± 0.1 |
| CV, %    | 2.5        | 2.0          | 2.1  | 2.9  |

Data are presented as a mean ± SD.

(PlateReader AF2200, Eppendorf, Madison, WI) at wavelengths of 540 nm excitation and 590 nm emission.

The dynamics of bacterial growth in milk protein suspension was assessed by optical density (OD) measurements. The 96 well plate (Becton Dickinson Co., Naperville, IL) was supplemented with 200 \(\mu\text{L}\) of milk protein suspensions in BHI (Table 1) and 20 \(\mu\text{L}\) of the bacterial inoculum \((1 \times 10^8 \text{CFU/mL})\). Two hundred microlitres of inoculated BHI was used as a control. The plates were placed in the microplate reader (Infinite 200 PRO NanoQuant, Tecan, Männedorf, Switzerland) and incubated at 37 °C for 8 h with shaking at 200 rpm. During incubation, the microbial growth in each well was monitored by OD measurements at 600 nm automatically every 1 h.
Table 4. Number of *S. aureus* CFU (× 10^2) in whole milk, milk fraction and BHI after incubation at 37°C for 6 h with shaking at 180 rpm.

| Sample | Whole milk | Cell-reduced | Skim | Whey |
|--------|------------|--------------|------|------|
| 1      | 123 ± 13.0 | 118 ± 6.6    | 95 ± 5.0 | 17 ± 4.1 |
| 2      | 201 ± 8.8  | 132 ± 10.1   | 100 ± 10.2 | 15 ± 4.4 |
| 3      | 146 ± 11.9 | 136 ± 7.1    | 110 ± 7.1 | 15 ± 3.2 |
| 4      | 136 ± 16.1 | 125 ± 13.0   | 116 ± 5.1 | 10 ± 3.6 |
| 5      | 111 ± 10.4 | 116 ± 9.7    | 69 ± 11.1 | 39 ± 4.0 |
| 6      | 193 ± 11.5 | 169 ± 7.8    | 115 ± 11.1 | 15 ± 3.6 |
| 7      | 192 ± 15.1 | 173 ± 10.1   | 132 ± 10.3 | 83 ± 10.2 |
| 8      | 419 ± 10.7 | 394 ± 31.4   | 171 ± 10.2 | 50 ± 5.5 |
| 9      | 258 ± 10.9 | 231 ± 20.1   | 113 ± 0.8 | 31 ± 3.6 |
| 10     | 190 ± 14.4 | 156 ± 16.0   | 99 ± 5.1 | 30 ± 3.6 |
| 11     | 216 ± 5.0  | 185 ± 12.6   | 67 ± 10.5 | 24 ± 5.1 |
| 12     | 225 ± 8.6  | 207 ± 10.2   | 107 ± 7.5 | 36 ± 5.0 |
| Average| 201 ± 81.6^a | 182 ± 73.4^ab | 107 ± 27.3^b | 30 ± 20.4^c |
| CV, %  | 40.6       | 43.1         | 25.3 | 67.3 |

The experiments were conducted in triplicate and repeated twice. The numbers of CFU are presented as a mean ± SD calculated using six values (three from each biological replicate); ^a^ ^b^ Means within a row with different superscripts differ (p < 0.05).

80% as compared with whole milk and 99.6% as compared with BHI (control). The growth rate of the bacteria cultivated in whey was significantly lower than that of bacteria cultivated in skim (p < 0.05) and cell-reduced (p < 0.001) fractions or in whole milk (p < 0.001). Bacterial growth in the skim fraction was significantly lower than the value for whole milk (p < 0.01), and did not differ significantly from the cell-reduced fraction (p ≥ 0.05). Furthermore, the growth of *S. aureus* did not vary significantly between whole milk and the cell-reduced fraction (p ≥ 0.05). The highest variance in the growth rate was recorded for whey fractions. Using backward linear regression analysis, no significant associations were found between whole milk, SCC and percentages of fat, protein and lactose as independent variables and bacterial growth (p ≥ 0.05).

The analysis involving the incubation of *S. aureus* in pure milk proteins suspension did not show any substantial bactericidal effect (Figure 1). Lactoferrin, at the mid-range concentration, was the only protein which caused a slight decrease in the viability of *S. aureus* (p < 0.05). Similar results were obtained in a bacteriostatic analysis, in which *S. aureus* was incubated in BHI medium with the addition of pure milk proteins (Figure 2).

**Discussion**

The hypothesis that milk from individual cows could differ in its growth medium properties for mastitis-causing pathogens was already confirmed for mastitis-causing *Escherichia coli* in the work by Kornalijnslijper et al. (2003). In agreement with the results obtained by these authors, also the present study demonstrated that the growth of mastitis-causing bacteria (*S. aureus* in the current study) was less stimulated by whole milk samples and their individual fractions in comparison to the nutrient BHI medium. Also of note is the observation that the growth of *S. aureus* did not vary significantly between whole milk and the cell-reduced fraction (p ≥ 0.05). This can be explained by the observations showing that cows infected with *S. aureus* do not necessarily have elevated SCC (Pettersson-Wolfe et al. 2010). On the other hand, the study was conducted *in vitro*, so the interactions occurring *in vivo*, e.g. with epithelial cells, were not involved. Although milk fat or its degradation products may have some antimicrobial activity (van Hooijdonk et al. 2000), the *S. aureus* strain used in the present study grew better in whole milk and in cell-reduced fractions containing milk fat than after its removal. The main protein in milk is casein which consists of αs1-, αs2-, β- and κ-caseins (Shah 2000). It was previously reported that milk collected from different cows differs in the concentration of casein (Hallen et al. 2008). Peptides derived from casein have been shown to possess various bioactive properties (Zucht et al. 1995). In the present study, the growth of *S. aureus* in the skim fraction, 70–80% (based on the literature data) of the nutritional requirements of *S. aureus* and *E. coli* as well as the humoral immune response against these microorganisms are different, which can explain the observed differences.

Most likely, the bactericidal effect observed in the current study can be attributed to the presence of α-lactalbumins, β-globulins, lactoferrin or immunoglobulins, because it is known that these proteins are not removed by the acidification and in general they all possess antibacterial activity (Bullen et al. 1972; Fang et al. 1998; Chaneton et al. 2011). Among whey proteins, except for immunoglobulins whose antibacterial activity is associated with specific immune responses, one of the most important protective role is attributed to lactoferrin. The direct bacteriostatic effect of lactoferrin is well established by *in vitro* experiments (Naidu & Arnold 1997). In addition to its bacteriostatic effect, lactoferrin also exhibits bactericidal activity (Arnold et al., 1980; Naidu & Arnold, 1997), which is
mediated by electrostatic interactions between the negatively charged lipid layer and the positively charged lactoferrin surface that cause changes in the permeability of the membrane (Valenti & Antonini 2005). Indeed, in the current study, when these proteins were tested alone, a slight reduction of bacterial growth was observed for lactoferrin, whereas in the case of other proteins, including immunoglobulins, the growth was not altered. The absence or low antibacterial activity of individual milk proteins could be explained by the additional synergistic effect that these substances may have on each other when present within a suspension. As reported by Chaneton et al. (2011), the antimicrobial activity of milk components is concentration-dependent and can be augmented by the presence of some proteins. For example, β-lactoglobulin and lactoferrin may complement each other in the mammary gland defences against bacterial infection. Moreover, the results obtained in the present study may be associated with the strain of *S. aureus* used in our experiment. Given that different *S. aureus* strains are able to utilise unique infection and survival strategies, it can be assumed that the influence of milk components on

**Figure 1.** Growth of *S. aureus* after 6 h of incubation in the presence of milk proteins suspended in PBS. The % of control values are presented as a mean ± SD calculated using six values (three from each biological replicate); *statistically significant difference between the milk proteins and control (p < .05).
the growth of this microorganism will also vary between strains. The variability in the growth rate, in the presence of milk proteins among different \textit{S. aureus} isolates was reported by Chaneton et al. (2011). Furthermore, it was also demonstrated that the variability in immune protein response against \textit{S. aureus} is strain-specific (Yunee et al. 2011).

Conclusions

Taking into account the relatively low-inoculum density and short-incubation period, which both reflected the early phase of intramammary infection, the present study adds further support to the hypothesis that growth medium and antimicrobial properties of milk could play an important role in the early pathogenesis of bovine mastitis (in the case of the current study caused by \textit{S. aureus}). Therefore, it can be concluded that depending on its growth medium and antimicrobial properties, milk may be one of the important factors impacting the incidence of this disease among individual cows.

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Disclosure statement

None of the authors have any conflicts of interest that could inappropriately influence their work.

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