



OCCURRENCE AND ANTIMICROBIAL RESISTANCE OF *STAPHYLOCOCCUS AUREUS* IN BULK TANK MILK AND MILK FILTERS

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ABSTRACT

This work is focused on the monitoring of *Staphylococcus aureus* prevalence in raw milk and milk filters, its antibiotic resistance and detection of methicillin resistant *Staphylococcus aureus* (MRSA). Samples of raw cow's milk and milk filters were collected in the period from 2012 till 2014, from 50 dairy farms in the Czech Republic. The total of 261 samples (164 samples of raw milk and 97 milk filters) were cultivated on Baird-Parker agar. Both the typical and atypical colonies were examined by plasmoagulase test and PCR method was used for detection of species specific fragment SA442 and *mecA* gene. Standard disk diffusion method was used to determinate resistance to antimicrobial agents. The bacterium *Staphylococcus aureus* was detected on 25 farms (50%). The antimicrobial resistance showed differences between the farms. Total of 58 samples were positive for *Staphylococcus aureus*, of which were 37 (14.2%) isolated from raw milk samples and 21 (8.1%) from milk filters. From these samples we isolated 62 *Staphylococcus aureus* strains, 41 isolates bacteria *S. aureus* from raw milk (66.1%) and 21 isolates *S. aureus* from milk filters (33.9%). The presence of antibiotic resistance in *Staphylococcus aureus* isolates was low, most of them were resistant to amoxicilin. According to the results obtained by the PCR method for the methicillin - resistant *S. aureus* (MRSA), the *mecA* gene was present in 6 strains (9.7%), 4 isolates obtained from milk samples (6.5%) and 2 isolates from milk filters (3.2%). These isolates can be considered as a possible source of resistance genes, which can be spread through the food chain. Nowadays, a globally unfavourable increasing trend of prevalence of methicillin resistant staphylococci strains especially *Staphylococcus aureus* is being observed worldwide. The improper hygiene and poor farm management practices contributed to the presence of *S. aureus* in the milk. This may have contributed to the high level of *S. aureus* isolated. Improving the hygienic conditions of the milking environment and utensils may reduce the prevalence of *S. aureus* in milk. Objective of this study was monitoring of *Staphylococcus aureus* prevalence and determine the prevalence rate of antimicrobial resistance of *S. aureus* isolated from raw milk and milk filters in the Czech Republic.

Keywords: *mecA* gene; MRSA; PCR; antibiotic resistance; disk diffusion method

INTRODUCTION

Milk and dairy products are basic components of human diet. However, raw milk consumption is accompanied by the risk of ingesting pathogenic bacteria that can pose an elevated health hazard (**Latorre et al., 2009**). One of the pathogen is *Staphylococcus aureus*, which recognized worldwide as one of the most important foodborne pathogens associated with food poisoning (**Gundogan et al., 2005**). The literature demonstrates the fact that it is considered the most common cause of illness caused due to consumption of raw milk (**Lee Loir et al., 2003**). *S. aureus* is the major causative agent of mastitis in cows (**Rabello et al., 2007**). Bacterial contamination of milk usually occurs during the milking process and it depends on the sanitary condition of the environment, the equipment used for milking and the milking personnel. It could also occur from microorganisms that enter the udder through the teat canal opening (**Kalsoom et al., 2004**). Therefore, milk and dairy products may pose a risk to consumers. Antibiotic resistant *S. aureus* isolates pose a major challenge for both veterinary and human medicine because they have a negative impact on treatment of

infections (**Brouillet et al., 2005**). Currently, it is detected adverse increasing trend worldwide prevalence of methicillin-resistant strains of staphylococci, especially *Staphylococcus aureus* (MRSA). The incidence of MRSA strains were found not only in humans but also in animals, including the food production ones (**Karpíšková et al., 2009**). The aim of this study was to confirm the presence of the bacteria *S. aureus* in the samples of raw milk and milk filters, occurrence of antibiotic resistance with a special focus on MRSA strains.

MATERIAL AND METHODOLOGY

Samples of raw milk and milk filters were collected in the period from 2012 till 2014, from 50 dairy farms in the Czech Republic. From 261 samples collected 164 samples were of raw milk and 97 samples of filters. The milk samples of the amount of 250 ml were taken in the sterile bottles and transported at 4 ± 1 °C to the laboratory where they were immediately processed. The milk filters were collected into sterile PE packet and transported at 4 ± 1 °C to the laboratory too where they were immediately processed.

Detection of *Staphylococcus aureus* was performed as follows: 25 ml of milk was diluted with 225 ml of buffered peptone water (Oxoid, UK) and milk filters were diluted with 225 ml also of buffered peptone water (Oxoid, UK) and homogenized. After enrichment at 37 °C overnight samples were cultivated on Baird - Parker agar (Oxoid, UK) supplemented with egg yolk-tellurite emulsion. From each plate, both the typical and atypical colonies were examined by plasmoagulase test and confirmation of suspected *S. aureus* strains was carried out by polymerase chain reaction (PCR) method of a species specific fragment SA442 and *mecA* gene which encodes the resistance to methicillin (Martineau et al., 1998, Bosgelmez-Tmaz et al., 2006).

Antibiotic resistance of *S. aureus* was tested by the standard disk diffusion method on Mueller - Hinton agar (Oxoid, UK) according to the CLSI methodology (2012a).

Strains were tested for resistance to 14 therapeutically significant antimicrobial agents: oxacillin (OX) (1 µg), tetracycline (TE) (30 µg), erythromycin (E) (15 µg), chloramphenicol (C) (30 µg), co-trimoxazole (SXT) (25 µg), amoxicillin/clavulanic acid (AMC) (20/10 µg) and clindamycin (DA) (2 µg), gentamicin (CN) (10 µg), ciprofloxacin (CIP) (15 µg), vancomycin (VA) (30 µg), teicoplanin (TEC) (30 µg), rifampicin (RD) (5 µg), cefotaxime (FOX) (30 µg) and cefotaxime (CTX) (30 µg) (Oxoid, UK).

Based on the inhibition zone size isolates were evaluated as sensitive, intermediately resistant or resistant, according to the criteria specified in CLSI (2012b).

More attention was dedicated on the occurrence of methicillin - resistant *S. aureus* strains, which were resistant to penicillins and other beta-lactam antibiotics (David et al., 2010).

RESULTS AND DISCUSSION

In this study we described the isolation and antibiotic susceptibility characterization of *S. aureus* from 50 dairy farms in the Czech Republic. From 261 samples collected 164 (62.8%) samples were of raw milk and 97 (37.2%) samples of filters. The bacterium *Staphylococcus aureus* was detected on 25 farms (50%). *S. aureus* was detected from 0% to 66.7% of the samples. The results reported in our study were similarly high when compared to the study by Gündoğan et al. (2006). According to his results, *S. aureus* was isolated from all raw milk samples.

According to our results, *S. aureus* was detected in 37 samples of raw milk (22.6%) and 21 samples of milk filters (21.6%). From these samples we isolated 62 *Staphylococcus aureus* strains, 41 isolates bacteria *S. aureus* from raw milk (66.1%) and 21 isolates *S. aureus* from milk filters (33.9%). Gran et al., (2003) reported similar results, and showed that *S. aureus* was found in 49 out of 60 milk samples (82%).

Similar results were obtained in a study by Karpíšková et al. (2011), where the detection of zoonotic foodborne pathogens in raw milk was described. In their work a total of 56.6% of raw cow's milk taken from vending machines were positive for the presence of *S. aureus* in the Czech Republic. This bacterial species is one of the major etiological agents causing mastitis in dairy cattle (Cretenet et al., 2011).

Data of the *Staphylococcus aureus* occurrence are shown in the Table 1.

Samples	No.	No. of <i>S. aureus</i> strains	%
Raw milk	164	37	14.2
Milk filters	97	21	8.1
Total	261	58	

Table 1 Number and percentage of *S. aureus* strains isolated from raw milk and milk filters

A further objective of the study was to characterize and compare the antibiotic resistance profiles of bacteria *S. aureus* isolated from the investigated samples. *S. aureus* strains were once nearly uniformly susceptible to semi-synthetic penicillinase-resistant β-lactams (e.g. methicillin, oxacillin), the most commonly used class of antibiotics for skin infection. These strains were termed methicillin resistant *Staphylococcus aureus*, or MRSA, a term that implied cross-resistance to all β-lactams including all penicillins and cephalosporins (Larsen et al., 2000). *Staphylococcus aureus* has an impressive ability to adapt to environmental conditions and it can fast become resistant to almost all antibiotics (McCallum et al., 2010). In the last few decades a large increase in bacterial dissemination of antibiotic resistance was reported, which becomes a major health challenge (Goñi et al., 2004). The spread of antibiotic resistance among *S. aureus* strains is very important, especially in the treatment of staphylococcal infections (Ito et al., 2003). Development of resistance to a particular antibiotic depends on the level of exposure to antimicrobial agents (Rychlík et al., 2006).

Teicoplanin and vancomycin were the drugs to which a large proportion of the isolates from bulk tank milk and filters were sensitive (Figure 1).

As showed in Figure 1, the largest amount of *S. aureus* isolates obtained from milk filters were resistant to oxacillin, tetracycline, erythromycin, clindamycin, rifampicin and cefotaxime about 3.3%, followed by amoxicillin/clavulanic acid (1.6%). The largest amount of *S. aureus* isolates isolated from raw milk were resistant to amoxicillin / clavulanic acid about 11.3%, followed by oxacillin, tetracycline, erythromycin and cefotaxime (6.5%), clindamycin and rifampicin (3.3%) and vancomycin and teicoplanin (1.6%).

The antimicrobial resistance profile of the tested *S. aureus* strains to different antibacterial agents revealed that 17.8% (n = 11) of the strains were resistant to at least one antibiotic.

The antimicrobial resistance showed differences between the farms. These isolates had identical resistance patterns to oxacillin, tetracycline and cefotaxime. These results are comparable with studies by Juhász-Kaszanyitzky et al. (2007) where the MRSA isolates from cows were highly resistant to ampicillin, cephalexin, erythromycin and tetracycline and susceptible to enrofloxacin, gentamicin and potentiated sulphonamides. After positive findings of MRSA, repeated sample collections were carried out as described above.

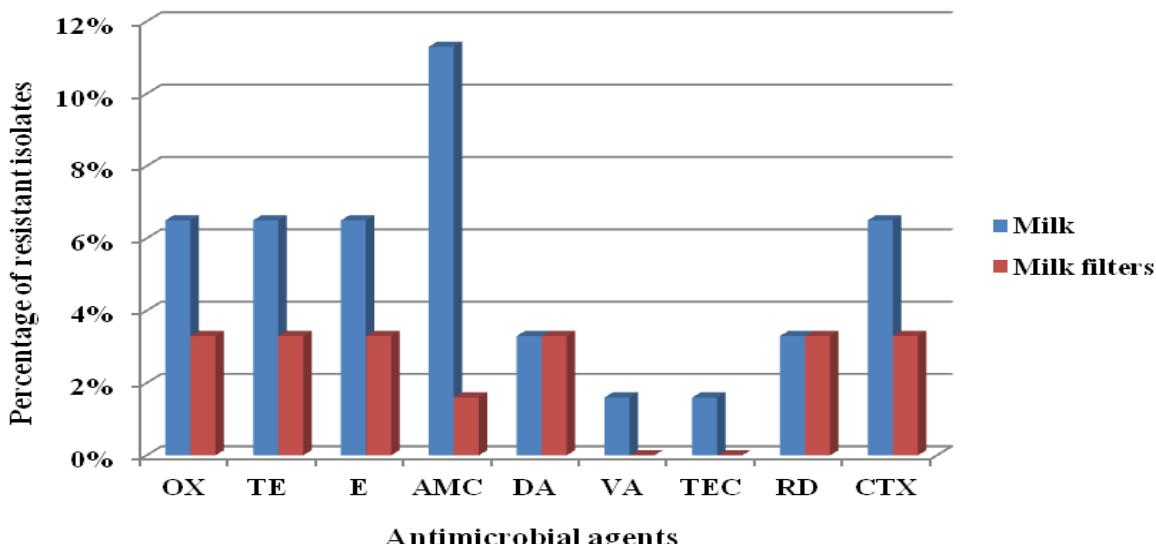


Figure 1 Antimicrobial resistance of *Staphylococcus aureus* in bulk tank milk and milk filters

Data of the *Staphylococcus aureus* resistance are shown in the Figure 1.

The altered transpeptidase called penicillin-binding protein (PBP2a) with low affinity for beta-lactams (**Ibrahem et al., 2005**) is determined by *mecA* gene encoding the resistance to oxacillin.

The emergence of resistant pathogens to commonly used antibiotics is worldwide fear of 21 century. One of the most important bacteria in this regard is *Staphylococcus aureus*, in particular methicillin-resistant strains. According to the results obtained by the PCR method for the methicillin - resistant *S. aureus* (MRSA), the *mecA* gene was present in 6 strains (9.7%), 4 isolates obtained from milk samples (6.5%) and 2 isolates from milk filters (3.2%). The detection of MRSA in milk samples was irregular. All the isolates from the study area were resistant to oxacillin and were also resistant to tetracycline, erythromycin, amoxicillin/clavulanic acid and cefotaxime. In other studies carried out in cows, MRSA were most frequently isolated from milk of animals showing signs of subclinical mastitis (**Lee, 2003**).

In addition, from all samples that were positive for the presence of *mecA* gene, were collected from 4 farms at different localities. These results are comparable with studies made by **Karpíšková et al. (2008)**, where the prevalence of MRSA was monitored in ruminants. There were 9 MRSA isolates (2%) detected in 444 samples (including raw milk, environmental and animal samples) collected from 12 farms in the Czech Republic.

Čížek et al. (2008) in their study showed that from 45 isolates of *S. aureus* bacteria obtained from 192 milk filters collected from Moravian farms were none of MRSA strains.

According to our results and findings as far as the occurrence of MRSA strains it is concerned that these

strains are presented in primary food production in the Czech Republic, but their frequency is still relatively low.

CONCLUSION

Results obtained in this study confirm the occurrence of *Staphylococcus aureus* in raw milk and milk filters. The occurrence of strains resistant to antimicrobials and MRSA in the environment of primary food production posses a potential risk of its spreading into the environment and colonization of personnels that are within the close contact with food during processing. MRSA strains appear rarely in the food of animal origin in the Czech Republic, however, there is a minimal potential risk that these strains can be transferred to humans via food chain.

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Acknowledgments:

This study was financially supported by research project NAZV KUS QJ 1230044 and QJ I210284.

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