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# THE BACTERIOLOGICAL QUALITY OF BULK TANK MILK IN THE CZECH REPUBLIC

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## ABSTRACT

This study was focused to map the occurrence of bacteriological risks in raw cow's milk. In total, 212 samples of bulk tank milk from 25 farms were collected and investigated in 2012 – 2014 in the Czech Republic. The occurrence of the following microorganisms: *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp. *Campylobacter* spp. and *Listeria monocytogenes* were observed. The presence of *E. coli* was detected in 197 (93%) samples, but none of the strains harboured the *stx* encoding genes. *S. aureus* was detected in 68 samples (32.1%) of raw cow's milk. The presence of *Salmonella* spp. and *Campylobacter* spp. was not detected in tested samples. Only one milk sample was positive for the presence of *L. monocytogenes* (0.5%). The further examination of obtained pathogens involved antimicrobial susceptibility, detection of enterotoxin encoding genes and resistance to oxacillin in *S. aureus*, determination of extended spectrum β-lactamases in *E. coli* and serotyping of *L. monocytogenes*. The results of this study confirm the presence of pathogenic bacteria in raw milk. Information on public health hazards associated with the consumption of raw Cabrita, milk should be extended to the potential consumers and drinking of untreated raw milk could be avoided.

**Keywords:** *bacteria; Escherichia coli; Staphylococcus aureus; Salmonella spp.; Campylobacter spp.; Listeria monocytogenes*

## INTRODUCTION

In recent years, there has been a growing interest in local food production. Consumers are looking for foods that have undergone the least processing. As a consequence, there is an increased tendency to consume raw milk. Cow's milk is the most consumed milk in the Czech Republic (Czech Statistical Office 2013). Consumption of raw milk represents a risk for the consumers, due to the possible presence of zoonotic pathogenic microorganisms in raw milk (Claeys, 2013).

Microorganisms in raw milk originate from different sources: the cow, the milking, storage and transportation equipment, and the environment. Current technology provides the means to collect milk with only small numbers of microorganisms. However, milk may contain a highly diverse microbial population. The types of microorganisms in milk of greatest interest are spoilage and pathogenic microorganisms for example shiga toxin-producing *E. coli* (STEC), *Staphylococcus aureus*, *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes* and others. Pathogenic bacteria in milk can be either infectious or toxin-producing. Milk is generally a good growth medium for microorganisms, so controls over storage temperature and production hygiene are critical for maintaining an acceptable product (Hassan, et al., 2011).

An overview of foodborne disease reports from different industrialized countries indicates that milk and milk products are implicated in 1–5% of the total bacterial foodborne outbreaks, with 39.1% attributed to milk (Claeys, 2013).

Nowadays, the consumer is offered a wide range of commercial types of milk including raw milk sold through milk vending machines or directly on the farm. There isn´t any legislation that would regulate the sale of raw milk and microbiological parameters in either EU, on the national level there are limits only for total plate and *S. aureus* counts.

Since consumption of raw milk may pose health risks to consumers, therefore the aim of this study was to investigate the bacteriological quality and safety of raw milk on dairy farms in the Czech Republic.

## MATERIAL AND METHODS

### SAMPLE COLLECTION

A total of 212 raw cow´s milk samples from 25 farms in the Czech Republic were collected in years 2012-2014. The sampling was done at regular time intervals during the whole year, with five to eight samples collected from each of the 25 dairy farms involved in the study. Farms were randomly selected to cover the whole area of

the Czech Republic. All milk samples were collected into sterile sampling bottles and transported in a cooler sampling case to the laboratory for immediate examination.

**ISOLATION AND IDENTIFICATION OF *ESCHERICHIA COLI* (*E. COLI*)**

Detection of *E. coli* was carried out according to ISO 16649-1 with slight modifications (Hassan, et al., 2011). The detection was performed after enrichment of 25 ml of milk in 225 ml of buffered peptone water (BPW, Oxoid, UK) at 37 °C for 24 hours followed by aerobic cultivation on Tryptone Bile X-glucuronide medium (TBX, Oxoid, UK) (44 °C for 24 hours) and on MacConkey agar containing cefotaxime (2 mg/L) (37 °C for 24 hours) for the isolation of ESBL (Extended-spectrum β-lactamase) producing strains. From each positive sample, one to three suspected *E. coli* isolates were included in the study for confirmation. Confirmation of suspected colonies from TBX agar consisted of the detection of oxidase (OXItest, Pliva-Lachema, CZ) and production of indole (COLItest, Pliva-Lachema, CZ).

**ISOLATION AND IDENTIFICATION OF *STAPHYLOCOCCUS AUREUS* (*S. AUREUS*)**

Detection of *S. aureus* was carried out according to (ISO, 2012).. The detection was performed after enrichment of 25 ml of milk was diluted with 225 ml of buffered peptone water (Oxoid, UK) and homogenized. After enrichment at 37 °C overnight samples were cultivated on Baird - Parker agar (B-P, Oxoid, UK) supplemented with egg yolk-tellurite emulsion. From each plate, both the typical and atypical colonies were examined by plasmacoagulase test (DENKA SEIKEN Co., LTD., Japan) and confirmation of suspected *S. aureus* strains was carried out by polymerase chain reaction (PCR) based on the detection of the species specific fragment SA442 (ISO, 2000).

**ISOLATION AND IDENTIFICATION OF *SALMONELLA* SPP**

Detection of *Salmonella* spp was carried out according to ISO 6579 (Martineau,1998). At first non-selective enrichment in buffered peptone water (Oxoid, UK) was performed. This was followed by selective enrichment in two types of media Rappaport-Vassiliadis Soya Peptone Broth (RVS) and Muller-Kauffmann Tetrathionate-Novobiocin Broth (MKTTn, Oxoid, UK) simultaneously. Then isolation on media RAMBACH (MERCK, D) and XLD (Oxoid, UK) was performed.

**ISOLATION AND IDENTIFICATION OF *CAMPYLOBACTER* SPP**

Detection of thermotolerant *Campylobacter* spp. was carried out according to ISO 10272, Microbiology of food and animal feeding stuffs, Horizontal method for the detection of *Salmonella* spp., 2003). After enrichment, which was done in Bolton medium with horse blood (Oxoid, UK) and after 48 hours of cultivation at 42 ° C suspension was inoculated on *Campylobacter* Selective Blood Free Agar (CCDA, Oxoid, UK) with incubation at 42 ° C for 48 hours at micro-aerophilic conditions.

**ISOLATION AND IDENTIFICATION OF *LISTERIA MONOCYTOGENES* (*L. MONOCYTOGENES*)**

Detection of *L. monocytogenes* was performed according to ISO 11290-1 with a modification in the primary multiplication step which was carried out in the buffered peptone water (Oxoid, UK) at 37 °C for 24 hours[9]. Secondary multiplication was done in Fraser broth (Oxoid, UK) followed by aerobic cultivation on ALOA agar medium (BIO-RAD, FR).

**ANTIMICROBIAL SUSCEPTIBILITY TESTING**

The susceptibility to antibiotics commonly used in clinical treatment was tested by disk diffusion method according to the CLSI protocol (2012) on Mueller-Hinton agar (Oxoid Ltd, UK). Antibiotic disks were obtained from Oxoid Ltd. (UK). Resistance of *E. coli*, *S. aureus* and *L. monocytogenes* strains was evaluated based on the size of the zones of inhibition and classified as susceptible, intermediate resistant, or resistant according to the CLSI (2012).

**ANTIMICROBIAL SUSCEPTIBILITY TESTING OF *ESCHERICHIA COLI***

Antimicrobial susceptibility was tested by the disk diffusion method according to the CLSI protocol (2012) on Mueller-Hinton agar (Oxoid Ltd, UK) using the following antimicrobials and concentrations: ampicillin - AMP (10 µg), amoxicillin/clavulanic acid - AMC (30 µg), cefotaxime - CTX (30 µg), chloramphenicol - C (30 µg), streptomycin - S (10 µg), kanamycin - K (30 µg), gentamycin - CN (10 µg), sulfamethoxazole/trimethoprim - SXT (25 µg), trimethoprim - TMP (5 µg), tetracycline - TE (30 µg), nalidixic acid - NA (30 µg), ciprofloxacin - CIP (5 µg), and colistin - CT (10 µg) (Oxoid, UK). *E. coli* strain ATCC 35218 was used as a quality control.

**ANTIMICROBIAL SUSCEPTIBILITY TESTING OF *STAPHYLOCOCCUS AUREUS***

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), amoxicilin/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), cefoxitin - FOX (30 µg) and cefotaxime - CTX (30 µg) (Oxoid, UK). *S. aureus* strain ATCC 25923 was used as a quality control.

**ANTIMICROBIAL SUSCEPTIBILITY TESTING OF *LISTERIA MONOCYTOGENES***

Antibiotic resistance of *L. monocytogenes* was tested by the standard disk diffusion method on Mueller - Hinton agar (Oxoid, UK) with 5% defibrinated sheep blood according to the CLSI methodology. The following panel of antimicrobial agents and concentrations were used; erythromycin - E (15 µg), gentamycin - CN (10 µg), penicillin - P (1 µg), tetracycline - TE (30 µg),

sulfamethoxazole/trimethoprim - SXT (25 µg), ampicillin - AMP (2 µg) and meropenem - MEM (10 µg) (Oxoid, UK). By 2012 CLSI (except MIC for penicillin and ampicillin) or EUCAST (the European Committee on Antimicrobial Susceptibility Testing) did not recommend any criteria for the evaluation of the antimicrobial susceptibility of listeria. In this study, the results were interpreted according to averages border zones of inhibition for *Staphylococcus* spp., with the exception of ampicillin and penicillin, for which criteria were used for *Enterococcus* spp.

**TYPING OF BACTERIA**

More attention has been concentrated to the occurrence of methicillin-resistant *S. aureus* strains (MRSA) and ESBL producing *E. coli* and genes encoding selected virulence factors.

For the determination of MRSA, in *S. aureus* isolates, PCR for the detection of the *mec*A gene, which is responsible for the resistance to methicillin, was used (ISO, 1999).

The attention was also paid to the ability of *S. aureus* strains to produce enterotoxins, which are responsible for foodborne intoxication and some allergic reactions (CLSI, 2012, Poulsen, 2003). For the detection of the genes encoding enterotoxins SEA-SEE and SEH, multiplex PCR according to Løvseth et al., (2004) was performed (Balaban, 2000).

Polymerase chain reaction (PCR) was used for the detection of genes of *E. coli* encoding selected virulence factors – *eae, hly, stx*$_1$, and *stx*$_2$, for resistance to *β*-lactams - *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{CTX-M,}$. In *E. coli* isolates multiplex PCR for the detection of genes encoding resistance to β-lactams *bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{CTX-m}$ was carried out (Sharma, 2000, Løvseth, 2004) .The detection of virulence genes was performed using multiplex PCR according to (Fagan et al., 1999, BriÑas, 2002).

Typical colonies for *Listeria monocytogenes* were confirmed and serotyped by slide agglutination using the commercially available antisera (DenkaSeiken, Japan) and verified by multiplex PCR( Lewis,2007) .

## RESULTS AND DISCUSSION

This study was focused to map the occurrence of bacteriological risks in raw cow´s milk. In total, 212 samples of bulk tank milk from 25 farms were collected and investigated in 2012 – 2014 in the Czech Republic. The detailed results are shown in Table 1. Differences were observed in the bacteriological quality of raw cow´s milk collected on the different dairy farms. While milk from some farms was bacteriological safe in milk samples from other farms pathogenic microorganisms were repeatedly detected. In our study, *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes* were detected.

**PREVALENCE OF *E. COLI* IN RAW COW´S MILK**

This study shows that the presence of *E. coli* in raw cow´s milk is very common, as confirmed by other studies (Fagan,1999, Doumith ,2004). Altogether, 197 (93%) positive milk samples were detected. From each sample, three *E. coli* colonies were isolated but for further testing, only isolates with different resistance phenotypes from one sample were included in the study. Therefore a total of 273 *E. coli* isolates were obtained for further testing.

**ANTIMICROBIAL SUSCEPTIBILITY IN *E. COLI* ISOLATES**

Overall, 38 (13.9%) isolates were resistant to one or more antimicrobial agents. Although the prevalence of resistant strains differed between farms, the resistance to ampicillin (8.8%) and tetracycline (7.0%) antibiotics was most commonly detected in most of the farms as can be seen in Figure 1. In the samples from 17 (68%) farms were detected resistant *E. coli* and on the 8 (32%) farms were detected sensitive *E. coli* strains. On farm P with the highest incidence of resistant strains, identical resistance phenotypes were observed, even after repeated sampling. A total of 7 (33.3%) *E. coli* isolates from 21 strains were resistant to antimicrobial agents on farm P. This farm is a conventional dairy farm.

Significant was the presence of multi-resistant strains which were found repeatedly. *E. coli* resistant to three or more groups of antimicrobials (multi-resistant *E. coli*) were found in 8 (3.7%) samples from 7 (28%) farms. Overall, 8 (2.9%) multi-resistant *E. coli* were obtained. These strains were resistant especially to tetracycline and ampicillin, but all so to streptomycin. High rates of resistance to tetracycline and ampicillin on dairy farms have also been reported by (Scaria et al., 2010, Altalhi, 2009).

**DETECTION OF RESISTANCE GENES IN *E. COLI* ISOLATES**

In the past few years, there has been growing concern in the scientific community about the emergence and dissemination of *E. coli* isolates producing ESBL being very frequently associated with community infection (Skočková,2015).ESBL are capable of hydrolyzing third-generation cephalosporins and monobactams, and are plasmid-mediated β-lactamases that are easily transferable between different bacteria (Scaria,2010). In our study, genes encoding the resistance to β-lactam antibiotics were present in 2 (0.7%) isolates, with *bla*$_{TEM}$ being the most frequent. No *bla*$_{SHV}$ and *bla*$_{CTX-m}$ genes were detected. Production of ESBL in any of the examined sample of raw cow´s milk was not detected.

The genes encoding selected virulence factors – *eae, hly, stx*$_1$, *stx*$_2$ were not detected and no STEC was identified.

**PREVALENCE OF *S. AUREUS* IN RAW COW´S MILK**

When investigating the incidence of pathogenic microorganisms, we recorded the highest detection rate of *Staphylococcus aureus*. Altogether, 68 (32.1%) positive milk samples were detected. From each sample, two *S. aureus* colonies were isolated but for further testing, only isolates with different phenotypes from one sample were included in the study.

**Table 1 Characterisation of farms, number of raw cow´s milk samples examined and results of bacteriological analysis**

| Farm characteristic | | *E. coli/bla* gene | *S. aureus/* MRSA | *L. monocytogenes* | *Salmonella* spp. | *Campylobacter* spp. |
|---|---|---|---|---|---|---|
| **A** | Ecological, 285 cows | 5 | 1 | 0 | 0 | 0 |
| **B** | Ecological, 284 cows | 12 | 0 | 0 | 0 | 0 |
| **C** | Ecological, 100 cows | 13 | 5 | 0 | 0 | 0 |
| **D** | Ecological, 60 cows | 2 | 0 | 0 | 0 | 0 |
| **E** | Ecological, 23 cows | 5 | 0 | 0 | 0 | 0 |
| **F** | Ecological, 154 cows | 11 | 5 | 0 | 0 | 0 |
| **G** | Ecological, 350 cows | 9 | 3 | 0 | 0 | 0 |
| **H** | Ecological, 70 cows | 11 | 8 | 0 | 0 | 0 |
| **I** | Ecological, 20 cows | 7/1 | 0 | 0 | 0 | 0 |
| **J** | Ecological, 60 cows | 4 | 3 | 0 | 0 | 0 |
| **K** | Conventional, 180 cows | 11 | 1/1 | 0 | 0 | 0 |
| **L** | Conventional, 15 cows | 7 | 1 | 0 | 0 | 0 |
| **M** | Conventional, 495 cows | 9 | 3 | 0 | 0 | 0 |
| **N** | Conventional, 450 cows | 8 | 10 | 0 | 0 | 0 |
| **O** | Conventional, 57 cows | 21 | 3 | 0 | 0 | 0 |
| **P** | Conventional, 710 cows | 21/1 | 1 | 0 | 0 | 0 |
| **Q** | Conventional, 270 cows | 12 | 3 | 0 | 0 | 0 |
| **R** | Conventional, 600 cows | 9 | 1 | 0 | 0 | 0 |
| **S** | Conventional, 131 cows | 0 | 1 | 0 | 0 | 0 |
| **T** | Conventional, 407 cows | 6 | 0 | 0 | 0 | 0 |
| **U** | Conventional, 90 cows | 8 | 4 | 0 | 0 | 0 |
| **V** | Conventional, 360 cows | 32 | 0 | 1 | 0 | 0 |
| **W** | Conventional, 600 cows | 4 | 3 | 0 | 0 | 0 |
| **X** | Conventional, 656 cows | 34 | 18 | 0 | 0 | 0 |
| **Y** | Conventional, 700 cows | 12 | 2 | 0 | 0 | 0 |
| | **Total** | 273/2 | 76/1 | 1 | 0 | 0 |

**Table 2 Characterisation of farms, number of positive milk samples and the enterotoxigennic potential of *Staphylococcus aureus***

| Farm characteristic | | No. of *S. aureus* | No. of *S. aureus* with genes encoding SEs | *Combination of toxins* |
|---|---|---|---|---|
| **A** | Ecological, 285 cows | 1 | 1 | C |
| **B** | Ecological, 284 cows | 0 | 0 | - |
| **C** | Ecological, 100 cows | 5 | 0 | - |
| **D** | Ecological, 60 cows | 0 | 0 | - |
| **E** | Ecological, 23 cows | 0 | 0 | - |
| **F** | Ecological, 154 cows | 5 | 5 | B(4x), A(1x) |
| **G** | Ecological, 350 cows | 3 | 0 | - |
| **H** | Ecological, 70 cows | 8 | 0 | - |
| **I** | Ecological, 20 cows | 0 | 0 | - |
| **J** | Ecological, 60 cows | 3 | 0 | - |
| **K** | Conventional, 180 cows | 1 | 0 | - |
| **L** | Conventional, 15 cows | 1 | 0 | - |
| **M** | Conventional, 495 cows | 3 | 0 | - |
| **N** | Conventional, 450 cows | 10 | 0 | - |
| **O** | Conventional, 57 cows | 3 | 0 | - |
| **P** | Conventional, 710 cows | 1 | 1 | A |
| **Q** | Conventional, 270 cows | 3 | 0 | - |
| **R** | Conventional, 600 cows | 1 | 1 | B |
| **S** | Conventional, 131 cows | 1 | 1 | C |
| **T** | Conventional, 407 cows | 0 | 0 | - |
| **U** | Conventional, 90 cows | 4 | 3 | D(1x), D,H(2x) |
| **V** | Conventional, 360 cows | 0 | 0 | - |
| **W** | Conventional, 600 cows | 3 | 0 | - |
| **X** | Conventional, 656 cows | 18 | 3 | D,H (1x), D,A,H(2x) |
| **Y** | Conventional, 700 cows | 2 | 0 | - |
| | **Total** | 76 | 15 | |

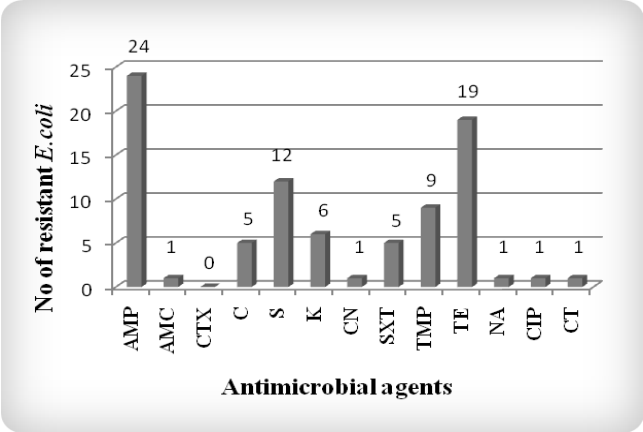


**Figure 1 Antimicrobial resistance in *Escherichia coli* from raw cow´s milk**
**AMP (ampicillin), AMC (amoxicillin/clavulanic acid), CTX (cefotaxim), C (chloramphenicol), S (streptomycin), K (kanamycin), CN (gentamycin), SXT (sulfamethoxazole/ trimethoprim), TMP (trimethoprim), TE (tetracycline), NA (nalidixic acid), CIP (ciprofloxacin), CT (colistin),(Oxoid, UK).**

Therefore a total of 76 *S. aureus* isolates were obtained for further antimicrobial resistance characterization and detection enterotoxins encoding genes. Similar results were obtained in a study of Jørgensen et al., (2005) who detected *S. aureus* not only in milk samples, but also in a farm environment and manufacturing equipment (Pitout,2004). Also Sobrinho et al., (2012) have detected *S. aureus* in 91.4% of samples of raw milk (Kanamori, 2011).

## ANTIMICROBIAL SUSCEPTIBILITY IN *S. AUREUS* ISOLATES

Differences in an antimicrobial resistance were observed between the investigated farms. In this study only 11.8% of isolated *S. aureus* strains were resistant to at least one of the antimicrobial agent tested. The phenotypic resistance profiles of the *S. aureus* isolates were as follows: amoxicilin/clavulanic acid (9.2%), tetracycline (6.6%), erythromycin, clindamycin and cefotaxime (2.6%) and oxacillin, chloramphenicol, rifampicin and ciprofloxacin (1.3%).

In the samples from 8 (32%) farms were detected resistant *S. aureus* and on the 17 (68%) farms were detected sensitive *S. aureus* strains. *S. aureus* resistant to three or more groups of antimicrobials (multi-resistant *S. aureus*) was found in 3 (1.4%) samples from 1 (4%) farm (farm Q). From this farm no other *S. aureus* strains were isolated. This farm is a conventional dairy farm. The results of antimicrobial susceptibility are shown in Figure 2.

## DETECTION OF *MEC*A GENES IN *S. AUREUS* ISOLATES

Global problem of the 21st century becomes the occurrence of pathogenic microorganisms resistant to routinely used antibiotics. The presence of MRSA and ESBL producing *Enterobacteriaceae* are the most significant (Skočková, 2015, Jørgensen, 2005).

In recent years, the increase of staphylococci strains that show resistance to methicillin/oxacillin has become a serious clinical and epidemiological problem. MRSA strains harbour the *mec*A gene, which encodes a modified PBP2 protein with a low affinity for methicillin and all β-lactam antibiotics (Sobrinho, 2012). Cows, chickens and pigs, are known reservoirs of MRSA and it was shown that the transmission of pathogens to humans occurs (Hososaka, 2007). Overall, one (1.3%) MRSA isolates were obtained in this study. A study in Switzerland did not find any MRSA in raw milk but they were found in milk samples from cows with mastitis (Velasco,2005). Methicillin-resistant *S. aureus* in milk are less important as a food safety issue, since milk is almost always heat treated before consumption. However, these exceptions and raw milk consumption, which is widely practiced by farmers and their families (Oliver et al., 2009), could expose people to MRSA (Schmid, 2009).

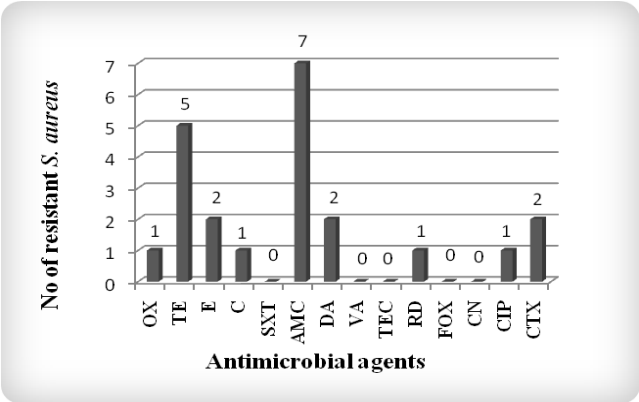


**Figure 2 antimicrobial resistances in *Staphylococcus aureus* from raw cow´s milk**
**OX (oxacillin), TE (tetracycline), E (erythromycin), C (chloramphenicol), SXT (co-trimoxazole), AMC (amoxicilin/clavulanic acid), DA (clindamycin), VA (vancomycin), TEC (teicoplanin), RD (rifampicin), FOX (cefoxitin), CN (gentamicin), CIP (ciprofloxacin), CTX (cefotaxime) (Oxoid, UK).**

## DETECTION OF ENTEROTOXINS IN *S. AUREUS* ISOLATES

*Staphylococcal* enterotoxin outbreak has always been an indispensable threat to farm dairies and the frequent reports of raw milk products contamination with different types of *S. aureus* clearly demonstrates the significance of this pathogen (Huber, 2009). In terms of risk of foodborne diseases there is a problem especially the ability of approximately 50-75% of *S. aureus* strains to produce under the suitable conditions the extracellular thermostable enterotoxins (SEs) (Oliver, 2009).

A total of 76 *S. aureus* isolates were obtained for further testing. Detection of the genes encoding enterotoxins SEA-SEE and SEH by multiplex PCR method was performed. A total 38 (50%) enterotoxin positive *S. aureus* were obtained. In our study, 15 (19.7%) of these isolates were positive for the production of classical enterotoxins SEA-SEE, which are the leading cause of foodborne diseases. No SEs were identified in 37 (48.7%) samples. In our study, 6 (7.9%) of these isolates were positive for the production of classical enterotoxins SEA-

SEE on an ecological farms. Four strains (5.3%) from the ecological farms were positive for SEB. A total 9 (11.8%) enterotoxin positive *S. aureus* were obtained on a conventional farms. Six strains (7.9%) from a conventional farms were positive for SED. Two strains (2.6%) were positive for SEA. One strain (1.3%) derived from organic and the other (1.3%) from conventional farms.

It should be noted that enterotoxin A-producing *S. aureus* is one of the main causes of food-borne diseases, SEA also predominates in milk from cows with mastitis (Schønberg, 2001). Therefore contamination of dairy products with *S. aureus* may be due to the presence of this pathogen in raw milk. Out of 38 strains (50%) investigated 5 strains (6.6%) were proven positive for *seb*, *sec* of one strain (1.3%) and *sed* of six strains (7.9%). Results are shown in Table 2. Similarly Oliveira et al., (2011), who examined 83 isolates of *S. aureus* from subclinical mastitis, discovered that 9.6% of *S. aureus* were carrying genes encoding production of SEs (Argudín,2010). Similarly with the data of other studies which reported that *seb*, *sec* or *sed* is mostly involved in food poisoning, the largest percentage of *sed* (19.5%) and *seb* (12.2%) genes was found in our study (Stephan,2001, Oliveira, 2011). Gene for enterotoxin B was determined in 5 (6.6%) raw cow´s milk samples from only two (8.0%) farms. Four (5.3%) of these *seb* positive isolates originated from one farm. This farm is an ecological farm.

On the farm F the highest incidence of enterotoxin-producing *S. aureus* was observed, even after repeated sampling. Four strains (5.3%) were positive for SEB and one strain (1.3%) was positive for SEA. On the other farms was the presence of enterotoxin-producing *S. aureus* only sporadic.

### PREVALENCE OF *L. MONOCYTOGENES* IN RAW COW´S MILK

*Listeria monocytogenes* was also detected in this work. The prevalence of *L. monocytogenes* in bulk tank milk is reported to range from 1 to 12% (Normanno,2005). Among the 13 known *L.monocytogenes* serotypes, the only one identified in this study was 1/2a. Ninety-five percent of *L. monocytogenes* strains associated with human listeriosis and food samples belong to serotypes 1/2a, 1/2b, and 4b (Adwan,2005). Serotype 1/2a has frequently been detected in different food matrices (Oliver, 2005). The same serotype (1/2a) was detected in association with raw milk food-borne diseases in the United States (Kathariou, 2002). None of the tested strains revealed resistance to the tested antimicrobial agents.

### PREVALENCE OF *SALMONELLA* SPP. AND *CAMPYLOBACTER* SPP. IN RAW COW´S MILK

The presence of *Salmonella* spp. and *Campylobacter* spp. was not detected in the tested samples. These results are equivalent with study of Kalmus et al., (2015) and similar to the results of Hill et al., 2012, who detected no *Salmonella* spp. and only a single sample (0.34%) of *Camplyobacter* spp. in raw milk samples (Martins, 2011, Cabrita, 2004).

## CONCLUSION

The results of this study confirm the presence of pathogenic bacteria in raw milk. The study shows the fact that the consumption of raw milk is not safe for the consumers, and that heat treatment of raw milk before the consumption has a positive meaning. Our results confirm that unpasteurized milk may be contaminated with different types of microorganisms and can be an important source of foodborne illnesses. This problem is critical especially when consumers are children, elderly or other persons belonging to risk groups. Pathogenic bacteria can be transmitted to milk through contact with contaminated sources on the farm and feces from infected animals. The results showed MRSA isolates in raw cow's milk samples, they still represent a possible threat for transmission of this multidrug resistant pathogen. The most effective tool for the microbiological safety of milk is pasteurization or other heat treatment and proper handling incuding cooling. It should be developed more accurate methods of detection in certain bacterial species in order to more accurately determine the amount and prevalence of these risks. Information on health hazards associated with contaminated raw milk should be extended to the public, so that consumption of untreated raw milk could be minimised or avoided. Furthermore, this study underlines the importance of educational training on food safety measures and the application of good practices for farmers selling raw milk through the vending machines.

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