RESEARCH ARTICLE

Prevalence of *Staphylococcus aureus* and *Streptococcus agalactiae* isolated from Raw Milk in Dakahlia Governorate, Egypt

Aya Emam1*, Mohamed El-Diasty1 and Adel Abdelkhalek2

1Mansoura provincial lab, Animal Health Research Institute, Agricultural Research Center 35511, Giza, Egypt
2Food hygiene and Control Department, Faculty of Veterinary Medicine, Badr University 11829, Egypt

Article History: Received: 26/02/2021  Received in revised form: 19/03/2021  Accepted: 26/03/2021

Abstract

This study was carried out on 200 random milk samples collected from different areas at Dakahlia Governorate for detection of prevalence and antimicrobial resistance of some zoonotic bacteria in milk as *Staphylococcus aureus* and Streptococci by using biochemical tests, antibiotic sensitivity test, PCR for confirmation and detection of some resistance and virulence genes. *Staphylococcus aureus* (*S. aureus*) was detected in 63%, 76%, and 42%, while *Streptococcus agalactiae* (*St. agalactiae*) was found in 8%, 10% and 4% of examined individual milk samples, retail milk samples, and milk of Bulk tank, respectively. *S. aureus* isolates revealed high levels of resistance to ampicillin (100%), nalidixic acid (90%), oxacillin (85%), penicillin (85%), cefoxitin (75%), rifampin (25%), tetracycline (20%) and erythromycin and sulphonamethoxazole/trimethoprim (10%). Whereas, *St. agalactiae* show high resistance to tetracycline (90%), ampicillin (80%), rifampin (60%), sulphonamethoxazole/trimethoprim (50%), gentamycin (45%), erythromycin and nalidixic acid (40%), chloramphenicol (30%), and streptomycin (25%). PCR results revealed that 4 out of 5 (80%) methicillin-resistant (MRSA) isolates had mecA and 2 (40%) had mecC, while 1 (20%) had Sea enterotoxin. Three isolates (100%) of *St. agalactiae* had sul1 gene and one out of three (33.3%) had tetK genes, while dfrA could not be detected. The main outcome of the current work is that milk can cause severe public health hazards to people because it had a variety of microorganisms. It is important to ensure using good hygienic practices in farms and prevent the haphazard abuse of antibiotics.

Keywords: Raw milk samples, *S. aureus*, *St. agalactiae*, Antibiotic resistant, Resistance genes

Introduction

Milk is considered an essential food commodity for humans. Milk contains essential elements for the human body such as protein, glucose, minerals, and vitamins. Moreover, milk is considered the cheapest source of animal protein [1].

The presence of food-borne pathogens in milk may be due to direct contact with contaminated sources in the dairy farm environment and excretion from the udder of an infected animal [2]. Milk and its products can cause severe public health hazards to people as they are highly susceptible to a variety of microorganisms because of their high nutritive value [3].

*Staphylococcus aureus* (*S. aureus*) is a common cause of food-borne disease worldwide which produces heat-stable enterotoxins that cause gastroenteritis, this causes an estimated 241,000 illnesses per year in the United States [4, 5]. *S. aureus* is considered one of the most common agents causing food poisoning [6]. *S. aureus* produces several virulence factors, including enterotoxins (SEG to SEQ and SEA to SEE), and other toxins, such as toxic shock syndrome toxin (TSST-1) and exfoliative toxin A and B.
Methicillin-resistant (MRSA) isolates could produce one or more staphylococcal enterotoxins (SEs) which are part of the main virulence factors of the pathogen. Members of these SEs play a vital role in outbreaks of food poisoning and other infections that are septic-related [8]. *Staphylococcal enterotoxins* are heat stable which able to survive high temperatures and able to thrive and maintain their activity in food previously contaminated with the pathogen [9].

Streptococci are one of the major mastitis pathogens which have a significant effect on dairy animals wellbeing, quality, and productivity of milk [10]. Streptococcus is the main cause of pharyngitis and tonsillitis in human especially in children and is the main bacteria transmitted from the milk to human and also can transmitted from the human to animals [11].

*Streptococcus agalactiae* (*St. agalactiae*) is associated with skin and soft tissue infections (SSTI), bacteremia, and urinary tract infections (UTI) and occasionally with arthritis, necrotizing fasciitis, toxic shock syndrome, endocarditis, or meningitis in adults [12]. *St. agalactiae* were detected in 4% of 100 bulk tank milk samples of cattle origin, collected from different dairy farms in Sharkia Governorate, Egypt [13].

Antibiotic resistance is a type of drug resistance where a microorganism is able to survive exposure to an antibiotic. Infections of human by resistant microorganisms often fail to respond to conventional treatment and lead to prolonged illness and greater risk of death [14]. Therefore, this study was designed to investigate the public health hazard of milk contaminated with zoonotic bacteria and to detect the resistance phenotype and antibiotic resistance genes of the isolated bacteria.

**Material and methods**

**Milk samples:**

This study included two hundred random raw milk samples (100 buffalo milk samples collected from owners in different localities, 50 raw milk samples from dairy cow farms, and 50 buffalo raw milk samples collected from different Markets, Dakhila Governorate, Egypt. The collected samples were directly transferred to the laboratory under refrigeration and hygienic conditions with minimum delay.

**Total Staphylococcus aureus count**

By using the surface plating technique, one-tenth (0.1) mL of prepared dilutions of each sample was dispended onto a dry surface of mannitol salt agar (Oxoid, UK) plates and evenly distributed. Inoculated plates were incubated at 37°C for 24-48h. The plates were examined for the presence of typical yellow colonies [15].

**Total count of Streptococci**

One tenth mL of prepared dilutions of each sample was dispended onto modified Edward’s medium (Oxoid, UK) plates and incubated at 35°C for 24h. *St. agalactiae* were identified as esculin negative (purple colonies) of 0.5 mm diameter and surrounded by a hemolytic zone, while group D streptococci (esculin positive) appear as black colonies [16, 17].

**Identification of the isolated staphylococci and streptococci**

*Microscopical examination:*

Films were made from the pure culture of the suspected colonies and examined by light microscope at 1000X magnification after staining by Gram’s stain for the presence of Gram-positive cocci arranged in grapes like clusters (*S. aureus*) or chains (Streptococci) [15].

*Biochemical reactions:*

*Staphylococcus aureus:*

*Staphylococcus aureus* isolates were subjected to catalase test, coagulase test, and growth at 10% NaCl [16].

*Streptococci species*

*Streptococcus* species were classified with CAMP test [17], oxidase test [16], bile esculin test [16], growth at 6.5% NaCl [16], detection of arginine decarboxylase (ADH) [16], hippurate hydrolysis test, hemolysis [16], and sugar fermentation test [16].
Antimicrobial susceptibility testing of the recovered isolates

By using the disc diffusion method [18] the antibiotic susceptibility of the isolates was determined following the guidelines of the Clinical Laboratory Standards Institute (CLSI) [19]. Antibiotic discs (Oxoid, UK) that were used in antibiotic sensitivity test of Staphylococci were ampicillin (10 mg), erythromycin (15 mg), oxacillin (1 mg), cefoxitin (30 mg), chloramphenicol (30 mg), streptomycin (10 mg), penicillin (10 mg), rifampicin (5 mg), gentamicin (10 mg), tetracycline (30 mg), nalidixic acid (30 mg), and sulphonamethoxazole / trimethoprim (25 mg). The inhibition zone diameters were read and interpreted according to the CLSI [19].

Molecular detection of antibiotic resistance and virulence genes

The recovered isolates were screened for some antibiotic resistance genes and virulence genes using oligonucleotide primers as previously described. Polymerase chain reaction amplification (PCR) of *Staphylococcus* resistance genes MecA [20], MecC [21], and MecI [22], and the *Sec, Sed* [26] virulence genes were performed. Streptococcus tetK, sul1 [24], and dfrA genes [25] were examined. The amplified products were separated on 1.5% agarose gel containing ethidium bromide 0.5µg/mL [26].

Results and Discussion

Table (1) illustrated that the total *S. aureus* count in the examined individual milk samples ranged from 2.0×10^2 to 3.46×10^6 CFU/g with a mean value of 1.30×10^3 ± 5.58×10^4 CFU/g, while in retail milk ranged from 4.0×10^2 to 6.0×10^5 with a mean value of 1.22×10^3 ± 2.54×10^4 CFU/g, but it ranged from 2.9×10^2 to 1.42×10^6 in bulk tank with a mean value of 9.33×10^4 ± 3.36×10^5 CFU/g. These findings agreed with Rall et al. [27] who found that *S. aureus* was detected in 70.4% of raw milk samples with maximum count of *S. aureus* were 8.9 x 10^3. While lower results were detected in a previous study [28] in which *S. aureus* was detected in 41.66% of the collected raw milk samples from different localities in Iran, while mean counts of *S. aureus* was at a range of 1.4×10^2 ± 78 to 2.3×10^3 ± 109. In a previous research, El-Leboudy and coauthors [29] showed that mean value of *S. aureus* counts in raw milk samples collected from governmental and private farms at Alexandria Governorate, Egypt were 5.26 ×10^3 ± 8.1 × 10 and 2.95 × 10^2 ± 7.7 × 10, respectively. *Staphylococcus aureus* is an important human pathogen which found in upper respiratory tract and skin wound of human [30]. Most staphylococcal foodborne intoxications are due to food contamination by food-handlers during food processing [31]. As presented in Table (1), *S. aureus* was detected at high percent in retail milk samples (76%) then individual milk samples (63%) and the lowest percent (42%) was found in bulk tank milk which gives indication on the sanitary condition. These findings agreed with Rall et al. [27] who found that *S. aureus* were detected in 70.4% of raw milk samples at concentrations of up to 8.9 x 10^3. Whereas, Haque and coworkers [32] reported that *S. aureus* was detected in 79.16% of raw cow’s milk samples.

The demonstrated results in Table (1) showed that total streptococci count of examined individual milk samples ranged from 2.0×10^2 to 5.0×10^4, while in retail milk ranged from 6.0×10^2 to 8.0×10^6, but it ranges from 2.9×10^2 to 1.4×10^6 in the bulk tank. Streptococci were detected in 80%, 90%, and 86% with mean value 9.66×10^3 ± 1.17×10^3, 1.12×10^2 ± 3.16×10^5, and 5.59×10^5 ± 1.88×10^5 in individual milk samples, retail milk samples, and bulk tank milk. Our results were higher than a previous study [33] in which the mean values of total streptococci counts in milk samples from three farms in Gharbia Governorate were 28.65 x 10^3 ± 5.75 x 10^3, 22 x 10^3 ± 5.7 x 10^3, and 27.1 x 10^3 ± 14.2 x 10^3 CFU/mL. The streptococci count in raw milk samples collected from El-Behera Governorate markets were 26 %, with mean values of 4.5x10^3± 0.7x10^3 CFU/mL [34].
Table (1): Total count of *Staphylococcus aureus* and *Streptococci* in the examined milk samples

<table>
<thead>
<tr>
<th>Examined samples (No.)</th>
<th>Positive samples for <em>S. aureus</em></th>
<th>Count /mL</th>
<th>Positive samples for <em>Streptococci</em></th>
<th>Count /mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>Min.</td>
<td>Max.</td>
</tr>
<tr>
<td>Individual milk (n=100)</td>
<td>63</td>
<td>63</td>
<td>2.0×10²</td>
<td>3.46×10⁶</td>
</tr>
<tr>
<td>Retail milk (n=50)</td>
<td>38</td>
<td>76</td>
<td>4.0×10²</td>
<td>6.0×10⁵</td>
</tr>
<tr>
<td>Bulk tank (n=50)</td>
<td>21</td>
<td>42</td>
<td>2.9×10²</td>
<td>1.42×10⁶</td>
</tr>
</tbody>
</table>
Enterococci especially *E. faecalis* and *E. faecium* can contaminate milk from human or animal feces, water sources, the farm environment, or from milking equipment, bulk storage tanks, and equipment used during milk harvesting or local processing [30].

**Table (3)** presented the antibiogram of 20 *S. aureus* isolates. High levels of resistance were observed to ampicillin (100%), nalidixic acid (90%), oxacillin (85%), penicillin (85%), cefoxitin (75%), rifampin (25%), tetracycline (20%), erythromycin, and sulphamethoxazole / trimethoprim (10%). These findings were in harmony with previous research [38] in which high rate of resistance was recorded to ampicillin (100%). The susceptibility of MRSA isolates to trimethoprim-sulphamethoxazole and rifampin was low [39]. *S. aureus* showed high resistance toward penillin G (86.04%), ampicillin (74.42%), and tetracycline (13.95%) [40]. Whereas in another study, *S. aureus* showed high resistance rate to ampicillin (95.2%) and penicillin (83.3%) [41]. Hoque and coauthors [42] reported that 8.2% of *S. aureus* isolates were resistant to erythromycin.

**Table (2)** showed that individual milk samples were detected in 8, 13, 9, 15, 4, 47, and 7% as *St. agalactiae*, *St. dysagalactiae*, *St. pyogenes*, *St. uberis*, *St. pneumoniae*, *E. fecalis* and *E. facieum*, respectively. While in retail milk were detected in 10, 32, 16, 18, 4, 58 and 8% as *St. agalactiae*, *St. dysagalactiae*, *St. pyogenes*, *St. uberis*, *St. pneumoniae*, *E. fecalis* and *E. faecium* respectively and in bulk tank were detected in 4, 32, 6, 4, 0, 56 and 8% as *St. agalactiae*, *St. dysagalactiae*, *St. pyogenes*, *St. uberis*, *St. pneumoniae*, *E. fecalis* and *E. faecium* respectively. the present study in accordance with previous study of Citak [35] who found *E. faecalis* and *E. faecium* in 54.2% and 29.0% of milk samples, respectively. *St. agalactiae* were detected in 4% of milk samples in Sharkia Province, Egypt [13]. Moreover, *St. agalactiae* and *St. uberis* were detected in 22.5%, 21.88% and 7.5%, 3.13% from smallholder farms and supermarkets in Ismailia city, respectively [36]. However, *St. agalactiae* and *St. pyogenes* were detected at percent of 70% and 30%, respectively from random samples of buffalo milk in different areas at Behera Governorate [37].

**Table (2): Prevalence of *Streptococcus species* in the examined milk samples.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Individual milk (n=100)</th>
<th>Retail milk (n=50)</th>
<th>Bulk tank milk (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td><em>St. agalactiae</em></td>
<td>8</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td><em>St. dysagalactiae</em></td>
<td>13</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td><em>St. pyogenes</em></td>
<td>9</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td><em>St. uberis</em></td>
<td>15</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td><em>St. pneumoniae</em></td>
<td>4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td><em>E. fecalis</em></td>
<td>47</td>
<td>47</td>
<td>29</td>
</tr>
<tr>
<td><em>E. facieum</em></td>
<td>7</td>
<td>7</td>
<td>4</td>
</tr>
</tbody>
</table>
Table (3): Phenotypic antimicrobial susceptibility of *S. aureus* isolates from milk samples.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Sensitive</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>2</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>5</td>
<td>25</td>
<td>ND</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>13</td>
<td>65</td>
<td>5</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>13</td>
<td>65</td>
<td>3</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>20</td>
<td>100</td>
<td>ND</td>
</tr>
<tr>
<td>Penicillin</td>
<td>3</td>
<td>15</td>
<td>ND</td>
</tr>
<tr>
<td>Rifampin</td>
<td>7</td>
<td>35</td>
<td>8</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>ND</td>
<td>ND</td>
<td>2</td>
</tr>
<tr>
<td>Sulphamethoxazole/Trimethoprim</td>
<td>17</td>
<td>85</td>
<td>1</td>
</tr>
</tbody>
</table>

ND: not determined

Table (4) showed that tetracycline was the least effective antibiotic on *St. agalactiae* since the resistance rate was 90%, followed by ampicillin (80%), rifampin (60%), sulphamethoxazole/trimethoprim (50%), gentamycin (45%), erythromycin and nalidixic acid (40%), chloramphenicol (30%), and finally streptomycin (25%). Our findings were in accordance with the results of Jain et al. [43] 33.3% of *St. agalactiae* isolates were resistant to erythromycin. While, 94.5% of isolates were resistant to tetracycline and 24.9% were resistant to chloramphenicol [44].

Figures 1 A and 1 B showed that 4 out of 5 (80%) MRSA isolates had *mecA* and 2 out of 5 (40%) carried *mecC* gene. While, 20% (1 out of 5) of isolates were positive to *sea* enterotoxin gene at 102 bp (Figure 2); *sec* and *sed* gene could not be detected. Our results were in accordance with the previous study of Cikman [45] who mentioned that *mecA* gene was found in 315 out of 494 (63.8%) isolates. However, 20% (29/145) of the tested MRSA isolates carried *mecA* gene [32].

Table (4): Antimicrobial sensitivity pattern of *Streptococcus agalactia* isolated from raw milk samples

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Sensitive</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>4</td>
<td>20</td>
<td>ND</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>8</td>
<td>40</td>
<td>4</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>2</td>
<td>10</td>
<td>ND</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>8</td>
<td>40</td>
<td>3</td>
</tr>
<tr>
<td>Sulphamethoxazole/trimethoprim</td>
<td>4</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>Rifampin</td>
<td>8</td>
<td>40</td>
<td>ND</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>12</td>
<td>60</td>
<td>ND</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>12</td>
<td>60</td>
<td>3</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>13</td>
<td>65</td>
<td>1</td>
</tr>
</tbody>
</table>

ND: not determined
Figure (1): Agarose gel electrophoresis of PCR amplified products from *S. aureus* isolates. Lane L: molecular size marker (size range 100-1000 bp), lanes 1, 3, 4, 5 showed positive results for the presence of *mecA* gene at 310 bp (A) and *mecC* gene at 304bp (B). Lane P: positive control, lane N: negative control.

Figure (2): Agarose gel electrophoresis of PCR amplified products from *S. aureus* isolates showed positive results in isolate 4 for the presence of *sea* gene at 102bp and absence of *sec* and *sed* genes. Lane L: molecular size marker (size range 100-1000 bp), lane P: positive control, lane N: negative control.

Figure (3): Agarose gel electrophoresis of PCR amplified products from *St. agalactiae* isolates. Lane L: molecular size marker (size range 100-1000 bp), Lane 1 showed positive results for the presence of *tetK* gene at 352 bp (A) and *SulI* gene at 433 bp (B), lane P: positive control, lane N: negative control.
Staphylococci pathogen was blamable for a huge scale of infections in human because of their invention of secreted and other cell-surface related virulence factors that regulate by various genes. The common serotypes are SEA, SEB, SEC, and SED. It is well acknowledged that bacterial components and products, including the capsule, surface-associated adhesins, secreted proteins and exotoxins, play a role in the process such as coagulase, hemolysins (encoded by hl genes), exfoliative toxin (ET, et genes), toxin of toxic shock syndrome 1 (TSST-1, tst gene), bicomponent leukotoxins (LukS–LukF, encoded by luk genes), enterotoxin-like toxins (SEIs, sel genes) and enterotoxins (SEs, se genes) [46]

As revealed in Figures (3 A and B) 3 out of 3 (100%) St. agalactiae isolates had sull gene and one out of three (33.3%) had tetK genes, while dfrA could not be detected. However, Emam et al. [47] reported that tetK gene was detected in 16% of St. agalactiae isolates. This difference could be due to variations in the use of antimicrobials in the area of study. It has been mentioned that sensitive strains refuge antibiotic resistance genes might express this resistance and generate strains that are likely to be resistant to those antibiotics [48].

Conclusion

Although milk is considered an essential food for humans due to its high nutritive value, it can cause severe public health hazards to people because it had a variety of microorganisms. Milk is considered the main cause of transmission of some zoonotic bacteria as S. aureus and St. agalactiae and increases the resistance of antibiotics between animals and humans who consume the milk of these animals. It is important to ensure using good hygienic practices in farms and prevent the haphazard use of antibiotics.

Conflict of interest

The authors declare no conflict of interest.

References


الملخص العربي

معدل انتشار الميكروب العنقودي الذهبى واستربتوميسين أجلاكتيا فى الحليب الخام في محافظة الدقهلية ، مصر

آية إمام 1 ، محمد الديسطي 1 و عادل عبدallah 2

1عمل المنصورة ، معهد بحوث صحة الحيوان ، مركز البحوث الزراعية 35511 ، الجيزة ، مصر
2قسم الرقابة الصحية على الأغذية ، كلية الطب البيطري ، جامعة بدر 11829 ، مصر

أجريت هذه الدراسة على 200 عينة حليب عشوائية تم جمعها من مناطق مختلفة بمحافظة الدقهلية للكشف عن انتشار ومقاومة مضادات الميكروبات لبعض البكتيريا حيوانية المصدر في الحليب مثل المكورات العنقودية الذهبية والعديدية باستخدام الاختبارات البيوكيميائية ، اختبار الحساسية للمضادات الحيوية ، اختبار تفاعل البلازما المتسلسل (PCR) لتأكيد واكتشاف الجينات المقاومة والضارة. استنتجت نتائجنا أن المكورات العنقودية الذهبية تم اكتشافها في 63% و 76% و 42% ، بينما تم اكتشاف الاستربت الاجلاكتيا 8% و 10% و 4% من عينات اللبن الفردية المفحوصة وعينات الحليب بالتجزئة وعينات الحليب المعزول من الخزان السابق على التوالي. أظهرت سلالات المكورات العنقودية الذهبية وجود مستويات عالية من المقاومة للأمبيسيلين (100%) ، حمض التاليدكسك (90%) ، أوكساسيلين (85%) ، البنسيلين (85%) ، سيفوكسيتين (75%) ، ريغامين (22%) ، تتراسيكلين (20%) و أيرثروميسين وسلفاميثوكسازول / تريميثوبريم (10%) وأظهرت الاستربت الاجلاكتيا مقاومة عالية (96%) ، تتراسيكلين (90%) ، أيرثروميسين (84%) ، سلفاميثوكسازول / تريميثوبريم (70%) ، جنتاميسين (45%) ، ريغامين (60%) ، أمبيسيلين (80%) ، سيفوكسيتين (75%) و أيرثروميسين ونايلديكسك (40%) ، كولورامفيكول (30%) . واكتشاف البلازم المتسلسل أن 4 ممزوجات من (80%) (المكورات العنقودية الذهبية المقاومة للفينيسلين) تحتوي على (جبن و 2 من 5 (4% كان (mecA) جين). في حين أن 1 من 5 (20%) كان لديه (Sea) جين، في حين أن 1 من 5 (20%) كان لديه (mecC) لديهم (تتراسيكلين (25%) و سيفوكسيتين (20%) و ريغامين (15%) و البنسيلين (10%) و أيرثروميسين (5%) و أوكساسيلين (3%) و أمبيسيلين (1%) و كولورامفيكول (1%) و سيفوكسيتين (1%) .

النتيجة الرئيسية للعمل الحالي هو أن الحليب ممكن أن يسبب مشاكل صحية عامة وشبهية إذا لم يحتوي على مجموعة من الكائنات الحية الدقيقة. من المهم التأكد من استخدام الممارسات الصحية الجيدة في المزارع ومنع سوء استخدام العشوائي للمضادات الحيوية.