Mastitis in dairy cow farms in canton Sarajevo and antimicrobial resistance of causative agents

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Abstract. Mastitis is frequent and costly disease in dairy farming, while antimicrobial resistance is an important public health threat. Increasing resistance among zoonotic pathogens led to more investigation among animal pathogens. Study, conducted on dairy farms in Canton Sarajevo, aimed to establish mastitis prevalence in dairy cows, causative bacteria and investigate antimicrobial resistance. Lactating animals (n=1214) were tested using the California Mastitis Test during November 2017. Milk from positive animals was microbiologically cultivated. The overall prevalence of mastitis was 9.9 %, while 19 out of 180 dairy farms had at least one mastitis case. In 49.2 % of samples, we identified S. aureus, 2.5% contained E. coli, 0.8% contained Enterobacteriaceae, 13.3 % had mixed infection and 34.2% samples had no growth. Using disk diffusion test highest resistances were observed to bacitracin (E. coli), trimethoprim/sulfamethoxazole (Enterobacteriaceae) and penicillin (S. aureus). Since oversight on antimicrobial use in farm animals is sporadic in the country, additional investigations of antimicrobial usage and trends in antimicrobial resistance causing agents are needed. Reducing mastitis rates on farms requires compliance with preventive measures alongside early detection, isolation of cases, culling of repeated cases, microbiological monitoring and testing for antimicrobial resistance before treatment.

1. Introduction
Mastitis in dairy farming is the most common and one of the most expensive diseases [1]. Both clinical and subclinical mastitis is found on all dairy farms regardless of the level of cow health management, technology of production, feeding, zoo-hygiene and other factors [2]. Since mastitis control programs primarily focus on contagious agents such as Streptococcus agalactiae and Staphylococcus aureus, environmental pathogens as causes of mastitis have become more important [3].

On the other hand antimicrobial resistance (AMR) of bacteria is identified globally as the most important public health threat, projected to be a leading cause of human death in the near future [4]. Increased rates and prevalences of antimicrobial resistance among human bacterial pathogens emphasize the need to investigate this phenomenon among animal bacterial pathogens, since many of the microorganisms found in animals are zoonotic.
This paper reports the results of a study conducted on dairy farms in the Canton of Sarajevo (Bosnia and Herzegovina) to establish the prevalence of mastitis, identify causative agents and investigate antimicrobial resistance of the cultured bacterial isolates.

2. Materials and methods
The list of dairy farms investigated was compiled using a register of commercial farms maintained by the Veterinary Inspection of the Canton of Sarajevo and expanded to hobby/sustenance farms identified through field surveys by veterinary inspectors. Therefore, we considered 104 registered dairy farms and 76 additional farms with 2707 animals in total. Four farms were excluded from investigation for the reasons of either being closed or the owner refusing to participate. In most of the registered farms, the number of animals was less than was recorded at the time of farm registration (overall 40% fewer animals). Therefore, out of 1472 animals on registered farms, 1011 were lactating cows, whereas of 266 enumerated cattle on additional farms, 203 were lactating cows and so considered for investigation.

All lactating cows (n=1214) were tested using the California Mastitis Test (CMT) during November 2017. From CMT-positive cows, pooled milk samples from all quarters were taken for microbiological cultivation, before any treatment was administrated. Microbiological isolation of bacteria was completed using standardized laboratory protocols [5]. Identified bacterial isolates were then investigated for antimicrobial resistance using the disk diffusion method including following antimicrobials: amoxicillin, amoxicillin/clavulanic acid, ampicillin, bacitracin, cefoxitin, clindamycin, cloxacillin, penicillin G, and trimethoprim/sulfamethoxazole. These results (diameter of inhibition zone) were interpreted using EUCAST recommendations (European Committee on Antimicrobial Susceptibility Testing) as resistant (R), susceptible (S) and intermediate (I).

Data management, analysis and graphical representation of study results were done using Excel (Microsoft Office).

3. Results and discussion
Based on our data, the average farm in the Canton of Sarajevo has 9.3 animals, out of which 6.7 are lactating cows. If the two largest farms are excluded (500 and 99 animals, out of which 430 and 59 were lactating cows, respectively), the average for the remaining farms is 6 animals, or 4 lactating cows per farm (Figure 1).

![Figure 1](image1.png)

**Figure 1.** Distribution of investigated dairy farms in Canton Sarajevo (n=176), based on number of animals on the farms

On the basis of CMT results, the overall prevalence of mastitis in dairy cows was 9.8 %, while 19 out of 176 dairy farms had at least one mastitis case (i.e. farm level prevalence was 10.7%). Farm level
mastitis prevalence was higher among larger farms (>50 animals), while within farm prevalence was higher among small family farms (Figure 2).

Even though the established prevalence figures are moderate compared to reported mastitis prevalences in dairy farms from other countries [6-9], there is still room for improvement, especially in small family farms where zoo-hygiene conditions are less adequate. Microbiological investigation of milks revealed 49.2% contained *S. aureus*, 2.5% contained *E. coli*, 0.8% contained *Enterobacteriaceae* (other than *E. coli*), 133% of milks contained mixed infections and in 34.2% of milks, no bacteria were able to be cultivated (Figure 3).

![Figure 2.](image)

**Figure 2.** Number of farms in the Canton of Sarajevo with mastitis cases (blue bars – primary axis), farm level prevalence (orange line – secondary axis) and average prevalence of mastitis within farms (grey line –secondary axis) per farm size (number of animals) group

![Figure 3.](image)

**Figure 3.** Results of the microbiological culturing of CMT-positive dairy cows
A review from 2016 reported the average prevalence of bacterial agents that cause mastitis per 100 cows worldwide [10]. *S. aureus* was responsible for 40-70% of these mastitis cases, while environmental pathogens (i.e. *E. coli*) caused 40% of these [10]. Also, typically 3%-40% of milks from animals with mastitis will not yield any bacterial growth. In animals with chronic or *E. coli* mastitis, the bacterial agent is eliminated by the white blood cells in milk [11].

The disk diffusion test was used to examine AMR in 95 of our bacterial isolates, and high percentages of isolates were resistant to ampicillin (among all three bacterial species/groups), bacitracin (among *E. coli* and other *Enterobacteriaceae* isolates) and penicillin G (among *S. aureus* isolates) (Table 1).

Table 1. Antimicrobial resistance in bacteria isolated from dairy cows, given as percentages (%) of resistant (R), intermediate (I) and susceptible(S) bacterial isolates per species/group

| Antimicrobial                      | *S. aureus* |  | *E. coli* |  | *Enterobacteriaceae* |  |
|-----------------------------------|-------------|---|-----------|---|----------------------|---|
|                                   | R          | I | S         | R | I | S | R | I | S |
| Amoxicillin                       | 13.6       | - | 86.4      |   | 46.6 | - | 53.4 | - | 57.2 |
| Amoxicillin/clavulanic acid       | 13.6       | - | 86.4      |   | 26.7 | - | 73.3 | 42.8 | 14.2 | 43 |
| Ampicillin                        | 39.7       | - | 60.3      |   | 60  | - | 40   | 42.8 | 57.2 |
| Bacitracin                        | 17.8       | 5.4| 76.8      |   | 86.6 | - | 13.4 |   |
| Cefoxitin                         | 17.8       | - | 82.2      |   | 33.3 | - | 66.7 | 28.5 | 71.5 |
| Clindamycin                       | 28.7       | 24.6| 46.7      |   |
| Cloxacillin                       | 21.9       | - | 74        |   |
| Penicillin G                      | 42.4       | - | 57.6      |   |
| Trimethoprim/sulfamethoxazole     | 26         | 16.4| 57.6      | 40 | - | 60 | 57.1 | - | 42.9 |

The primary importance of AMR in mastitis-causing bacteria is the resultant human health risk, but simultaneously, reduced efficiency and options for mastitis treatment. Studies report the percentage of penicillin-resistant *S. aureus* is from 17% to 52% (out of the total number of *S. aureus* isolates from milk of mastitis cases) [6,7]. Penicillin-resistant *S. aureus* isolates are found in only 4% of mastitis cases in Norway, where legislation prescribes that only veterinarians decide on and administer antibiotic treatment to animals [12]. In countries where farmers are also legally able to decide on and administer antibiotics, the percentage of resistant bacteria is much higher, up to the point where the antibiotics most commonly used in mastitis treatment are utterly ineffective [12]. Given that Bosnia and Herzegovina is a developing country where oversight of antibiotic use in farm animals is less comprehensive than elsewhere, additional investigations should be conducted to establish trends in AMR in mastitis-causing agents in dairy farms.

Mastitis in dairy cows is a complex disease occurring as a result of the interactions of many factors related to host, causative agent and the environment. Investigations aimed to recognize and specifically target contributing factors has led to the establishment and widespread use of simple mastitis prevention measures such as teat disinfection after milking and dry cow treatments [10]. In order to reduce the occurrence of mastitis in our farms, these standard preventive measures should be fully implemented alongside ensuring early detection of mastitis, isolating diseased animals, culling of repeat cases, microbiological monitoring and testing for antimicrobial resistance before treatment is administered.

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