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# Mastitis Pathogens Isolated from Samples of Milk in Dairy Cows and Their Resistance against Antimicrobial Agents

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**Abstract:** The objective of this study was to evaluate the effectiveness of different antibiotics against mastitis causing microorganisms during first phase of lactation in herd of 320 Holstein cows in east of Slovakia. Milk samples from quarters were cultured and identified bacteria were subjected to antimicrobial susceptibility test by disc diffusion method to a large number of antibiotics. The results revealed higher sensitivity against tetradelta (100% of *Strep. Agalactiae* and *Strep. uberis*, Coagulase Negative Staphylococci), (94.4% of *Stap. aureus*) with highest number of bacterial isolates, followed by cefalexin + kanamycin amoxicillin plus clavulanat acid and ceftiotur (100% of *Strep. agalactiae and Strep. uberis*). Resistance was observed against penicillin (22.2% of *Stap. aureus*) amoxicillin (22.2% of *Stap. aureus* and 10.5% of *Strep. uberis*) and streptomycin (22.2% of *Stap. aureus* and 52.7% of *Strep. uberis*).

**Key words:** Mastitis, dairy cows, resistance, *Staphylococcus aureus*, *Streptococcus agalactiae*.

## 1. Introduction

Mastitis is inflammation of the parenchyma of mammary gland, characterized by physical, chemical and bacteriological changes in milk and pathological changes in glandular tissues. It is a most devastating disease condition in terms of economic losses occurring throughout the world [1, 2].

This disease is mainly caused by microorganisms usually bacteria, including gram-negative and gram-positive bacteria, mycoplasmas, yeasts and algae [2, 3].

The majority of mastitis incidences are caused by only a few common bacterial pathogens involved: *Staph.* spp. (*Stap. aureus* and *Stap. epidermidis*), *Strep.* spp. (*Strep. agalactiae*, *Strep. dysgalactiae*, *Strep. uberis* and *Strep. bovis*), coliforms (mainly *E. coli* and *Klebsiella pneumoniae)* and *Actinomyces pyogenes* [4].

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Coagulase Negative Staphylococci (CNS) and *Corynebacterium bovis*, two other highly prevalent pathogens, are historically considered to be of limited importance and are therefore often described as minor pathogens. The impact of CNS is increasing probably because prevalence of major pathogens is decreasing [5, 6].

Antimicrobials are routinely used for treatment of dairy cattle affected with clinical and subclinical infections. Vasiľ et al. [3] noticed on increasing occurrence of subclinical and latent mastitis caused by CNS resistant to antibiotics. For this reason, control of antibiotic resistance bacterial pathogens of mastitis should be the starting point for ensuring the effectiveness of control methods applied.

Recently, it has been recognised that antimicrobial susceptibility of CNS and *Streptococcus* spp., which represent the majority of organisms isolated from bovine milk, is important for the early recognition of newly emerging resistant milk-borne bacterial agents [7].

The use of antimicrobials have, over time, increased the number of antimicrobial resistant microbes globally, and any use of these agents will to some extent benefit the development of resistant strains and also inappropriate usage of antimicrobials such as wrong dose, drug or duration may contribute the most to the increase in antimicrobial resistance without improving the outcome of treatment [7, 8].

The present work aimed to study the effectiveness of different antibiotics against isolated microorganisms from milk samples of dairy cows.

## 2. Materials and Methods

### *2.1 Characteristic of Herd and Samples Collection*

The study was conducted in herd of 320 Holstein dairy cows in east of Slovakia. The average milk yield of the animals was 7,500 ± 40 kg/lactation. Dairy cows were kept freely housed in three cowsheds and milked twice a day in the tandem milking shed Boumatic 2 × 10 Xpressway (Wisconsin, USA). A total of 1,280 milk samples were collected from udder quarters and cultivated pathogenic bacteria according to Vasil' et al. [3] were examined and sensitivity of microorganisms against antibiotics had been tested.

### *2.2 Resistance to Antibiotics*

All isolated pathogens were tested by *in vitro* test on Mueller-Hinton agar by zone disc methods [9] after 24 h incubation at 37 °C on resistance to twelve antibiotics: amoxicillin, amoxicillin + clavulanat acid, cefalexin + kanamycin, ceftiofur, cloxcillin, enrofloxacin, lincomycin, nafpenzal, neomycin, penicillin, rifaximin, streptomycin and tetradelta (Oxoid Ltd. Basingstoke, Hants, UK). Resistance or sensitivity of the bacteria tested was interpreted by reference zones in accordance with the instructions [9].

Statistical evaluation of data was done by Excel program.

## 3. Results and Discussion

A total of 1,280 milk samples from udder quarters were investigated, 242 (18.9%) samples were positive. No pathogens were isolated from 1,031 (80.5%) milk samples as given in Table 1. The study of the frequency of susceptibility of *Stap. Aureus* (n = 18) to antibiotics has revealed a higher sensitivity to the tetradelta (94.4% to each), combinations of amoxicillin plus clavulanat acid and cefallexin plus

**Table 1 Results of microbiological culture of milk samples collected from dairy cows.**

| Isolated microorganisms | Total No. | % |
|---|---|---|
| *Staphylococcus aureus* | 18 | 1.4 |
| *Streptococcus agalactiae* | 23 | 1.0 |
| *Streptococcus uberis* | 19 | 1.5 |
| *E. coli* | 25 | 3.2 |
| *Enterococcus* spp. | 14 | 1.1 |
| *Corynebacterium pyogenes* | 7 | 0.5 |
| *Pseudomonas aeruginosa* | 11 | 0.9 |
| CNS* | 108 | 8.4 |
| Others (bacteria and mould) | 17 | 1.3 |
| Infected quarters | 242 | 18.9 |
| Non-infected quarters | 1,031 | 80.5 |
| Reject quarters | 7 | 0.5 |
| Total quarters | 1,280 | 100 |
| Total dairy cows in herd | 320 | |

Legend: Total No.—total number of isolate; %—percentage of total No. bacteria; CNS*—*S. epidermidis*, *S. chromogenes*, *S. schleiferi*, *S. intermedius*, *S. xylosus*.

Source: authors.

**Table 2 Frequency of susceptibility of *Staphylococcus* spp. and *Streptococcus* spp. to antibiotics.**

| Bacterial strains | *Staph. aureus* (18) | | CNS (108) | | *Strep. agalactiae* (23) | | *Strep. uberis* (19) | |
|---|---|---|---|---|---|---|---|---|
| Antibiotic agent | S (%) | R (%) | S (%) | R (%) | S (%) | R (%) | S (%) | R (%) |
| Amoxicillin | 77.8 | 22.2 | 94.4 | 5.6 | 100 | 0 | 89.5 | 10.5 |
| Amox. + clavulanat | 94.4 | 5.6 | 98.9 | 1.1 | 100 | 0 | 100 | 0 |
| Cephal. + kanamyc. | 94.4 | 5.6 | 98.9 | 1.1 | 100 | 0 | 100 | 0 |
| Ceftiofur | 94.4 | 5.6 | 96.2 | 3.7 | 100 | 0 | 100 | 0 |
| Cloxacillin | 83.3 | 16.7 | 96.2 | 3.7 | 91.3 | 8.7 | 79 | 21 |
| Enrofloxacin | 88.9 | 11.1 | 95.4 | 4.6 | 100 | 0 | 100 | 0 |
| Lincomycin | 83.3 | 16.7 | 95.4 | 4.6 | 82.6 | 17.4 | 100 | 0 |
| Nafpenzal | 88.9 | 11.1 | 95.4 | 4.6 | 100 | 0 | 100 | 0 |
| Penicillin | 77.8 | 22.2 | 93.5 | 6.5 | 100 | 0 | 100 | 0 |
| Rifaximin | 88.9 | 11.1 | 98.9 | 1.1 | 47.8 | 52.2 | 100 | 0 |
| Streptomycin | 77.8 | 22.2 | 92.6 | 7.4 | 69.6 | 30.4 | 47.3 | 52.7 |
| Tetradelta | 94.4 | 5.6 | 100 | 0 | 100 | 0 | 100 | 0 |

Legend: Amox.—Amoxicillin; Amox. clavulanat—Amoxicillin clavulanat acid; Cephal. + kanamyc.—Cephalexin + kanamycin; CNS—Coagulase negative staphylococci; n—number of bacteria strains; S—Sensitivity; R—Resistant.
Source: authors.

kanamycin (94.4% to each) and ceftiotur (94.4%). A certain resistance has been noted to amoxicillin, streptomycin and penicillin (22.2% to each) (Table 2).

Staphylococci were mostly susceptible to antimicrobials tested but, Muhamed et al. [10] found that *Stap. aureus* was resistant to penicillin and streptomycin (41.44% and 25.65% respectively). Similar results were obtained by Sumathi et al. [11] where *Staphylococcus* spp. and *Streptococcus* spp. were resistant to streptomycin and penicillin. In contrast, CNS (n = 108) have been found to show a complete sensitivity to the tetradelta (100%), and higher sensitivity to amoxicillin combination plus clavulanat acid, cefallexin plus kanamycin and rifaximin (98.9% to each).

Foltys and Kirchnerová [8] tested 60, 62 and 77 strains of *Stap. aureus*, *Strep. agalactiae* and *E. coli*, respectively to various antibiotics and they reported that *Stap. aureus* was sensitive to all antibiotics except lincomycin and streptomycin, whilst *Strep. agalactiae* was 100% sensitive to amoxicillin and ampicillin and resistant to streptomycin, neomycin and tetracycillin and *E. coli* was resistant to all antibiotics.

Increasing the consumption of antibiotics in veterinary and human medicine is in the last period, accompanied by the phenomenon of an increase in bacterial resistance. The use of antimicrobials in animal nutrition, in the production of plants, feed and food prices can have a negative impact on public health through the increase in resistant bacteria or bacteria producing resistant genes that pass into the organism of people directly or indirectly [12, 13].

## 4. Conclusion

Antibiotic susceptibility tests should be done to determine the effectiveness of drug that can be used for successful treatment of diseases. Proper isolation and identification of the causative organism play significant role in prevention and control of the diseases. In our study combinations of amoxicillin plus clavulanat acid, cefalexin plus kanamycin, enrofloxacin and tetradelta were the most effective antibiotics for control of bovine mastitis. Antibiotic resistance is one of the important problems encountered in the treatment and control of mastitis.

Mastitis caused by resistant bacteria is difficult to cure and has severe consequences. Thus, determination of the antibiotic susceptibilities of pathogens causing mastitis is of crucial importance for the treatment and control of mastitis in dairy cows.

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