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ANTIBACTERIAL PROFILE OF STAPHYLOCOCCAL ISOLATES ASSOCIATED WITH THE BUBALINE MASTITIS

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Summary

Between January and May 2017, 68 samples of milk were collected from bubalines reared for milk production in the south-western part of Arad County, Pecica, in a herd summing a total of 43 Bubalines. The samples were examined using indirect tests for the detection of mastitis, California Mastitis Test. From the positive tests to indirect tests, 38 samples of milk were processed for the bacteriological examination and antibiogram. The identification of the bacterial agents was realized according to standard methodology by studying the cultural, morphological and biochemical characters. The antibiotic susceptibility test was performed using Kirby-Bauer diffusion method using the following antibiotics: methcillin, ampicillin with sulbactan, tetracycline, doxycycline, gentamycin, kanamycin, erythromycin, vancomycin, ciprofloxacin, polymyxin B, novobicin, rifampicin, ceftrioxone, cefoxitin, cefaclor. Following the evaluation of the milk samples, 26 strains of staphylococcus were have been isolated, 19 of the coagulase-positive (*S. hyicus* and *S. aureus*) and 7 coagulase-negative strains (*S. haemolyticus*, *S. sciuri* and *S. epidermidis*). The antibiotic susceptibilty of these staphylococcus strains isolated from the mastitic milk was variable depending on the antibiotic groups. For the β-lactams used (methcillin, ceftriaxone, cefoxitin, cefaclor, ampicillin with subactan), the antibiotic sensitivy was maximal, except methcillin where resistant strains were isolated from. All the isolated strains were resistant to polymyxin B, and sensitive to ciprofloxacin.

Keywords: bubaline, mastitis, Staphylococcus, antibacterial

Staphylococci, and in particular *Staphylococcus aureus*, are significant bacterial pathogens associated with bubaline mastitis. Over the past decade, infections associated with methicillin-resistant *S. aureus* (MRSA) have been described in several animal species, including bubaline.

Materials and methods

It has been taken into account a farm belonging to a household, from the south-western part of Arad county, in Pecica town in which buffalos were grown for dairy production, amounting a total of 43 buffalos of common breed, in which indirect diagnostic tests of clinical and subclinical mastitis were performed with R-

Mastitest. The experiment was carried out over a period of 5 months, between January and May 2017. A number of 68 samples of milk were taken; all of these samples were examined by indirect mastitis detection tests using the California Mastitis Test (3). The samples that showed a positive reaction a number of 38 samples were taken which were processed and prepared for the bacteriological examination, and for the antibiogram.

For the bacteriological examination the milk samples were processed in the Laboratory of Research on Bacterial Infectious Diseases (B.6.e) from the Department of Infectious Diseases and Preventive Medicine of the Faculty of Veterinary Medicine Timisoara.

The identification of the bacterial agents in the milk samples involved the study of the cultural, morphological and biochemical characteristics of the isolated bacterial agents, including the following steps:

- Sampling;
- Direct bacterioscopic examination of the milk sampled;
- Isolation of the bacterial agents in pure culture;
- Typification of the isolated bacterial agents by establishing the morphological, cultural, and biochemical characters;

In order to obtain accurate results, from a bacteriological point of view, the sampling was been done under special care, so as to be less exposed to contamination with environmental bacteria, in the following way:

- The udder and the mamelon have been washed and dried well with paper towels;
- The containers used for sampling were pre-sterilized. After the containers were closed, each sample got a serial number;
- At the time of harvesting the containers were held in near-horizontal position to avoid contamination of the milk sample with impurities from the skin, or hair of the examined cow;

To isolate the bacteria in pure culture the milk samples have been seeded on liquid culture (broth) or solid culture (enriched with 5% sterile bovine blood).

The insemination of the milk on the respective media was performed under aseptic conditions, under the protection of the flame from the gas lamp, using sterile instruments (Pasteur pipettes, and bacteriological loops) and containers (Petri dishes and plates) with sterile culture media.

After 24 hours of incubation the samples were examined. From the colonies existing in the Petri dishes and in broth Gram stained smears were performed to reveal some of the morphological characters of the bacterial agents(form, grouping, etc.). Purification of the bacterial etiological agent was accomplished by replicating a single colony using the bacteriological loop and passing in onto 5% agar with blood.

Subsequently, after this partial identification and purification the typification of the isolated bacterial agents was made by the determination of metabolic and biochemical characters.

Initially, for each culture two types of reactions or tests were studied evidencing the presence of oxidizing or reducing enzymes, namely oxidase and catalase. These tests indirectly differentiate the genuses: *Staphylococcus spp*. (which are oxidase-negative and catalase-positive, except *Staphylococcus aureus*, subspecies anaerobius), *Streptococcus spp*. (which are oxidase and catalase – positive).

After performing these tests, for further study of the biochemical characters of the isolated bacteria and for their taxonomic classification microtests were used.

For the taxonomic classification of the isolated bacteria in milk the API Staph tests, produced by BioMerieux SA, France, were used.

The antibiotic susceptibility test for the isolated staphylococci strains was performed using the Kirby-Bauer diffusion method using the following ingredients: Mueller-Hinton broth and agar, Petri plates and biodisks impregnated with antibiotics, produced by Oxoid company. The following antibiotics were used: methicillin, ampicillin with sulbactan, tetracycline, doxycycline, gentamycin, kanamycin, erythromycin, vancomycin, ciprofloxacin, polymyxin B, novobiocin, rifampicin, pristinamycin, lincomycin, ceftrioxone, cefoxitin, and cefaclor. The antibiotic susceptibility test was also performed in order to establish the therapeutic conduit to the respective clinical cases, to identify the methcillin resistant strains, and to identify the resistance phenotypes.

Results of the bacteriological examination

During the study 26 strains belonging to the *Staphylococcus* genus were isolated.

Morphology. All 26 Gram-stained bacterial strains were Gram-positive, having the form of cocci with a size of 0.8-1.5 mm in diameter, and the predominant grouping was in bunch, and occasionally in pair, or solitary bacterial cells.

In this study 26 strains of *Staphylococcus* were isolated, of which 19 coagulase-positive strains (CoP, represented by *S. hyicus* and *S. aureus*) and 7 coagulase-negative strains (CoN, represented by *S. haemolyticus*, *S. sciuri* and *S. epidermidis*) isolated from milk from cows suffering from clinical and subclinical mastitis.

Results of the antibiotic sensitivity test

The *Staphylococcus* strains that haven’t been exposed to antibiotic pressure are susceptibe to these substances, however, the strains isolated from dogs and cats with various conditions under antibiotic pressure due to the therapy may present multiple resistance phenomenos.

Analyzing the results it can be observed that the sensitivity to antibiotics was variable depending on the antibiotic groups.

In the case of antibiotics: novobiocin, rifampicin, pristinamycin, ciprofloxacin, vancomycin, ceftriaxone, cefoxitin, cefaclor and ampicillin/sulbactan, being considered *Staphylococcus* elective antibiotics, the percentage of the susceptible strains was 100%. This suggests that the isolated and tested strains originated from animals where these antibiotics were not used. It can also be said that all these antibiotics constitute the kit for staphylococci, or are usually used in humans in the therapy of infections with *Staphylococcus*, respectively in animals.

To beta-lactamins used (methicillin, ceftriaxone, cefoxitin, cefaclor, ampicillin with sulbactan), the sensitivity to the antibiotics was maximal, except methicillin where 7 resistant strains were isolated. Of these, two strains of *S. hycus* were resistant to methicillin, 4 strains of *S. aureus* and one strain of *S. epidemidis*. The strains tested were largely sensitive sensitive to beta-lactamins as a result of previous correctly done treatments.

The antibiotic resistance phenomenon, in the case of beta-lactamins, is based on plasmid and chromosomal genetic determinants that govern the synthesis of beta-lactamins, with a broad spectrum thus providing staphylococci resistance.

The resistance to methicillin is transmitted by plasmids (factor R) having a common pattern over other beta-lactamins. For this reason, methicillin-resistant staphylococci strains are considered strains with a special zoonotic risk, having a complex circuit, namely man-animal-man (1, 5, 7, 8, 10).

To aminoglycosides (gentamicin, kanamycin) and macrolides (erythromycin, and vancomycin) the antibiotic susceptibility was different, being maximal for vancomycin. In this case, different strains have been isolated, 8 strains were resistant to gentamicin, 7 strains showed resistance to kanamycin, 9 to tetracycline, 9 to doxycycline, 11 strains to erythromycin, and 14 to lincomycin.

Most strains showed resistance to polymyxin B (24 strains) due to the use of locally applied preparations containing this antibiotic, in the past.

The antibiotic resistance to tetracycline (tetracycline, doxycycline) was reduced, with 18 strains resistant to this group of antibiotics, where the resistance phenomenon is plasmidic and chromosomial (9 strains of tetracycline and 9 of doxycycline). All strains tested were showed sensitivity to ciprofloxacin because this quinolone is not used in the therapy of diseased bovines.

The development of staphylococcal resistance to various antibiotics is a consequence of the unreasonable use in the therapy of certain diseases in cattle, especially those of the mammary gland. The non-ionic antibiotics used create a selection pressure selecting and transmitting genetic determinants of the plasmid and chromosomal type. Consequently, the phenomenon of multiple resistance occurs which is transmitted in- and interspecific. The resistance to methicillin shows highly importance for it can be associated with the resistance to beta-lactamins and other antibiotic groups (5, 9, 10). After testing the strains isolated from milk, coming from bovine suffering from mastitis, a resistance to 17 antibiotics has been identified, methicillin-resistant strains and several resistotypes, to beta-lactamins, tetracycline, macrolides, polymyxin B. The data on resistance to methicillin and the identified resistotypes are similar to the results reported by other authors, on the antibiotic resistance phenomenon.

Zhang et al. (11) have investigated, during a study, the strains of *S. aureus* isolated from milk coming from cows that have mastitis, in eastern China. Of the 200 samples of milk analyzed 58 were positive for *S. aureus*, of which 11 were strains of *S. aureus* (MRSA) methicillin-resistant. Hata (4), in a study conducted in Japan on mastitis outbreaks in cows, produced by *Staphylococcus aureus* methicillin resistant strains (MRSA), out of 78 cows tested, 31 cows were MRSA-bearing, and they were detected by testing the milk coming from lactating cows.

In Egypt, El-Ashker et al. (2), a detailed study has been carried out on the structure of the *Staphylococcus aureus* population in a modern farm of dairy cows (Gamasa) and buffaloes in Dakahlia Governorate, Egypt. A number of 872 milk samples, coming from 218 animals with clinical and subclinical mastitis, have been investigated. *Staphylococcus aureus* has been identified in 5.6% of all collected samples, while the methicillin-resistant strains (MRSA) accounted for 24.5% of all identified *S. aureus* strains (12/49).

Nemeghaire et al. (6) reported a prevalence of MRSA strains in females of 19.8%. All the strains isolated were multidrug resistant, to at least two antimicrobial agents, in addition to cefoxitin and penicillin, with an average resistance to various microbial agents of 9.5. The isolated strains showed a wide range of antibiotic resistance genes, and virulence genes.

Over the past 25 years there has been a worldwide increase in the prevalence of methicillin-resistant Staphylococcus aureus strains. Furthermore, in many cases, MRSA infections occurred outside the hospitals, independently, caused by strains of community associated MRSA (CA-MRSA). In Germany, at least 10% of these sporadic infections are due to MRSA strains associated with animal breeding (LA-MRSA).

Conclusions and recommendations

It has been confirmed that staphylococci are an important cause in the occurrence of mastitis in buffaloes, and the existence of methicillin-resistant strains, and the multi-resistance phenomenon have been demonstrated.

The antibiotic susceptibility of strains isolated from milk coming from diseased animals, was variable, depending on the antibiotic groups:

- to beta-lactamins used (methicillin, ceftriaxone, cefoxitin, cefaclor, ampicillin with sulbactan), the antibiotic susceptibility was maximal, except to methicillin, as resistant strains were isolated;
- as regards aminoglycosides (gentamicin, kanamycin) and macrolides (erythromycin and vancomycin), the antibiotic susceptibility was different, being maximal for vancomycin. A phenomena of resistance have been described for gentamicin, kanamycin, erythromycin and lincomycin, respectively;
- the antibiotic susceptibility to tetracycline (tetracycline, doxycycline) has been increased;
- all the isolated strains were resistant to polymyxin B and sensitive to ciprofloxacin.

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