

## **ANTIBIOTIC SUSCEPTIBILITY OF PATHOGENS ISOLATED FROM MASTITIC MILK IN CATTLE**

**MIHAELA BRÎNDA**

Faculty of Veterinary Medicine Timisoara  
Calea Aradului No. 119, 300645, Timisoara, Romania

### **Summary**

There was tested the antibiotic susceptibility of etiological agents isolated from mastitic milk samples, based on bacteriological examination and antibiogram, in order to find the most efficient medication, to decrease the bacteriological load of milk and, finally, to decrease economical losses due to bovine mastitis. The antibiotic susceptibility of pathogens was tested to tetracycline, neomycin, novobiocin, cephalotin, amoxyclave, ampicillin, oxacillin, penicillin G (only for *Streptococcus spp.*), streptomycin, erythromycin and there was also tested the resistance to sulfonamides.

**Key words:** antibiotic susceptibility, bovine mastitis, antibiogram.

Usually bovine mastitis can be caused by a series of pathogens, differentiated into two broad categories: those causing contagious mastitis (*S. aureus*, *Str. agalactiae*, *Mycoplasma bovis*, etc.) widespread from the infected quarters, primarily during milking (man hands, milking machines), and those causing environmental mastitis (*Str. uberis*, *Str. dysgalactiae*, coliforms, etc.) present in the environment (bedding, flooring, droppings) generally transmitted in any time of cow's life: during milking, between milking, during the dry period, especially at first calving, in heifers (6).

Resistance to antibiotics is most commonly installed along genetic mechanisms of mutation and recombination, as a result of selection pressures, in terms of frequent use of antibiotic therapy.

Mastitis can cause economic losses by decline in milk production, effective elimination of cows with chronic mastitis, destruction of large quantities of contaminated milk and milk from treated cows, high cost of preventive and curative treatments.

The present research aimed at testing antibiotic sensitivity by performing antibiograms for efficient implementation of a mass treatment in the herd.

### **Materials and methods**

Research has been performed on a dairy farm from Caras-Severin County. Whole herd of cows was tested by indirect method (R-Mastitest). Milk samples were collected for bacteriological examination from 18 cows with positive reactions in R-Mastitest, in milk of one or more mammary quarters. The susceptibility to antibiotics was established by difusimetric method (1) with

antibiotics commonly used in mastitis treatment, after the isolation of etiological agents was made.

Interpretation of the results was made according to inhibition diameter. Bacteria tested were classified in the following categories of sensitivity: sensitive, intermediate sensitive or resistant.

In that study we used the following antibiotics: tetracycline, neomycin, novobiocin, cephalotin, amoxyclave, ampicillin, oxacillin, penicillin G (only for *Streptococcus spp.*), streptomycin, erythromycin and we also tested the resistance to sulfonamides.

### Results and discussions

Eighteen cows were diagnosed with clinical and subclinical mastitis by indirect method (R-Mastitest) and from them were collected milk samples for bacteriological examination. The developed mastitis have affected usually one or two mammary quarters, rarely three and never whole mammary glands, as described also in literature (2). Totally from that cows were collected 27 milk samples.

From two mammary quarters belonging to 2 different cows there were isolated concomitantly one *Staphylococcus spp.* strain and one *Streptococcus spp.* strain, and therefore it was considered as having 29 samples. After other authors (6) concomitant mammary infection with streptococci and staphylococci is unusual, and removing one of them can lead to increased incidence of other.

Of all the isolated strains, 12 were *Staphylococcus spp.*, 4 were *Streptococcus spp.* and the remaining samples were either sterile (8 samples) or, in 5 cases, could not identify the etiological agent. Further, in the *Streptococcus spp.* and *Staphylococcus spp.* strains were realized antibiograms, using the main antibiotics used to treat mastitis.

The sensitivity and resistance of the isolated strains were different depending of the bacterial genus and the antibiotics tested (Table 1).

Table 1

#### Results of antibiograms

No.	Antibiotic	No. of <i>Staphylococcus spp.</i> strains				No. of <i>Streptococcus spp.</i> strains			
		S	I	R	Total	S	I	R	Total
1	Tetracycline	-	2	10	12	-	-	4	4
2	Neomycin	3	5	4	12	-	-	4	4
3	Novobiocin	1	3	8	12	-	-	4	4
4	Cephalotin	9	-	3	12	2	-	2	4
5	Amoxyclave	4	-	8	12	1	-	3	4
6	Ampicillin	4	1	7	12	3	-	1	4
7	Oxacillin	-	-	12	12	-	-	4	4
8	Sulfonamides	-	1	11	12	-	-	4	4

LUCRĂRI ȘTIINȚIFICE MEDICINĂ VETERINARĂ VOL. XLII (1), 2009, TIMIȘOARA

9	Streptomycin	5	2	5	12	-	-	4	4
10	Erythromycin	9	3	-	12	2	-	2	4
11	Penicillin G	It was not tested.				2	-	2	4

S – sensitive, I – intermediate sensitive, R – resistant

Because of prolonged treatments with same antibiotics frequently is noticed the emergence of resistant variants of bacterial strains. Analyzing the antibiograms' results of *Staphylococcus spp.* it was observed that in the isolated strains was revealed the phenomenon of multiple resistance to three or more antibiotics, none being resistant to only one or two antibiotics. In literature (7) multiple antimicrobial resistance is defined as resistance to four or more antimicrobials.

In cases of *Staphylococcus spp.* strains it could be noticed that:

- the highest sensitivity was to cephalotin and erythromycin, followed by the sensitivity to streptomycin, amoxyclave and ampicillin, neomycin and, only in one case, to novobiocin;
- the highest resistance was to oxacillin (all the staphylococcal strains), followed by sulfonamides tetracycline, novobiocin, amoxyclave, ampicillin, streptomycin, neomycin and cephalotin;
- intermediate sensitivity was to neomycin, novobiocin, erythromycin, tetracycline, streptomycin, sulfonamides and ampicillin.

The antibiograms' results were similar to data from literature (3, 4, 5).

All of streptococcal strains presented multiple resistance. The sensitivity of *Streptococcus* strains to penicillin G was different from other authors (5, 7) with mention that the two streptococcal strains resistant derived from the two mammary quarters with polymicrobial mastitis (both staphylococcal and streptococcal).

### Conclusions

In most cases of bovine mastitis the isolated pathogens were staphylococci.

All most of staphylococcal strains isolated had an increased susceptibility to cephalotin and erythromycin (75%) and all of them are resistant to oxacillin (100%).

All the streptococcal strains isolated presented multiple antibiotic resistance.

All of the isolated strains of *Staphylococcus spp.* revealed the phenomenon of multiple resistance to three or more antibiotics.

**References**

1. **Buiuc, D., Neagu, M.**, Tratat de microbiologie clinică, Ed. Medicală, București, 1999.
2. **Grădinaru, A.C., Voloșeniu, M.S., Sofronie, Mariana, Ruginosu, Elena, Runceanu, L. Gh., Popescu, O.**, Rev. Rom. Med. Vet. 2007, 17, 3, 161.
3. **Guterbock, W.M.**, Rational treatment of clinical mastitis, Proc 33rd Ntl Mastitis Couc, Olando FL, Natl Mastitis Counc Inc, Madison WI, 1994, 40-52.
4. **Nichita, Ileana, David, Carmen, Trif, R., Tîrziu, E., Gros, R.V.**, Lucrări științifice medicină veterinară Timișoara, 2007, XL, 676-682
5. **Prescott, J.F., Baggot, J.D., Walker, R.D.**, eds. Antimicrobial therapy in veterinary medicine 3<sup>rd</sup> ed. Ames: Iowa State Univ.Press, 2000, 134-159.
6. **Radostits, M.O., Gay, C.C., Blood, D.C., Hinchcliff, K.W.**, Veterinary Medicine. A Textbook of the Diseases of Cattle, Sheep, pigs, Goats and Horses, 9<sup>th</sup> Edition, W. B. Saunders Company Ltd, 2000.
7. **Teale, C.J., Cobb, S., Martin, P.K., Watkins, G.**, VLA Antimicrobial Sensitivity Report 2002, Norwich, 19-21.

Antibiotic resistance and pathogenicity factors in *Staphylococcus aureus* isolated from mastitic Sahiwal cattle. *J Biosci.* 2011 Mar;36(1):175-88. doi: 10.1007/s12038-011-9004-6. The characterizations pathogenic factors (adhesin and toxin genes) and antibiotic susceptibility of isolates were carried out using gene amplification and disc diffusion assays, respectively. A high prevalence of MRSA was observed in the tested isolates (13.1%). A few isolates showed similar antibiotic-resistance patterns, which could be due to identical strains or the dissemination of the same strains among animals. These findings can be utilized in mastitis treatment programmes and antimicrobials strategies in organized herd. Publication types. Research Support, Non-U.S. Isolation, identifications and antimicrobial susceptibility pattern of coagulase positive *Staphylococcus* from subclinical mastitic dairy cattle in and around Haramaya University. Firaol Tafa<sup>1</sup>, Yitagele Terefe\*<sup>1</sup>, Nateneal Tamerat<sup>1</sup>, Endrias Zewdu<sup>2</sup>. <sup>1</sup>Haramaya University, College of Veterinary Medicine, P. O.BOX 138, Dire Dawa, Ethiopia <sup>2</sup>Ambo University, College of Agriculture and Veterinary Sciences, Department of Veterinary Laboratory Technology, P.O.Box 19, Ambo, Ethiopia \*Corresponding author; E-mail:yitagele@yahoo.com yterefe@haramaya.edu.et. A total of 210 dairy cattle from seven farms were screened for SCM by CMT. The prevalence of SCM in the study areas was 71.4% (150/210). Antibiotic susceptibility of enterobacteriaceae species isolated from mastitic milk in A. Igeria.pdf. Content available from CC BY-NC-ND 4.0: 5671a98908ae3aa2fcedad40.pdf. The major pathogens isolated from the milk samples were *S.aureus*, *E.coli*, coagulase negative staphylococci (CNS), *Streptococcus* species, *Bacillus subtilis*, *Serratia marcescens* and other *Bacillus* species. This study investigated the current status of clinical mastitis among dairy cattle in and around Bangalore. The prevalence of mastitis was assessed by the results of bacteriological evaluation of milk samples collected from clinical mastitis cases. A total of seventy five bacterial isolates were recovered from sixty clinical cases of mastitis affected cows. A total of 829 mastitic milk samples, both clinical and subclinical, were collected from 21 farms by veterinarians and submitted to the laboratory for testing from which 229 *S. aureus* isolates were recovered, an isolation rate of 28.1%. These isolates were tested for susceptibility to the antibiotics penicillin, erythromycin and clindamycin. Antibiotic susceptibility testing showed that 53.7% of the 80 randomly selected isolates were resistant to at least one of the three antibiotics tested. To our knowledge, this study represents the first large scale molecular studies on *S. aureus* isolates from dairy farms in Argentina. Keywords. *Staphylococcus aureus* bovine mastitis antimicrobial resistance molecular typing. The antibiotic susceptibility testing was performed by broth microdilution method with the MICRONAUT system for Gram-positive bacteria using commercial 96-well microtiter plates (MICRONAUT-S MRSA/GP; Merlin, Bornheim, Germany), according to the manufacturer's recommendations. Table 4. Antimicrobial susceptibility of *Staphylococcus* isolates from milk. Non-*Staphylococcus aureus* isolates showed high resistance rates to erythromycin, erythromycin/clindamycin, fosfomicin, fusidic acid, clindamycin, daptomycin, moxifloxacin and gentamicin, with 96.7%, 93.3%, 93.3%, 90.0%, 83.3%, 83.3%, 80.0% and 80.0%, respectively.