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Susceptibility to antibiotics of bacterial microorganisms most commonly isolated from clinical and subclinical forms of *mastitis* in cows

Daniel Radzikowski

Faculty of Natural Sciences, Siedlce University of Natural Sciences and Humanities, Siedlce, Poland

E-mail address: daniel18-1994@wp.pl

ABSTRACT

Mastitis is an increasing problem in dairy farms and in the dairy industry. The paper presents changes in susceptibility of strains of bacteria isolated from quarter milk cows from *mastitis* in 2011, 2012, 2013, 2014, 2015 in the Factory Laboratory of Dairy Cooperative in south-eastern Poland. In total, 3409 strains of staphylococci, 2774 strains of streptococci and 423 strains of *E. coli* were isolated in these years. Susceptibility to antibiotics was examined by the disc diffusion method on Mueller-Hinton base. On the basis of the research it can be concluded that the highest sensitivity of staphylococci in all study periods is found for cefguin (from 91.2% to 96.1%) and cefapirin (92.2% to 96.2%), and the smallest for tetracycline (18 %), streptomycin (11.9%) and amoxicillin (9.3%). The highest sensitivity of bacteria from the Streptococcus family are cefapirin (97%), cefquinome (96.1%), amoxycycline (95.6%) and cefalexin (89.9%) on average in all years of research. The highest resistance was found for neomycin and streptomycin. They show that during the period under study over 90% of *Escherichia coli* were sensitive to streptomycin. Sensitivity at the level of 70%-80% was noted for amoxicillin, neomycin and cefapirin. The highest resistance of *E. coli* bacilli evolved for cefallaxin and tetracycline. Accordingly, early diagnosis of a pathogenic pathogen, and then selecting the appropriate form of treatment, gives you the opportunity to minimize the loss of *mastitis* in dairy cows.

***Keywords*:** dairy cows, antibiotics, sensitivity, resistance, antibiotic therapy, mastitis

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1. INTRODUCTION

Mastitis, together with metabolic diseases and infertility, is a serious problem in farms and the dairy industry [13]. According to estimates in Poland, the clinical form of mastitis affects 6.5% of the udder quarters, while the sub-clinical form was observed in as many as 43.4% of the cases studied [18]. The problem of mastitis affects many European countries, where milk production is one of the key sectors of agriculture. The studies showed that about 80% of cows in Denmark, Finland, Sweden and Norway had a clinical mastitis, whereas in about 15% of cows, there was a recurrence of this disease [19]. The factors that cause mastitis include mainly bacteria, but also fungi and algae. The basic form of treatment and prevention of mastitis is antibiotic therapy. The use of antibiotics plays an important role in controlling the occurrence of mastitis in cows by reducing the number of infections in the herd and preventing new infections.

The main effect of mastitis in a herd of cows is the increase in the number of somatic cells (LKS) in milk. This carries a loss for both producers and milk processors. Research indicates that the increase in the number of somatic cells in milk by 100,000 / ml, above 200,000 / ml, causes a reduction in milk production by 2.5%. In the Polish herds subjected to the study, the average content of LKS in milk was approximately 464 thousand in ml, causing PLN 1164 per share of losses annually [20]. In addition, the increase in LKS above 200,000 / ml results in a decrease in cheese productivity by at least 2%, and assuming the value of cheese at 2 euro / kg and a daily throughput of 500,000 kg of milk, causes a loss of approx. euro per day. In addition to economic losses, there is also a deterioration of animal welfare due to inflammation of the mammary gland, which is associated with painfulness, redness and increased body temperature of cows. Cows and milk secrete leukocytes, which is the body's immune response. There is also a change in the color of milk, there is a decrease in dry matter in milk, total protein, lactose, fat, and the content of whey proteins increases.

There are two forms of *mastitis*: clinical with marked physical, chemical and bacteriological changes in milk and pathological in the milk gland and subclinical, in which there are no changes in milk and udder [15]. The factors that cause mastitis include mainly bacteria, but also fungi and algae. Bacteria that cause mastitis in terms of the epidemiological link to the disease are divided into infectious and environmental pathogens. Infectious pathogens, which have the ability to survive in the mammary gland and cause *mastitis* manifesting with a significant increase in somatic cells in milk, are classified as bacteria: *Staphylococcus aureus* and *Streptococcus agalactiae* [1]. Environmental pathogens are not adapted to living in the udder of a cow, occur in close to its surroundings and as a result of the creation of suitable conditions for infection, they cause inflammation of the mammary gland. Included in this group are bacteria such as streptococci *Streptococcus uberis* and *Streptococcus dysgalactiae* and *Escherichia coli* [12].

The basic form of treatment and prevention of *mastitis* is antibiotic therapy. The use of antibiotics plays an important role in controlling the occurrence of *mastitis* in cows by reducing the number of infections in the herd and preventing new infections [5]. It is confirmed that the effectiveness of antibiotic therapy depends on the sensitivity of a given pathogen to antibiotics and the ability of the immune system of the cow [9]. In recent years, an increase in the antibiotic resistance of many strains of bacteria has been observed. The basic reason for this phenomenon is the abuse or unjustified use of antibiotics in the treatment of animals without carrying out research identifying a given pathogen and determining its sensitivity to a given antibiotic [16].

In recent years, more and more attention has been devoted to this problem and new methods are being sought to combat antibiotic-resistant mastitis-causing bacteria [7].

Inflammation of the mammary gland in dairy cows can be caused by a broad spectrum of bacteria from the group of both Gram-positive (including *Staphylococcus aureus*, *Streptococcus agalactiae*, *Corynebacterium bovis*, *Streptococcus dysgalactiae*) and Gram-negative bacteria (including *Enterococcus* spp., *Escherichia coli, Enterobacter* sp.) And pathogenic fungi (*Candida, Trichosporon, Geotrichum, Rhodotorula and Cryptococcus),* and *even algae (Prototheca zopfii* and *Prototheca wickerhamii*). In the scientific literature one can find information about more than 200 different organisms that can cause the cow's udder to become inflamed. Some microorganisms that cause mastitis are always present in the animal habitat, while others may only be present in the udder of an infected cow (*eg Streptococcus agalactiae*) (Table 1).Due to the origin of infectious agents, there are two main types of mastitis: environmental and infectious [9].

Table 1. Division of pathogens causing mastitis in dairy cows.

Microorganisms	
Environmental	**Infectious**
Streptococcus uberis	*Staphylococcus aureus*
Escherichia coli	*Streptococcus agalactiae*
Kliebsiella	*Streptococcus dysgalactiae*
Pseudomonas aeruginosa	*Corynebacterium bovis*
Bacillus cereus	*Mykoplazma*
Streptococcus faecalis	-
Fungi	-
Algae	-

The aim of the study was to determine the sensitivity to antibiotics of bacterial strains isolated from clinical and subclinical *mastitis* in dairy cows [2].

2. MATERIALS AND METHODS

The research material were bacterial strains isolated in 2011, 2012, 2013, 2014 and 2015 at the Laboratory of the Dairy Cooperative in south-eastern Poland. In total, 3409 strains of staphylococci, 2774 strains of streptococci and 423 strains of *E. coli* were milked from mastitis milk. Quarter milk samples were provided by dairy suppliers and tested to identify pathogens in milk according to commonly accepted methods [10]. Subsequently, antibiotic susceptibility

was tested using the Mueller-Hinton diffusion method on the Mueller-Hinton substrate as recommended by the Clinical Laboratory Standard Institute. Amoxicillin (Amc; 30 μg) discs, neomycin (N; 30 μg), streptomycin (S; 10 μg), tetracycline (T; 30 μg), cefalexin (Cl; 30 μg), cefquinome (CEQ; 30 μg) and cefapirin (CEF, 30 μg).

3. RESULTS

The sensitivity of bacteria from the *Staphylococcus* family to antibiotics is presented in Table 2. The data presented show that the highest sensitivity of staphylococci in all study periods is found for cefguinom (from 91.2% to 96.1%) and cefapirin (from 92.2% to 96.2%). The most resistant results were found for tetracycline and streptomycin. Analysing the sensitivity of staphylococci in 2011-2015, for all antibiotics used in the study, an increase in bacterial resistance to antibiotics may be observed. The largest increase in resistance of bacteria from the *Staphylococcus* family developed between 2011 and 2015 for tetracycline (18%), streptomycin (11.9%) and amoxicillin (9.3%). In 2015, resistance to tetracycline occurred in 62.1% of staphylococci.

Table 2. Susceptibility to selected antibiotics of staphylococci isolated from mastitis cows.

Antybiotics	2011 (n = 697)		2012 (n = 702)		2013 (n = 668)		2014 (n = 667)		2015 (n = 675)	
	S %	R %	S %	R %	S %	R %	S %	R %	S %	R %
Amoxicillin	92,4	7,6	91,5	8,5	96,2	3,8	88,9	11,1	83,1	16,9
Neomycin	93,2	6,8	92,1	7,9	89,9	10,1	78,5	21,5	87,1	12,9
Streptomycin	90,7	9,3	91,5	8,5	90,1	9.9	72,8	27,2	78,8	21,2
Tetracycline	55,9	44,1	60,1	39,9	49,9	50,1	62,3	37,7	37,9	62,1
Cephalexin	82,1	17,9	83,9	16,1	80,1	19,9	75,6	24,4	78,8	21,2
Cefquinome	95,8	4,2	92,3	7,7	96,1	3,9	92,3	7,7	91,2	8,8
Cephapirin	95,5	4,5	96,2	3,8	94,4	5,6	92,2	7,8	93,9	6,1

S – susceptible, R – resistant

Table 3 presents the sensitivity of streptococci isolated from milk from mastitis to antibiotics. The highest sensitivity of bacteria from the *Streptococcus* are cephapirin (97%), cefquinome (96.1%), amoxycycline (95.6%) and cefalexin (89.9%) on average in all years of research. Neomycin and streptomycin showed the highest resistance as well as the results of the tests Hawari and Al-Dabbas (2008). The decrease in streptococcal sensitivity, taking into account the first and last year of research, for antibiotics was noted for streptomycin from 30.9% to 12.9%, and neomycin from 33.1% to 18.9%. At the same time, increased sensitivity to

amoxcykline, cefalaxin and cefquinum was observed. Similar results were stated by Wawron et al. (2008), in which the highest streptococcal activity was demonstrated for amoxclinin

Table 3. Susceptibility to selected antibiotics of streptococci isolated from mastitis cows.

Antybiotics	2011 (n = 576)		2012 (n = 588)		2013 (n = 599)		2014 (n = 546)		2015 (n = 465)	
	S %	R %	S %	R %	S %	R %	S %	R %	S %	R %
Amoxicillin	92,2	7,8	93,8	6,2	98,1	1,9	96,7	3,3	97,0	3,0
Neomycin	33,1	66,9	27,9	72,1	23,8	76,2	24,5	75,5	18,9	81,1
Streptomycin	30,9	69,1	8,0	92	17,3	82,7	35,7	64,3	12,8	87,2
Tetracycline	49,9	50,1	38,9	61,1	54,5	45,5	53,6	46,4	42,1	57,9
Cephalexin	87,2	12,8	91,6	8,4	88,8	11,2	89,5	10,5	92,3	7,7
Cefquinome	94,9	5,1	97,2	2,8	96,6	3,4	95,9	4,1	95,7	4,3
Cephapirin	98,1	1,9	97,7	2,3	96,1	3,9	97,2	2,8	95,9	4,1

S – susceptible, R – resistant

Table 4. Susceptibility to selected antibiotics of *E. coli* isolated from mastitis cows.

Antybiotics	2011 (n = 59)		2012 (n = 101)		2013 (n =109)		2014 (n = 64)		2015 (n = 90)	
	S %	R %	S %	R %	S %	R %	S %	R %	S %	R %
Amoxicillin	78,2	21,8	81,2	18,8	64,5	35,5	75,6	24,4	77,7	22,3
Neomycin	72,8	27,2	83,1	16,9	83,4	16,6	75,0	25,0	83,3	16,7
Streptomycin	93,2	6,8	96,0	4,0	95,5	4,5	85,9	14,2	91,1	8,9
Tetracycline	67,7	32,3	61,3	38,7	68,3	31,7	70,3	29,7	66,6	33,4
Cephalexin	47,4	52,6	61,3	38,7	55,0	45,0	60,9	39,1	68,8	31,2
Cefquinome	67,7	32,3	57,4	42,6	65,1	34,9	60,9	39,1	67,7	32,3
Cephapirin	76,3	33,7	68,3	31,7	67,8	32,2	60,9	39,1	78,8	21,2

S – susceptible, R – resistant

Data on the resistance of *E. coli* to antibiotics are presented in Table 4. They show that during the period under study over 90% of *Escherichia coli* were sensitive to streptomycin.

Sensitivity at the level of 70-80% was noted for amoxicillin, neomycin and cefapirin. The highest resistance of *E. coli* bacilli evolved for cefallaxin and tetracycline. There was also no significant increase in the resistance of *Escherichia coli* to antibiotics over the entire study period. The conducted research indicates continuous changes in the sensitivity of bacterial strains to antibiotics over many years, which increases or decreases depending on the existing factor. Observations obtained are similar to those obtained by many domestic and foreign researchers [4, 8, 14, 20]. According to the research, there is currently no antibiotic that guarantees the effectiveness of therapy and some cure of the cow, and the preparations that are currently used show very variable effectiveness against bacterial strains. In addition, more and more often the use of antibiotics, which was distinguished by high sensitivity to the isolated strain of the organism, does not bring the expected results [16]. As a result of the analysis, changes in the percentage of susceptible bacterial strains to antibiotics over the following years are noticeable. Similar observations were made by other authors [3, 11]. Such periodic changes in the growth and decrease in sensitivity of a given antibiotic to bacteria may be caused by changes in the intensity of the antibiotic's use in a given area and an increase in resistance produced by bacterial microorganisms [7]. Bacteria produce resistance mechanisms through the production of enzymes that inactivate the action of antibiotics. *Staphylococcus* and *E. coli* strains produce β-lactamases that hydrolyze the β-lactam ring in β-lactam antibiotics. This feature is revealed in a way that is independent of bacterial contact with the antibiotic or the frequency of its use [17].

4. CONCLUSIONS

Mastitis is an increasing problem in dairy herds. The basic form of treatment is antibiotic therapy. In order to select a suitable preparation, a pre-existing antibiogram test should be used to determine the sensitivity of a given antibiotic to strains of *mastitis* causing microorganisms. This is very important because there is an increase in the resistance of bacteria to antibiotics, which is caused by the abuse and inappropriate use of these substances. Early diagnosis of pathogenic pathogen, choosing the right form of treatment gives the opportunity to minimize economic losses caused by *mastitis*.

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