

Article

Sensitivity and Treatment of Infectious Mastitis Pathogens in Cows to Antibiotics

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Abstract: The article presents data on the sensitivity of pathogens (staphylococci, streptococci, enterococci, Escherichia, Proteus, and Pseudomonas) to antibacterial drugs prior to the treatment of cows suffering from infectious mastitis. The sensitivity of these microorganisms to penicillin, streptomycin, sulfadiazine, chloramphenicol, tetracycline, enrofloxacin, kanamycin, monomycin, polymyxin, erythromycin, oleandomycin, tylosin, as well as anti-mastitis preparations Mastisan and Mastisept, was determined by the indicator disk method. When the diameter of the growth inhibition zone of the culture was 15–25 mm, sensitivity was evaluated as moderate; more than 25 mm as high; and the absence of an inhibition zone indicated resistance of the microorganism to the corresponding antibiotic. The results of sensitivity studies of the above-mentioned staphylococci, streptococci, enterococci, Escherichia, Proteus, and Pseudomonas to antibacterial drugs are presented. It was established that the average overall effectiveness of treatment of serous, catarrhal, fibrinous, purulent, and subclinical mastitis with Mastisan was about 80%. The Mastisept preparation showed an average overall treatment effectiveness of 85% for the indicated forms of mastitis, while in 14% of cows incomplete recovery of milk productivity was observed.

Keywords: Cattle, Infectious Mastitis, Strain, Antibiotics, Disease, Fibrosis, Staphylococci, Streptococci, Enterococci, Escherichia Coli, Proteus, Pseudomonas, Penicillin, Streptomycin, Sulfadiazine, Chloramphenicol, Tetracycline, Enrofloxacin, Kanamycin, Monomycin, Polymyxin, Erythromycin, Oleandomycin, Tylosin, Pathogen, Catarrhal, Subclinical, Purulent Mastitis

Introduction

To meet the population's demand for food products and ensure food security, one of the main tasks is the intensive development of livestock production: increasing the number of highly productive cows, extending their productive lifespan, and producing environmentally safe milk and dairy products. The incidence of infectious mastitis among highly productive cows averages 22–60%, which leads to a decrease in milk productivity and milk quality, increased morbidity in calves born from such cows, and additional economic costs for farms due to veterinary and sanitary measures[1].

Relevance of the topic

Timely diagnosis, effective treatment, and prevention of mastitis and subclinical mastitis caused by opportunistic microorganisms in cattle—staphylococci, streptococci, *Escherichia*, pseudomonads, and *pasteurella*—are urgent problems requiring rapid solutions [2].

In most countries of the world, the incidence of mastitis in livestock production is 20–25% with manual milking and 35–40% with machine milking .

In cows withdrawn from lactation, mastitis occurs in 17.5%, and during the dry period in 20–23.7%. Due to these diseases, the total amount of milk produced decreases by 15–20%, and its fat content by 0.8–1%. In the USA, up to 70% of economic losses in dairy cattle are associated with mastitis. The lifespan of highly productive animals is reduced to 6–8 years. The sanitary quality of milk deteriorates, and products made from such milk become low quality. Cows often become infertile. In young animals, dyspepsia (diarrhea) develops, and when children consume such milk, scarlet fever may occur. Economic losses are caused by reduced milk productivity, forced slaughter or death of cows, and increased veterinary costs[3].

In many cases, unfavorable environmental factors reduce the resistance of farm animals and contribute to the spread of mastitis pathogens. Determining the epizootic situation of infectious mastitis and developing and implementing effective methods of diagnosis, treatment, and prevention considering ecological conditions and zoohygienic management of animals are urgent tasks .

Infectious mastitis is widespread in all livestock farms of our republic, and as known from scientific literature, although certain aspects of the course, diagnosis, and treatment of the disease were studied in the 1980s–1990s, under modern livestock management conditions comprehensive epizootological study of infectious mastitis and the development and implementation of modern control measures are required. The global incidence of infectious mastitis among 211 million head of cattle is about 40%, while in farms of our country it ranges from 8 to 20%. The disease leads to decreased milk productivity, deterioration of technological properties of milk, and often atrophy of udder quarters, making cows unsuitable for further use[4].

Objectives and tasks of the research

Treatment of infectious diseases is carried out using various antibiotics, sulfonamides, and other drugs. However, before their use, it is necessary to determine the sensitivity of pathogens to antibacterial agents. The use of drugs without considering the sensitivity of microorganisms often does not provide the desired effect.

In this regard, in our studies, before treating cows suffering from infectious mastitis, the sensitivity of pathogens (staphylococci, streptococci, enterococci, *Escherichia*, *Proteus*, and *Pseudomonas*) to antibacterial drugs was studied. The sensitivity of these microorganisms to penicillin, streptomycin, sulfadiazine, chloramphenicol, tetracycline, enrofloxacin, kanamycin, monomycin, polymyxin, erythromycin, oleandomycin, tylosin, as well as anti-mastitis preparations Mastisan and Mastitsid, was determined by the indicator disk method[5].

With a diameter of the growth inhibition zone of 15–25 mm, sensitivity was evaluated as moderate; more than 25 mm as high; the absence of a growth inhibition zone indicated resistance of the microorganism to the corresponding antibiotic. The results of the sensitivity study of the above-mentioned staphylococci, streptococci, enterococci, *Escherichia*, *Proteus*, and *Pseudomonas* to antibacterial drugs are presented below.

Materials and Methods

Scientific research work was carried out in 2015–2024 in the Tashkent region: in the Tashkent district at the breeding farm “Rohatoy naslchilik fermer xo’jaligi”, in the Kibray district at the breeding farm “Rihsiboy chorva baraka”, in the Kuyichirchik district at the dairy farm “Sut bulog’i”, in the Yangiyul district at the dairy farm “Yuksalish”, as well as in the educational and experimental farm of the Tashkent State Agrarian University, in the laboratory of the Department of Veterinary Medicine, and in private farms of the population of the Tashkent region. Milk samples obtained from lactating cows kept in these farms and private households were used for diagnosing infectious (clinical and subclinical) forms of mastitis and conducting scientific studies [6].

During the scientific research, experimental work was carried out on 280 head of cattle and 12 rabbits.

Clinical examination of lactating cows was performed by organoleptic inspection of the general condition of the udder and its quarters. Attention was paid to the color of the udder skin, its shape, condition of the teats, presence of pain reaction, udder consistency, local temperature, and the condition of supramammary lymph nodes. The tone and patency of the teat canal, the nature, uniformity, and composition of the secretion, and the presence of flakes and foreign masses in it were determined [7].

To isolate the pathogenic agent in cows suspected of clinical and subclinical mastitis, before sampling milk the teats were treated with 70% ethyl alcohol. From the affected quarter of the udder, 2 ml of pathological material or 4–5 ml of milk were taken into sterile “Eppendorf” tubes. The samples were placed in a thermos with ice for further bacterioscopic and bacteriological examination (isolation of pure culture).

When isolating pure cultures from pathological milk, staphylococci were inoculated onto mannitol-salt agar and salt agar with phenol red, salt agar with egg yolk, milk-salt agar, sucrose agar, agar D, and blood agar; streptococci were cultured on broth with trypan blue or crystal violet (according to the method of N.G. Shatokhin), lactose broth, blood serum, broth with neomycin and bromocresol purple (according to the method of V.M. Kartashova), sugar broth, and alkaline Chesbro-Evans media; *Escherichia*, *Pseudomonas*, *Proteus*, *Citrobacter*, and *Enterobacter* were cultured on MPB, MPA, Endo medium, and Ploskirev medium.

After inoculation of pathological material onto nutrient media, the cultures were incubated in a thermostat at 37°C for 1–3 days, after which the results of inoculation, growth characteristics, and other cultural properties of microorganisms were evaluated [8].

Results and Discussion

In 2015–2018, quarterly diagnostic examinations were carried out in 120–125 lactating cows using dimastin, mastidin, mastitodiagnost, and mastoprim preparations.

In the districts of the Tashkent region (Tashkent, Kibray, Kuyichirchik, and Yangiyul), during 2015–2024, 300–450 cows were examined for infectious clinical and subclinical mastitis, and treatment of sick animals was carried out. In dairy farms where infectious mastitis was recorded, the etiology of the disease was studied in 125 cows, including 25 animals with clinical and 100 with subclinical forms of mastitis. Diagnosis was carried out using the “Mastomer” device.

When studying clinical forms of mastitis in 25 cows, it was found that 5 heads (25.00%) had fibrinous mastitis, 3 heads (11.43%) serous, 4 heads (20.41%) catarrhal, 3 heads (11.35%) hemorrhagic, and 10 heads (40.00%) purulent mastitis.

During bacteriological examination of mastitis, the biological and biochemical properties of 95 strains of staphylococci (25—*Staphylococcus aureus*, 60—*Staphylococcus epidermidis*, 16—*Staphylococcus saprophyticus*), 80 strains of streptococci (40—*Streptococcus dysgalactiae*, 23—*Streptococcus agalactiae*, 6—*Streptococcus faecalis*—enterococci), and 27 strains of other microorganisms (12—*Pseudomonas aeruginosa*, 2—*Escherichia coli*, 2—*Proteus vulgaris*, 5—*Citrobacter*, 6—*Enterobacter*) were studied [9].

Preparations "Mastisan" and "Mastitsid" were studied under test-tube conditions. The sensitivity of microorganisms isolated from udder secretion of cows with mastitis to antibiotics (penicillin, streptomycin, monomycin, tylosin, sulfadiazine, tetracycline, kanamycin, chloramphenicol, oleandomycin, enrofloxacin, polymyxin, erythromycin), as well as to Mastisan and Mastisept, was determined by agar diffusion method using antibiotic-containing indicator disks in accordance with current instructions.

In total, 180 cows infected with infectious mastitis from the above-mentioned regions were treated with antibiotics: 100 cows with Mastisan and 80 cows with Mastisept, and their effectiveness was studied.

For the prevention of infectious mastitis during the dry period, preparations "Mastisan", "Mastisept", and antibiotics were used in 214 cows (Table 1).

Table 1. Sensitivity of bacteria isolated from milk samples of cows with mastitis to antibiotics, as well as to the preparations "Mastisan" and "Mastisept"

Types of microbes	Number of strains	Sensitivity of microorganisms								
		Sensitivity of microorganisms		Moderate sensitivity		Moderate resistance		Resistance		
		Quantity	%	Quantity	%	Quantity	%	Quantity	%	
1	2	3	4	5	6	7	8	9	10	
Ampicillin (HIMEDIA SD077)										
Staphylococcus	120	75	61,53	38	29,85	6	5,02	2	1,6	
Streptococcus	89	33	38,35	32	28,90	13	12,10	19	18,09	
Enterococcus	35	-	-	-	-	2	5,40	35	93,60	
Escherichia	4	-	-	-	-	1	15,70	5	82,55	
Proteus	8	-	-	1	10,11	2	21,26	6	64,77	
Pseudomonas	30	6	21,42	4	09,88	7	24	12	41,88	
Streptomycin (SD091)										
Staphylococcus	120	11	7,85	45	34,60	33	25,33	37	28,89	
Streptococcus	89	56	54,47	36	33,45	75	68,88	2	1,08	
Enterococcus	35	-	-	-	-	3	9,01	34	89,08	
Escherichia	4	-	-	-	-	2	31,44	4	64,86	
Proteus	8	-	-	-	-	3	32,25	6	63,78	
Pseudomonas	30	-	-	6	20,42	7	23	15	52,48	
Sulfadiazine (SD092)										
Staphylococcus	120	-	-	-	-	2	1,44	124	96,55	
Streptococcus	89	-	-	-	-	5	4,89	94	92,99	
Enterococcus	35	-	-	-	-	-	-	37	98,95	
Escherichia	4	-	-	-	-	-	-	6	97,99	
Proteus	8	-	-	-	-	2	21,04	7	76,44	
Pseudomonas	30	-	-	-	-	-	-	28	97,65	
Chloramphenicol (SD006)										
Staphylococcus	120	74	56,44	29	22,44	20	13,56	3	1,99	
Streptococcus	89	11	11,11	31	31,31	21	21,21	36	35,56	
Enterococcus	35	-	-	-	-	11	29,72	26	68,77	
Escherichia	4	6	98,76	-	-	-	-	-	-	
Proteus	8	7	75,88	2	21,82	-	-	-	-	
Pseudomonas	30	9	29,77	8	26,99	5	16,85	6	20,89	
Tetracycline										
Staphylococcus	120	-	-	-	-	10	7,93	116	92,06	
Streptococcus	89	13	12,56	31	30,11	43	42,22	12	11,12	
Enterococcus	35	-	-	-	-	5	11,99	32	85,44	

Escherichia	4	6	99	-	-	-	-	-	-
Proteus	8	4	43,34	3	32,35	1	10,22	1	10,31
Pseudomonas	30	-	-	-	-	2	6,77	26	92,85
Enrofloxacin (SD150))									
Staphylococcus	120	105	83,12	10	7,13	6	3,99	4	3,01
Streptococcus	89	82	80,89	8	6,99	7	7,01	2	1,99
Enterococcus	35	28	74,66	5	12,55	3	8,01	1	2,10
Escherichia	4	6	100	-	-	-	-	-	-
Proteus	8	7	76,67	1	10,01	1	10,71	-	-
Pseudomonas	30	23	81,44	2	6,44	1	3,01	2	6,14
Kanamycin									
Staphylococcus	120	88	68,11	25	18,01	7	4,90	6	4,06
Streptococcus	89	67	66,66	20	19,20	8	7,78	5	4,99
Enterococcus	35	32	84,48	4	10,01	-	-	1	1,70
Escherichia	4	4	65,67	2	32,22	-	-	-	-
Proteus	8	6	64,65	3	32,93	-	-	-	-
Pseudomonas	30	10	33,71	6	20,42	8	27,57	4	13,88
Monomycin									
Staphylococcus	120	115	91,66	4	2,97	4	2,97	2	1,18
Streptococcus	89	95	95,95	3	2,73	-	-	1	1,01
Enterococcus	35	34	90,89	2	5,40	1	2,50	-	-
Escherichia	4	4	65,65	2	33,03	-	-	-	-
Proteus	8	7	76,99	1	10,09	1	10,08	-	-
Pseudomonas	30	23	79,89	3	09,96	-	-	2	6,77
Polymyxin									
Staphylococcus	120	-	-	2	1,58	3	2,22	121	95,47
Streptococcus	89	45	43,52	32	31,44	19	17,87	3	3,03
Enterococcus	35	-	-	2	5,40	3	8,10	32	84,57
Escherichia	4	-	-	-	-	-	-	6	99,89
Proteus	8	-	-	-	-	-	-	9	98,33
Pseudomonas	30	-	-	1	3,57	5	16,85	22	77,64
Erythromycin (SD013)									
Staphylococcus	120	119	92,11	6	4,76	-	-	1	0,79
Streptococcus	89	95	91,44	3	3,03	-	-	1	1,01
Enterococcus	35	32	86,48	3	8,10	-	-	2	5,40
Escherichia	4	-	-	-	-	2	31,24	4	63,67
Proteus	8	-	-	2	21,28	2	21,26	5	54,48
Pseudomonas	30	12	41,85	8	27,67	-	-	8	27,67
Oleandomycin									
Staphylococcus	120	11	7,73	9	7,14	5	3,96	101	79,45
Streptococcus	89	56	53,64	8	8,08	15	14,15	20	19,85
Enterococcus	35	-	-	-	-	-	-	37	95,79
Escherichia	4	-	-	-	-	-	-	6	95,79
Proteus	8	-	-	-	-	-	-	9	95,82
Pseudomonas	30	1	3,57	3	10,71	-	-	24	83,93
Tylosin (SD013)									
Staphylococcus	120	2	1,58	11	8,73	10	7,93	103	80,74
Streptococcus	89	28	28,28	17	17,17	9	9,09	46	44,46
Enterococcus	35	-	-	-	-	-	-	37	95,00
Escherichia	4	-	-	-	-	-	-	6	95,03
Proteus	8	-	-	-	-	-	-	9	95,06
Pseudomonas	30	2	7,14	-	-	4	13,28	21	73,00

Mastisan									
Staphylococcus	120	118	92,65	8	6,34	-	-	-	-
Streptococcus	89	81	80,89	18	18,18	-	-	-	-
Enterococcus	35	31	82,78	6	16,21	-	-	-	-
Escherichia	4	4	64,67	2	32,33	-	-	-	-
Proteus	8	5	53,56	4	41,44	-	-	-	-
Pseudomonas	30	26	91,85	2	7,14	-	-	-	-
Mastisept									
Staphylococcus	120	124	92,51	2	10,68	-	-	-	-
Streptococcus	89	81	83,87	18	17,18	-	-	-	-
Enterococcus	35	32	85,74	5	12,51	-	-	-	-
Escherichia	4	4	64,69	2	31,43	-	-	-	-
Proteus	8	6	61,67	3	31,37	-	-	-	-
Pseudomonas	30	26	89,83	2	7,14	-	-	-	-

The results of scientific studies showed that the sensitivity of staphylococci and streptococci, which are causative agents of infectious mastitis in cows, to antibiotics, as well as to the preparations "Mastisan" and "Mastisept", varies. It was established that about 90% of the isolated strains were sensitive or moderately sensitive to antibiotics, while low sensitivity was observed to ampicillin, streptomycin, sulfadiazine, chloramphenicol, tetracycline, oleandomycin, and tylosin[10].

Results of treatment of cows with infectious mastitis using the preparation "Mastisan"

For the treatment and prevention of infectious mastitis in cows, the preparation "Mastisan" was tested in the above-mentioned livestock farms. Using the preparation "Mastisan", 170 cows with mastitis of staphylococcal and streptococcal etiology were treated, and prevention of possible mastitis in the postpartum period was carried out in 152 cows.

The preparation "Mastisan" was administered at a dose of 5–10 ml using a milk catheter directly into the teat canal of the udder at intervals of 24–36 hours for 7–8 days[11].

Table 2. Results of treatment with Mastisan

No.	Types of mastitis	Number of animals (heads)	Results of treatment with Mastisan			
			Recovery of animals (%)			
			Recovery of productivity (%)	of milk (recovered)	Productivity loss (%)	
			10%	20%		
1	Serous	35	32/80.00		3/7.50	5/12.5
2	Catarrhal	13	13/75.45		3/17.64	1/6.88
3	Fibrinous	20	15/65.21		4/17.39	4/17.39
4	Purulent	12	9/50.00		4/22.22	5/27.77
5	Subclinical	90	86/90.50		5/5.31	3/3.19
	Total	170	155/80.72		19/9.90	18/9.38

In the conducted scientific studies, it was established that the effectiveness of complete treatment of cows with serous mastitis using the preparation "Mastisan" was 80.00%, with catarrhal mastitis – 75.45%, and with purulent mastitis – 50.00%. In cases of subclinical mastitis, the treatment effectiveness reached 90.50%. Thus, the highest effectiveness of the preparation was observed in subclinical mastitis, and the lowest in purulent mastitis.

During the treatment of cows with serous mastitis receiving the preparation "Mastisan", in 10–20% of animals, due to pathological changes of the teats, recovery of milk productivity was incomplete. At the same time, in 50% of cows milk productivity was fully restored, while in the remaining 50% recovery was partial[12].

The average effectiveness of complete treatment of mastitis of various etiologies (serous, catarrhal, fibrinous, purulent, and subclinical) with the preparation "Mastisan" was about 80%[13].

Results of treatment of cows with infectious mastitis using the preparation "Mastisept"

For the treatment and prevention of infectious mastitis in cows, the preparation "Mastisept" was tested in the above-mentioned dairy farms[14]. The results of the use of the preparation are presented in Table 3.

Table 3. Results of treatment of cows with infectious mastitis using the preparation "Mastisept"

No.	Types of mastitis	Number of treated cows (heads)	Treatment with Mastisept		
			Number of animals and degree of recovery (%)		
			Recovery of productivity (%)	Productivity loss (%)	
1	Serous	21	17/80.80	2/9.40	2/9.52
2	Catarrhal	10	8/80.10	1/10.00	1/10.00
3	Fibrinous	9	6/65.66	2/21.20	1/11.9
4	Purulent	6	4/65.60	1/14.60	1/14.60
5	Subclinical	44	42/95.45	1/2.27	1/2.27
	Total	90	77/85.55	7/7.72	6/6.67

Results of the conducted studies and observations[15].**Conclusion**

The effectiveness of complete treatment of cows with the preparation "Mastisept" was:

- in serous mastitis – 80%;
- in catarrhal mastitis – 80.10%;
- in fibrinous mastitis – 65.66%;
- in purulent mastitis – 65.60%.

In cases of subclinical mastitis, the effectiveness reached approximately 85.55%. Thus, the highest effectiveness of the preparation was observed in subclinical mastitis, and the lowest in purulent and fibrinous mastitis (65.60%).

When treating cows with serous mastitis using the preparation "Mastisept", in 10–20% of animals, due to pathological changes of the teats, milk productivity was not fully restored. In purulent mastitis, complete restoration of milk productivity was observed in 65.60% of cows, whereas in the remaining 33% recovery was partial.

The average effectiveness of complete treatment of various forms of mastitis (serous, catarrhal, fibrinous, purulent, and subclinical) with the preparation "Mastisept" was 85.55%, while in 14% of cows milk productivity was not fully restored.

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