



Antibiotic resistance of microorganisms isolated from cows with subclinical mastitis

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Abstract. Cow mastitis is a polyetiological disease involving pathogenic microorganisms. Antibiotics are used to treat sick animals, which contributes to the development of resistant clones of bacteria, and consumption of the corresponding milk leads to the spread of antibiotic-resistant microorganisms. The purpose of the work is to determine strains of antibiotic-resistant bacteria in the milk of cows suffering from subclinical mastitis. Bacteriological studies of mammary gland secretion samples were performed by inoculating the sediment obtained after centrifugation of the examined material on an agarised nutrient medium (with 5% ram erythrocytes). The study of antimicrobial sensitivity was performed by the disk diffusion method. It was established that pathogens with multiple resistance to antibiotics circulate among cows suffering from mastitis on the farm, which indicates their indiscriminate use in animal therapy schemes, in particular mastitis. It was found that the overall level of phenotypic resistance to antimicrobial agents of the examined isolates was within 19-33% (in *Staphylococcus spp.* – 19.9%, in *E. coli* – 33.2%, in *Enterococcus spp.* – 32.5%). The analysis of the results allowed assigning the culture study to the category of multiresistant: 31.6% of cultures of *Staphylococcus spp.* demonstrated resistance to five groups of antibiotics; 54.5% of *Escherichia* cultures – up to five groups of antibiotics; 37.5% of cultures of *Enterococcus spp.* resistant to three groups of antibiotics. It was established that 100% of the studied cultures of *Staphylococcus spp.* are sensitive to netilmicin, sparfloxacin, gatifloxacin and clarithromycin. Therewith, 100% of the examined cultures of *Enterococcus spp.* sensitive to gentamicin, norfloxacin, and nitrofurantoin, and 100% of the examined *E. coli* cultures were sensitive to cephalothin, meropenem, gentamicin, tobramycin, amikacin, nitroxoline, and gatifloxacin. Thus, dairy cows suffering from subclinical mastitis are a source of bacteria with multiple resistance to antibiotics and carry risks for consumers due to the consumption of dairy products contaminated with pathogens of zoonotic diseases, which requires proper control by veterinary service specialists

Keywords: antimicrobial drugs; disk diffusion; antibiotic-resistant bacteria; antibiotic therapy dairy cows; milk

Introduction

One of the key objectives of the animal husbandry industry is to ensure the demand of consumers with safe dairy products of appropriate quality, and the basis for their production is, accordingly, raw milk that corresponds to the requirements of current standards.

In Ukraine, the requirements for the quality of raw milk are harmonised with international standards, which define the microbiological criteria for the quality of raw milk: $\leq 1.0 \times 10^5$ – for milk of the extra grade; $\leq 3.0 \times 10^5$ for high-quality milk; $\leq 5.0 \times 10^5$ for milk of the first grade (DSTU 3662:2018, 2019).

However, in many cases, the sustainable production of livestock products is hindered by diseases, in particular mastitis in lactating cows. Mastitis in cows is an inflammation of the mammary gland under the action of, as a rule, a complex etiological factor with a prominent role of microbial agents (Amer *et al.*, 2018). It is important to consider that the course of mastitis, as indicated by the authors (Dittrich *et al.*, 2019, Blackmon *et al.*, 2023), is associated with a deterioration of the animal's welfare – painful sensations (redness, pain on palpation, swelling and hardening of the skin of the udder) and

deterioration of well-being (fever, anorexia, depression, lethargy, etc.).

The development of the disease is accompanied by significant losses of productivity and a decrease in milk quality indicators (Romero *et al.*, 2018). Significant economic losses are caused both by a decrease in productivity and by low reproduction rates and possible premature culling of cows (Cheng & Han, 2020).

According to many authors, the subclinical form of mastitis is most often registered among lactating cows around the world (Cheng & Han, 2020; Danchuk *et al.*, 2021); (Sharun *et al.*, 2021; Pascu *et al.*, 2022; Saeed *et al.*, 2022), which is accompanied by an increase in the content of somatic cells and changes in its physical and chemical qualities, in particular, the fatty acid composition.

Among the etiological factors of mastitis in cows, microorganisms play an important role, thus, some authors indicate that more than 140 types of microorganisms can be causative agents of mastitis (Goulart & Mellata, 2022). However, the development of mastitis is most often caused by *Staphylococcus aureus*, coagulase-negative *Staphylococcus* (non-aureus), *Escherichia coli*, *Corynebacterium bovis*, *Streptococcus uberis* and *Streptococcus dysgalactiae*, which are considered the most common pathogens of the mammary gland (Heikkilä *et al.*, 2018).

As the authors note (Heikkilä *et al.*, 2018; Goulart & Mellata, 2022), economic losses due to mastitis in cows in the USA are in the range of \$2 billion, with losses from each case estimated at \$326. The course of clinical mastitis caused by pathogenic *Escherichia coli* causes Holstein cows to decrease daily milk yield by almost 15%, which is considered an average milk loss of approximately 200 litres per cow during the 305-day lactation period.

As a result of the analysis of losses during milk production in Finland (Heikkilä

et al., 2018) it was established that mastitis caused by *Escherichia coli* caused the loss of 10.6% of milk from a 305-day milking per day (~3.5 kg/day); lack of milk as a result of mastitis caused by *Staphylococcus aureus* was 7.1% (2.3 kg/day); mammary gland disease caused by *Streptococcus uberis* and *Streptococcus dysgalactiae* led to a shortage of milk in the range of 3.7% (1.2 kg/day) – 6.6% (2.1 kg/day); losses in mastitis caused by *Corynebacterium bovis* amounted to 7.4% (2.4 kg/day); losses in mastitis caused by *Staphylococcus non-aureus* – 5.7% (1.8 kg/day). The authors conclude that systematic measures to prevent and combat mastitis lead to a significant increase in the efficiency of dairy farms. Among the measures designed to combat the spread of mastitis, a particular place is given to specific prevention and immunotherapy (Vasileiou *et al.*, 2019; Chobyryka *et al.*, 2020; Rozos *et al.*, 2022).

Notably, in addition to the risks of reducing the economic efficiency of dairy farms and losses of livestock products, mastitis carries risks to human health – both in an individual aspect due to the risks of consuming dairy products contaminated with zoonotic microorganisms with a high pathogenic potential (Sugrue *et al.*, 2019; Maity & Ambatipudi, 2020), and public health in general – potential spread with milk and dairy products of antibiotics residues and antibiotics resistant to microorganisms, the development of which is caused by the indiscriminate use of antibiotics for the treatment of mastitis (Boireau *et al.*, 2018; Singh *et al.*, 2018), which are critically important for humans, for example – cephalosporins and fluoroquinolones, colistin, tetracycline and macrolides (McEwen & Collignon, 2018).

The purpose of the work was to explore the signs of resistance to antibiotics in microorganisms isolated from cows with subclinical mastitis.

Materials and Methods

The diagnosis of subclinical mastitis was made based on the results of milk studies on the farm from the farms of the Poltava regions using the California mastitis test (Avita test). For microbiological research, udder secretion samples were taken from 23 cows with subclinical mastitis. The study complied with ARRIVE recommendations and was conducted without violating the guidelines of EU Directive 2010/63/EU on the protection of animals used for scientific purposes (2010).

Culture media were prepared and controlled according to ISO 11133:2014 “Microbiology of food, animal feeding stuffs and water” (2014). Preparation, production, storage and performance testing of culture media. The nutrient media, commercial tests, and discs with antimicrobial drugs manufactured by HiMedia were used in the study.

Bacteriological studies of mammary gland secretion samples were conducted by sowing the sediment obtained after centrifugation of the examined material on an agarised nutrient medium (with 5% ram erythrocytes and 0.05% esculin), which ensures the vegetation of most microorganisms that cause the development of mastitis. The isolated microorganisms were subjected to further differentiation according to cultural-morphological, tinctorial signs and the ability of bacteria to metabolise substrates. In addition, for the identification of isolated

bacterial cultures, the relevant recommendations presented in ISO 16649-2:2015 “Microbiology of food and animal feeding stuffs”. Horizontal method for the enumeration of -glucuronidase-positive *Escherichia coli*; SSU 8534: 2015 “Food products. Method for detection and determination of Enterococci” (2017).; ISO 6888-1:2021 (2021) “Microbiology of food and animal feeding stuffs” – Horizontal method for the enumeration of coagulase-positive staphylococci.

Antimicrobial susceptibility testing was performed by the disc diffusion method (considering some provisions of EUCAST, 2021). The results of the study were recorded and interpreted using an automatic colony counter Scan® 500 manufactured by INTERSCI.

Results and Discussion

This research presents the results of the study of selected cultures of zoonotic microorganisms from milk samples obtained from cows with signs of subclinical mastitis. As a result of the study, 23 samples of udder secretion samples were isolated: 19 cultures of *Staphylococcus spp.* (13 coagulase-positive staphylococci (CoPS) and 6 coagulase-negative staphylococci (CoNS), 8 cultures of *Enterococcus spp.* (*Enterococcus faecalis* and *Enterococcus faecium*) and 11 cultures of *Escherichia coli*.

The results of sensitivity/resistance studies of isolates of *Staphylococcus spp.* are presented in Table 1.

Table 1. The sensitivity of *Staphylococcus spp.* isolates from milk samples to antibacterial drugs (n=19)

Antibiotics	Cultures			ATU, mm
	S (n%)	R (n%)	ATU (n%)	
Penicillins				
Benzylpenicillin	9/47.4%	10/52.6%	0	26/26
Ampicillin	7/36.8%	12/63.2%	0	18/18
Oxacillin	3/15.8%	13/68.4%	3/15.8%	17/18

Table 1. Continued

Antibiotics	Cultures		ATU (n%)	ATU, mm
	S (n%)	R (n%)		
Aminoglycosides				
Gentamicin (I)	17/89.5%	2/10.5%	0	22/22
Kanamycin	14/73.7%	3/15.8%	2/10.5%	13/18
Tobramycin (II)	18/94.7%	1/5.3%	0	22/22
Netilmycin	19/100%	0	0	12/15
Amikacin (III)	16/84.2%	3/15.8%	0	22/22
Tetracyclines				
Tetracycline	11/57.9%	6/31.6%	2/10.5%	19/22
Doxycycline	13/68.4%	1/5.3%	5/26.3%	12/16
Quinolones				
Sparfloxacin	19/100%	0	0	15/19
Norfloxacin (II)	17/89.5%	1/5.3%	1/5.3%	17/17
Ciprofloxacin (II)	1/5.3%	5/26.3%	13/68.4%	24/50
Ofloxacin (II)	0	6/31.6%	13/68.4%	17/18
Levofloxacin (II)	0	4/21.1%	15/78.9%	24/50
Pefloxacin (II)	16/84.2%	0	3/15.8%	15/22
Gatifloxacin (IV)	19/100%	0	0	14/18
Lomefloxacin	16/84.2%	0	3/15.8%	18/22
Other antibiotics				
Nitrofurantoin	14/73.7%	5/26.3%	0	13/13
Chloramphenicol	18/94.7%	0	1/5.3%	18/18
Fusidic acid	12/63.2%	0	7/36.8%	24/24
Rifampicin	16/84.2%	1/5.3%	2/10.5%	23/26
Lincomycin	17/89.5%	1/5.3%	1/5.3%	17/21

Note: “S” is sensitive; “R” – resistant; “ATU” – Area of Technical Uncertainty

Source: author’s development

The analysis of the obtained data demonstrated that among the examined staphylococci, 61.5% of the isolates were resistant to penicillins; resistant to aminoglycosides were: 15.7% of isolates – to amikacin and 5.2% – to tobramycin; resistant to tetracycline drugs was 31.6% – to tetracycline and 5.3% – to doxycycline; resistant to drugs of the quinolone group were found: 26.3% – to ciprofloxacin, 31.6% – to ofloxacin, 21.1% – to levofloxacin. 36.8% of

isolates were resistant to fusidic acid, 26.3% to nitrofurantoin, 15.8% to lincomycin, and 5.3% to chloramphenicol. Thus, the circulation of staphylococci with multiple resistance to antimicrobial agents was detected, which indirectly indicates their indiscriminate use for antibacterial therapy of animals on this farm.

Results of sensitivity/resistance studies of isolates of *Enterococcus spp.* are presented in Table 2.

Table 2. The sensitivity of isolates of *Enterococcus spp.* from milk samples to antibacterial preparations (n=8)

Antibiotics	S (n/%)	Cultures		ATU (n/%)	ATU, mm
		R (n/%)			
Penicillins					
Benzylpenicillin	3/37.5%	4/50%		1/12.5%	14/15
Ampicillin	5/62.5%	2/25%		1/12.5%	8/10
Carbapenems					
Imipenem	0	6/75%		2/25%	21/50
Aminoglycosides					
Gentamicin (I)	8/100%	0		0	6/10
Streptomycin	6/75%	1/12.5%		1/12.5%	6/10
Tetracyclines					
Tetracycline	4/50%	3/37.5%		1/12.5%	14/19
Doxycycline	4/50%	2/25%		2/25%	12/16
Quinolones					
Norfloxacin (II)	8/100%	0		0	12/12
Ciprofloxacin (II)	6/75%	2/25%		0	15/15
Levofloxacin (II)	6/75%	2/25%		0	15/5
Gatifloxacin (IV)	3/37.5%	0		5/62.5%	14/18
Other antibiotics					
Nitrofurantoin	8/100%	0		0	15/15
Chloramphenicol	6/75%	1/12.5%		1/12.5%	12/18
Vancomycin	5/62.5%	3/37.5%		0	12/12
Erythromycin	0	2/25%		6/75%	13/23

Note: "S" – sensitive; "R" – resistant; "ATU" – Area of Technical Uncertainty

Source: author's development

The results of determining the antibiotic profile of *Enterococcus* isolates identify resistance to antibiotics from the penicillin group: 25% of isolates to ampicillin and 50% to benzylpenicillin; 75% of the examined isolates were resistant to imipenem; resistance to quinolones was found in: 25% of isolates to

ciprofloxacin and levofloxacin, respectively; resistant to drugs of the tetracycline group, therewith, to tetracycline – 37.5%, to doxycycline – 25%; resistance to chloramphenicol was found in 25% of the examined isolates; 37.5% of enterococci were resistant to vancomycin, 25% were resistant to erythromycin.

Table 3. Sensitivity of *E. coli* isolates from milk samples to antibacterial drugs (n=11)

Antibiotics	S (n/%)	Cultures		ATU (n/%)	ATU, mm
		R (n/%)			
Penicillins					
Ampicillin	4/36.4%	7/63.6%		0	14/14
Ampicillin/sulbactam	10/90.9%	1/9.1%		0	14/14

Table 3. Continued

Antibiotics	Cultures		ATU (n/%)	ATU, mm
	S (n/%)	R (n/%)		
Amoxicillin-clavulanic acid	0	11/100%	0	19/19
Ticarcillin	5/45.5%	4/36.4%	2/18.2%	17/20
Ticarcillin / clavulanic acid	0	6/54.5%	5/45.5%	20/23
Piperacillin	7/63.6%	0	4/36.4%	17/20
Cephalosporins				
Cefalotin (I)	11/100%	0	0	14/18
Cephalexin (I)	11/100%	0	0	14/14
Cefazolin (I)	10/90.9%	1/9.1%	0	14/18
Cefaclor (II)	2/18.2%	5/45.5%	4/36.4%	14/18
Cefuroxime (II)	0	11/100%	0	
Cefamandole (II)	7/63.6%	1/9.1%	3/27.3%	14/18
Cefixim (III)	9/81.2%	2/18.2%	0	17/17
Cefoperazone (III)	8/72.3%	0	3/27.3%	15/21
Cefotaxim (III)	10/90.9%	1/9.1%	0	17/20
Cefepim (IV)	0	10/90.9%	1/9.1%	24/27
Cefpodoxime (III)	11/100%	0	0	21/21
Carbapenems				
Meropenem	11/100%	0	0	16/22
Aminoglycosides				
Gentamicin (I)	11/100%	0	0	17/17
Kanamycin (I)	3/27.3%	0	8/72.3%	13/18
Tobramycin (II)	11/100%	0	0	17/17
Netilmicin	11/100%	0	0	12/15
Amikacin (III)	11/100%	0	0	18/18
Tetracyclines				
Tetracycline	0	11/100%	0	19/19
Doxycycline	1/9.1%	10/90.9%	0	12/16
Quinolones				
Nalidixic acid (I)	8/72.3%	0	3/27.3%	13/19
Nitroxoline (I)	11/100%	0	0	15/15
Ciprofloxacin (II)	10/90.9%	0	1/9.1%	22/25
Ofloxacin (II)	8/72.3%	1/9.1%	2/18.2%	22/24
Lomefloxacin (II)	10/90.9%	0	1/9.1%	18/22
Gatifloxacin (IV)	11/100%	0	0	14/18
Other antibiotics				
Chloramphenicol	11/100%	0	0	17/17

Note: “S” – sensitive; “R” – resistant; “ATU” – Area of Technical Uncertainty

Source: author’s development

Among the studied *Escherichia coli* (Table 3), an average of 43.6% was resistant to Penicillins, 34.7% – from the group of Cephalosporins, 14.5% – from the Aminoglycosides group, 95.5% of *E. coli* cultures were resistant to Tetracyclines, 11.8% – were resistant to Quinolones.

According to the results of the study, it was found that pathogens with multiple resistance to antibiotics are circulating among the cows on this farm, which indicates their indiscriminate use for the antibacterial therapy of animals on this farm.

Thus, as a result of studies of cultures isolated from cows with signs of subclinical mastitis, it was established that 100% of the examined cultures of *Staphylococcus spp.* were sensitive to Netilmicin, Sparfloxacin, Gatifloxacin, Clarithromycin; 100% of the studied cultures of *Enterococcus spp.* were sensitive to Gentamicin, Norfloxacin, Nitrofurantoin; 100% of the studied *E. coli* cultures were sensitive to Cefalotin, Cephalexin, Cefpodoxime, Meropenem, Gentamicin, Tobramycin, Netilmicin, Amikacin, Nitroxoline, Gatifloxacin, Chloramphenicol, and resistant in 100% of cases to Amoxicillin-clavulanic acid, Cefuroxime (II), Tetracycline.

Many researchers have proven that mastitis is one of the most common diseases of dairy cattle, which causes economic losses due to a decrease in milk yield and poor milk quality. It is believed that the main cause of mastitis in cattle is the striking effect of microorganisms with a high pathogenic potential, which causes the development of inflammation of the udder. Measures designed to combat the spread of infectious mastitis have been identified (Bradley, 2002; Blowey & Edmondson, 2010), which include: identification and treatment of clinical cases, teat disinfection after milking, dry cow therapy (DCT), timely culling of cows when chronic cases of mastitis are registered, proper ongoing maintenance of milking equipment.

However, practice demonstrates that the proposed actions are not sufficiently effective. Accordingly, there is an objective need to improve existing mastitis prevention and treatment measures, in particular, the improvement of etiotropic therapy schemes (Down et al., 2016). The data obtained during the analysis of the epizootic situation with mastitis of cows in the studied farm indirectly testify to the imperfection of the implemented preventive and curative measures.

Currently, several microorganisms have been identified as causative agents of mastitis (Heikkilä et al., 2018; Goulart & Mellata, 2022). But, more often, specialists identify representatives of the coccal microflora (*Staphylococcus spp.*, *Streptococcus spp.*, *Enterococcus spp.*) and a prominent representative of enterobacteria – *Escherichia coli*, as factors in the occurrence of mastitis, and, accordingly, in many cases, antibiotics are included in mastitis treatment regimens. The results of this research are consistent with the evidence of other authors regarding (Cheng & Han, 2020) the causative agents of cow mastitis.

Escherichia coli is a microorganism that can have in its arsenal a combination of pathogenicity factors (toxins, adhesins, invasins, capsule development, the ability to resist serum complement and iron absorption, resistance to antibiotics and the ability to colonise, the ability to produce biofilms), which provides the ability to persist in the dairy gland, causing recurrent mastitis (Kaper et al., 2004; Fernandes et al., 2011; Gomes et al., 2016; Cheng & Han, 2020). *Enterococcus spp.* (primarily *E. faecalis* and *E. faecium*) – are frequently obligate microorganisms of organic litter in livestock premises, which have a set of pathogenic factors (toxicity, adhesiveness, antibiotic resistance, ability to form a biofilm, etc.), which provides microorganisms with the opportunity to colonise the epithelium of the mammary gland and persist

causing inflammation (Elhadidy & Zahran, 2014; Rózańska *et al.*, 2019). Representatives of *Staphylococcus spp.*: coagulase-positive staphylococci are recognised causative agents of mastitis in a wide range of animals, and coagulase-negative staphylococci (*S. simulans*, *S. chromogens*, *S. hyicus*, *S. epidermis*) are a relatively “new” causative agent of mastitis – according to researchers (Vasudevan *et al.*, 2003; Taponen & Pyörälä, 2009; Sijojoki *et al.*, 2012; Hamid *et al.*, 2017) diseases caused by them have a less severe course, causing mainly the subclinical form of mastitis and have a set of pathogenicity factors (adhesiveness, resistance to antibiotics, toxigenicity) and are environmental pathogens. Data obtained by the authors in previous years (Ushkalov *et al.*, 2021, Vishovan, *et al.*, 2021) indicate the presence of a high pathogenic potential in potential mastitis pathogens and are consistent with the cited authors.

Thus, one of the important factors in the pathogenicity of mastitis pathogens is resistance to antibiotics. It is known that improper use of antibacterial therapy contributes to the development of antibiotic-resistant clones of microorganisms. Milk obtained from cows suffering from subclinical mastitis potentially poses a threat to human health, as it can be a source of zoonotic pathogens for a long time (asymptomatic course of the disease), causing diseases of the skin, upper respiratory tract, etc. (Schamberger *et al.*, 2004; Maity & Ambatipudi, 2020). In this regard, it is the problem of subclinical mastitis that needs particular attention since bacterial etiological factors have the opportunity for a long time (before the diagnosis and the beginning of therapeutic measures) to pollute the environment, including livestock products and have the potential to spread to people (such as service personnel and on product consumers). Thus, there are objective risks of the spread of zoonotic microorganisms with high pathogenic potential, in particular with

multiple resistance to antibiotics in the human population (Cheng & Han, 2020; Maity & Ambatipudi, 2020; Goulart & Mellata, 2022).

In the presented research, sensitivity to antibiotics in cultures of *Staphylococcus spp.*, *Escherichia coli*, *Enterococcus spp.* was explored within a small sample size, isolated from udder secretion samples of animals suffering from subclinical mastitis on a commodity farm.

It was established that 100% of the examined cultures of *Staphylococcus spp.* were sensitive to Netilmycin, Sparfloxacin, Gatifloxacin, Clarithromycin; 100% of the studied cultures of *Enterococcus spp.* were sensitive to Gentamicin, Norfloxacin, Nitrofurantoin; 100% of *E. coli* cultures tested were sensitive to Cefalotin, Cephalexin, Cefpodoxime, Meropenem, Gentamicin, Tobramycin, Netilmicin, Amikacin, Nitroxoline, Gatifloxacin, Chloramphenicol.

Analysis of the obtained data presented that the overall level of phenotypic resistance of the examined isolates was within 19-33% (19.9% was found among *Staphylococcus spp.*, 33.2% among *E. coli*, 32.5% among *Enterococcus spp.*). When determining sensitivity to antibiotics, EUCAST recommends determining both indicators of sensitivity and resistance and the zone of technical uncertainty – “Area of Technical Uncertainty”. Accordingly, within the “Area of Technical Uncertainty” there were 6.36% of *E. coli* cultures, 12.8% – *Staphylococcus spp.*, 16.3% – *Enterococcus spp.* Thus, the level of resistance among bacterial populations associated with subclinical mastitis may have the corresponding potential for growth.

Further, evaluating the obtained data, an attempt was made to characterise the examined cultures of microorganisms according to the degree of resistance to antibiotics. An international group of experts (Magiorakos *et al.*, 2012) under the auspices of the European Centre for Disease Prevention and Control (ECDC) and the Centres for Disease Control and Prevention

(CDC) of the USA proposed a special terminology to describe acquired resistance profiles. The following resistance profiles are defined: multidrug resistance (MDR) – acquired lack of sensitivity to at least one agent in three or more categories of antimicrobial drugs; extensive drug resistance (XDR) – lack of sensitivity to at least one drug in all but two or fewer categories of antimicrobial drugs (ie, bacterial isolates remain sensitive to only one or two categories); pan-resistance (PDR) – defined as insensitivity to all agents in all categories of antimicrobials. The analysis of the results (Tables 1-3) allowed assigning the study of the culture to the category of MDR. Among the group of *Enterococcus spp.* 37.5% of cultures were resistant to three groups of antibiotics, 25% of cultures were resistant to two groups of antibiotics. Among *Staphylococcus spp.* 31.6% of cultures were resistant to five groups of antibiotic groups, 5.3% of cultures were resistant to four groups. Among *E. coli*, 54.5% of cultures were resistant to five groups of antibiotics, 18.2% of cultures were resistant to four groups of antibiotics.

Considering the small size of the sample, the presented results are indicative and will determine the field of further research on the study of pathogenicity (phenotypic and genotypic determinants) and virulence in microorganisms associated with subclinical mastitis.

Conclusions

It was established that cultures of *Staphylococcus spp.* isolated from milk from cows with signs of subclinical mastitis showed sensitivity to Netilmycin, Sparfloxacin, Gatifloxacin, Clarithromycin in 100% of cases; cultures of *Enterococcus spp.* in 100% of cases were sensitive to Gentamicin, Norfloxacin, Nitrofurantoin; *E. coli* cultures showed sensitivity to Cefalotin, Cephalexin, Cefpodoxime, Meropenem, Gentamicin, Tobramycin, Netilmicin, Amikacin, Nitroxoline, Gatifloxacin, Chloramphenicol in

100% of cases. The level of resistance of the examined isolates was in the range of 19-33%, the analysis of the results allowed classifying the study isolates into the MDR category. Thus, milk from cows with subclinical mastitis is a risk factor for the spread of antibiotic-resistant bacterial clones. Measures at the level of individual livestock farms designed to reduce the risks of the spread of antibiotic-resistant microorganisms, in particular, etiologically significant in mastitis in cows, focus on ensuring the well-being of animals, increasing the level of biosecurity and introducing the technology of production of livestock products, elements of permanent monitoring of the condition of the udder, timely diagnosis of mastitis, if necessary – laboratory and it is possible to use means of extemporaneous formulation and autogenous immunobiological drugs – as an alternative to antimicrobial agents. There is reasonable evidence that preventive measures using autogenous vaccines, sera and globulins can be of significant importance for reducing the level of use of antimicrobial substances in the production of livestock products, which will contribute to the disruption of the food route of transmission of resistant populations of bacteria and metabolites of antimicrobial drugs. It is believed that autogenous immunobiological drugs can be used both for specific prevention and for the treatment of persistent infections.

Further research can be designed to establish a collection of strains of microorganisms – the causative agents of mastitis, studying the signs of pathogenicity in them and their possible use for the production of an autovaccine for the prevention of this dangerous disease.

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Conflict of Interest

None.

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Анотація. Мастит корів – поліетіологічне захворювання за участю патогенних мікроорганізмів. Для лікування хворих тварин використовуються антибіотики, що сприяє утворенню стійких клонів бактерій, а споживання відповідного молока призводить до поширення стійких до антибіотиків мікроорганізмів. Мета роботи – визначити штами антибіотикорезистентних бактерій у молоці корів, хворих на субклінічний мастит. Бактеріологічні дослідження проб секрету молочної залози проводили шляхом посіву осаду, отриманого після центрифугування досліджуваного матеріалу, на агаризоване живильне середовище (з 5 % еритроцитів барана). Дослідження антимікробної чутливості здійснювали методом дискової дифузії. Встановлено, що серед хворих на мастит корів у господарстві циркулюють збудники з множинною стійкістю до антибіотиків, що вказує на їх невибіркове застосування в схемах терапії тварин, зокрема маститу. Виявлено, що загальний рівень фенотипової стійкості до антимікробних засобів досліджуваних ізолятів знаходився у межах 19-33 % (у *Staphylococcus spp.* – 19.9 %, у *E. coli* – 33.2 %, у *Enterococcus spp.* – 32.5 %). Аналіз

результатів дав можливість віднести дослідженні культури до категорії мультирезистентних: 31.6 % культур *Staphylococcus spp.* виявляли стійкість до п'яти груп антибіотиків; 54.5 % культур ешерихій – до п'яти груп антибіотиків; 37.5 % культур групи *Enterococcus spp.* стійкі до трьох груп антибіотиків. З'ясовано, що 100 % досліджених культур *Staphylococcus spp.* є чутливими до нетилміцину, спарфлоксацину, гатифлоксацину та кларитроміцину. При цьому, 100 % досліджених культур *Enterococcus spp.* чутливі до гентаміцину, норфлоксацину та нітрофурантоїну, а 100 % досліджуваних культур кишкової палички виявилися чутливими до цефалотину, меропенему, гентаміцину, тобраміцину, амікацину, нітроксоліну та гатифлоксацину. Таким чином, дійні корови, хворі на субклінічний мастит, є джерелом бактерій з множинною стійкістю до антибіотиків і несуть ризики для споживачів внаслідок вживання молочних продуктів, контамінованих збудниками зоонозних захворювань, що потребує належного контролю з боку фахівців ветеринарної служби

Ключові слова: антимікробні препарати; дискова дифузія; антибіотикорезистентні бактерії; антибіотикотерапія; дійні корови; молоко