Pathogens isolated from bovine clinical mastitis and their antimicrobial resistance

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Received 27.05.2021

Accepted 30.09.2021

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Summary

This study aimed to isolate aerobic and microaerophilic bacteria from mastitis milk samples, as well as to determine their antibiotic resistance. A total of 196 bovine mastitis milk samples were tested by standard bacteriological methods and with API identification test kits. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method. The results revealed that the predominant isolate was *S. aureus*, with an isolation rate of 28%, followed by *Streptococcus* spp. (27%) and *E. coli* (19%). Isolation rates for *Corynebacterium* spp., *Mycoplasma* spp., and *Pseudomonas aeruginosa* were 11%, 6%, and 4%, respectively. Compared to the bacteria mentioned above, lower percentages were observed for *Trueperella pyogenes* (2%), *Pasteurella multocida* (2%), and *Klebsiella pneumoniae* (1%). A broad evaluation of antimicrobial resistance showed that the pathogens were resistant to tetracycline (68.63%), oxytetracycline (41.57%), ampicillin (39.08%), ceftiofur (38.1%), cephalexin (32.26%), penicillin (31.25%), amoxicillin/clavulanic acid (24.53%), enrofloxacin (24.44%), gentamycin (23.68%), and trimethoprim/sulfamethoxazole (22.09%). This study demonstrated that the sources of bacteria isolated from mastitis bovine milk samples were both contagious and environmental. More importantly, the present results demonstrate a critically high antimicrobial resistance in dairy cattle. For instance, *E. coli* isolates showed a crucial resistance to commonly used and recommended antimicrobials, including ceftiofur (100%), cephalexin (83.33%), and tetracycline (94.44%). The results of this study may provide valuable information about clinical aspects of bovine mastitis infections and current antimicrobial resistance levels in dairy cattle.

Keywords: mastitis, antimicrobial resistance, dairy cattle, bacterial strains

Mastitis is an inflammation of the mammary gland (3, 21). It decreases the amount of milk produced as well as milk quality, thus leading to significant economic losses. This disease is the most common and costly disease of dairy cows, and it is also one of the main reasons for the use antimicrobials in dairy herds (23).

A wide variety of microorganisms (over 135) have been associated with mastitis in dairy cattle. In general, mastitis agents can be categorized as contagious or environmental according to their primary sources. While *Staphylococcus aureus* (*S. aureus*), *Streptococcus agalactiae* (*Str. agalactiae*), and *Mycoplasma* spp. can be classified as contagious, *Escherichia coli* (*E. coli*), *Streptococcus dysgalactiae* (*Str. dysgalactiae*), *Klebsiella* spp., *Pseudomonas aeruginosa*, and *Trueperella pyogenes* are considered as environmental (3, 13).

Antibiotic usage is one of the most common treatment approaches to reduce intramammary infection

and, thus, the prevalence of mastitis in the herd. However, non-essential use of antibiotics for the treatment of mastitis or any other infection may result in the emergence of bacteria resistant to these antimicrobials. Thus, the knowledge of bacteria responsible for mastitis and of their profile of susceptibility to antimicrobials should be improved to control the disease (21).

The development of resistance by the bacteria that cause mastitis has a negative impact on the effectiveness of common treatment options and their capacity to control the disease. Hence, the identification of agents and determination of their antimicrobial susceptibility may play a major role in effective treatment (23). The levels of antimicrobial resistance vary over the years, as indicated by many previous studies, and there is a strong need for up-to-date information on resistance levels to develop an effective treatment of mastitis and to re-evaluate the antimicrobial choices (4, 20). Therefore, the main objective of the present study was to isolate bacterial agents from bovine mastitis

milk samples and to determine their antimicrobial resistance.

Material and methods

Samples. In this study, 196 bovine mastitis milk samples were examined for the presence of aerobic and microaerophilic bacteria (especially *Mycoplasma* species). All samples were obtained from Holstein-Friesian cows ($n = 196$) with signs of clinical mastitis. It was confirmed that no antimicrobial treatment had been given close to the sampling time. The samples to be tested were kept in sterile tubes and were transported to the laboratory in a cold chain.

Culture. The samples were cultured by the standard methods (22). Briefly, for bacteria culture, a 50 μ l sample was streaked on a pair of 5% sheep blood agar (GBL, Istanbul, Turkey), MacConkey agar (Merck, Darmstadt, Germany), and Eosine Methylene Blue (EMB) agar plates (Merck, Darmstadt, Germany). Afterwards, one series of plates were incubated at 37°C, aerobically, while the other series of plates were allowed to incubate in a 10% CO₂ atmosphere for 2 to 3 days. Colonies on agar plates were identified using an identification test kit (API, bioMérieux S.A., Marcy-l'Etoile, France). Catalase-positive Gram-positive cocci, coliform bacteria, Gram-negative bacilli, and Gram-negative cocci were identified using API Staph, API 20 E, API NH, and API NE, respectively. *Streptococcus* spp. and *Corynebacterium* spp. were identified by their gross morphology, Gram staining, catalase, and further biochemical tests were performed according to Quinn et al. (22). For *Mycoplasma* isolation, 50 μ l of each milk sample was plated on Mycoplasma Agar (Oxoid, Basingtoke, Hampshire, England), which was incubated at 37°C in a 10% CO₂ atmosphere for up to seven days. *Mycoplasma* was identified at the genus level by the appearance of a typical colony on Mycoplasma agar plates examined under a stereomicroscope (Olympus-SZ61, Tokyo, Japan).

Antimicrobial susceptibility test. The antimicrobial susceptibility test was performed by the Kirby-Bauer disk diffusion method with Mueller-Hinton Agar (Oxoid, Basingtoke, Hampshire, England). The results were evaluated according to the breakpoints of the Clinical and Laboratory Standards Institute (CLSI) (30). The antimicrobial susceptibility test was carried out for the following antibiotics,

which are currently used in veterinary practice: penicillin G (10 IU), ampicillin (10 μ g), amoxicillin/clavulanic acid (30 μ g), ceftiofur (30 μ g), cephalexin (30 μ g), gentamycin (10 μ g), oxytetracycline (30 μ g), tetracycline (10 μ g), trimethoprim/sulfamethoxazole (25 μ g), and enrofloxacin (5 μ g). All discs were obtained from Oxoid (Oxoid, Wade Road, Basingtoke, England). Reference bacterial strains, *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923), were used as control strains.

Data analysis. The prevalence rate for every pathogen isolated and the presence of multiple infections were calculated according to the diagnosis records. Antibiotics to which most of the pathogens were resistant were determined by *in vitro* antimicrobial susceptibility tests. Minitab Software (v17.1.0, the U. S.) was used to analyze the data.

Results and discussion

Mastitis is an economically important, prevalent, and complex disease, which, in dairy cattle, is caused mostly by infectious agents, such as bacteria, algae, and yeast. Given the high demand for milk worldwide, bovine mastitis has been increasingly important because not only does it decrease the milk yield, but also reduces milk quality. Therefore, it is essential to monitor the causative agents in order to choose an adequate antimicrobial, and thus protect herds from infection by mastitis pathogens (32). In this study, bacterial strains and their respective antimicrobial resistance were evaluated in milk samples obtained from dairy cows with signs of clinical mastitis. Out of a total of 196 milk samples, 36 samples (18.36%) were found to be culture negative. According to Kuehn et al. (15), approximately 10-40% of clinical mastitis cases yield "no significant growth" in routine clinical culture assays, and the reason for this situation seems to be contentious. Factors related to laboratory protocols, such as the low concentration of bacteria, the expertise of the technician, and the duration of incubation, can affect the sensitivity of culture (14). On the other hand, samples with no bacterial growth derived from cows with mastitis confirm the fact that bacterial infection is not the only cause of mastitis. Different factors in mastitis etiology, including fungi, viruses, and *Chlamydia*, have been reported by several researchers. Mastitis may occasionally also be related to traumatic or toxic factors (13, 31).

Culture results revealed that 160 out of the 196 milk samples (81.63%) were positive, while no growth was observed in the cultures of 36 samples (18.37%). The number of isolates from the 160 samples and their percentages are shown in Table 1. The predominant isolate from milk samples was *S. aureus*, which is a contagious mastitis agent. The other bacteria were *Streptococcus* spp., *E. coli*, *Corynebacterium* spp., *Mycoplasma* spp., *Pseudomonas aeruginosa*, *Trueperella pyogenes*, *Pasteurella multocida*, and *Klebsiella pneumoniae*. Among the 160 samples, 32 samples (20%) contained mixed bacterial infections, whilst 128 (80%) showed

Tab. 1. Number and percentage of bacteria from 160 mastitis milk samples

Isolate	Number of isolates	Culture percentage
<i>Staphylococcus aureus</i>	56	28
<i>Streptococcus</i> spp.	54	27
<i>Escherichia coli</i>	38	19
<i>Corynebacterium</i> spp.	22	11
<i>Mycoplasma</i> spp.	12	6
<i>Pseudomonas aeruginosa</i>	8	4
<i>Trueperella pyogenes</i>	4	2
<i>Pasteurella multocida</i>	4	2
<i>Klebsiella pneumoniae</i>	2	1
Total number	200	100

Tab. 2. Mixed bacterial agents isolated from mastitis milk samples

Bacterial strains	Number of samples
<i>Staphylococcus aureus</i> + <i>Corynebacterium</i> spp.	2
<i>Staphylococcus aureus</i> + <i>Mycoplasma</i> spp.	2
<i>Staphylococcus aureus</i> + <i>Streptococcus</i> spp.	2
<i>E. coli</i> + <i>Klebsiella pneumoniae</i>	2
<i>E. coli</i> + <i>Trueperella pyogenes</i>	2
<i>E. coli</i> + <i>Pasteurella multocida</i>	2
<i>E. coli</i> + <i>Streptococcus</i> spp.	2
<i>E. coli</i> + <i>Corynebacterium</i> spp.	2
<i>Streptococcus</i> spp. + <i>Corynebacterium</i> spp.	6
<i>Streptococcus</i> spp. + <i>Trueperella pyogenes</i>	2
<i>Staphylococcus aureus</i> + <i>Streptococcus</i> spp. + <i>Corynebacterium</i> spp.	2
<i>Staphylococcus aureus</i> + <i>E. coli</i> + <i>Pseudomonas aeruginosa</i>	2
<i>Mycoplasma</i> spp. + <i>E. coli</i> + <i>Pseudomonas aeruginosa</i>	2
<i>Mycoplasma</i> spp. + <i>Pseudomonas aeruginosa</i> + <i>Corynebacterium</i> spp.	2
Total number	32

mono-bacterial infections. A diagnosis of mixed etiology was made in 32 cases: 24 of them were dual, and 8 of them were triple mastitis pathogens, as shown in Table 2.

S. aureus and *Streptococcus* spp. have been reported as common bacterial factors involved in the development of clinical (9) and subclinical mastitis (27). In this study, *S. aureus* (28%) was the predominant pathogen in clinical mastitis milk samples, which is in agreement with studies conducted in Jordan (53.7%) (1) Canada (21.7%) (24), Tanzania (25.7%) (13), Ethiopia (28.81%) (16), and Turkey (34.3% and 26.5%) (12, 25). *S. aureus* is the most frequent major contagious pathogen transmitted by direct contact with infected milk. The presence of high numbers of these isolates may be due to poor milking hygiene associated with contaminated milkers' hands and equipment. Another agent with a high isolation rate was *Streptococcus* spp. (27%). Lakew et al. (16) also report that the second most dominant bacteria in their study were *Streptococcus* spp. (25.42%). These bacteria can be transmitted through both lateral and environmental contagion. Management factors concerning biosecurity procedures in dairy herds should be considered to prevent mastitis caused by these bacteria.

In dairy cows, *E. coli* is one of the most important agents that cause mastitis, during calving period and early lactation. It may induce local and sometimes severe systemic clinical signs in high producing cows (7). Therefore, the evaluation of *E. coli* prevalence may play an important role in understanding the etiology of mastitis. In this study, the percentage of *E. coli* in the clinical mastitis milk samples was 19%. In the literature, the frequency of *E. coli* in herds suffering from mastitis varies widely. Malinowski et al. (17) showed that *E. coli* dominated among Gram-negative

bacilli in herds located in Poland. Bradley et al. (5) reported that agents isolated most frequently from clinical mastitis samples were *Str. uberis* (23.5%) and *E. coli* (19.8%). Similarly, Breen et al. (6) indicated that predominant isolates were *E. coli* and *Str. Uberis*, with frequencies of 26.7% and 18.9%, respectively. It is worth noting that environmental and individual cow factors influence mastitis etiology independently, and the severity of mastitis caused by *E. coli* is determined primarily by cow factors (7). The results of the present study and many previous studies show that bacterial strains isolated from mastitis milk samples are similar, but the percentage of bacteria may vary noticeably because of differences in farm conditions, milking management, and prophylactic procedures (5, 6).

Corynebacterium spp. is a minor pathogen associated with moderate infections and rarely with clinical signs (3). In this study, however, *Corynebacterium* spp. made up 11% of all isolates. It is difficult to compare our proportion of *Corynebacterium* spp. with those in other studies, in particular, studies on clinical mastitis, because *Corynebacterium* spp. is seldom reported (29) and usually isolated from sub-clinical cases (28).

Other important aetiological factors in clinical mastitis are *Mycoplasma* spp., *Pseudomonas aeruginosa*, *Trueperella pyogenes*, *Pasteurella multocida*, and *Klebsiella pneumoniae*. In this study, the percentages of these bacteria (6%, 4%, 2%, 2%, and 1%, respectively) were remarkably low compared to those of major mastitis agents, such as *S. aureus*, *Streptococcus* spp., and *E. coli*. Comparable to our results, Breen et al. (6) reported the corresponding frequencies as follows: 2.3% for *Corynebacterium* spp., 0.5% for *Klebsiella* spp., 0.3% for *Pseudomonas* spp., and 0.2% for *Trueperella pyogenes*. On the other hand, the percentages determined in clinical mastitis samples by Riekerink et al. (24) were 9.1% for *Klebsiella* spp., 2.6% for *Trueperella pyogenes*, 1.6% for *Pseudomonas* spp., 0.4% for *Corynebacterium* spp., and 0.1% for *Pasteurella multocida*.

Apart from the isolation and identification of mastitis agents, an important point that must not be overlooked is that the determination of antimicrobial resistance plays an essential role in evaluating the prognosis for the mastitis infection process. Adequate antimicrobial innovation requires a multifaceted approach that includes both avoidance of unnecessary use of antibiotics and making the best choice of available antimicrobial agents (20, 32). The results of antimicrobial susceptibility tests for the isolates are shown in Table 3. The tests on *E. coli* strains revealed 100% resistance to

Tab. 3. Number and percentage of isolates resistant to antimicrobial agents

Isolates	Antimicrobial agents																			
	P		AMP		AMC		EFT		CL		CN		ENR		OT		TE		SXT	
	TA	NR	TA	NR	TA	NR	TA	NR	TA	NR	TA	NR	TA	NR	TA	NR	TA	NR	TA	NR
<i>Staphylococcus aureus</i>	46	16	46	16	32	0	52	2	42	0	36	12	48	12	54	22	30	14	56	0
	34.78%		34.78%		0		3.84%		0		33.33%		25%		40.74%		46.67%		0	
<i>Streptococcus</i> spp.	52	16	52	18	24	0	50	16	24	0	44	12	54	10	54	24	22	16	50	16
	30.77%		34.61%		0		32%		0		27.27%		18.52%		44.44%		72.73%		32%	
<i>Escherichia coli</i>	NT		38	20	32	18	36	36	36	30	38	12	38	10	36	10	36	34	32	6
	NT		52.63%		56.25%		100%		83.33%		31.58%		26.32%		27.78%		94.44%		18.75%	
<i>Corynebacterium</i> spp.	20	2	20	2	8	0	18	0	8	0	18	0	22	6	20	10	6	0	20	6
	10%		10%		0		0		0		0		27.27%		50%		0		30%	
<i>Pseudomonas aeruginosa</i>	4	4	8	8	4	4	8	8	8	8	8	0	8	2	8	6	4	4	8	6
	100%		100%		100%		100%		100%		0		25%		75%		100%		75%	
<i>Trueperella pyogenes</i>	4	0	4	0	NT		NT		NT		4	0	4	0	NT		NT		NT	
	0		0		NT		NT		NT		0		0		NT		NT		NT	
<i>Pasteurella multocida</i>	2	2	4	2	4	2	2	2	4	2	2	0	4	2	4	0	2	0	4	2
	100%		50%		50%		100%		50%		0		50%		0		0		50%	
<i>Klebsiella pneumoniae</i>	NT		2	2	2	2	2	0	2	0	2	0	2	2	2	2	2	2	2	2
	NT		100%		100%		0		0		0		100%		100%		100%		100%	

Explanations: P – penicillin G; AMP – ampicillin; AMC – amoxicillin/clavulanic acid; EFT – ceftiofur; CL – cephalixin; CN – gentamycin; ENR – enrofloxacin; OT – oxytetracycline; TE – tetracycline; SXT – trimethoprim/sulfamethoxazole; TA – total number of isolates tested by antibiogram; NR – number of resistant bacteria; NT – not tested

ceftiofur, 94.44% to tetracycline, and 83.33% to cephalixin. The other predominant bacteria, *S. aureus*, and *Streptococcus* spp., showed lower antimicrobial resistance compared to *E. coli*. It is worth noting that the resistance of *Streptococcus* spp. and *S. aureus* strains to tetracyclines (tetracycline and oxytetracycline) was higher than it was to the other antibiotics.

Most of the isolates were resistant to antimicrobials used in this study: tetracycline (68.63% of isolates), oxytetracycline (41.57%), ampicillin (39.08%), ceftiofur (38.1%), cephalixin (32.26%), penicillin (31.25%), amoxicillin/clavulanic acid (24.53%), enrofloxacin (24.44%), gentamycin (23.68%), and trimethoprim/sulfamethoxazole (22.09%). One hundred and six isolates were resistant to two or more antimicrobial agents (Tab. 4). Among those isolates, 12 isolates of *S. aureus* had the highest rate of resistance to the penicillin/ampicillin/tetracycline/oxytetracycline/enrofloxacin/gentamycin combination. Resistance to ceftiofur/cephalexin/tetracycline was found in 10 isolates of *E. coli*.

The present results highlight the importance of potential antimicrobial resistance in bovine mastitis. In this study, a high percentage of resistance to tetracyclines (46.67% and 40.74% for tetracycline and oxytetracycline, respectively) was observed in *S. aureus* isolates, which were comparable to penicillin (34.78% for penicillin and ampicillin) and gentamycin (33.33%). The percentages of resistance to enrofloxacin (25%) and ceftiofur (3.84%) were relatively low

compared to those for the aforementioned antibiotics. McDougall et al. (18) and Muhamed et al. (19) determined similar resistance percentages for penicillin (28% and 41%, respectively). In contrast, Aleksh et al. (1), Gurler et al. (10), and Suleiman et al. (26) reported noticeably high percentages of resistance to penicillin (87-100%). The results of the present study suggest that the number of Gram-positive bacteria isolated from mastitis that are resistant to tetracyclines is increasing, whereas β -lactam resistant strains were few. Widespread resistance to tetracyclines may be due to their widespread usage for treating mastitis. Therefore, the determination of tetracycline resistance levels is especially important for effective treatment of mastitis.

In this study, *E. coli* isolates were highly resistant to cephalosporins (100% for ceftiofur and 83.33% for cephalixin) and tetracycline (94.44%), and moderately resistant to penicillins (52.63% for ampicillin and 56.25% for amoxicillin/clavulanic acid). One of the most critically important antibiotics in the treatment of *E. coli* mastitis is cephalosporins. There is some notable scientific evidence that these drugs are effective against mastitis caused by *E. coli*, which has considerably low resistance to them. Previous studies have reported very low or nil resistance to ceftiofur in European countries (2, 11). However, it is important to note that *E. coli* isolates from dairy cattle show an increase in resistance, especially, to third-generation cephalosporins (4). Our results are in agreement with the above findings. This situation may cause more seri-

Tab. 4. Resistance profiles of isolates to at least two antimicrobial agents

Antimicrobial agents	NI	Isolates
AMP+P	4	<i>Staphylococcus aureus</i>
TE+SXT	2	<i>Streptococcus</i> spp.
OT+TE	8	<i>Streptococcus</i> spp.
OT+ENR	4	<i>Streptococcus</i> spp.
OT+ENR	4	<i>Corynebacterium</i> spp.
EFT+CL+TE	10	<i>Escherichia coli</i>
EFT+AMP+AMC	2	<i>Escherichia coli</i>
SXT+AMP+P	2	<i>Streptococcus</i> spp.
SXT+AMP+P	2	<i>Corynebacterium</i> spp.
SXT+OT+ENR	2	<i>Corynebacterium</i> spp.
AMP+P+CL+OT	4	<i>Streptococcus</i> spp.
AMP+P+SXT+CN	2	<i>Streptococcus</i> spp.
EFT+AMP+TE+CL	4	<i>Escherichia coli</i>
EFT+AMP+TE+AMC	2	<i>Escherichia coli</i>
EFT+CL+TE+AMC	4	<i>Escherichia coli</i>
EFT+CL+TE+AMC+CN	4	<i>Escherichia coli</i>
EFT+CL+OT+SXT+AMP	4	<i>Pseudomonas aeruginosa</i>
EFT+TE+OT+P+CN	2	<i>Streptococcus</i> spp.
EFT+TE+OT+SXT+AMP	4	<i>Streptococcus</i> spp.
EFT+SXT+AMP+P+ENR+CN	6	<i>Streptococcus</i> spp.
TE+OT+AMP+P+ENR+CN	12	<i>Staphylococcus aureus</i>
EFT+CL+TE+AMP+AMC+P	2	<i>Pseudomonas aeruginosa</i>
TE+OT+SXT+AMP+AMC+ENR	2	<i>Klebsiella pneumoniae</i>
EFT+CL+TE+OT+AMP+ENR+CN	4	<i>Escherichia coli</i>
EFT+CL+SXT+AMP+P+AMC+ENR	2	<i>Pasteurella multocida</i>
EFT+TE+OT+SXT+AMP+AMC+ENR	2	<i>Escherichia coli</i>
EFT+CL+TE+OT+SXT+AMP+AMC+ENR	2	<i>Escherichia coli</i>
EFT+CL+TE+OT+SXT+AMP+AMC+ENR+CN	2	<i>Escherichia coli</i>
EFT+CL+TE+OT+SXT+AMP+AMC+ENR+P	2	<i>Pseudomonas aeruginosa</i>

Explanations: P – penicillin G; AMP – ampicillin; AMC – amoxicillin/clavulanic acid; EFT – ceftiofur; CL – cephalixin; CN – gentamycin; ENR – enrofloxacin; OT – oxytetracycline; TE – tetracycline; SXT – trimethoprim/sulfamethoxazole; NI – number of isolates

ous problems in treatment for bovine *E. coli* mastitis in the future. Furthermore, the present results reveal a remarkable rise in resistance to many drugs that are relatively cheap and easily accessible. Indeed, two of the most important factors in resistance dynamics are the extensive usage and the prices of drugs. The use of antibiotics in animals intended for human consumption should be limited to specific indications and based on bacteriological diagnosis. On the other hand, lower levels of resistance were observed for gentamycin (31.58%), oxytetracycline (27.78%), enrofloxacin (26.32%), and trimethoprim/sulphamethoxazole (18.75%). Comparable results have been reported by Aleksh et al. (1) and Suleiman et al. (26). On the other hand, Giannechini et al. (8) and Aleksh et al. (1) have

reported considerably higher resistance to oxytetracycline and trimethoprim/sulphamethoxazole. Various levels of resistance to commonly used antimicrobials are reported in the literature, but the main point is that there has been a remarkable increase in antimicrobial resistance in *E. coli* mastitis cases over the years (4). The present study has revealed alarming levels of resistance of *E. coli* to antimicrobial agents commonly used in the field. More studies are needed to obtain reliable information about the current resistance dynamics of *E. coli* and future clinical perspectives in dairy cattle.

Multidrug-resistant pathogens have been a growing concern not only for livestock science/animal breeding, but also for clinical procedures in veterinary medicine (32). In this study, a remarkable multi-drug resistance was observed for various combinations of antibiotics. As many as 53% of all strains showed resistance, and among them, *S. aureus* and *E. coli* isolates seemed to have high levels of multi-drug resistance. For instance, 10 isolates of *E. coli* showed resistance to at least seven different types of antibiotics (Tab. 4). Similar results were obtained for *S. aureus* (12 isolates were resistant to six different antimicrobials). It is important to note that multi-drug resistance is a very important threat to the control of mastitis because it reduces the number of applicable antibiotics (20). The occurrence of multi-drug resistance may be related to unnecessary and injudicious use of antibiotics and the adaptation of pathogens to different antimicrobials. The results of the present study highlight a very crucial problem in mastitis medication because the above-mentioned resistance can influence the effectiveness of treatment in dairy cattle.

In this study, a detailed microbiological analysis of bovine mastitis milk samples was performed to detect the presence of aerobic and microaerophilic bacteria and to determine their antimicrobial susceptibility. Gram-positive cocci were the most common species detected in the mastitis milk samples. The importance of environmental pathogens, such as *E. coli* and *Pseudomonas aeruginosa*, was also undeniable. *S. aureus* was the most frequent contagious pathogen, and it was found to be highly resistant to tetracycline and oxytetracycline. Another important finding is that 53% of isolates showed multi-drug resistance to up to nine different antimicrobial agents. Overall, our results indicate a critical rise in antimicrobial resistance in dairy cattle. According to bacteriological diagnosis, the main sources of pathogens were both contagious and environmental. Moreover, the results of our study indicate an alarming resistance of *E. coli* to commonly used and recommended antimicrobials, including ceftiofur, cephalixin, and tetracycline. The knowledge of drug resistance trends in mastitis cases can promote more effective and responsible use of antibiotics, which is required for effective treatment of mastitis in dairy cattle.

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