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Incidence rate of clinical mastitis and antibiotic susceptibility of mastitis-producing pathogens in dairy cattle from Northern Antioquia, Colombia*

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Abstract

Introduction: Mastitis is defined as the inflammation of the mammary gland causing great economic losses in dairy herds, which merits investigation. *Objective:* To determine the incidence rate of clinical mastitis IRCM, associated etiology, and antibiotic sensitivity of pathogens isolated from cases of mastitis in dairy cattle. *Materials and methods:* 37 herds were followed during 12 months for clinical mastitis (CM) diagnosis. Milk samples from CM cases were bacteriologically cultured, which underwent, along with isolates from subclinical mastitis cases from a previous study, antibiotic susceptibility testing. For data analysis, standard descriptive statistics were used. *Results:* The IRCM was 13.8 cases per 100 cows-year at risk. A total of 188 pathogens of CM cases were isolated, among which *Streptococcus agalactiae*, *Streptococcus pyogenes*, and *Corynebacterium* spp. were the most frequent, with 29.8%, 11.7%, and 5.9%, respectively. A high susceptibility of contagious bacteria was found for cloxacillin and cefoperazone. *Conclusions:* The present study found an IRCM of 13.8 cases per 100 cows-year at risk. The most prevalent pathogens identified in cases of CM were contagious. A high sensitivity of contagious bacteria was found for most β-lactam antibiotics.

Keywords: cattle, milk, mastitis, antibiotic susceptibility test, antimicrobial resistance, incidence rate of clinical mastitis.

Tasa de incidencia de mastitis clínica y susceptibilidad antibiótica de patógenos productores de mastitis en ganado lechero del norte de Antioquia, Colombia

Resumen

Introducción: la mastitis se define como la inflamación de la glándula mamaria causante de grandes pérdidas económicas en hatos lecheros, lo que amerita su investigación. *Objetivo:* determinar la tasa de incidencia de mastitis clínica TIMC, la etiología asociada y la sensibilidad antibiótica de patógenos aislados de casos de mastitis en ganado lechero. *Materiales y métodos:* se siguieron 37 hatos por 12 meses para el diagnóstico de MC. A las muestras de leche de los casos de MC se les realizó cultivo bacteriológico y a estas, así como a los aislamientos de casos de mastitis subclínica de un estudio previo, se les efectuó prueba de susceptibilidad antibiótica. Para el análisis de la información se utilizó estadística descriptiva estándar. *Resultados:* TIMC fue de 13,8 casos por 100 vacas-año a riesgo. Se aislaron 188 patógenos totales de casos de MC, entre los cuales el *Streptococcus agalactiae*, el *Streptococcus pyogenes* y el *Corynebacterium* spp. fueron los más frecuentes, con 29,8; 11,7 y 5,9 %, respectivamente. Se halló una alta sensibilidad de las bacterias

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contagiosas para los antibióticos cloxacilina y cefoperazone. *Conclusiones:* en el presente estudio se encontró una TIMC de 13,8 casos por 100 vacas-año a riesgo. Los patógenos más prevalentes identificados en casos de MC fueron contagiosos. Se encontró una alta sensibilidad de las bacterias contagiosas para la mayoría de los antibióticos β-lactámicos.

Palabras clave: bovinos, leche, mastitis, prueba de sensibilidad antibiótica, resistencia antimicrobiana, tasa de incidencia de mastitis clínica.

Taxa de incidência de mastite clínica e susceptibilidade antibiótica de patógenos produtores de mastite em gado leiteiro do norte de Antioquia, Colômbia

Resumo

Introdução: a mastite se define como a inflamação da glândula mamária causante de grandes perdas econômicas em rebanhos de gado leiteiro, o que justifica a sua pesquisa. *Objetivo:* determinar a taxa de incidências de mastite clínica TIMC, a etiologia associada e a sensibilidade antibiótica de patógenos isolados de casos de mastite em gado leiteiro. *Materiais e métodos:* seguiram-se 37 rebanhos bovinos durante 12 meses para o diagnóstico de MC. Com as amostras de leite dos casos de MC realizou-se cultivo bacteriológico e a estas, assim como aos isolamentos de casos de mastite subclínica de um estudo prévio, efetuou-se prova de susceptibilidade antibiótica. Para a análise da informação se utilizou estatística descritiva padrão. *Resultados:* TIMC foi de 13,8 casos por 100 vacas-ano a risco. Foram isolados 188 patógenos totais de casos de MC, entre os quais o *Streptococcus agalactiae*, o *Streptococcus pyogenes* e o *Corynebacterium* spp. foram os mais frequentes, com 29,8; 11,7 e 5,9 %, respectivamente. Constatou-se uma alta sensibilidade das bactérias contagiosas para os antibióticos cloxacilina e cefoperazone. *Conclusões: neste* estudo evidenciou-se uma TIMC de 13,8 casos por 100 vacas-ano a risco. Os patógenos mais prevalentes identificados em casos de MC foram contagiosos. Encontrou-se uma alta sensibilidade das bactérias contagiosas para a maioria dos antibióticos β-lactâmicos.

Palavras chave: bovinos, leite, mastite, prova de sensibilidade antibiótica, resistência antimicrobiana, índice de incidência de mastite clínica.

Introduction

Bovine mastitis is the inflammation of the mammary gland, which is considered the most common infectious disease in dairy cows (1). According to the International Dairy Federation A2 Group of Experts on Mastitis, the clinical form of mastitis is characterized by inflammation with heat, pain, redness, and enlargement of the mammary gland or changes in the appearance of milk, or all the above symptoms (2). One way of measuring the frequency of clinical mastitis (CM) in dairy herds is by calculating the incidence rate of clinical mastitis (IRCM), which has been reported 14.4 cases per 100 cows-years at risk (3), 41.95 cases per 100 cows-years at risk (4), and 43.3 cases per 100 cows-years at risk (5). The authors did not find any published study that reported IRCM in Colombia.

More than 200 mastitis-causing microorganisms have been registered (6). The most frequent microorganisms associated with bovine mastitis can be divided into four categories: contagious, environmental, opportunistic, and others (7); among these, the first two are the most frequently associated categories with infectious mastitis. The main contagious pathogens are *Staphylococcus aureus* and *Streptococcus agalactiae*; this group of contagious microorganisms also includes *Corynebacterium bovis*, *Mycoplasma bovis* (7), and *Streptococcus dysgalactiae* (6,8), although the latter is the only pathogen with the characteristics of contagious and environmental microorganisms (9). The environmental pathogens that are most frequently isolated from cases of mastitis include *Streptococcus* spp., also known as environmental streptococci (different from S. *agalactiae*), and coliform bacteria (10). The environment of the cow is the main reservoir for pathogens that cause mastitis of environmental origin, which is usually associated with clinical cases and is the predominant type of mastitis in well-managed herds with low somatic cell counts (11).

The etiology of mastitis may vary between countries, which may be due, among other reasons, to different management factors. For example, regarding the prevalence of specific pathogens that consider only samples with positive culture per udder quarter, a positivity of 31, 27, 15, 14, 4.8, and 3.1% was reported for *S. aureus*, CNS, *S. dysgalactiae*, *S. uberis*, *E. coli*, and *Streptococcus* spp., respectively (12). Another study conducted in Israel found that clinical cases of mastitis were associated with coliform bacteria (60.2%), environmental streptococci (18.6%), and coagulase-negative Staphylococcus (CNS, 8.7%) (13). A study based on reports of CM treatments performed by veterinarians found that *S. aureus* and *S. dysgalactiae* were the most prevalent bacteria in first-calf cows (12). Other researchers isolated pathogenic microorganisms in 61% of the samples, with the highest prevalence for *Corynebacterium* sp. (45.0%), *Staphylococcus* sp. (29.6%), and *Streptococcus* sp. (14.6%) in manual milking; and *Staphylococcus* sp. (36.4%), *Corynebacterium* sp. (27.6%), *Micrococcus* sp. (15.6%), and *Streptococcus* sp. (12.9%) in mechanical milking (14). In Colombia, the most recent study reported that the most frequently isolated pathogens for subclinical mastitis were *S. agalactiae*, CNS, and *Corynebacterium* sp., with a prevalence of 34.4, 17.6, and 13.2%, respectively (15). In other studies on subclinical mastitis, the most frequently isolated bacteria were *S. agalactiae* and *S. aureus*, with a prevalence of 35 to 45% and 14 to 33% (16,17), respectively, as well as *S. aureus* with a prevalence of 29% in the infections (18).

Therapy with antibiotics is considered an important tool in the control of mastitis, and surveillance programs for the acquisition of antibiotic resistance by bacteria are important to ensure optimal results in the use of antimicrobials and to minimize selection risk and the dissemination of resistance (19). There are few published studies on the susceptibility of bacteria isolated from cases of bovine mastitis in dairy herds in Colombia. Only three studies were found; one of them reported that the resistance rate of *S. agalactiae* to antibiotic penicillin was 19.4% (20), while other authors registered a sensitivity of 100% (21,22). Two of the studies found that the *S. aureus* bacteria showed a high frequency of resistance to penicillin (20,22).

To expand our knowledge about bovine mastitis in Colombia, it is necessary to have studies on IRCM and the pathogens associated with its presentation, as well as research that seeks to update information on the antibiotic susceptibility of bacteria isolated from bovine mastitis cases. All this aiming to provide useful information for veterinary medical professionals and milk producers, in order to improve the condition of dairy herds regarding this disease.

The objective of this work was to estimate IRCM and to determine the pathogens involved in the presentation of CM, as well as the antibiotic susceptibility of pathogens associated with clinical and subclinical mastitis in dairy herds from six municipalities of the dairy production micro-basin of the northern highlands in Antioquia.

Materials and methods

Ethical considerations

This research was approved by the Ethics Committee for Animal Experimentation at the Universidad de Antioquia, Colombia (Minutes 48 of December 12, 2008).

Herds

Inclusion criteria

In each municipality, herds were selected taking into account the following criteria: facility of access, due either to short distance from the urban area or existing drivable roads; milk storage in cooling tanks; adequate identification of cows; and permission of the owner or steward to collect samples from the animals, as well as their commitment to provide the necessary information.

Sample

The sample consisted of 37 herds from six municipalities in the dairy production micro-basin of the northern highlands of Antioquia, Colombia: San Pedro de los Milagros, Santa Rosa de Osos, Donmatías, Belmira, San José de la Montaña, and Entrerríos. The herds correspond to a sample selected for convenience among 3042 herds registered in the mentioned six municipalities, which produce 75% of the milk and where 70% of the cows is concentrated in the area (23). The municipalities are located at a height between 2200 and 2581 m. a. s. l. with an average temperature of 14.5 °C. Of the 3042 herds, 120 were invited to participate in the study: 99 accepted, while 21 declined acceptance; a group of 32 herds was formed for the study. The distribution of herds by municipality was proportional to the number of herds each one had: Belmira 9% (3 herds), Donmatías 11% (3 herds), Entrerríos 19% (6 herds), San José 5% (2 herds), San Pedro de los Milagros 24% (8 herds), and Santa Rosa de Osos 32% (10 herds). Five herds had to be replaced throughout the study for different reasons; replacements were selected from the list of eligible farms with similar characteristics. The information regarding the time of participation of replaced herds was also analyzed; thus, in the end, the data of 37 herds was studied.

Protocol for visits and sample collection

Cases of CM were detected in monthly visits, which were carried out during 12 months, in the period between July 1, 2009 and June 30, 2010, or whenever the producer informed the researchers about its occurrence during this period. Milk sampling was always done by a veterinarian participating in the study, who collected the sample after the owner or the person in charge of milking had filed a report. In the udder quarter that presented CM, the tip of the nipple was disinfected with cotton swabs soaked in 70% alcohol, and milk samples were collected (approximately 5 ml). Milk samples were transported to the laboratory in refrigerators at 4 °C and kept refrigerated until the culture was carried out in the following 24 hours.

Bacteriological culture

Samples were cultured using standard laboratory methods (15). The presence of three or more species of bacteria in the same culture was considered contamination, and it was rejected for this study (9).

In vitro *antimicrobial sensitivity test*

As mentioned before, for these tests, subclinical mastitis isolates from a previous study (15) and isolates from the CM cases of the present study were used. The number of isolates analyzed varied according to the bacterium species and the antibiotic used. This number ranged between 959 for cloxacillin in the case of the bacterium *S. agalactiae* and 2 for cephalexin in the case of *Klebsiella* spp. The antibiotic susceptibility of these bacteria was determined using the disc-agar diffusion technique or Kirby-Bauer test (24). The isolates were classified as sensitive (S), intermediate (I), and resistant (R), according to the measurement of the diameter of the inhibition zone, for which we followed the Clinical and Laboratory Standards Institute (CLSI) guidelines (25). The following antibiotic sensi-discs were used: cloxacillin (5 μg), spiramycin (100 μg), penicillin G (10 IU), lincomycin (2 μg), ampicillin (10 μg), cefoperazone (75 μg), amoxacillin (10 μg), cephalexin (30 μg), and trimethoprim-sulfamethoxazole (25 μg).

Case definition

An udder quarter with CM was defined as the one with visibly abnormal milk and/or general signs of inflammation (2). A cow with CM was defined as the one that presented at least one udder quarter with CM. An udder quarter was considered infected when it presented CM, and one or two pathogens were isolated from the respective sample.

Statistical analysis

Information was stored in Excel sheets and then exported to the statistical software Stata® 12.0 (26). Data was examined to detect erroneous entries, and when improbable data was detected, it was removed. Data was analyzed through descriptive statistics using standard methods. The prevalence of CM was calculated per udder quarter. To calculate IRCM, days at risk per cow were defined as proposed by other authors (4), where a case of mastitis was defined as a report of a cow with CM or a series of reports separated by a minimum of 14 days. When CM was reported after at least 14 days of a previous case in the same cow, it was considered the beginning of a new case of mastitis. Cows were not considered at risk of presenting a new case of mastitis when they were sick of this or after 14 days after the last report of mastitis. To this effect, the following formula was applied: (# new events of mastitis/total number of cows/days at risk) $\times$ 365 $\times$ 100 (4). The number of days at risk of presenting a new case of mastitis was calculated individually for each animal during the period between July 1, 2009 and June 30, 2010. Herds that did not report cases of CM during the period were removed from the sample for the calculation of IRCM.

Results

General characteristics of the sample

Eighty percent of the cows in the sample were of the Holstein breed and 20% from other breeds or crosses. Average age was 5.7 ± 2.3 years, average milk production was 17.8 ± 6.32 L/cow/d. 43% of the farms had between 1 and 25 animals, 49% between 26 and 75, and 8% had more than 76 animals. 26% of the cows had one calving, 19% two, 17% three, and 37% four or more calvings. 30.3% of the cows were in the first third of lactation (0-100), 30.5% in the second third (101-200), 24.5% in the last third (201-300), and the 14.7% had more than 300 days in lactation.

Incidence rate of clinical mastitis and the etiology of mastitis

In total, 257 observations (cases) of CM in udder quarter were made. IRCM in the study period was 13.8 cases per 100 cows-year at risk. Although 257 cases of CM were reported, only 180 of them could be cultured given that in the other cases they had already undergone treatment when the veterinarian was able to visit to collect the sample. A total of 180 cultures were obtained from samples of udder quarters with CM; in 50 of them (27.7%) there was no isolate, while in 130 (72.2%) isolates were obtained. In the latter case, two pathogens were isolated in eight of the cases. The most frequently isolated pathogens among the 138 isolates were *S. agalactiae*, followed by *S. pyogenes* and *Corynebacterium* spp., with a prevalence of 40.6, 15.9, and 8.0%, respectively (table 1).

In vitro antimicrobial sensitivity

A high percentage of *S. agalactiae* isolates showed sensitivity when the β-lactam antibiotic group—cloxacillin (87.6%), penicillin (85.3%), ampicillin (91.8%), and amoxacillin (96%)—was used, as well as with the antibiotic cefoperazone (98.4%), but not with cephalexin (45.4%). This bacterium was resistant to lincomycin (50.7%), spiramycin (33.7%), trimethoprim sulfa (27%), and penicillin (14.7%).

In relation to the other streptococcal species, the study observed a behavior similar to that evidenced for *S. agalactiae*, specifically regarding sensitivity to β-lactams, except for *S. uberis*, which showed a lower sensitivity to cloxacillin (74.1%) and penicillin (74.1%). Both this bacterium and *S. pyogenes* showed a greater sensitivity to trimethoprim-sulfa (84.6% and 89.7%, respectively), in comparison to *S. agalactiae*. A pattern of resistance similar to the bacterium *S. agalactiae* was observed for the other streptococcal species. *S. pyogenes* and *S. dysgalactiae* showed lower resistance to spiramycin (11.6 and 18.3%, respectively). S. uberis showed greater resistance to penicillin (25.9%) (table 2).

In the case of ECPs, the bacteria *S. aureus* and *S. intermedius* showed a high percentage of isolates sensitive to antibiotics cloxacillin (95.4 and 96.5%), cefoperazone (98 and 88.6%), and trimethoprim-sulfa (98 and 98.6%). Additionally, *S. aureus* presented a high percentage of sensitivity to cephalexin (81.3%). It is important to highlight the high resistance of *S. aureus* and *S. intermedius* to penicillin (38.8 and 74.3%, respectively), and the resistance rate of these same bacteria to ampicillin (35.5 and 75.5%, respectively) (table 3).

Table 1. Pathogens isolated from 130 culture-positive samples from udder quarters presenting clinical mastitis in dairy cows from the northern highlands of Antioquia, Colombia

Pathogen	No. of isolates	Percentage
S. agalactiae	56	40.6
S. pyogenes	22	15.9
Corynebacterium spp.	11	8.0
S. dysgalactiae	8	5.8
S. intermedius	8	5.8
E. coli	8	5.8
S. aureus	7	5.1
S. uberis	6	4.3
S. epidermidis	4	2.9
S. haemolitycus	4	2.9
Others[a]	3	2.2
Candida spp.	1	0.7
Total	138	100.0

[a] This group includes the bacteria *Geotrichum* spp., *Trichosporum* spp., *Citrobacter freundii*, *Pseudomonas fluorescens*.

Table 2. Antibiotic susceptibility test results of *Streptococcus* isolates from udder quarters affected with clinical and subclinical mastitis in cows from the northern highlands of Antioquia, Colombia

Bacterium	Result	Penic	Ampi	Amoxa	Cloxa	Cephale	Cefope	Lincom	Spir	Tri-sul
S. agalactiae	S (%)	817 (85.3)	879 (91.8)	921 (96.0)	840 (87.6)	186 (45.4)	554 (98.4)	138 (48.9)	176 (65.9)	287 (72.5)
	I (%)	0	0	0	2 (0.2)	10 (2.4)	0	1 (0.4)	1 (0.4)	2 (0.5)
	R (%)	141 (14.7)	79 (8.2)	38 (4.0)	117 (12.2)	214 (52.2)	9 (1.6)	143 (50.7)	90 (33.7)	107 (27.0)
	Total	**958**	**958**	**959**	**959**	**410**	**563**	**282**	**267**	**396**
S. pyogenes	S (%)	310 (87.6)	324 (91.5)	336 (94.9)	323 (91.2)	154 (74.0)	149 (98.7)	47 (45.6)	38 (88.4)	182 (89.7)
	I (%)	0	0	0	0	2.0 (1.0)	0	1.0 (0.0)	0	0
	R (%)	44 (12.4)	30 (8.5)	18 (5.1)	31 (8.8)	52 (25)	2 (1.3)	55 (53.4)	5 (11.6)	21 (10.3)
	Total	**354**	**354**	**354**	**354**	**208**	**151**	**103**	**43**	**203**
S. dysgalactiae	S (%)	101 (87.1)	107 (92.2)	115 (98.3)	109 (93.2)	25 (61.0)	73 (97.3)	7 (41.2)	49 (81.7)	29 (70.7)
	I (%)	0	0	0	0	0	1,0 (1,3)	0	0	0
	R (%)	15 (12.9)	9 (7.8)	2.0 (1.7)	8 (6.8)	16 (39.0)	1.0 (1.3)	10 (58.8)	11 (18.3)	12 (29.3)
	Total	**116**	**116**	**117**	**117**	**41**	**75**	**17**	**60**	**41**
S. uberis	S (%)	60 (74.1)	72 (90.0)	79 (97.5)	60 (74.1)	11 (78.6)	64 (94.1)	13 (36.1)	23 (71.9)	11 (84.6)
	I	0	0	0	0	0	0	0	0	0
	R (%)	21 (25.9)	8 (10.0)	2 (2.5)	21 (25.9)	3 (21.4)	4 (5.9)	23 (63.9)	9 (28.1)	2 (15.4)
	Total	**81**	**80**	**81**	**81**	**14**	**68**	**36**	**32**	**13**

S = sensitive; I = intermediate; R = resistant.
Penic = penicillin; Ampi = ampicillin; Amoxa = amoxacillin; Cloxa = cloxacillin; Cephale = cephalexin; Cefope = cefoperazone; Lincom = lincomycin; Spir = spiramycin; Tri-sul = trimethoprim-sulfamethoxazole.

Table 3. Antibiotic susceptibility test results of coagulase-positive staphylococcal isolates to several antibiotics in samples from udder quarters affected with clinical and subclinical mastitis in cows from the northern highlands of Antioquia, Colombia

Bacterium	Result	Penic	Ampi	Amoxa	Cloxa	Cephale	Cefope	Lincom	Spir	Tri-sul
S. aureus	S (%)	212 (60.9)	225 (64.5)	266 (76.0)	333 (95.4)	126 (81.3)	195 (98.0)	74 (63.8)	60 (74.1)	148 (98.0)
	I (%)	1 (0.3)	0.0	0.0	0.0	0.0	3 (1.5)	2 (1.7)	1 (1.2)	0.0
	R (%)	135 (38.8)	124 (35.5)	84 (24.0)	16 (4.6)	29 (18.7)	1 (0.5)	40 (34.5)	20 (24.7)	3 (2.0)
	Total	**348**	**349**	**350**	**349**	**155**	**199**	**116**	**81**	**151**
S. intermedius	S (%)	36 (25.0)	35 (24.5)	63 (43.8)	139 (96.5)	55 (74.3)	62 (88.6)	22 (56.4)	17 (53.1)	73 (98.6)
	I (%)	1 (0.7)	0.0	0.0	0.0	0.0	1 (1.4)	0.0	0.0	0.0
	R (%)	107 (74.3)	108 (75.5)	81 (56.3)	5 (3.5)	19 (25.7)	7 (10.0)	17 (43.6)	15 (46.9)	1 (1.4)
	Total	**144**	**143**	**144**	**144**	**74**	**70**	**39**	**32**	**74**

S = sensitive; I = intermediate; R = resistant.
Penic = penicillin; Ampi = ampicillin; Amoxa = amoxacillin; Cloxa = cloxacillin; Cephale = cephalexin; Cefope = cefoperazone; Lincom = lincomycin; Spir = spiramycin; Tri-sul = trimethoprim-sulfamethoxazole.

In the case of CNS bacteria (*S. epidermidis*, *S. haemolitycus*, and *S. saprofiticus*), a high sensitivity was found for *S. epidermidis* when antibiotics cloxacillin (93.5%), cefoperazone (100%), amoxacillin (80.6%), cephalexin (82.1%), and trimethoprim-sulfa (93.9%) were used. In the case of *S. haemolitycus*, a high sensitivity was found when cloxacillin (94.9%), cefoperazone (97.6%), amoxacillin (84.7%), and trimethoprim-sulfa (88.2%) were used. In the case of *S. saprofiticus*, a high sensitivity was found when antibiotics cloxacillin, spiramycin, penicillin, ampicillin, cefoperazone, and amoxacillin were used (100%). For *S. epidermidis*, a high percentage of resistance was observed to antibiotics penicillin (55.4%), lincomycin (53.1%), and ampicillin (48.2%). S. haemolitycus showed resistance to antibiotics penicillin (61%), lincomycin (77.8%), and ampicillin (52.5%). *S. saprofiticus* only showed resistance to lincomycin (100%) (table 4).

With respect to gram-negative bacteria, *E. coli* showed high sensitivity to the antibiotic cefoperazone (98%), and moderate sensitivity to neomycin (59%), ampicillin (70%), amoxacillin (67%), and trimethoprim-sulfa (55%), as well as resistance to spiramycin, lincomycin, cephalexin, and trimethoprim-sulfa (74, 92, 69, and 45%, respectively). Although sensitivity tests were performed for the bacteria of the genera *Klebsiella* spp. and *Enterobacter* spp., their number was very small. These results are shown in table 5.

Table 4. Antibiotic susceptibility test results of coagulase-negative staphylococcus (ECN) isolates in samples from udder quarters affected with clinical and subclinical mastitis in cows from the northern highlands of Antioquia, Colombia

Bacterium	Result	Penic	Ampi	Amoxa	Cloxa	Cephale	Cefope	Lincom	Spir	Tri-sul
S. epidermidis	S (%)	62 (44.6)	72 (51.8)	112 (80.6)	130 (93.5)	55 (82.1)	73 (100.0)	15 (46.9)	26 (65.0)	62 (93.9)
	I (%)	(0.0)	0.0	0.0	(0.0)	1 (1.5)	0.0	(0.0)	1 (2.5)	0.0
	R (%)	77 (55.4)	67 (48.2)	27 (19.4)	9 (6.5)	11 (16.4)	0 (0.0)	17 (53.1)	13 (32.5)	4 (6.1)
	Total	139	139	139	139	67	73	32	40	66
S. haemolyticus	S (%)	23 (39.0)	28 (47.5)	50 (84.7)	56 (94.9)	14 (77.8)	41 (97.6)	2 (22.2)	25 (78.1)	15 (88.2)
	I (%)	(0.0)	0.0	0.0	(0.0)	0.0	0.0	(0.0)	(0.0)	0.0
	R (%)	36 (61.0)	31 (52.5)	9 (15.3)	3 (5.1)	4 (22.2)	1 (2.4)	7 (77.8)	7 (21.9)	2 (11.8)
	Total	59	59	59	59	18	42	9	32	17
S. saprofiticus	S (%)	6 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)	NE	6 (100.0)	0 (0.0)	5 (100.0)	NE
	I (%)	(0.0)	0.0	0.0	(0.0)	NE	0.0	(0.0)	0 (0.0)	NE
	R (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	NE	0 (0.0)	1 (100.0)	0 (0.0)	NE
	Total	6	6	6	6	NE	6	1	5	NE

S = sensitive; I = intermediate; R = resistant.
Penic = penicillin; Ampi = ampicillin; Amoxa = amoxacillin; Cloxa = cloxacillin; Cephale = cephalexin; Cefope = cefoperazone; Lincom = lincomycin; Spir = spiramycin; Tri-sul = trimethoprim-sulfamethoxazole; NE = Not estimated.

Table 5. Antibiotic susceptibility test results of *Escherichia coli*, *Klebsiella* spp., and *Enterobacter* spp. isolates in samples from udder quarters affected with clinical and subclinical mastitis in cows from the northern highlands of Antioquia, Colombia

Bacterium	Result	Ampi	Amoxa	Cephale	Cefope	Lincom	Spir	Tri-sul	Neomy
E. coli	S (%)	44 (70)	42 (67)	4 (31)	62 (98)	4 (8)	8 (26)	6 (55)	37 (59)
	I (%)	0	2 (3)	0,0	0	0	0	0	2 (3)
	R (%)	19 (30)	19 (30)	9 (69)	1 (2)	48 (92)	23 (74)	5 (45)	24 (38)
	Total	63	63	13	63	52	31	11	63
Klebsiella spp.	S (%)	2 (40)	2 (40)	2 (100)	5 (100)	5 (100)	NE	NE	3 (60)
	I (%)	0	0	0	0	0	NE	NE	0
	R (%)	3 (60)	3 (60)	0 (0)	0 (0)	0 (0)	NE	NE	2 (40)
	Total	5	5	2	5	5	NE	NE	5
Enterobacter spp.	S (%)	7 (88)	7 (88)	1 (14)	NE	NE	NE	NE	2 (29)
	I (%)	0	0	0	NE	NE	NE	NE	0
	R (%)	1 (13)	1 (13)	5 (71)	NE	NE	NE	NE	5 (71)
	Total	8	8	6	NE	NE	NE	NE	7

S = sensitive; I = intermediate; R = resistant.
Ampi = ampicillin; Amoxa = amoxacillin; Cephale = cephalexin; Cefope = cefoperazone; Lincom = lincomycin; Spir = spiramycin; Tri-sul = trimethoprim-sulfamethoxazole; Neomy = neomycin; NE = Not estimated.

Discussion

No data was found from studies carried out in Colombia on IRCM in order to compare with the findings of this work. A review of international studies showed that the IRCM results of this study agree with a report from Uruguay, which found 14.4 cases per 100 cows-year at risk in a follow-up year (3). This was considered low when compared to 41.95 cases per 100 cows-year at risk reported in Denmark (4) and 43.3 cases per 100 cows-year at risk reported in Tanzania (27). However, caution must be taken when comparing these findings with the results of studies carried out in other countries, since IRCM is associated with factors such as climate, race, production, and management (3).

The IRCM found in the herds studied is considered low, which could be partly explained by an under-notification of cases, due to the fear of milk producers to diminish the prestige of their herds if they revealed the real number of cases. The incomplete report of mastitis cases by milk producers is due to the fact that some of them are apathetic to matters related to controlling this disease or they lack time to keep full records (4).

In general, in the frequency of isolation, there was a predominance of contagious bacteria over environmental bacteria. It is worth noting the high frequency of the bacterium *S. agalactiae*, which is transmitted mainly during milking (28), since it can be found on surfaces that have had recent contact with contaminated milk, such as milking equipment and the milker's hands (7); similarly, poor hygiene conditions during the milking routine favor its presentation (6). The characteristics of herd management in this study were previously described by the authors (23); they identified that handwashing before milking, pre-sealing, sealing, and washing the nipples was done by the 18.8, 40.6, 78.1, and 21.9% of milkers, respectively. Therefore, the high prevalence of the bacterium *S. agalactiae* could be associated with previously diagnosed deficiencies in hygiene in the herds of the area. The prevalence of *S. agalactiae* was consistent with the findings of another study conducted in the Bogotá savanna (17), in which this bacterium was more prevalent in manual milking and it had the highest percentage of infection with respect to mechanic milking (61.7 and 50%, respectively) (16). *S. agalactiae* was also the most frequently isolated pathogen in cases of subclinical mastitis in a study conducted in this same area (15), as well as in previous studies on subclinical mastitis in San Pedro de los Milagros and the Bogotá savanna, with 47 and 44.9%, respectively (17,20). In contrast, in a study carried out in the highlands of Cundinamarca and Boyacá, a prevalence of only 6.84% for *S. agalactiae* was found in cases of subclinical mastitis (18), and a prevalence of 6.4% in a study conducted in dairy herds in the eastern part of Antioquia (21).

To evaluate the susceptibility of bacteria to antibiotics, the agar diffusion test was used, due to its ease of execution, low cost, interlaboratory reproducibility, and flexibility in the type and number of antibiotics that can be analyzed (29). It should be noted that the interpretation results of susceptibility to antibiotics used by the agar diffusion method have as reference parameters plasma pharmacokinetic data in humans, which is extrapolated to domestic animals. Although these data serve as a reference point for veterinary medicine, there may be variations between the human and cattle species. In summary, for the specific case of interpreting these results, it is important that the veterinarian has specific consideration for the pharmacokinetics of antibiotics in cattle, and even more so in the specific compartment of the mammary gland.

This study reported that 14.7% of *S. agalactiae* isolates were resistant to penicillin, a result slightly lower than 19.4%, which was found in another study carried out in the area (20) and which contrasts with the 100% sensitivity reported by other studies in Colombia (21,22). Reports of *S. agalactiae* strains resistant to β-lactam antibiotics are scarce. A study carried out in New York reported *S. agalactiae* strains multi-resistant to β-lactam antibiotics (30). It is worth to mention the finding of a significant percentage of *S. agalactiae* isolates resistant to antibiotics spiramycin (33.7%), lincomycin (50.7%), and cephalexin (52.2%). This result agrees with what was found by Gao et al. (31), who reported resistance of *S. agalactiae* isolates to erythromycin, an antibiotic from the group of macrolides, such as spiramycin, and also evidenced multi-resistance to antibiotics gentamicin, amikacin, and tetracycline. Although the percentage of *S. agalactiae* isolates resistant to β-lactam antibiotics is relatively low, it is worrisome, given that a very important therapeutic option could be lost for the control of this bacterium, since penicillin is the antibiotic of first choice in cases of bovine mastitis (28).

Both ECP bacteria (*S. aureus* and *S. intermedius*) showed resistance to β-lactamase sensitive penicillins (penicillin and ampicillin), and *S. intermedius* showed resistance to amoxacillin. This resistance of ECP bacteria to β-lactam antibiotics could be explained by the production of β-lactamase, which is the most common resistance mechanism in staphylococci (19). High frequency of resistance to penicillin has been reported by other studies for bacteria isolated from udder quarters with mastitis (20,22,32). For the case of a few *S. aureus* isolates resistant to β-lactam antibiotics obtained in this study, the finding could be associated with the presence of methicillin-resistant *S. aureus* (MRSA) strains, which must be considered resistant to all penicillins, cephalosporins, cephems, and other β-lactams. However, there are only few reports of MRSA in this group of animals and in their milk (33-36).

ECN bacteria, specifically *S. epidermidis* and *S. haemolitycus*, showed high resistance to lincomycin, penicillin, and ampicillin. These results are in agreement with what was found by other authors, who reported a 66.7% resistance rate of ECN bacteria to antibiotics ampicillin and penicillin (22), and resistance of 75% to penicillin (20).

In relation to gram-negative bacteria, it was possible to obtain a significant number of antibiotic susceptibility tests for the *E. coli* bacterium, although the frequency of isolation for *Klebsiella* spp. and *Enterobacter* was very low. A high sensitivity of *E. coli* to broad-spectrum antibiotics such as cefoperazone was observed, and, although to a lesser degree, to β-lactam ampicillin and amoxacillin. According to Petri (37), cefoperazone is a third-generation broad-spectrum cephalosporin with good action against gram-negative bacteria. However, other authors have reported a 5.9% resistance of *E. coli* to ampicillin in samples from cases of subclinical mastitis (19). Regarding this last aspect, the finding of some *E. coli* isolates resistant to the analyzed group of β-lactam antibiotics (ampicillin, amoxacillin, cephalexin, cefoperazone) makes us suspect of the production of extended-spectrum β-lactamases (ESBL) in some of these isolates. However, since this was beyond the objectives of this study, it was not confirmed whether these isolates were indeed ESBL producers. ESBL production by bacteria reduces the efficacy of a wide range of β-lactam antibiotics, such as third generation cephalosporins and monobactams (38), and it has been documented that food-producing animals are important reservoirs of ESBL-producing Enterobacteriaceae (39). Multi-resistant *E. coli* has been isolated from bovine milk in several previous studies (40-46), which requires further studies to establish the importance of this phenomenon at the national level.

Conclusions

This study found an incidence rate of IRCM of 13.8 cases per 100 cows-year at risk. The most prevalent pathogens identified in cases of CM were contagious. A high sensitivity of contagious bacteria to most β-lactam antibiotics was evidenced.

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References

1. De Pinho Manzi M, Nóbrega DB, Faccioli PY, Troncarelli MZ, Menozzi BD, Langoni H. Relationship between teat-end condition, udder cleanliness and bovine subclinical mastitis. Res Vet Sci. 2012;93(1):430-4. https://doi.org/10.1016/j.rvsc.2011.05.010
2. Griffin, TK, Morant SV, Dodd FH. Bovine mastitis. Definition and guidelines for diagnosis. En: Bulletin of the International Dairy Federation. Bruselas: International Dairy Federation; 1987. p. 3-4.
3. Gianneechini R, Concha C, Rivero R, Delucci I, Moreno J. Occurrence of clinical and sub-clinical mastitis in dairy herds in the West Littoral Region in Uruguay. Acta Vet Scand. 2002;43(4):221-30. https://doi.org/10.1186/1751-0147-43-221
4. Bartlett PC, Agger JF, Houe H, Lawson LG. Incidence of clinical mastitis in Danish dairy cattle and screening for non-reporting in a passively collected national surveillance system. Prev Vet Med. 2001;48(2):73-83. https://doi.org/10.1016/S0167-5877(00)00192-6
5. Kivaria FM, Noordhuizen JP, Msami HM. Risk factors associated with the incidence rate of clinical mastitis in smallholder dairy cows in the Dar es Salaam region of Tanzania. Vet J. 2007;173(3):623-9. https://doi.org/10.1016/j.tvjl.2006.01.009
6. Blowey R. Mastitis control in dairy herds. Winslow, UK: Farming Press Books; 1995.
7. Philpot N, Nickerson S. Mastitis: counter attack. Nickerson S, Philpot N, editores. Illinois: Babson Bros; 1991. p. 29-33.
8. Fox LK, Gay JM. Contagious mastitis. Vet Clin North Am Food Anim Pract. 1993;9(3):475-87. https://doi.org/10.1016/S0749-0720(15)30615-0
9. National Mastitis Council. Laboratory handbook on bovine mastitis. Revised edition. Madison: National Mastitis Council; 1999. p. 1-11; 31-9.
10. Oliver SP. Frequency of isolation of environmental mastitis-causing pathogens and incidence of new intramammary infection during the nonlactating period. Am J Vet Res. 1988;49(11):1789-93.

11. Archbald L. Reproductive diseases. En: Anderson DE, Ring D, editores. Current veterinary therapy food animal practice. St. Louis, MO: Saunders; 1999. p. 563-8.
12. Persson K, Bengtsson B, Lindberg A, Nyman A, Ericsson Unnerstad H. Incidence of mastitis and bacterial findings at clinical mastitis in Swedish primiparous cows-Influence of breed and stage of lactation. Vet Microbiol. 2009;134(1-2):89-94. https://doi.org/10.1016/j.vetmic.2008.09.004
13. Shpigel NY, Winkler M, Ziv G, Saran A. Clinical, bacteriological and epidemiological aspects of clinical mastitis in Israeli dairy herds. Prev Vet Med. 1998;35(1):1-9. https://doi.org/10.1016/S0167-5877(98)00052-X
14. Ruiz AK, Ponce P, Gomes G, Mota RA, Sampaio E, Lucena ER, Benone S. Prevalencia de mastitis bovina subclínica y microorganismos asociados: comparación entre ordeño manual y mecánico, en Pernambuco, Brasil. Rev Salud Anim. 2011;33(1):57-64.
15. Ramírez NF, Keefe G, Dohoo I, Sánchez J, Arroyave O, Cerón J, et al. Herd- and cow-level risk factors associated with subclinical mastitis in dairy farms from the High Plains of the northern Antioquia, Colombia. J Dairy Sci. 2014;97(7):4141-50. https://doi.org/10.3168/jds.2013-6815
16. Rodríguez G, Contreras D, Ordóñez M. Caracterización de la mastitis bovina en el Valle de Ubaté. Rev Med Vet (Bogotá). 2002;2(4):57-66.
17. Rodríguez G. Comportamiento de la mastitis bovina y su impacto económico en algunos hatos de la Sabana de Bogotá, Colombia. Rev Med Vet (Bogotá). 2006;(12):35-55. https://doi.org/10.19052/mv.2052
18. Calderón A. Rodríguez V. Prevalencia de mastitis bovina y su etiología infecciosa en sistemas especializados en producción de leche en el altiplano cundiboyacense (Colombia). Rev Colomb Cienc Pecua. 2008;21(4):582-9.
19. Persson K, Aspán A, Nyman A, Persson Y, Andersson UG. CNS species and antimicrobial resistance in clinical and subclinical bovine mastitis. Vet Microbiol. 2011;152(1-2):112-6. https://doi.org/10.1016/j.vetmic.2011.04.006
20. Ramírez N, Gaviria G, Arroyave O, Sierra B. Prevalencia de mastitis en vacas lecheras lactantes en el municipio de San Pedro de los Milagros, Antioquia. Rev Colomb Cienc Pecua. 2001;14(1):76-87.
21. Trujillo C, Gallego A, Ramírez N, Palacio L. Prevalence of mastitis in dairy herds in Eastern Antioquia. Rev Colomb Cienc Pecua. 2011;24(1):11-8.
22. Ruiz D, Ramírez N, Arroyave O. Determinación de concentraciones inhibitorias mínimas a algunos antibióticos de las bacterias aisladas de glándula mamaria bovina en San Pedro de los Milagros, Antioquia. Rev Colomb Cienc Pecua. 2001;14(2):143-54.
23. Ramírez N, Cerón J, Jaramillo M, Palacio Luis. Arroyave O. Diagnóstico de mastitis en el Norte de Antioquia. En: VII Seminario Internacional Competitividad en carne y leche. Colanta; 2010. p. 69-78.
24. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966;45(4):493-6.
25. Clinical and Laboratory Standars Intitute. Performance Standards for Antimicrobial Disk Susceptibility Tests. 2006.
26. StataCorp. 2011. Stata Statistical Software. Release 12. StataCorp LP, College Station, TX.
27. Kivaria FM, Noordhuizen J, Nielen M. Interpretation of California mastitis test scores using Staphylococcus aureus culture results for screening of subclinical mastitis in low yielding smallholder dairy cows in the Dar es Salaam region of Tanzania. Prev Vet Med. 2007;78(3-4):274-85. https://doi.org/10.1016/j.prevetmed.2006.10.011
28. Pyörälä S. Staphylococcal and Streptococcal mastitis. En: Sandholm M, editor. The bovine udder and mastitis. Universidad de Helsinki; 1995. p. 143-148.
29. Rubin JE. Antimicrobial susceptibility testing and interpretation of results. En: Giguère S, Prescott J, Dowling P, editores. Antimicrobial therapy in veterinary medicine. Nueva Jersey: John Wiley & Sons; 2006. p. 11-20.
30. Berghash SR, Davidson JN, Armstrong JC, Dunny GM. Effects of antibiotic treatment of nonlactating dairy cows on antibiotic resistance patterns of bovine mastitis pathogens. Antimicrob Agents Chemother 1983;24(5):771-6. https://doi.org/10.1128/AAC.24.5.771

31. Gao J, Yu F-Q, Luo L-P, He J-Z, Hou R-G, Zhang H-Q, et al. Antibiotic resistance of Streptococcus agalactiae from cows with mastitis. Vet J. 2012;194(3):423-4. https://doi.org/10.1016/j.tvjl.2012.04.020
32. Gao J, Ferreri M, Yu F, Liu X, Chen L, Su J, Han B. Molecular types and antibiotic resistance of Staphylococcus aureus isolates from bovine mastitis in a single herd in China. Vet J. 2012;192(3):550-2. https://doi.org/10.1016/j.tvjl.2011.08.030
33. Türkyılmaz S, Tekbıyık S, Oryasin E, Bozdogan B. Molecular epidemiology and antimicrobial resistance mechanisms of methicillin-resistant Staphylococcus aureus isolated from bovine milk. Zoonoses Public Health. 2010;57(3):197-203. https://doi.org/10.1111/j.1863-2378.2009.01257.x
34. Fessler A, Scott C, Kadlec K, Ehricht R, Monecke S, Schwarz S. Characterization of methicillin-resistant Staphylococcus aureus ST398 from cases of bovine mastitis. J Antimicrob Chemother. 2010;65(4):619-25. https://doi.org/10.1093/jac/dkq021
35. Spohr M, Rau J, Friedrich A, Klittich G, Fetsch A, Guerra B, et al. Methicillin-Resistant Staphylococcus aureus (MRSA) in three dairy herds in Southwest Germany. Zoonoses Public Health. 2011;58(4):252-61. https://doi.org/10.1111/j.1863-2378.2010.01344.x
36. Kreausukon K, Fetsch A, Kraushaar B, Alt K, Müller K, Krömker V, et al. Prevalence, antimicrobial resistance, and molecular characterization of methicillin-resistant Staphylococcus aureus from bulk tank milk of dairy herds. J Dairy Sci. 2012;95(8):4382-8.
37. Petri W. Antimicrobianos, penicilinas, cefalosporinas y otros antibióticos β-lactámicos. En: Las bases farmacológicas de la terapéutica. México: McGraw-Hill; 2001. p. 1207-36.
38. Bush K. Proliferation and significance of clinically relevant β-lactamases. Ann N Y Acad Sci. 2013;1277:84-90. https://doi.org/10.1111/nyas.12023
39. Carattoli A. Animal reservoirs for extended spectrum beta-lactamase producers. Clin Microbiol Infect. 2008;14(supl. 1):117-23. https://doi.org/10.1111/j.1469-0691.2007.01851.x
40. Saini V, McClure J, Léger D, Keefe G, Scholl D, Morck D, Barkema H. Antimicrobial resistance profiles of common mastitis pathogens on Canadian dairy farms. J Dairy Sci. 2012;95(8):4319-32. https://doi.org/10.3168/jds.2012-5373
41. Dahmen S, Métayer V, Gay E, Madec JY, Haenni M. Characterization of extended-spectrum beta-lactamase (ESBL)-carrying plasmids and clones of Enterobacteriaceae causing cattle mastitis in France. Vet Microbiol. 2013;162(2-4):793-9.
42. Ghatak S, Singha A, Sen A, Guha C, Ahuja A, Bhattacharjee U, et al. Detection of New Delhi metallo-beta-lactamase and extended-spectrum beta-lactamase genes in Escherichia coli isolated from mastitic milk samples. Transbound Emerg Dis. 2013;60(5):385-9. https://doi.org/10.1111/tbed.12119
43. Metzger S, Hogan J. Short communication: antimicrobial susceptibility and frequency of resistance genes in Escherichia coli isolated from bovine mastitis. J Dairy Sci. 2013;96(5):3044-9. https://doi.org/10.3168/jds.2012-6402
44. Liu Y, Liu G, Liu W, Liu Y, Ali T, Chen W, et al. Phylogenetic group, virulence factors and antimicrobial resistance of Escherichia coli associated with bovine mastitis. Res in Microbiol. 2014;165(4):273-7. https://doi.org/10.1016/j.resmic.2014.03.007
45. Timofte D, Maciuca IE, Evans NJ, Williams H, Wattret A, Fick JC, Williams NJ. Detection and molecular characterization of Escherichia coli CTX-M-15 and Klebsiella pneumoniae SHV-12 β-lactamases from bovine mastitis isolates in the United Kingdom. Antimicrob Agents Chemother. 2014;58(2):789-94. https://doi.org/10.1128/AAC.00752-13
46. Odenthal S, Akineden Ö, Usleber E. Extended-spectrum β-lactamase producing Enterobacteriaceae in bulk tank milk from German dairy farms. Int J Food Microbiol. 2016;238:72-8. https://doi.org/10.1016/j.ijfoodmicro.2016.08.036