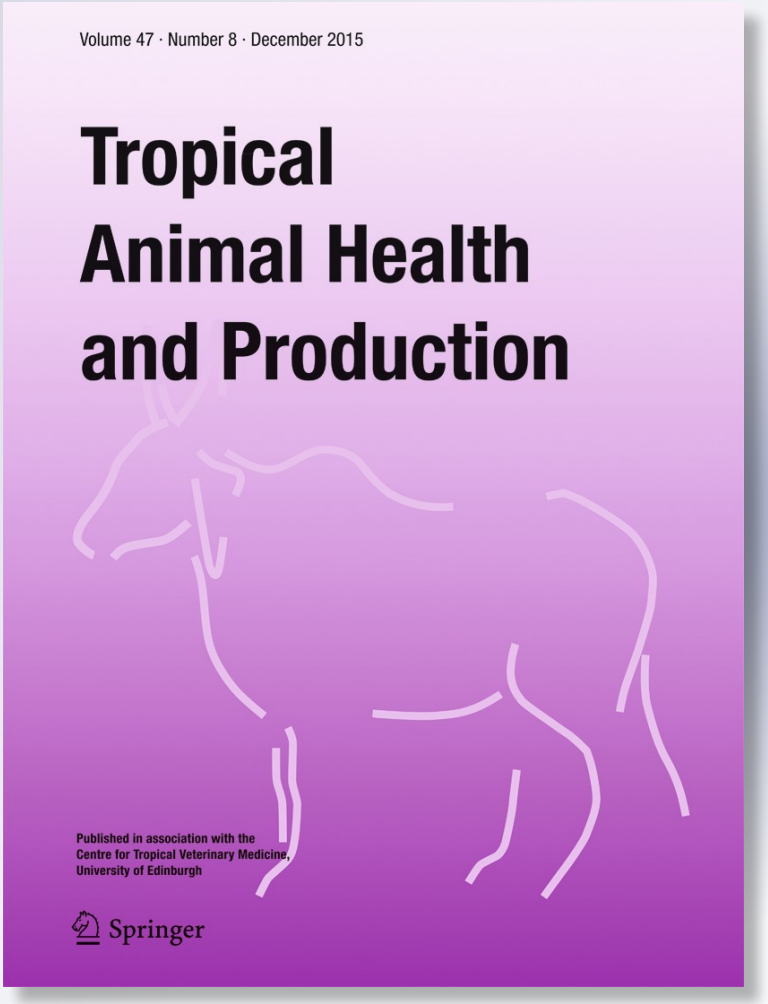
Prevalence of bovine subclinical mastitis, its etiology and diagnosis of antibiotic resistance of dairy farms in four municipalities of a tropical region of Mexico

Jaime Olivares-Pérez, Ahmed Eid Kholif, Saul Rojas-Hernández, Mona Mohamed Mohamed Yasseen Elghandour, Abdelfattah Zeidan Mohamed Salem, et

Tropical Animal Health and Production

ISSN 0049-4747
Volume 47
Number 8

Trop Anim Health Prod (2015)
47:1497-1504
DOI 10.1007/s11250-015-0890-8



Springer

Trop Anim Health Prod (2015) 47:1497–1504
DOI 10.1007/s11250-015-0890-8

REGULAR ARTICLES

Prevalence of bovine subclinical mastitis, its etiology and diagnosis of antibiotic resistance of dairy farms in four municipalities of a tropical region of Mexico

Jaime Olivares-Pérez[1] · Ahmed Eid Kholif[2] · Saul Rojas-Hernández[1] · Mona Mohamed Mohamed Yasseen Elghandour[3] · Abdelfattah Zeidan Mohamed Salem[3] · Adrian Zaragoza Bastida[4] · David Velázquez-Reynoso[1] · Moisés Cipriano-Salazar[1] · Luis Miguel Camacho-Díaz[1] · María Uxúa Alonso-Fresán[3] · Nicolas DiLorenzo[5]

Received: 23 October 2014 / Accepted: 14 July 2015 / Published online: 9 August 2015

Abstract A region-wide survey was conducted in the tropical area of Tierra Caliente, State of Guerrero, Mexico to estimate the prevalence of subclinical bovine mastitis (SCM), distribution of mastitis pathogens, and in vitro antimicrobial susceptibility of different mastitis pathogens in dairy farms. In total, 1036 quarter milk samples were obtained from 259 cows at 87 different dairy farms. Collected quarter milk samples were submitted for California Mastitis Test (CMT), bacteriological examination, and testing for antimicrobial susceptibility. Overall prevalence of SCM in the studied area was 20.5 %. Prevalence in the different regions was as follows: 28 % in Arcelia municipality, 21 % in Tlalchapa municipality, 19.4 % in Pungarabato municipality, and 14.3 % in Finch Cutzamala municipality. Of all positive isolates, 97.5 % were Gram-negative bacteria. Moreover, of all positive isolates, 37.5 % were *Proteus vulgaris*, 25 % *Salmonella* spp., 12.5 % *Enterobacter aerogenes*, and 10 % *Escherichia coli. Klebsiella pneumonia* and *E. coli* were sensitive for netilmicin antimicrobial. However, *E. coli* was sensitive for pefloxacin and gentamicin with a sensitivity for pefloxacin for *E. aerogenes*, while *Staphylococci* were sensitive for gentamicin and dicloxacillin. It could be concluded that practices such as the implementation of mastitis control programs, improved milking hygiene together with an intramammary treatment with netilmicin, pefloxacin, and gentamicin antimicrobials should be considered for mastitis prevention in the study area of Tierra Caliente, in the tropical area of Guerrero, Mexico.

Keywords Antibiotics susceptibility · Dairy farms · Mexico · Subclinical mastitis

Abbreviations

CMT	California Mastitis Test
I	Intermediate resistance
R	Resistant
S	Sensitive
SCM	Subclinical bovine mastitis

Introduction

Mastitis is defined as an inflammatory reaction of the mammary gland induced when pathogenic microorganisms in the udder produce toxins that are harmful to the mammary gland (Fratini et al. 2014). Mastitis is primarily caused by bacterial infection and is a major cause of economic loss in dairy cattle production (Fragkou et al. 2014; Hosseinzadeh and Saei 2014). As a result of the inflammation, milk composition is

✉ Abdelfattah Zeidan Mohamed Salem
asalem70@yahoo.com

[1] Unidad Académica de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Guerrero, AltamiranoP.O. 40660, Mexico

[2] Dairy Science Department, National Research Centre, 33 Bohouth St. Dokki, Giza, Egypt

[3] Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Estado de México, Mexico

[4] Universidad Autónoma del Estado de Hidalgo, Instituto de Ciencias Agropecuarias, Rancho Universitario, Av. Universidad Km. 1, Ex-Hda. de Aquetzalpa AP 32, CP 43600 Tulancingo, Hidalgo, Mexico

[5] North Florida Research and Education Center, Department of Animal Sciences, University of Florida, Marianna, FL, USA

altered due to a decrease in the synthesis of caseins and lactose, and fat quantity and quality (Botaro et al. 2015). Mastitis also has negative effects on the quality of the milk and manufactured milk products, decreasing the shelf life of the liquid milk products (Guerin-Faublee et al. 2002). Intramammary infections with mastitis has negative effects resulting in the reduction of milk yield (Souto et al. 2010; Fragkou et al. 2014) which is considered to be the most important cause of worldwide economic losse in the dairy industry. The most frequently isolated microorganisms are staphylococci, streptococci, and coliforms, but other microorganisms may infect the udder (Blum et al. 2014; Hosseinzadeh and Saei 2014). The frequency of infection by these udder pathogens varies per country; therefore, preventive measures and milking procedures is different according to local husbandry conditions (Bradley 2002). The most common treatment of mastitis is based on intramammary infusion of antibacterial agents.

Antimicrobials are an important tool in mastitis control programs. However, the widespread use of antimicrobial agents in the treatment and control of mastitis can cause a problem of antibiotic residues in milk leading to the risk of developing resistance in humans who consume milk or milk products (Oliver and Murinda 2012) and also in calves fed with waste milk containing antimicrobial residues (Duse et al. 2014). Moreover, the widespread use of antimicrobials can cause sensitization of the normal pathogens and lead to the development of strains of bacteria resistant to antibiotics (Paterna et al. 2014).

In the tropical region of Guerrero, Mexico, the dairy activity participates in the economic livelihood of many families. So, it is very important to consider the problems that mastitis may cause and the importance of a timely diagnosis to gain knowledge about the prevalence of the disease in the region, as well as the pathogens involved in their etiology. This information can be used to comprehensively assess the resistance and/or sensitivity of these pathogens to the antibiotics used in the treatment of this disease, using the California Mastitis Test (CMT) for the diagnosis of subclinical mastitis (SCM) as it was shown to be a useful and quick diagnostic test for field applications (Paterna et al. 2014). The objective of this study was to report the prevalence of SCM in several dairy farms of a tropical region of Mexico, and the causative pathogens, as well as the sensitivity of mastitis pathogens to several antibiotics.

Materials and methods

Study location

The study was conducted in four municipalities (Tlalchapa, Cutzamala Finch, Arcelia, and Pungarabato) of the tropical hot region of the State of Guerrero, Mexico located at 18° 20′ 30″ north latitude and 100° 39′ 18″ west longitude in the left margin of the Cutzamala river which is one of the main tributaries of the Rio Balsas River.

Size and selection of the sample

A total of 1036 quarter milk samples were obtained from 259 dairy cows at 87 different farms from four municipalities (81 from Tlalchapa, 72 from Pungarabato, 56 from Cutzamala, and 50 from Arcelia). Breed was recorded when samples were collected. Quarter foremilk samples were collected aseptically for bacteriological assay as described by Honkanen-Buzalski (1995). Before sampling, the first streams of milk were discarded, and teat ends were disinfected with cotton swabs soaked in 70 % alcohol and allowed to dry. The sampling sites determination was based on aspects such as availability of livestock, safety, and accessibility for sampling. The inclusion criteria for sampling cows were mainly cows in milking; however, cows that showed inflammation, injury, and/or abnormal characteristics of the milk were excluded.

Evaluation of the prevalence of mastitis

The diagnosis of the prevalence of mastitis was carried out by the CMT, using 10 % Teepol (Leucocytest®; Synbiotics Corporation, France) according to manufacturer's instructions. Briefly, in each well of the test plate, 2 ml of milk was stripped from individual teats, and an equal amount (2 ml) of 10 % Teepol was added to the milk. To mix the reagent and milk, the plate was circularly moved for 10 s, and changes observed at 20 s. The formation of milk clots upon addition of the reagent was recorded and considered as positive.

Bacterial isolation

Milk samples (20 ml) from cows with SCM (positive CMT) were collected and aseptically placed into sterile glass tubes and on the same day were taken as soon as possible under cooling conditions to the Microbiology Laboratory of the Academic Unit of Veterinary Medicine, University of Guerrero for bacterial isolation and antibiotics sensitivity test. The collected milk samples were cultured on different agar media: blood agar, Mac Conkey agar, and Eosin methylene blue agar, as 0.01 ml of milk/agar plate. Samples were incubated at a temperature of 37 °C for 18 to 24 h; however, the plates with no growth were left up to 48 h. The organisms were identified on the basis of colony morphology, Gram staining results, and biochemical tests (Balows et al. 1991; Murray et al. 1994). Gram staining was performed as described by Murray et al. (1994). The color and morphology of the bacteria were observed by microscopy with immersion lens (100×).

Cultivation on blood agar allows the growth of all bacteria in each sample. The selective Mac Conkey agar identifies the presence of *E. coli* (dry, flat, pink-lactose positive colonies)

and *Klebsiella* (dark pink resembling fish-eye, lactose positive) from other strains whose colony growth does not produce pink coloration and, thus, are lactose-negative (*Serratia*, *Enterobacter*, *Pseudomonas* or *Proteus*); also, *Staphylococcus* (pinpoint strains, opaque and limited pale pink coloration) and *Enterococcus* were identified (pinpoint strains, red, opaque) and isolated. Eosin methylene blue agar was used to identify *E. coli* (shiny metallic green colonies) from other *Enterobacteriaceae* (*Salmonella* spp., *Shigella*, *Serratia*, *Enterobacter*, *Pseudomonas*, and *Proteus*) (Jayne et al. 2001).

Two biochemical tests (catalase and indole) were used to classify strains as *Staphylococcus* or *E. coli*. Catalase test was performed to distinguish *Staphylococcus* from *Streptococcus* (Gram-positive bacteria), while indole test was performed to identify *E. coli* (formation of a red ring on the surface of the broth after 24–48 h of incubation; indole positive) from other bacterial species (*Klebsiella*, *Serratia*, *Enterobacter*, *Proteus*, or *Pseudomonas*: indole negative). *Staphylococcus* was identified as catalase-positive colonies (which appeared as opaque white) and the *Streptococcus* as catalase-negative (which appeared as colonies of yellow color). The isolates were maintained frozen at −70 °C in brain heart infusion broth containing 15 % glycerol.

Susceptibility testing

The in vitro susceptibility of isolates to some antimicrobials was determined on milk samples from cows with positive CMT, using twelve antibiotics (BIO RAD, Eugenia, Mexico City, Mexico) as: CAT.71080180 (Gram-positive); CAT.71080280 (Gram-negative) and CAT.71080380 (Combined). Each sample was sub-cultured on blood agar, Mac Conkey agar, and Eosin methylene blue agar. For Gram-positive bacteria, the sensitivity was tested to ceftazidime (30 μg), cefuroxime (30 μg), dicloxacillin (1 μg), erythromycin (15 μg), penicillin (10 UI), and tetracycline (30 μg).

For Gram-negative bacteria, the sensitivity was tested to amikacin (30 μg), carbenicillin (100 μg), ceftriaxone (30 μg, chloramphenicol (30 μg), netilmicin (3 μg), and nitrofurantoin (300 μg).

Antibiotics with action against Gram-positive and Gram-negative bacteria were the following: ampicillin (10 μg), cephalothin (30 μg), cefotaxime (30 μg), gentamicin (10 μg), pefloxacin (5 μg), and trimetropim-sulfametoxazol (25 μg).

Each sample was sub-cultured in Mueller Hinton agar plates (Difco, le Pont de Claix, France), where antibiotic discs were placed at the time of the reculture of the bacterial colonies, and then Petri dishes were incubated at 37 °C for 24 h. After incubation, the degree of inhibition of the bacteria to each antibiotic was measured with the diameter of the inhibition halo taking into consideration the distance in millimeters of a circle at the position of each antibiotic that inhibited the growth of bacteria. Based on this criterion, there were three options: resistant (R), intermediate resistance (I), and sensitive (S), considering the diameter of the inhibitory halo of each antibiotic as shown in Table 1.

Table 1 Interpretation of test results to the sensitivity of bacteria causing mastitis in dairy cows to different antimicrobial agents

Antimicrobial agent	Diameter of inhibition halo (mm)		
	Resistant	Intermediate resistance	Sensitive
Amikacin	≤14.0	15.0–16.0	≥17.0
Ampicillin	≤13.0	14.0–16.0	≥17.0
Carbencilina	≤13.0	14.0–16.0	≥17.0
Ceftriaxone	≤13.0	14.0–20.0	≥21.0
Ceftazidime	≤14.0	15.0–17.0	≥18.0
Cefuroxime	≤14.0	15.0–22.0	≥23.0
Dicloxacillin	≤10.0	11.0–12.0	≥13.0
Erythromycin	≤13.0	14.0–22.0	≥23.0
Pefloxacin	≤14.0	15.0–22.0	≥23.0
Penicillin	≤14.0	15.0–22.0	≥23.0
Tetracycline	≤14.0	15.0–18.0	≥19.0
Chloramphenicol	≤12.0	13.0–17.0	≥18.0
Netilmicin	≤12.0	13.0–14.0	≥15.0
Nitrofurantoin	≤14.0	15.0–16.0	≥17.0
Cephalotin	≤14.0	15.0–17.0	≥18.0
Cefotaxime	≤14.0	15.0–22.0	≥18.0
Gentamicin	≤12.0	13.0–14.0	≥15.0
Trimetropim-Sulfametoxazol	≤10.0	11.0–15.0	≥16.0

Adapted from the following: Performance Standers for Antimicrobial Disk Susceptibility Test

Calculations and data analysis

The prevalence of mastitis, the bacterial genera involved, and the sensitivity to antibiotics was analyzed by descriptive statistics and presented in tables of prevalence estimated by the following equation:

Mastitis prevalence (%)=total positive tests÷total tested samples×100

Results

The current study dealt with a total of 1036 quarter milk samples from 259 cows at 87 different dairy farms from four municipalities. Prevalence of SCM in the studied area was 20.5 %. Prevalence in the different regions was as follows: 28.0 % in Arcelia, 21.0 % in Tlalchapa, 19.4 % in Pungarabato, and 14.3 % in Finch Cutzamala (Table 2).

Of all analyzed samples, the ones obtained from *Bos taurus* genotype had the highest number and percentage of the positive CMT (20.7 %), together with *B. Taurus*×*Bos indicus* genotype (20.7 %) and finally *B. indicus* genotype (19.6 %) which had the lowest number and percentage (Table 3).

Results in Table 4 indicate the diversity of bacterial agents responsible for SCM in dairy cows of the study. Between the causative agents of SCM that grew on the different culture media (n=40), about 97.5 % were Gram-negative bacteria (39 out of 40 isolates) and the 2.5 % were Gram-positive (1 out of 40 isolates). Of all positive isolates, 37.5 % were *Proteus vulgaris*, 25.0 % *Salmonella* spp., 12.5 % *Enterobacter aerogenes*, 10.0 % *E. coli*, 7.5 % *Proteus mirabilis* 5.0 % *Klebsiella pneumonia*, and 2.5 % *Staphylococcus*.

None of *Salmonella* spp., *P. vulgaris*, and *P. mirabilis* showed any sensitivity to the tested antimicrobials. However, *E. aerogenes* were only sensitive to pefloxacin. Moreover, *P. vulgaris* showed resistance to gentamicin, chloramphenicol, cefotaxime, and carbenicillin, with an intermediate resistance to other antimicrobials (Table 5). *Salmonella* spp. showed an intermediate resistance to pefloxacin and chloramphenicol.

Table 2 Prevalence of mastitis in dairy cows in four municipalities of the Tierra Caliente region of the State of Guerrero, Mexico

Municipality	Positive samples[a]		Negative samples		Total
	n	%	*n*	%	
Finch Cutzamala	8	14.3	48	85.7	56
Tlalchapa	17	21.0	64	79.0	81
Pungarabato	14	19.4	58	80.6	72
Arcelia	14	28.0	36	72.0	50
Total	53	20.5	206	79.5	259

[a] Card test

Table 3 Prevalence of mastitis in dairy cows by genetic group in four municipalities of the Tierra Caliente region of the State of Guerrero, Mexico

Genetic group	Positive		Negative		Total
	n	%	*n*	%	
Bos taurus[a]	25	20.7	96	79.3	121
Bos indicus[b]	9	19.6	37	80.4	46
B.Taurus×*B.indicus*	19	20.6	73	79.3	92
Total	53	20.5	206	79.5	259

[a] The cow *Bos taurus* genotype is the native genotype of native breed of Creole, Swiss and American Holstein Friesian

[b] The cow *Bos indicus* genotype is the native genotype of the Brahman breed

Both *K. pneumonia* and *E. coli* were sensitive to netilmicin antimicrobial. However, *E. coli* was sensitive also to pefloxacin and gentamicin. On the contrary, *Staphylococci* were sensitive to gentamicin and dicloxacillin (Table 6).

Discussion

From all tested samples, 20.5 % were positive for CMT. Arcelia municipality had the highest prevalence of SCM in comparison to other municipalities with lower prevalence for Finch Cutzamala municipality. Obtained results show that mastitis prevalence in the studied area seems to be caused by environmental origin rather than contagious pathogens. In the environmental mastitis, *Staphylococcus* spp. and *E. coli* are the main pathogens responsible for the inflammation (Baskaran et al. 2009). However, these differences between regions may be explained by different management factors such as specific dry period management strategies (Green et al. 2007), leaking milk, and previous udder infection (Mungube et al. 2005). In addition, different management systems between farms, stage of lactation (Regassa et al. 2013), parity, breed (Almaw et al. 2008), feeding regimes, and heifer replacement rates (McDougall 1999) are important factors. Moreover, the poor milking hygiene conditions and poor hygiene of the udder, in the studied area, without the use of antiseptics, sealants, and sanitary napkins are different reasons (Haltia et al. 2006). All of previous factors were observed in the current study in Tierra Caliente of Guerrero; however, housing system and herd size, management variation, and lack of experience for veterinary surveillance in the areas of study are factors that might be of importance (Regassa et al. 2013). Ahmad et al. (2012) revealed that animals with poor hygiene in milking process had a high prevalence of mastitis. This might be due to absence of udder washing, milking of animals with common milkers which could be vectors of spread especially for contagious mastitis. Ruiz et al. (2011) observed that the milking system was the determining factor in the

Table 4 Results of the cultured milk samples (n=53 positive card) on blood agar, Mac Conkey, and Eosin methylene blue, and Gram stain

Culture medium	Without growth		With growth					
			Gram-positive[a]		Gram-negative		Total	
	n	%	*n*	%	*n*	%	*N*	%
Blood agar	32	60.4	1	1.9	20	37.7	21	52.5
Mac Conkey agar	42	79.3	0	0.0	11	20.8	11	27.5
Eosin methylene blue	45	84.9	0	0.0	8	15.1	8	20.0
Total			1	1.9	39	97.5	40	

[a] Card test

prevalence of SCM between ranches, reporting a greater prevalence in the mechanized system (49.4 %) versus the manual system (hand milking, 33.4 %).

However, the prevalence of SCM was higher with about 5.3 % in *B. taurus* cattle and *B. Taurus*×*B. indicus* cattle than in *B. indicus* cattle; the difference seems not to be marked. The cattle of *B. taurus* and thus *B. taurus*×*B. indicus* are genetically specialized for high milk production. Rupp and Boichard (2003) noted a negative relationship between milk production and the degree of resistance to mastitis in dairy cattle. This has given rise in many countries to the inclusion of mastitis in their programs for animal selection, as a feature to improve resistance to infections caused by intramammary pathogens (Rupp and Boichard 2003). In Mexico, Muñoz Santiago et al. (2012) compared four genetic groups of cows: ¾ Holstein, ¾ Swiss, Zebu, Swiss/Zebu, and reported that ¾ Swiss group was the most affected by different SCM causing agents (53.3 % of cows of the breed), while the Swiss/Zebu group was the least affected. They did not discussed any probable reasons for different prevalence between different genetic groups.

In general, it can be seen that the total prevalence of mastitis in dairy cows in the Tierra Caliente region of the State of Guerrero is 20.5 %. This figure is lower than that reported by Calderón and Rodriguez (2008) and Ruiz et al. (2011) who obtained a prevalence between 33.4 and 49.4 % in dairy cattle farms. In humid tropics in Mexico, Insua et al. (2008) reported SCM prevalence between 2.8 to 42.0 %. Moreover, in Marquelia town, Guerrero, Mexico, Muñoz Santiago et al. (2012) found that the prevalence of SCM was 45.9 %. However, the relatively low SCM prevalence found in this study is also a sign of problems in the cow's udder health which might negatively influence milk production devoid of safety for the health of consumers, especially in populations that traditionally uses raw milk for consumption and cheese production.

Table 5 Prevalence of mastitis bacterial agents in 21 milk samples showed growth in different culture media

Bacterial isolate	Culture medium in agar (*n*)			Total	
	Blood	Mac Conkey	Eosin methylene blue	*N*	%
Salmonella spp.	5	3	2	10	25.0
Proteus vulgaris	9	3	3	15	37.5
Proteus mirabilis	3	0	0	3	7.5
Klebsiella pneumoniae	2	0	0	2	5.0
Escherichia coli	2	1	1	4	10.0
Enterobacter aerogenes	3	2	0	5	12.5
Staphylococcus[a]	1	0	0	1	2.5
Total	25	9	6	40	

[a] The identification was made by the catalase test

As was mentioned before, only 53 cows were positive to CMT. Of the positive samples (i.e., 53 samples), only 52.5 % grew up on the culture medium of blood agar, 27.5 % grew up on Mac Conkey agar, and 20.0 % grew up on Eosin methylene blue agar. Compared to other studies, the current percentages of grown pathogenic agents from SCM are low. About 61.0 % (Ruiz et al. 2011) and 88.0 % (Persson et al. 2011) from SCM positive milk samples were grown when milk samples were cultured in blood agar medium. In addition, Islam et al. (2011) observed that from the total number of positive CMT, only 39.0 % grew in blood agar medium and 61.0 % in Eosin methylene blue medium.

Bacteriological culture is routinely used to diagnose SCM in dairy cows, and culture results are often the basis for treatment or culling decisions. Moreover, in SCM control programs, detection of the responsible etiological agent is a tool that will help make appropriate decisions according to the epidemiological situation of each herd. In the current study, 97.5 % of the mastitis-causing agents were Gram-negative bacteria. These results show that the prevalent causes of SCM in the studied area are mainly due to environmental mastitis and not due to contagious pathogens. Gram-negative bacilli are the main cause of environmental mastitis and are mainly found in the environment in which cows are housed, such as the ground, milking equipment, and perhaps the hands of the milkers, indicating an association with poor hygiene during milking protocols (Guízar et al. 2008). In Mexico, grazing and tie stalls are still the most common

Table 6 In vitro susceptibility (*R* resistant, *I* intermediate resistance, *S* sensitive) of mastitis bacterial agents to antimicrobials in dairy cows of four municipalities in the Tierra Caliente region of the State of Guerrero, Mexico

Antimicrobial agent	Bacterial agent						
	Salmonella spp.	*P. vulgaris*	*P. mirabilis*	*K. pneumoniae*	*E. coli*	*E.aerogenes*	*Staphylococci*
Pefloxacin	I	I	R	I	S	S	I
Amikacin	R	I	R	R	R	R	–
Gentamicin	R	R	R	R	S	S	S
Chloramphenicol	I	R	R	I	R	R	–
Nitrofurantoin	R	I	R	I	R	R	–
Cephalotin	R	I	R	R	R	R	I
Ceftriaxone	R	I	I	I	R	R	–
Trimethoprim-Sulfamethoxazole	R	I	R	R	I	I	I
Cefotaxime	R	R	R	R	I	I	R
Netilmicin	R	I	R	S	S	R	–
Carbenicillin	R	R	R	R	R	R	–
Erythromycin	–	–	–	–	–	–	I
Cefuroxime	–	–	–	–	–	–	R
Ampicillin	R	I	R	R	R	R	R
Penicillin	–	–	–	–	–	–	R
Ceftazidime	–	–	–	–	–	–	R
Dicloxacillin	–	–	–	–	–	–	S
Tetracycline	–	–	–	–	–	–	R

feeding systems for dairy cattle. A relationship between such feeding systems and predominant bacteria causing both clinical and sub-clinical mastitis was reported by Unnerstad et al. (2009) and Persson et al. (2011) in Sweden. Islam et al. (2011) identified that Gram-positive and Gram-negative bacteria responsible for SCM in sheep and goats were cocci and rod-shaped, respectively. However, Regassa et al. (2013) stated that Gram-positive cocci were the main cause of camel mastitis and accounted for 74.6 % of the total isolates.

Proteus vulgaris was the most commonly isolated bacterial group causing mastitis in the dairy cows in our study. The reasons are unknown; however, this may be related with the geographical area, animal management system, climate, and many other factors, including antimicrobial resistance. Muñoz Santiago et al. (2012) found, in another place in the State of Guerrero, Mexico, that in all cows with SCM, 26.7 % were Gram-negative bacilli and 13.3 % were Gram-positive bacilli. Antimicrobial resistance may result from increased levels of antimicrobial exposure (Jamali et al. 2014). Therefore, prudent antimicrobial use guidelines which address strategies that may influence the development of antimicrobial resistance should be developed and strictly followed. Dairy producers are encouraged to work with and obtain help from veterinary practitioners to develop new and improved strategies for prudent use of antimicrobials (Barlow 2011).

There are other reports of the participation of other agents in the development of SCM such as *E. coli* with 14.7 to 27.6 % of isolates (Islam et al. 2011) or *Klebsiella* spp. with 0.8 % (Persson et al. 2011). The results of the current study indicate the diversity of bacterial agents responsible for SCM in dairy cows, which may represent a risk in certain circumstances to public health.

Contaminated milk obtained from cows affected by SCM is a potential source of staphylococcal food poisoning to consumers (Zecconi and Hahn 2000). Because most udder inflammation with mastitis is subclinical, it is easy for farmers to neglect the disease resulting in infection persistency, and mastitis progresses to chronic mastitis. Most of dairy farmers have introduced dry cow antibiotic therapy to control mastitis, which in many cases has proven to be cost effective and satisfactory (Islam et al. 2011). Mastitis can be overcome by antibiotic treatment after identification of the causative agents. Antibiotic sensitivity tests can be performed to ensure adequate treatment (Islam et al. 2011). However, the administration of antimicrobials can negatively affect the calf's gut flora and increase the level of antimicrobial resistant gut bacteria if they are fed waste milk; therefore, waste milk of cows with SCM and treated with such antibiotics should not be used. Our results showed that only *E. coli*, *E. aerogenes*, *Staphylococci*, and *K. pneumonia* were fully sensitive to some antimicrobials, particularly gentamicin, netilmicin, and dicloxacillin. These results showed some similarities with previous studies, but higher rates of resistance to the same antibiotics are reported here. Limited knowledge about the prudent use of

antimicrobial agents and lack of veterinarian surveillance in the studied region are likely the main reasons of the highly observed resistance of antimicrobial agents. Almost no studies are found in the study area dealing with sensitivity and resistance of mastitis-causing agents to different antimicrobial agents. To our knowledge, limited information is available about the susceptibility of SCM-causing agents to antimicrobials in Mexico. Therefore, we will only cite studies from different regions and countries. In agreement with our study, Persson et al. (2011) reported some resistance of *E. coli* to ampicillin and tetracycline-trimethoprim with high sensitivity to gentamicin. The variability of antibiotic susceptibility between individual species may arise from several reasons. For example, the geographical variations in resistance profiles of coagulase-negative staphylococci species have a considerable impact on antimicrobial prescription (Kenar et al. 2012). Moreover, differing health education for farmers and unregulated use of antibiotics in some places may be another reason for different susceptibility and resistance of different bacterial agents. Generally, differences from our study and other cited studies may be due to different environments, different sampling conditions, different SCM-causing microbial agents with different resistance levels, different cultural practices about the prudent use of antimicrobial agents, and many other factors.

Conclusions

The prevalence of SCM was 20.5 % in the area of Tierra Caliente, Guerrero, Mexico. Pungarabato municipality had the highest prevalence percentage (28.0 %). Of the causing bacterial agents, 97.5 % were Gram-negative. About three fourths of the SCM was caused by *P. vulgaris*, *Salmonella* spp., and *E. aerogenes*. Moreover, the mastitis-causing agents showed sensitivity to netilmicin, pefloxacin, and gentamicin which could be considered for mastitis treatment in the study area. However, pefloxacin antibiotic has been classified as a highly critical antibiotic according to WHO for humans and animals. All types of antibiotic can cause resistance in calves and the environment. Increased antimicrobial resistant gut bacteria is the most important outcome of feeding calf's waste milk from cows treated with antibiotics. Initially, waste milk of cows with SCM and treated with such antibiotics should not be used, and more studies are required. The obtained results showed that the SCM in the studied area is mainly of environmental origin. Therefore, and under the hot tropical region of Guerrero State, Mexico, it is recommended that in order to reduce the prevalence of mastitis in the area, some standards need to be developed and be followed. Mastitis control programs, improved milking hygiene, culling of chronic mastitis carriers, and treating of clinically and subclinically infected cows should be practiced.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the official Mexican standard of animals care (NOM-051-ZOO-1995).

Conflict of interest All authors declare that there are no present or potential conflicts of interest among the authors and other people or organizations that could inappropriately bias their work.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

Ahmad, S., Yaqoob, M., Bilal, M.Q., Muhammad, G., Yang, L.G., Khan, M.K. and Tariq, M., 2012. Risk factors associated with prevalence and major bacterial causes of mastitis in dromedary camels (*Camelus dromedarius*) under different production systems. Tropical Animal Health and Production, 44, 107–112.

Almaw, G., Zerihun, A. and Asfaw, Y., 2008. Bovine mastitis and its association with selected risk factors in smallholder dairy farms in and around Bahir Dar, Ethiopia. Tropical Animal Health and Production, 40, 427–432.

Balows, A., Housher, W.Jr., Herrman, K.L., Isenberg, H.D. and Shadoms, H.J., 1991. Manual of clinical microbiology, (American Society of Microbiology, Washington DC).

Barlow, J., 2011. Mastitis therapy and antimicrobial susceptibility: a multispecies review with a focus on antibiotic treatment of mastitis in dairy cattle. Journal of Mammary Gland Biology and Neoplasia, 16, 383–407.

Baskaran, S.A., Kazmer, G.W., Hinckley, L., Andrew, S.M. and Venkitanarayanan, K., 2009. Antibacterial effect of plant-derived antimicrobials on major bacterial mastitis pathogens *in vitro*. Journal of Dairy Science, 92, 1423–1429.

Blum, S.E., Heller, E.D. and Leitner, G., 2014. Long term effects of *Escherichia coli* mastitis. The Veterinary Journal, 201, 72–77.

Botaro, B.G., Cortinhas, C.S., Dibbern, A.G., e Silva, L.F.P., Benites, N.R. and dos Santos, M.V., 2015. *Staphylococcus aureus* intramammary infection affects milk yield and SCC of dairy cows. Tropical Animal Health and Production, 47, 61–66.

Bradley, A.J., 2002. Bovine Mastitis: an evolving disease. Veterinary Journal, 164, 116—28.

Calderón, A. and Rodríguez, V.C., 2008. Prevalencia de mastitis bovina y su etiología infecciosa en sistemas especializados en producción de leche en el altiplano cundiboyacense (Colombia). Revista Colombiana de Ciencias Pecuarias, 21, 582–589.

Duse, A., Waller, K.P., Emanuelson, U., Unnerstad, H.E., Persson, Y. and Bengtsson, B., 2014. Risk factors for antimicrobial resistance in fecal *Escherichia coli* from preweaned dairy calves. Journal of Dairy Science, 98, 500–516.

Fragkou, I.A., Boscos, C.M. and Fthenakis, G.C., 2014. Diagnosis of clinical or subclinical mastitis in ewes. Small Ruminant Research, 118, 86–92.

Fratini, F., Casella, S., Leonardi, M., Pisseri, F., Ebani, V.V., Pistelli, L. and Pistelli L., 2014. Antibacterial activity of essential oils, their blends and mixtures of their main constituents against some strains supporting livestock mastitis. Fitoterapia, 96, 1–7.

Green, M.J., Bradley, A.J., Medley, G.F. and Browne, W.J., 2007. Cow, farm, and management factors during the dry period that determine the rate of clinical mastitis after calving. Journal of Dairy Science, 90, 3764–3776.

Guerin-Faublee, V., Tardy, F., Bouveron, C. and Carret G., 2002. Antimicrobial susceptibility of *Streptococcus* species isolated from

clinical mastitis in dairy cows. International Journal of Antimicrobial Agents, 19, 219–226.

Guízar, F.P., Bedolla, C.J.I. and Luis, C.J., 2008. Determinación de la Prevalencia de Mastitis Bovina en el Municipio de Tímbaro, Michoacán, Mediante la Prueba de California. Revista Electrónica Veterinaria, 9(10), 1–34.

Haltia, L., Honkanen-Buzalski, T., Spiridonova, I., Olkonen, A. and Myllys, V., 2006. A study of bovine mastitis, milking procedures and management practices on 25 Estonian dairy herds. Acta Veterinaria Scandinavica, 48, 22–27.

Honkanen-Buzalski, T., 1995. Sampling technique, transportation and history. In: M. Sandholm, T. Honkanen-Buzalski, L. Kaartinen, S. Pyörälä (eds), The bovine udder and mastitis, (Gummerus kirjapaino Oy, Jyväskylä, Finland), 112.

Hosseinzadeh, S. and Saei, H.D., 2014. Staphylococcal species associated with bovine mastitis in the North West of Iran: Emerging of coagulase-negative staphylococci. International Journal of Veterinary Science and Medicine, 2, 27–34.

Insua, A.D., Pérez, G.C. and Silveira, P.E., 2008. Evaluación Epizootiológica de la Mastitis Bovina en Cuatro Vaquerías. Revista Electrónica de Veterinaria, 9, 1–9.

Islam, M.A., Samad, M.A. and Anisur Rahman, A.K.M., 2011. Bacterial pathogens and risk factors associated with mastitis in Black Bengal goats in Bangladesh. Bangladesh Journal of Veterinary Medicine, 9, 155 – 159.

Jamali, H., Radmehr, B. and Ismail S., 2014. Prevalence and antibiotic resistance of *Staphylococcus aureus* isolated from bovine clinical mastitis. Journal of Dairy Science, 97, 2226–2230.

Jayne, L.D., Russel, R.J. and Elvinger, F., 2001. Use of eosin methylene blue agar to differentiate *Escherichia coli* from other gram-negative mastitis pathogens. Journal of Veterinary Diagnostic Investigation, 13, 273–275.

Kenar, B., Kuyucuoğlu, Y. and Şeker, E., 2012. Antibiotic susceptibility of coagulase-negative staphylococci isolated from bovine subclinical mastitis in Turkey. Pakistan Veterinary Journal, 32(3), 390–393.

McDougall, S., 1999. Prevalence of clinical mastitis in 38 Waikato dairy herds in early lactation. New Zealand Veterinary Journal, 47, 143–149.

Mungube, E.O., Tenhagen, B.A., Regassa, F., Kyule, M.N., Shiferaw, Y., Kassa, T. and Baumann, M.P., 2005. Reduced milk production in udder quarters with subclinical mastitis and associated economic losses in crossbred dairy cows in Ethiopia. Tropical Animal Health and Production, 37, 503–512.

Muñoz Santiago, J., Arrieta B.E., Camacho Díaz, L.M. and Hernández Valenzuela, D., 2012. Aislamiento bacteriano en bovinos de doble propósito con mastitis subclínica, en la costa de guerrero, México. REDVET Revista elecrtonica de Veterinaria, 13, 1–11.

Murray, R.G.E., Doetsch, R.N. and Robinow, C.F., 1994. Determinative and cytological light microscopy. In: Gerhardt, P., R.G.E. Murray, W.A. Wood and N.R. Krieg (eds), Methods for General and Molecular Bacteriology (ASM Press, Washington DC, USA), 21–41.

Oliver, S.P. and Murinda, S.E., 2012. Antimicrobial resistance of mastitis pathogens. Veterinary Clinics of North America: Food Animal Practice, 28, 165–185.

Paterna, A., Contreras, A., Gómez-Martín, A., Amores, J., Tatay-Dualde, J., Prats-van der Ham, M., Corrales, J.C., Sánchez, A. and De la Fe, C., 2014. The diagnosis of mastitis and contagious agalactia in dairy goats. Small Ruminant Research, 121, 36–41.

Persson, Y., Nyman, A.J. and Grönlund-Andersson, U., 2011. Etiology and antimicrobial susceptibility of udder pathogens from cases of subclinical mastitis in dairy cows in Sweden. Acta Veterinaria Scandinavica, 53, 36–43.

Regassa, A., Golicha, G., Tesfaye, D., Abunna, F. and Megersa, B., 2013. Prevalence, risk factors, and major bacterial causes of camel mastitis in Borana Zone, Oromia Regional State, Ethiopia. Tropical Animal Health and Production, 45, 1589–1595.

Ruiz, A.K., Ponce, P., Gomes, G., Mota, R.A., Sampaio, E., Lucena, E.R. and Benone, S., 2011. Prevalencia de mastitis bovina subclínica y microorganismos asociados: comparación entre ordeño manual y mecánico, en Pernambuco, Brasil. Revista de Salud Animal, 33 (1), 57–64.

Rupp, R. and Boichard, D., 2003. Genetics of resistance to mastitis in dairy cattle. Veterinary Research, 34, 671–688.

Souto, L.I., Minagawa, C.Y., Telles, E.O., Garbuglio, M.A., Amaku, M., Melville, P.A., Dias, R.A., Sakata, S.T. and Benites, N.R., 2010. Correlation between mastitis occurrence and the count of microorganisms in bulk raw milk of bovine dairy herds in four selective culture media. Journal of Dairy Research, 77, 63–70.

Unnerstad, H.E., Lindberg, A., Waller, K.P., Ekman, T., Artursson, K., Nilsson-Ost, M. and Bengtsson, B., 2009. Microbial aetiology of acute clinical mastitis and agent-specific risk factors. Veterinary Microbiology, 137, 90–97.

Zecconi, A. and Hahn, G., 2000. *Staphylococcus aureus* in raw milk and human health risk. Bulletin of the IDF, 345, 15–18.