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ORIGINAL RESEARCH

Prevalence, bacterial causes, and antimicrobial susceptibility profile of mastitis isolates from cows in large-scale dairy farms of Northern Ethiopia

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Abstract The study was undertaken to determine the prevalence of bovine mastitis, isolate mastitis causing bacteria, assess the association of some risk factors, and determine the antibiotic resistance pattern of bacterial isolates in cows in large-scale dairy farms of Northern Ethiopia. A total of 305 lactating and nonlactating cows were included in the present study. The overall prevalence of clinical and subclinical mastitis was 3.6 and 33.8 %, respectively. The quarter level prevalence was 15.4 %; from which, 11.9 and 1.1 % were subclinical form and blind teat, respectively, while the remaining 2.4 % were of clinical form. *Staphylococcus aureus* accounted for 36 % of the isolates followed by *Escherichia coli* (27.3 %). Risk factors including age ($p<0.001$), parity ($p<0.001$), and lactation stage ($p=0.02$) showed significant association with the occurrence of mastitis. Higher prevalence was observed in both groups of older cows (i.e., 6–9 years (odds ratio (OR)=4.65, 95 % confidence interval (CI)=2.74–7.89) and >9 years (OR=3.63, 95 % CI=1.42–9.25)), cows with four to seven calves (OR=3.39, 95 % CI=2.06–5.60), and cows in late lactation stage (OR=3.79, 95 % CI=1.64–8.75). In multivariable logistic regression analysis, age ($p=0.005$) and lactation stage ($p=0.027$) showed statistically significant association with the occurrence of mastitis. The antimicrobial susceptibility pattern showed high susceptibility of *S. aureus* to nalidixic acid (82.4 %) followed by chloramphenicol (58.8 %); however, these species were resistant to the rest of the antimicrobials tested. Highest resistance was observed against clindamycin and ampicillin. Coliform bacteria (*E. coli* and *Klebsiella pneumoniae*) showed resistance to most of the antimicrobials used. Detailed investigation is needed to identify the interplay of managemental and environmental risk factors to design appropriate control measures.

Keywords Antimicrobial susceptibility · Dairy cows · Mastitis · Northern Ethiopia · Prevalence · Risk factors

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Introduction

Milk is one of the most important foods of human beings. It is universally recognized as a complete diet due to its essential components (Javaid et al. 2009). However, health risk to consumers can be associated with milk, due to the presence of zoonotic pathogens and antimicrobial drug residues (Bradley 2002). The quality of milk may be lowered by a numbers of factors such as adulteration, contamination during and after milking, and the presence of udder infections (Esron et al. 2005).

Mastitis, inflammation of the mammary gland, is a highly prevalent problem in dairy cattle and is one of the most important threats affecting the world's dairy industry (Wallenberg et al. 2002). Mastitis has been known to cause a great deal of loss or reduction of productivity, influence the

quality and quantity of milk yield, and cause culling of animals at an unacceptable age. The disease generally involves the interplay between management practice and infectious agents.

Ethiopia has the largest cattle population in Africa with an estimated population of 49.3 million (CSA 2008). Around 42 % of the total cattle heads are milking cows. Given the considerable potential for smallholder income and employment generation from high-value dairy products, development of the dairy sector in Ethiopia can contribute significantly to poverty alleviation and nutrition in the country. Nevertheless, the quality and quantity of milk produced in the country deteriorate because of various causes (Matios et al. 2009). Undoubtedly, mastitis is the single most important disease that significantly affects the dairy sector.

Several studies in the country have documented prevalence ranging from 1.8 to 21.1 % for clinical and 22.3 to 46.6 % for subclinical mastitis with significant economic losses associated with the disease (Workineh et al. 2002; Kerro and Tareke 2003; Biffa et al. 2005; Hunderra et al. 2005; Mungube et al. 2005; Getahun et al. 2008; Almaw et al. 2008; Bitew et al. 2010).

Mastitis is one of the major diseases of crossbred cows in Addis Ababa milk shed next to reproductive-associated problems (Lemma et al. 2001). Mungube et al. (2005) and Tesfaye et al. (2010) estimated the economic losses from mastitis in the urban and periurban areas of Addis Ababa, to be US$ 58 and 78.65 per cow and per lactation, respectively.

Although several studies have revealed the significance of the diseases in various scales of dairy farms, all of them focused in Central Ethiopia mainly Addis Ababa and its environs and small-scale dairy farms. Only few studies have investigated the prevalence of mastitis in large-scale dairy farms. There is paucity of information on the status of mastitis in Tigray Regional State, Northern Ethiopia, which accounts for around 10 % of the national cattle population. High-yielding cattle breeds are being introduced to households and small businesses to satisfy the increasing demand for milk and dairy products. On the other hand, with intensification of dairy cows, mastitis could be devastating warranting a coordinated epidemiological surveillance system. To our knowledge, this is the first report from Mekelle and its surroundings, Northern Ethiopia. This work was designed to determine the prevalence and some associated risk factors for bovine mastitis, to isolate bacterial causative agents, and to determine the antibiotic susceptibility profile of bacterial isolates against the commonly used antibiotics.

Materials and methods

Study area

The study was conducted in different large-scale dairy farms in and around Mekelle, Northern Ethiopia. Mekelle is the capital city of Tigray Regional State and is located at 783 km north of Addis Ababa at 38.5° east longitude and 13.5° north latitude and an altitude of 2,200 m above sea level. The mean annual rainfall of the area is 628.8 mm. The annual minimum and maximum temperature is 11.8 and 29.94 °C, respectively. The cattle population of Tigray Region is estimated above 4.5 million heads, and the cattle population of the study area is about 0.94 million; of which, the dairy cattle population (cross breed and local breeds) of the area is about 36,500. The average milk production of the local breeds and crossbred (local zebu×Friezian) cows is about 2.9 l/day and 8.5 liter/day, respectively (RSTBARD 2009).

Farms and study design

The investigation was planned as an 8-month long (October 2009 to May 2010) cross-sectional study. The target population was all large market-oriented dairy farms. General descriptions of the Ethiopian improved breed-based dairy farms were published previously (Workineh et al. 2002; Shiferaw et al. 2003; Mungube et al. 2005).

The farms are market-oriented specialized dairy farms. Milk is sold to the city's cafeteria and households. The study farms have exclusively crossbred cattle (Friesian with local zebu breeds) with blood proportions above 70 % exotic. Hand milking is practiced twice a day. Calves are allowed either to suckle before and after milking or bucket fed. A variety of feed stuffs are used in the diets, such as hay supplemented with urea, wheat barn, sesame cake, cotton-seed cake, and in some farms clover and alfalfa. The farms use modern houses with concrete floor with well-ventilated and rain-proof roof system. The farms receive artificial insemination, pregnancy diagnosis, and veterinary services from the city's veterinary services.

For the purpose of this study, large dairy farm was defined as one with herd size of 50 dairy cows and above according to Shiferaw et al. (2003). Between October 2009 and May 2010, 13 large dairy farms were identified in the study region, and samples were collected. The mean size of herds included in the study was 71 (range 52–157). No information was available for prevalence estimates of larger herds in the region. Based on the resources available, 20 to 30 animals were randomly selected from each farm in proportion to the size of the farm. Accordingly, 305 lactating crossbred (Holstein-Friesian×zebu) cows with healthy or inflamed udder were included in the study.

To determine the association of risk factors, data including age, parity, and physiological status of the cow were collected from the record sheet of the farms and by observation. Age of the study animals was determined from birth records and dentition characteristics and categorized as young adults (≥3 to 5 years), adults (≥6 to 9 years), and

old (>9 years). Parity was also categorized as few (with one to three calves), moderate (four to seven calves), and many (>7 calves). Lactation stage of the cow was categorized as early lactation (1–120 days), mid-lactation (121–240 days), and late lactation (above 240 days).

Clinical examination of the udder

Following clinical examination, clinical mastitis was diagnosed at the quarter level based on visible and palpable signs (hard and swollen quarter, kicking up on touching the udder, heat) as previously described (Kivaria et al. 2007). In addition, milk from each quarter was withdrawn and examined for any change (watery secretions, clots in milk, and blood-tinged secretions). The size and consistency of mammary quarters were inspected for the presence of any anatomical malformation, such as disproportional symmetry, swelling, firmness, and blindness.

California mastitis test

The California mastitis test (CMT) was conducted to diagnose the presence of subclinical mastitis. This screening test was performed according to the procedure given by Quinn et al. (2002). The result was scored as 0, +1, +2, or +3 depending on the intensity of reaction. Samples with CMT result score of 0 and +1 were considered as negative, while those with a score of +2 or +3 were taken as positive. If at least one quarter was positive by the CMT, the cow was considered positive.

Milk sample collection

Milk samples were collected aseptically from quarter's diagnosed with CMT≥+2 and clinical cases and were submitted for bacteriological examination and tests for antibiotic susceptibility. Briefly, the udder of the cow was thoroughly cleaned with water and dried with a clean towel. After disinfecting the teats with swabs with 70 % ethyl alcohol, milk was collected. The first three to four streams of milk were discarded, and then, 5–10 ml of milk was collected from each teat aseptically in separate universal bottles. Tubes were sealed properly and transported on ice to Veterinary Microbiology laboratory in Mekelle University, where samples were immediately cultured or kept in a refrigerator at 4 °C for a maximum of 24 h until cultured on standard bacteriological media.

Bacteriological examination of milk samples

Bacteriological examination was done with some modification according to Quinn et al. (2002). A loopful of milk sample was streaked on tryptose blood agar base enriched with 7 % defibrinated sheep blood (Oxoid, UK) and MacConkey agar (Oxoid, UK) plates using the quadrant streaking method. Both agar plates were incubated aerobically at 37 °C for 24–48 h and examined for characteristic bacterial colonies. Pure culture colonies were selected and subcultured on general purpose medium, nutrient agar (Oxoid, UK), and incubated aerobically at 37 °C for 24–48 h for further biochemical identification. After this incubation on nutrient agar (Oxoid, UK), bacteria were identified according to their Gram reaction and morphology. Further identification of the organisms was done by implementing biochemical tests, catalase, oxidase, CAMP test, IMViC tests, triple sugar iron agar test, nitrate reduction, and urease test. In addition, mannitol salt agar was used to differentiate *Staphylococcus aureus* from other *Staphylococcus* spp.

Antimicrobial susceptibility test

Antimicrobial susceptibility test was conducted on a total of 48 randomly selected bacterial isolates, *Staphylococcus* species (n=26) and coliforms (*Escherichia coli* and *Klebsiella* pnuemoniae (n=22), which were frequently recovered during the study period. The isolates were tested for their susceptibility to six antimicrobials using the Kirby–Bauer disk diffusion method (Cappucino and Sherman 2005). The following antimicrobial disks (Oxoid, UK) with their corresponding concentration were used: ampicillin (10 μg), erythromycin (15 μg), nalidixic acid (30 μg), clindamycin (2 μg), sulfamethoxazole (25 μg), and chloramphenicol (30 μg). The cutoff values for the evaluation of the susceptibility of isolates were when the inhibition zone was greater than or equal to 29 and 17 mm for ampicillin (for staphylococci and Gram-negative enteric organisms, respectively) and 23, 19, 21, 16, and 18 mm for erythromycin, nalidixic acid, clindamycin, sulfamethoxazole, and chloramphenicol, respectively. As a plating medium, Mueller–Hinton agar was used. Antibiotic-impregnated paper discs and plates were incubated at 37 °C for 16–18 h. Interpretation was made as per the zone size inhibition chart provided by the manufacturer of the antibiotic disks. The antimicrobial susceptibility was scored as resistant, intermediate, and sensitive based on sensitivity pattern.

Statistical analysis

Data obtained both from bacteriology and questionnaire were stored in Microsoft Excel spreadsheet (Microsoft Corp.). Prevalence of mastitis related to specific risk factors was determined as the proportion of affected cows out of the total examined. These data were analyzed by descriptive statistics, univariable and multivariable regression using the SPSS 11.5 statistical package (SPSS 2002). To see the contribution of the various risk factors for the occurrence of

Table 1 Relative occurrences of bacteria isolated from clinical and subclinical mastitis

Bacterial	Clinical no.[a] (%[b])	Subclinical no. (%)	Total no. (%)
S. aureus	3 (10.7)	43 (43.0)	46 (36.0)
Other staphylococci	1 (3.6)	10 (10.0)	11 (8.6)
E. coli	16 (57.1)	19 (19.0)	35 (27.3)
K. pneumoniae	2 (7.1)	9 (9.0)	11 (8.6)
Pseudomonas auroginosa	–	3 (3.0)	3 (2.3)
Proteus species	–	4 (4.0)	4 (3.1)
S. agalactiae	3 (10.7)	6 (6.0)	9 (7.0)
S. dysgalactiae	3 (10.7)	1 (1.0)	4 (3.1)
Other streptococci	–	3 (3.0)	3 (2.3)
Corynebacterium species	–	2 (2.0)	2 (1.6)
Total	28 (100)	100 (100)	128 (100)

[a] Number of milk samples positive for the specific bacterial isolate

[b] Proportion from the total of the same column

mastitis, we conducted chi-square (χ^2) test using the cross tabulation feature of the software. Univariable logistic regression was applied to measure the strength of that association. The logistic regression model was fitted with individual cow CMT result (positive/negative) as the outcome. The model was built using the forward stepwise (conditional) selection procedure by applying the iterative maximum likelihood estimation procedure, while the statistically significant contribution of individual predictors to the models was tested using the Wald's test and likelihood-ratio tests. Any interaction between variables was assessed by constructing a multivariable model as previously described by Hailessilasie et al. (2010). The logistic model was checked for goodness-of-fit using the Hosmer and Lemeshow test. $p<0.05$ was taken as significant.

Results

Prevalence

Of the total 305 cows examined during the study period, 114 (37.4 %) of the cows were positive for mastitis. Out of these, 3.6 % (11/305) and 33.8 % (103/305) showed clinical and subclinical mastitis, respectively. The quarter level prevalence was found to be 15.3 % (187/1220); from which, 11.9 % (145/1220) and 1.1 % (13/1220) were found to be of subclinical form and blind teat, respectively. The remaining 2.4 % (29/1220) were of clinical form revealing active cases of mastitis with visible signs of inflammation on the udder and changes in milk quality. From the total of 114 cows found positive with CMT, 56.1 % (64/114), 30.7 % (35/114), 6.1 % (7/114), and 7 % (8/114) were found positive for single, two, three, and four quarters, respectively.

Bacteriological examination result

Milk samples from 174 quarters, 103 CMT positive and 11 clinically infected cows, were cultured. Milk samples from 67 dairy cows showed growth only on blood agar whereas samples from 47 dairy cows showed growth on both MacConkey and blood agar. A total of 128 bacterial isolates were isolated from the milk samples processed. Out of these isolates, 28 (21.9 %) were from clinical mastitis, and the other 100 (78.1 %) isolates were from subclinical mastitis. *S. aureus* accounted for 46 (36 %) of the isolates (3 and 43 isolates from clinical and subclinical mastitis, respectively)

Table 2 Univariable and multivariable logistic regression analyses (LR) of risk factors for the occurrence of mastitis in large dairy farms of Mekelle City, Northern Ethiopia, OR odds ratio, CI confidence interval

Risk factors	Category level	Number	Prevalence (%)	Univariable LR analyses results				Multivariable LR analysis result			
				p value	OR	95 % CI of OR		*p* value	OR	95 %CI of OR	
						Lower	Upper			Lower	Upper
Age	3–5 years	141	19.9	0.001	–	–	–	0.005	–	–	–
	6–9 years	142	53.5	0.001	4.65	2.74	7.89	0.01	3.11	2.08	6.73
	9 years and above	22	45.5	0.008	3.63	1.42	9.25	0.022	1.89	0.87	3.41
Parity	1–3	167	25.1	0.001	–	–	–	0.001	–	–	–
	4–7	122	53.3	0.001	3.39	2.09	5.60	0.056	2.73	1.47	8.66
	>7	16	43.8	0.109	2.32	1.1	6.61	0.18	0.98	0.72	2.44
Lactation stage	Early	141	36.9	0.06	–	–	–	0.102	–	–	–
	Mid	137	34.3	0.08	0.98	0.56	1.67	0.204	1.02	0.69	3.21
	Late	27	66.7	0.02	3.79	1.64	8.75	0.027	3.62	2.43	7.89

Table 3 Antimicrobial susceptibility profiles of *S. aureus* (n=17) and other *Staphylococcus* species (n=9)

Antimicrobial	*S. aureus*			Other staphylococci		
	Resistant (%)	Intermediate (%)	Susceptible (%)	Resistant (%)	Intermediate (%)	Susceptible (%)
Ampicillin	14 (82.4)	1 (5.9)	2 (11.8)	8 (88.9)	0 (0.0)	1 (11.1)
Erythromycin	10 (58.8)	6 (35.3)	1 (5.9)	5 (55.6)	1 (11.1)	3 (33.3)
Nalidixic acid	1 (5.9)	2 (11.8)	14 (82.4)	2 (22.2)	0 (0.0)	7 (77.8)
Clindamycin	15 (88.2)	1 (5.9)	1 (5.9)	5 (55.6)	1 (11.1)	3 (33.3)
Trimethoprim–sulfamethoxazole	9 (52.9)	5 (29.4)	3 (17.6)	5 (55.6)	1 (11.1)	3 (33.3)
Chloramphenicol	3 (17.6)	4 (23.5)	10 (58.8)	2 (22.2)	1(11.1)	6 (66.7)

followed by *E. coli* which accounted for 35 (27.3 %) of the bacterial isolates (16 and 19 isolates from clinical and subclinical mastitis, respectively). *E. coli* was the most common isolate from clinical mastitis cases 16 (66.7 %) followed by *S. aureus*, *Streptococcus agalactiae*, and *Streptococcus dysgalactiae* 10.7 % each whereas *S. aureus* 43 (41.3 %) was the predominant isolate from subclinical cases followed by *E. coli* 19 (18.3 %) (Table 1).

Risk factors

Association of the investigated risk factors and the occurrence of mastitis in large-scale dairy farms of Mekelle City are shown in Table 2. Univariable logistic regression analysis indicated that all the risk factors assessed showed significant association with the occurrence of mastitis. Cows older than 6 years were more affected (53.5 %) with mastitis compared to young adult cows (19.9 %). Similarly, there was a higher occurrence of mastitis in cows with four to seven calves (53.3 %, odds ratio (OR)=3.39, 95 % confidence interval (CI)=2.06–5.60) than those cows with one to three calves. In relation to lactation stages of the cow, a higher prevalence of mastitis was recorded in cows at late stage of lactation (66.7 %, OR=3.79, 95 % CI=1.64–8.75) than cows in early and mid-lactation stages. In multivariable logistic regression analysis, age (p=0.005) and lactation stage (p=0.027) showed a statistically significant association with the occurrence of mastitis.

Antimicrobial susceptibility test result

Antimicrobial susceptibility tests were performed on a total of 48 frequently isolated bacterial isolates (*S. aureus* (n=17), other *Staphylococcus* species (n=9), *E. coli* (n=15), and *Klebsiella pneumoniae* (n=7)). *S. aureus* showed high susceptibility to nalidixic acid (82.4 %) followed by chloramphenicol (58.8 %). However, this organism was resistant to clindamycin (88.2 %), ampicillin (82.4 %), erythromycin (58.8 %), and trimethoprim–sulfamethoxazole (52.9 %). The other *Staphylococcus* species were also susceptible to nalidixic acid (77.8 %) and chloramphenicol (66.7 %) but showed resistance to the rest of the antimicrobials (Table 3). The coliform bacteria (*E. coli and K. pneumoniae*) were resistant to most of the antimicrobials used (Table 4).

Discussion

In the present study, the overall prevalence of mastitis in crossbreed cows owned by large-scale dairy farms was 37.4 % and is in close agreement with previous findings, 33.6 % by Getahun et al. (2008), 34.9 % by Biffa et al.

Table 4 Antimicrobial susceptibility profiles of *E. coli* (n=15) and *K. pneumoniae* (n=7)

Antimicrobial	*E. coli*			*K. pneumoniae*		
	Resistant (%)	Intermediate (%)	Susceptible (%)	Resistant (%)	Intermediate (%)	Susceptible (%)
Ampicillin	6 (40.0)	1 (6.7)	8 (53.3)	3 (42.9)	0 (0.0)	4 (57.1)
Erythromycin	11 (73.3)	3 (20.0)	1 (6.7)	4 (57.1)	3 (42.9)	0 (0.0)
Nalidixic acid	8 (53.3)	6 (40.0)	1 (6.7)	1 (14.3)	4 (57.1)	2 (28.6)
Clindamycin	11 (73.3)	1 (6.7)	3 (20.0)	5 (71.4)	1 (14.3)	1 (14.3)
Trimethoprim–sulfamethoxazole	7 (46.7)	4 (26.7)	4 (26.7)	4 (57.1)	2 (28.6)	1 (14.3)
Chloramphenicol	8 (53.3)	1 (6.7)	6 (40.0)	3 (42.9)	0 (0.0)	4 (57.1)

(2005), and 40.4 % by Kerro and Tareke (2003) in urban and periurban smallholder production systems of Ethiopia. However, the finding by Workineh et al. (2002) (59.7 %) in two major Ethiopian dairies is much higher than the present report. Similarly, the prevalence report of both clinical and subclinical mastitis is lower than the findings of Workineh et al. (2002) who reported 21.5 % clinical and 38.2 % subclinical mastitis. Data on large dairy farms in the country are few. Differences have been observed in the prevalence of mastitis in the present study and other reports, and the reasons for these variations need to be analyzed in further studies that include more data on management variables. Blind quarters (1.1 %) reported in this study might be associated with the seriousness of mastitis problem and absence of culling chronically infected animals in those farms.

The high prevalence of *S. aureus* followed by coliforms (mainly *E. coli*) in this study is in accordance with other workers (Hunderra et al. 2005; Abera et al. 2010). *S. aureus* is the most important and prevalent mastitis-causing organism globally, including Ethiopia. High prevalence of *S. aureus* points to poor milking time hygiene as this pathogen is mainly spread during milking via milkers' hands and towels (Bradley 2002). Higher incidence of coliform mastitis is an indication of poor hygienic practices in dairy environment, as these organisms originate from the cow's environment and infect the udder through the teat canal. Contamination of end of the teat is a major predisposing factor in the development of environmental mastitis (Bradley 2002). The most commonly used antibiotic for the treatment of mastitis in the study area is penicillin in combination with streptomycin, and this may explain for the lower rate of isolation of streptococcal organisms in the present study.

The prevalence of infected quarters increases with age, peaking at 7 years and with an increasing parity of cows. This may be attributed to the increased opportunity of infection with time and the prolonged duration of infection, especially in a herd without a mastitis control program. The observed higher prevalence of mastitis with increasing age and parity is in accordance with the work of other investigators (Almaw et al. 2008; Abera et al. 2010). In the present study, late lactation stage was also found to increase the occurrence of mastitis significantly ($p<0.05$). These infections could be the result of chronic contagious mastitis which transferred from lactation to lactation. The chance of occurrence of contagious mastitis would be high toward the end of lactation due to repeated exposure.

The antimicrobial susceptibility tests carried out in this study indicated for the high resistance of *Staphylococcus* species to ampicillin followed by clindamycin and erythromycin. The resistance of *S. aureus* to ampicillin may be attributed to the production of betalactamase, an enzyme that inactivates penicillin and closely related antibiotics. This could be associated with the predominant use of penicillin for treatment of mastitis cases in the area. Coliform bacteria (*E. coli* and *K. pneumoniae*) were resistant to most of the antimicrobials tested. The probable reason for the development of resistance by these organisms to most of the antimicrobials tested is prolonged and indiscriminate usage of these antimicrobials in the study area.

In conclusion, the present study indicated considerable prevalence of the disease with the isolation of major pathogenic microorganisms from both clinical and subclinical mastitis in larger dairy farms of Mekelle and its surrounding. To reduce the impact of the disease on the dairy sector, appropriate control measures targeting the specific causative agent (contagious or environmental pathogens) should be in place, and awareness needs to be created to dairy producers on the impact of the disease. The antimicrobial susceptibility test showed resistance of the major bacterial isolates to most of the antimicrobial agents tested. Therefore, dairy farm owners need to be advised to avoid the indiscriminate use of one type of antimicrobial for a long period and the need to consult animal health professionals for prescription and administration of drugs. Further work aimed at determining the interplay of management and environmental risk factors should help to design appropriate control measures.

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