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Dairy cows mastitis survey in Adama Town, Ethiopia

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A total of 102 smallholder dairy farms and 303 cross bred milking cows were examined to determine the overall prevalence of mastitis, to identify risk factor, to isolate and identify bacterial pathogens and to conduct *in vitro* antimicrobial susceptibility test in Adama Town, Ethiopia using pre-tested questionnaires, California mastitis test (CMT), microbial isolation and identification and *in vitro* antibiotic susceptibility test. The overall mastitis prevalence was 73.4% (at herd), 39.5% (at cow) and 23.7% (at quarter) level of which (15.6 and 57.8%) at herd, (5.9 and 33.6%) at cow and (2.9 and 20.8%) at quarter level were clinical and sub-clinical mastitis respectively. The major isolates of subclinical mastitis cases were *Staphylococcus aureus* (26.8%), *Staphylococcus intermedius* (2.5%), Coagulase Negative *Staphylococcus* (CNS, 18.7%), *Streptococcus agalactiae* (5.8%), *Streptococcus dysgalactiae* (2.0%), *Streptococcus uberis* (4.5%) and *Escherichia coli* (6.5%) and from clinical mastitis were *S. aureus* (2.5%) and *S. agalactiae* (3.8%). Among the risk factors stage of lactation, parity and presence of teat lesions have been shown statistically significant ($p<0.05$) difference in the prevalence of mastitis. In the present study, out of the nine *in vitro* antimicrobials used, Gentamycine (3.1%), Chloramphinicol (4.2%), Vancomycin (17.6%), Streptomycin (25.4%), Kanamaycin (26.4%), Polymyxin B (48.6%), Penicillin (48.9%), Amoxicillin (68.7%) and Bacitracin (74.9%) showed resistance to mastitic pathogens. Gentamycine and Chloramphinicol were found to be more effective antibiotic among all the tested antibiotics. The main reasons for the occurrence of a high number of resistant strains in this study were the use of sub-therapeutic level of antibiotics and/or short treatment episodes and the long-term presence of infected cows in herds. Finally, due to the high resistance levels detected in the present study, it was believed that it is necessary to set up antimicrobial resistance (AMR) surveillance programs in the country.

Key words: Mastitis, bacteria, isolates, antibiotic, susceptibility, prevalence, risk factors.

INTRODUCTION

Ethiopia holds large potential for dairy development. The country currently manages the largest livestock population in Africa, estimated to be about 52.13 million head of cattle, 24.2 million sheep, 22.6 million goat, 8.73 million equine, 0.99 million of camel and 44.89 million poultry (CSA, 2012). Even though Ethiopia has huge number of livestock, the productivity has always been sub-optimal due to low genetic potential of the animals, poor nutrition and prevailing diseases. Mastitis is one of the most important economically devastating diseases of dairy cattle particularly for the backyard farmers in developing world, with different levels of economic losses (Hogeveen et al., 2011; Atyabi et al., 2006). Mastitis (Mast: breast, itis: inflammation) is one of the prevailing diseases characterized by inflammation of the mammary gland (udder) that causes physical and chemical changes in milk and leads to pathological condition of the glandular tissue, which may result due to microbial,

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thermal, chemical or physical causes (Fox, 2005). Although it may be caused by thermal or chemical or physical agents, the causes are almost entirely infectious and mostly bacterial. It is generally associated with poor hygienic and husbandry practices. The infection rate of mastitis in cows with pendulous udder is higher than those having non-pendulous udder (Hundera et al., 2005). In recent years, acquired antimicrobial resistance in bacteria is an increasing threat in human as well as in veterinary medicine. Hence, monitoring antimicrobial susceptibility in pathogenic as well as in commensal bacteria in animals is recommended by World Organisation for Animal Health (OIE) (Acar and Rostel, 2001). Such monitoring generates data of importance for therapeutic decisions and provides information on trends in resistance that might be cause for interventions regarding antimicrobial use.

In Ethiopia even if some studies have been conducted so far on the prevalence and the major cause of bovine mastitis in the country by Workineh et al. (2002), Biffa et al. (2005), Hunidera et al. (2005), Getahun et al. (2008), Mekibib et al. (2010), Mekonen et al. (2012), Bedane et al. (2012), Bekele et al. (2012), Alemu et al. (2013) and Zeryehun et a. (2013), it is insufficiently investigated and information related to magnitude of the disease, risk factor and antimicrobial susceptibility are scanty. Such information is important to envisage when designing appropriate strategies that would help to reduce its prevalence and effects. Therefore, the objectives of this study were to determine the prevalence of mastitis, to isolate and identify major mastitis pathogens, to perform *in vitro* antimicrobial susceptibility test and to assess risk factors.

MATERIALS AND METHODS

Study design

Study type

Cross sectional type of study was carried out from September 2008 to April 2009 in and around Adama town of Oromia regional state, Ethiopia. The prevalence rate of sub-clinical and clinical mastitis at cow level was calculated using the formula described by Wasserstein (1995).

Sampling

From in and around Adama town, 102 smallholder dairy farms (those had Holestein-Frisian zebu cross breed cows) were randomly selected for this study. Simple random sampling was carried out to select 303 lactating crossbreed cows from the total of smallholder's dairy farms in the study areas. The consideration during sample size determination includes 95% confidence interval, 5% precision and 60% prevalence from the previous studies in similar study areas (Workneh et al., 2002). Sample size was calculated using the formula described by Thrusfield (2005). Milk samples were taken in sterile universal bottles and closed with screw caps. The universal bottles were marked with a permanent marker, so that the markings were easy to read when the universal bottles were placed in a rack. The universal bottles were marked before sampling. The surface of the teat ends were cleaned by wiping with clean cotton dipped in 70% alcohol. Scrubbing with alcohol pads falls way short of sterilizing teat skin. An insulated cool box was used for transporting samples (Quinn et al., 2004).

Risk factor analysis

A questionnaire was developed, pre tested and administered to the smallholders' dairy owners of the animals. Data on each cow was collected in a format designed for this purpose. The animal level factors considered were parity numbers, herd size, stage of lactation and presence of teat lesion. The farm level factors were housing, farm hygiene, milking hygiene and milking sequence.

Clinical and subclinical analyses

California mastitis test (CMT)

CMT was carried out to screen sub-clinical mastitis and for selection of samples for bacterial culture. A small amount of milk from each quarter is squired into shallow cups in the CMT paddle, an equal amount of 3% CMT reagent was added to each cup and mixed well. A gentle circular motion was applied to a mixture in a horizontal plane for 15 s. Finally, the reactions were graded as negative, trace, 1+, 2+, and 3+, as described by Quinn et al. (2004). Cows and herds were considered positive for subclinical mastitis, when at least one quarter of a cow and one cow from the herd became positive for CMT, respectively. Definition of quarter was: one teat together with the part of cow's udder that it drains.

Clinical observation

Gross abnormalities indicated the clinical form of the disease was detected by physical examination of the udder for the presence of swelling, pain, hotness, disproportional symmetry, fibrosis, visible injury, tick infestation, atrophy and teat blindness. It was also recognized based on abnormalities in milk including flakes, clots and watery secretion.

Analysis of pathogens and their antimicrobial susceptibility

Microbial investigation was performed according to Quinn et al. (2004). The isolates were exposed to antimicrobial sensitivity using Kanamayacin (K 30), Streptomycin (S 10), Penicillin (P 10), Amoxicillin (Aml 2), Gentamycine (CN10), Chloramphenicol (C 30), Polymyxin (PB 300), Bacitracin (B 10) and Vancomycin (VA 30) discs *in vitro* disc diffusion (Kirby-Baur test method) was done based on Clinical and Laboratory Standards Institute (CLSI, 2010) at Microbiology Laboratory of Faculty of Veterinary Medicine of Addis Ababa University.

Statistical analysis

The data collected during the study periods were entered into MS-Excel spread sheet and analyzed using STATA software (STATA 2001). The effect of risk factors with possible association of the disease was analyzed using Chi-square. The associations between dependent and independent variables were tested by logistic regression model. For all the analysis performed, $p<0.05$ was taken as statistically significant (Snedecor and Cochran, 1989). Prevalence of bovine mastitis related to specific risk factors was determined as the proportion of affected cows out of the total

Table 1. Prevalence of clinical and subclinical mastitis at herd, cow and quarter levels based on clinical observation and culture.

Observation level	N	Prevalence of clinical mastitis		Prevalence of subclinical mastitis	
		Clinical observation in % (N)	Culture in % (N)	CMT in % (N)	Culture in % (N)
Herd level	102	15.6 (16)	100 (16)	57.8 (59/102)	98.3 (58)
Cow level	303	5.9 (18)	100 (18)	33.6 (102/303)	90.19 (92)
Quarter level	1172	2.98 (35)	100 (35)	20.8 (244/1172)	93.85 (229)

N: Number of observation; n: number of positive.

examined (Thrusfield, 2005). The prevalence of clinical and subclinical mastitis at herd, cow, and quarter level as defined by CMT score and bacteriological result was dependent variables. The independent variable at herd level included farm hygiene, barn floor status, milking hygiene and milking sequence. Stage of lactation was classified into three in such a way that the beginning of lactation referred to the first two months of lactation period, middle of lactation referred to the next five months period and end of lactation referred to the last weeks of lactation. A farm was considered to have good barn floor status, if the floor is made of concrete and bad if the floor is muddy. A farm was regarded as having good milking hygiene, if it practiced any one of the specific practices considered during the analysis.

RESULTS

Prevalence at quarter level

The results of this study showed that out of 2012 quarters, 47 (2.34%) were blinded. The overall quarter level prevalence of clinical and subclinical mastitis was 2.39% (n=47) and 88.01% (n=345), respectively. The individual quarter level prevalence of subclinical mastitis was 20.48% (n=103), 22.00% (n=110), 16.39% (n=78) and 20.78% (n=101) for the front right, front left hind right and hind left quarters, respectively. The results of univariate logistic regression revealed that quarter level prevalence of subclinical mastitis was not significantly different between the hind (21.44%) and front (26.03%) quarters and also the right (25.63%) and left quarters (21.96%).

Prevalence of clinical and subclinical mastitis

On the bases of clinical observation, 15.6% (n=16) herds, 5.9% (n = 18) cows and 35 (2.98%) quarters had clinical mastitis based on clinical observations and all the clinically mastitic positive herds, cow and quarter were 100% positive on bacterial culture. The prevalence of sub clinical mastitis was determined by CMT and microbiological cultures as presented in Table 1. From a total of 102 herds, 59 (57.8%) were positive based on CMT test and 98.3% of them were bacteriologically, culture positive. From the total 303 dairy cows, 102 (33.6%) of them were CMT positive and among this 90.19% were culture positive. From the total 1172 quarters, 244 (20.8%) quarters were CMT positive, 93.5% of them were culture positive (Table 1).

Risk factors affecting the prevalence of subclinical mastitis at cow level

The results of a univariate logistic regression revealed that the cow level prevalence of sub-clinical mastitis in the study area was significantly affected by stage of lactation and parity ($p<0.05$). All the cows (n=8) with teat lesion had subclinical mastitis. The prevalence of subclinical mastitis was significantly higher in cows at the end of lactation (78.82%) and in those with high parity number (65.69%). When the factors with p-value less than 0.25 were fitted in the multivariate model, only stage of lactation had significant effect on cow level prevalence ($p<0.05$). In the case of farm (herd) level prevalence of subclinical mastitis, only the practice of milking mastitic cow last had significant effect on the prevalence of subclinical mastitis ($p<0.05$). The prevalence was significantly higher (86.42%) in those which were not milking mastitic cows last (Table 2). Risk factors with p-value less than 0.25 were fitted in a multivariate model and only the practices of milking mastitic cow last had significant effect on the prevalence of subclinical mastitis ($p<0.05$). Risk factors with p-value less than 0.25 were fitted in a multivariate model and only the practices of milking mastitic cow last had significant effect on herd level prevalence of subclinical mastitis.

Bacterial isolates

From the total 118 lactating cows, 279 quarters of them were positive for mastitis either clinically or CMT tests. A total of 173 bacterial isolates were found, as presented in Table 3. Generally, the most important pathogens isolated from clinical cases were *Streptococcus agalactiae* (31.8%), *Staphylococcus aureus* (22.7%) and Coagulase Negative *Staphylococcus* (CNS, 13.6%). In case of subclinical mastitis, *S. aureus* (33.5%), CNS (24.2%), *S. agalactiae* (7.5%) and *Escherichia coli* (8.1%) were the most frequently isolated pathogens (Table 3).

From the total isolates, *S. aureus* (32.2%) and *S. agalactiae* (10.4%) were the major contagious pathogens and *E. coli* (8.7%), *Klebsiella* species (3.3%), *Enterobacter* species (2.2%), *Streptococcus uberis* (6.0%),

Table 2. Risk factors affecting the prevalence of subclinical mastitis at cow and herd level.

Factor	Categories	N	n (%)	P value	OR	95% CI of OR
At cow level						
Stage of lactation	Beginning	40	6 (15.00)	-	-	-
	Middle	178	43 (24.16)	0.237	1.80	0.68-4.81
	End	85	67 (78.82)	0.000	21.09	7.00-63.66
Parity	1-3	201	49 (24.38)	0.000	5.93	3.41-10.32
	> 3	102	67 (65.69)			
Teat lesions	-	8	8 (100%)	-	-	-
At herd level						
Herd size	1-5	94	66 (70.21)	0.776	1.27	0.24-6.70
	>5	8	6 (75.00)			
Udder washing before milking	Yes	62	39 (62.90)	0.38	0.36	0.14-0.94
	No	40	33 (82.50)			
Milking mastitic cow last	Yes	21	2 (9.52)	0.000	0.02	0.00-0.08
	No	81	70 (86.42)			
Hand washing before milking	Yes	6	4 (66.67)	0.828	0.82	0.14-4.76
	No	96	68 (70.83)			
Drainage structure	Good	60	41 (68.33)	0.551	1.30	0.54-3.14
	Bad	42	31 (73.81)			
Barn floor status	Good	63	41 (65.08)	0.125	2.08	0.82-5.29
	Bad	39	31 (79.48)			

N: Number of observation; n: number of positives; OR: odd ratio; CI: confidence interval.

Streptococcus dysgalactiae (3.3%) and *Arcanobacterium pyogenes* (1.6%) were the major environmental pathogens isolated. Other minor pathogens isolated included were CNS (23.0%), *Staphylococcus intermedius* (3.0%) and *Micrococcus* (2.5%) as shown in Table 3.

In vitro antimicrobial susceptibility test result

Antimicrobial sensitivity test was done for all isolates and the results of antimicrobial sensitivity tests are presented in Table 4. *S. aureus* was sensitive to Gentamycine (100%), Chloramphenicol (92%), Kanamayacin (90%), Vancomycin (80%) and Streptomycin (54%) and was resistant to Amoxicillin (62%), Penicillin (65.3%), Polymyxin B (89%) and Bacitracin (100%). In this study, Gentamicine, Chloramphenicol, Kanamayacin and Vancomycin were the most effective on *S. aureus* isolates. *S. intermedius* were sensitive to almost all antimicrobial disks applied. CNS was sensitive to Chloramphenicol (100%), Streptomycin (93%), Gentamycine (92%) and Vancomycin (70.2%) and was resistant to Penicillin (50%), Kanamayacin (72%), Amoxicillin (72%), Polymyxin B (80%) and Bacitracin (85%). *S. agalactiae* was sensitive to Gentamycine (100%), Chloramphenicol (100%) Vancomycin (84.2%), Penicillin (80%), and Streptomycin (52%) and was resistant to amoxicillin (100%) and Polymyxin B (92%) and *S. dysgalactiae* was sensitive to Polymyxin B (91%) and Amoxicillin (80%), but resistant to many of the other antimicrobial disks. *S. uberis* was sensitive to all other antimicrobial disks applied except to Bacitracin (70%) and Amoxicillin (65%) which were resistant. *E. coli* was sensitive to all antimicrobial discs except Bacitracin (80%), Penicillin (79%), Amoxicillin (75%) and Polymyxin B (65%) which were resistant. *Klebsiella* spp. Was sensitive to all antimicrobial discs except Bacitracin (75%), Polymyxin B (75%) and Amoxicillin (65%) which were resistant. *Enterobacter* spp. was sensitive to all antimicrobial discs except Bacitracin (80% resistance). *Micrococcus* species was sensitive to all antimicrobial discs applied except Streptomycin (62%), Polymyxin B (65%), Amoxicillin (75%) and Penicillin (75%), which were resistant.

Table 3. Bacteria species isolated from dairy cows clinical and subclinical mastitis.

Species of Bacteria Identified	Clinical		Subclinical		Total	
	N	%	N	%	N	%
S. aureus	5	22.7	54	33.5	59	32.2
CNS	3	13.6	39	24.2	42	23
S. intermedius	1	4.5	5	3.1	6	3.3
S. agalactaie	7	31.8	12	7.5	19	10.4
S. dysgalactaie	2	9.1	4	2.5	6	3.3
S. uberis	2	9.1	9	5.6	11	6
E. fesalis	1	4.5	5	3.1	6	3.3
E. coli	-	-	13	8.1	13	8.7
Enterobacter spp.	-	-	4	2.5	4	2.2
Klebssela spp.	-	-	6	3.7	6	3.3
Micrococcus	-	-	5	3.1	5	2.7
C. bovis	1	4.5	2	1.2	3	1.6
A. pyogens	-	-	3	1.9	3	1.6
Total	22	100	161	100	183	100

DISCUSSION

Prevalence and associated risk factors

This study showed that the overall prevalence of mastitis in crossbred cows in and around Adama was 73.4% at herd level, 39.4% at cow level and 23.7% at quarter levels of which 15.6 and 57.8% at herd level, 5.9 and 33.6% at cow level and 2.9 and 20.8% at quarter level were clinical and subclinical, respectively. The present overall cow level mastitis prevalence result (39.5%) is in close agreement with previous studies by Melesse et al. (2011), Bifa et al. (2005) and Bekele et al. (2012) who reported prevalence of 37.1, 34.9 and 34.3%, respectively. However, overall mastitis prevalence reported in the present study is relatively lower than the previous studies by Mekibib et al. (2010), Zeryehun et al. (2013), Bedane et al. (2012), Nibret et al. (2011) and Mekonnen et al. (2012) who reported prevalence of 71, 74.3, 59.1, 60.9 and 62.9%, respectively, but higher than the previous studies by Getahun et al. (2008) who reported 24.1%. The variability in the prevalence of bovine mastitis between reports could be attributed to difference in management of the farms. In this study, the clinical mastitis prevalence accounted for 5.9% whereas the subclinical mastitis was 33.6% of the share. The clinical prevalence of 5.9% in this study was comparable with that of Nibret et al. (2011), Melesse et al. (2011) and Benta and Habtamu (2011) who reported prevalence of 4.9, 8.5 and 5.3%, respectively. The present findings were lower than the findings of Mekibib et al. (2010), Zeryehun et al. (2013), Bedane et al. (2012), and Bifa et al. (2005) who reported prevalence of 22.4, 19.6, 21.1, 15.1 and 16.11%, respectively and higher than the findings of Mekonnen et al. (2012), Getahun et al. (2008) and Bekele et al. (2012) who reported prevalence of 3.9, 1.8 and 3.3%, respectively. The present subclinical bovine mastitis finding (33.6%) is in close agreement of Bekele et al. (2012), Bedane et al. (2012), Melesse et al. (2011) and Hundera et al. (2005) who reported prevalence of 31, 38, 28.6 and 34.6%, respectively. The present findings were lower than the findings of Alemu et al. (2013), Mekibib et al. (2010), Zeryehun et al. (2013), Mekonnen et al. (2012), Nibret et al. (2011) and Kerro and Tareke (2003) who reported prevalence of 41.2, 48.6, 55.1, 54.4, 56 and 62.9%, respectively and higher than the findings of Bifa et al. (2005) and Getahun et al. (2008) who reported prevalence of 23 and 22.3%, respectively. In this study similar to previous studies by Mekibib et al. (2010), Bedane et al. (2012), Zeryehun et al. (2013), Bekele et al. (2012), Getahun et al. (2008), Nibret et al. (2011), Mekonnen et al. (2012), Melesse et al. (2011), Bifa et al. (2005), kerro and tareke (2003), Workeneh et al. (2002), and Hussein (1999), the overall prevalence of clinical mastitis is lower than subclinical mastitis.

In Ethiopia, the subclinical form of mastitis (account high economic loss) was neglected and efforts have been concentrated on the treatment of clinical cases (Kerro and Tareke, 2003). According to Radostits et al. (2000), an affected quarter suffers on average 30% of reduction in productivity and an affected cow is estimated to lose 15% of its production for the lactation. As usual, the owners of smallholder dairy farms in the study areas were not well informed about the invisible loss from subclinical mastitis since dairy farming is mostly a sideline business in them.

In the present study, parity number 3 and above, late lactation stage and teat lesions were also found to increase occurrence of mastitis significantly ($p<0.05$). According to Erskine (2001), primiparous cows have more effective defense mechanism than multiparous

Table 4. Antibiotic sensitivity test.

Bacteria isolate	No. tested	K30%			S10%			P10%			Am12%			CN10%			C30%			PB300%			B10%			VA30%		
		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
S. aureus	59	-	10	90	46	-	54	65	6	29	62	16	22	-	-	100	7.9	-	92.1	8	-	92	100	-	-	16	4	80
S. intermedius	5	-	4	96	3	-	97	-	-	100	8	-	92	-	-	100	-	-	100	8	10	82	17	-	83	-	-	100
CNS	46	72	8	20	-	7	93	50	-	50	72	-	28	4	4	92	-	-	100	80	3	17	85	15	-	29.2	-	70.8
S. agalactiae	19	28	-	72	40	8	52	20	-	80	100	-	-	-	-	100	-	-	100	92	-	8	31	-	69	15.8	-	84.2
S. dysgalactiae	6	21	-	79	-	-	100	-	-	100	80	-	20	-	-	100	11	-	89	91	-	9	24	-	76	7	-	93
S. uberis	10	24	8	68	7	-	93	35	-	65	65	-	35	11	-	89	7	-	93	12	-	88	70	-	30	-	-	100
E. coli	13	25	-	75	35	-	65	79	-	21	75	-	25	8	-	92	-	-	100	65	-	35	80	-	20	5	5	90
Klebsiella spp.	6	4	-	96	11	-	98	25	-	75	65	-	35	-	-	100	-	-	100	75	-	25	75	-	25	20	-	80
Enterobacter	4	-	-	100	-	-	100	10	-	90	25	-	75	-	-	100	-	-	100	25	-	75	80	-	20	25	-	75
Micrococcus	5	-	-	100	62	7	31	75	-	25	75	-	25	28	-	72	25	-	75	65	-	35	24	-	76	25	-	75

S: Susceptible, I: intermediate, R: resistance, K30%: Kanamayacin, S10%: Streptomycin, P10%: Pencilline, AML12%: Amoxicillin, CN10%: Gentamycine, C30%: Chloramphenicol, PB300%: Polymyxin B, B10%: Bacitracin, VA30%: Vancomycin.

cows. The prevalence of subclinical infection increases as the stage of lactation progresses. In the case of farm (herd) level prevalence of subclinical mastitis, only the practice of milking mastitic cow last had significant effect on the prevalence of subclinical mastitis ($p<0.05$). The prevalence was significantly higher (86.42%) in those, which were not milking mastitic cows last (Table 4).

Bacterial isolation and identification

Klebsiella (3.3%), *Enterobacter* spp. (2.2%), *S. uberis* (6.0%), *S. dysgalactiae* (3.3%) and *A. pyogenes* (1.6%) were the major environmental pathogens isolated. Other minor pathogens isolated included were CNS (23.0%), *S. intermedius* (3.0%) and *Micrococcus* (2.5%) as shown in Table 3.

In the present study, *S. aureus* was the predominant pathogen (32.2%) of the area and this finding was comparable with the reports of Zingeser et al. (1991) (27%) and Barbuddhe et al. (2001) (23.2%). However, it was higher than the reports made by Hussein (1999) (10.6%). The reports of Kerro and Tareke (2003) (40.5%) and Hunderra et al. (2005) (44.4%) were higher than the present finding. The relative high prevalence of *S. aureus* in this study could be associated with lack of effective udder and hand washing before milking, use of separate clothes for drying, post milking teat dipping and disinfection of milking areas. The result of CNS (23.0%) in the current study is much lower than the finding of Hussein (1999) (42%). However, this result was much higher than the result of Miline et al. (2002), which was reported as 10%. CNS is a minor pathogen and normally considered as normal inhabitants of bovine udder (Gentilini et al., 2002). *S. agalactiae* prevalence (10.4%) in this study was lower than the finding of Kerro and Tareke (2001) (13.1%) and Bishi (1998) (27%). The 6.0% isolation result of *S. uberis* was comparable with Kerro and Tareke (2003) finding which was 5.1% and much lower than that of Miltenburg et al. (1996), that is 12.1%. Isolates of *S. dysgalactiae* (3.3%) were lower than the report of Kerro and Tareke (2003), which was 5.6%, *E. coli* (6.46%) was the predominant environmental pathogen isolated in the present study. The prevalence of environmental *E. coli* may be associated with poor farm hygiene and poor of stable areas. In this study, environmental pathogens were isolated, however a common understanding with increasing herd size, manure disposal and sanitation problem high to build up to bacterial population (coliform and environmental streptococcus) in the cows immediate environment.

Antibiotics sensitivity test

The antimicrobial sensitivity test results of this study are closer to the previous authors (Edward et al., 2002; Gentilini, 2002; Nesru, 1998; Kang, 2007; Sanmartin et al., 2007; Shakuntala et al., 2003).

The results of sensitivity tests of the organisms

isolated to antibiotics (Table 4) show that 3.1% of the strains were resistant to Gentamycin, 4.2% to Chloramphinicol, 17.6% to Vancomycin, 25.4% to Streptomycin, 26.4% to Kanamaycin, 48.6% to Polymyxin B, 48.9% to Penicillin, 68.7% to Amoxicillin and 74.9% to Bacitracin. Gentamycine and chloramphinicol were found to be more effective antibiotic among all the tested antibiotics. The main reasons for the occurrence of a high number of resistant strains in this study are the use of sub-therapeutic level of antibiotics and/or short treatment episodes and the long-term presence of infected cows in herds. Finally, due to the high resistance levels detected in the present study, it is believed that it is necessary to set up permanent resistance surveillance programs in the country.

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