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Epidemiology of Mastitis in Dairy Cattle with Special Reference to Some Associated Risk Factors

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ABSTRACT

Mastitis is one of the most important health problems of dairy cattle as it causes physical, chemical and bacteriological changes in the produced milk, so the aim of this study is investigating the epidemiology of mastitis pathogens of dairy cattle and their associated risk factors. A total of 397 samples were gathered from mastitic cows and their surrounding environment including 127 milk samples from sub-clinically mastitic cows, 60 milk samples from clinically mastitic cows, 60 teat swabs, 50 milking machines swabs, 50 worker's hand swabs and 50 bedding samples from different farms containing small and large dairy herds located in El Dakhalia and Menoufiya Governorate. These samples were examined bacteriologically and biochemically for isolation and biochemical identification of *E. coli* and *S. aureus*. Our results revealed that the prevalence of *E.coli* was 33.33% (11/33), 21.74% (20/92), 16.67% (4/24), 18.52% (5/27), 13.33% (2/15) and 13.33% (4/30) in milk samples from clinically mastitic cows, milk samples from subclinically mastitic cows, teat swabs, milking machines swabs, worker's hand swabs and bedding samples, respectively, while the prevalence of *Staphylococcus aureus* was 23.08% (9/39), 17.43% (19/109), 11.9% (5/42), 10.53% (4/38), 17.07% (7/41) and 10.53% (4/38), in the same groups respectively. A significant relationship was found between of the number of these bacteria and cows age, herd size and hygienic condition of the farm. Moreover, PCR was done on 14 isolates of *E. coli* for detection of ESBL producing *E. coli*. The results of PCR revealed that the prevalence of blaTEM and blaSHV was 64.3% (9/14) and 92.9% (13/14), respectively, while none of the isolates carried blaCMY-2. We concluded that *Staphylococcus aureus* and *E.coli* are considered two main pathogens of mastitis and the prevalence of mastitis increases in old cattle, inadequate hygienic condition, lack of post milking teat dipping and absence of udder washing which are all predisposing risk factors of mastitis in the study area.

Keywords: *Mastitis, S. aureus, E. coli, blaTEM, blaSHV.*

INTRODUCTION

Mastitis is an inflammatory process of the mammary gland parenchyma which can be caused by infection with a pathogen, allergy, injury and neoplasm and it is one of the most costly disease of dairy cattle worldwide. (Kibebew, 2017). More than 80 species of pathogenic microorganisms have been accounted as causative agents of mastitis (Philpot, 1979). Contagious pathogens are found on the teat surface of infected cattle and they are considered the primary sources of infection between dairy cattle, this category include many pathogens as *Staphylococcus aureus (CPS), Streptococcus agalactiae* (Eriksson *et al.*, 2005).

Staphylococcus aureus exists at the top and colonizes the nipple evolving through the mammary gland canal into the gland. The intramammary infection with *Staphylococcus aureus* mainly cause subclinical mastitis

resulting in a chronic mastitis that can last lifelong (Shearer & Harris, 2008). *Staphylococcus aureus* is difficult to be excluded from the mastitic cow due to the ability of the *Staphylococcus aureus* to produce different enzymes and toxins which cause extensive damage in the udder tissue and allow the bacteria to penetrate the udder tissue; survive in the keratin layer of the teat canal which in normal conditions acts as inhibitory factor to *Staphylococcus aureus* and prevent phagocytosis due to the presence of the protein A in some strains (Jayarao *et al.*, 2006).

Clinical mastitis is diagnosed in cattle based on apparent signs and symptoms and palpation of udder (Sharif *et al.*, 2009), while subclinical mastitis can be detected by applying California mastitis test (CMT). (Hamadani *et al.*, 2014).

Economic losses due to mastitis is attributable to loss in milk production, discarded abnormal milk, decreasing quality and price of the produced, cost of drugs, , herd replacement, costs of increaded labor and increased drug residues in produced milk (Varshney & Naresh, 2005). Globally, the losses due to mastitis amount to about 53 billion dollars annually (Ratafia, 1987). Treatment for mastitis, if performed by farmers themselves, they usually use a sub-therapeutic dose of antimicrobial agents, this especially increase antibiotic resistant bacteria (Falkow & Kennedy, 2001). Enterobacteriaceae produce β-lactamase enzymes that stop the action of β-lactams antibiotics. There are more than one thousand β-lactamase enzymes that can be categorized into 4 classes (A-D) (Bush & Jacoby, 2010). The main Class A enzymes in Enterobacteriaceae are known as (ESBLs) extended spectrum β-lactamases. It can provide resistance to various β-lactams, including penicillin, monobactams and 2nd, 3rd and 4th generation cephalosporins but usually not the cephamycins or carbapenems. There are three classical types of ESBLs, i.e., TEM , SHV and CTX-M (Hald & Baggesen, 2011). So, the aims of our study are isolation and biochemical identification of *S. aureus* and *Escherichia coli* from milk samples of clinical and subclinical mastitis cases as well as environmental samples including teat swabs, milking machines swabs, worker's hand swabs and bedding materials, evaluation of some hygienic factors associated with occurrence of mastitis in dairy cattle and detection of some antibiotic resistance genes in isolated *E.coli* using multiplex PCR.

METHODOLOGY

Sampling

A total of 397 samples were collected from mastitic cows and their surrounding environment including 127 milk samples from subclinically mastitic cows, 60 milk samples from clinically mastitic cows, 60 teat swabs, 50 milking machines swabs, 50 worker's hand swabs and 50 bedding samples from different dairy farms in Dakhalia and Menoufiya Governorate, during the period extended from September 2018 till June 2019. These samples were collected under aseptic condition and transported immediately to the laboratory for examination.

Isolation and biochemical identification of bacteria from different samples:

Isolation and biochemical identification of *S. aureus* was carried out according to Singh and Prakash (2008). Isolation and biochemical identification of *E.coli* was carried out according to (Cruickshank *et al.*, 1975).

Molecular identification of isolated E. coli:

DNA Extraction:

DNA Extraction was performed according to I-genomic BYF DNA Extraction mini Kit, Cat.No.17361, Korea (INtRON)®.

Amplification of DNA:

The primer used for PCR amplification is tabulated in (Table 2). The multiplex PCR mix (25µL) for each sample consisted the following: 3 µL extracted DNA, 12.5 µL Master Mix, 1 µL from each forward primer, 1 µL from each reverse primer and 3.5 µL nuclease free water. The reaction mixture was incubated in the thermal cycler as follows: The first initial cycle: 94℃ for 5 minutes (initial denaturation). The subsequent 30 cycles: 95℃ for 1 minute (denaturation), 55℃ for 1 minute (annealing) and 72℃ for 1 minute (extension). The final extension step was at 72℃ for 10 minutes then kept at 4℃ as the holding temperature.

Detection of amplified products:

Expected fragments providing visible bands of appropriate size of 247bp, 393bp and 1000bp were considered positive for blaTEM, blaSHV and blaCMY-2, respectively.

Statistical analysis:

Data were collected, tabulated, and statistically analyzed with SPSS version 25 by using the Chi-Square analysis test (X2) to compare between two qualitative variables. The level of significance was set at 0.05.

Table (1): Primers used for PCR amplification:

Target gene	Primer sequence	Amplified product (bp)	Reference
blaSHV	(F) AGGATTGACTGCCTTTTTG (R) ATTTGCTGATTTCGCTCG	393	**Kozak *et al*., (2009)**
blaTEM	(F) TTAACTGGCGAACTACTTAC (R) GTCTATTTCGTTCATCCATA	247	**Colom *et al*., (2003)**
blaCMY-2	(F) GACAGCCTCTTTCTCCACA (R) TGGACACGAAGGCTACGTA	1000	**Kozak *et al*., (2009)**

RESULTS

Table (2): Frequency of isolation of *Staphylococcus spp.* and Coliforms from milk samples according to bacteriological examination:

Isolated mo.	Clinical mastitis (n=60)		Subclinical mastitis (n=127)		Total (n=187)	
	NO.	%	NO.	%	NO.	%
Staphylococcus spp.	39	65.00	109	85.83	148	79.14
Coliforms	33	55.00	92	72.44	125	66.84

Table (3): Frequency of isolation of *Staphylococcus spp.* and Coliforms from different environmental samples of dairy farms according to bacteriological examination:

Isolated mo.	Teat swabs (n=60)		Milking machines swabbs (n=50)		Worker's hand swabs (n=50)		Bedding samples (n=50)	
	NO.	%	NO.	%	NO.	%	NO.	%
Staphylococci spp.	42	70.00	38	76.00	41	82.00	38	76.00
Coliforms	24	40.00	27	54.00	15	30.00	30	60.00

Table (4): Frequency of isolation of *Staphylococcus spp.* from different examined samples according to biochemical examination:

Type of samples	No. of isolated samples	*Staphylococcus spp.*			
		Staph. aureus		*CNS*	
		No.	%	No.	%
Milk samples (clinical mastitis)	39	9	23.08	30	76.92
Milk samples (subclinical mastitis)	109	19	17.43	90	82.57
Teat swabs	42	5	11.90	37	88.09
Milking machines swabs	38	4	10.53	34	89.47
Worker's hand swabs	41	7	17.07	34	82.93
Bedding samples	38	4	10.53	34	89.47
Total	307	48	15.64	259	84.36

CNS (Coagulase Negative Staphylococci)

Table (5): Frequency of isolation of *E.coli and* other Coliforms from different examined samples according to biochemical examination:

Type of samples	No. of isolated samples	Coliforms			
		E. coli		Other Coliforms	
		No.	%	No.	%
Milk samples (clinical mastitis)	33	11	33.33	22	66.67
Milk samples (subclinical mastitis)	92	20	21.74	72	78.26
Teat swabs	24	4	16.67	20	83.33
Milking machines swabs	27	5	18.52	22	81.48
Worker's hand swabs	15	2	13.33	13	86.67
Bedding samples	30	4	13.33	26	86.67
Total	221	46	20.81	175	79.19

Table (6): The association between age of dairy cows and *Staphylococcus spp.* infection according to bacteriological and biochemical examination of milk samples:

Age	Results of *Staphylococcus spp.* infection in milk samples			
	+ve	%	-ve	%
Old age (>4-8 years) (n=115)	96	83.48	19	16.5
Young age (2-<4 years) (n=72)	52	72.22	20	27.8
Chi-square	3.3987 (NS)			
P-value	0.065			

NS (Non-significant)

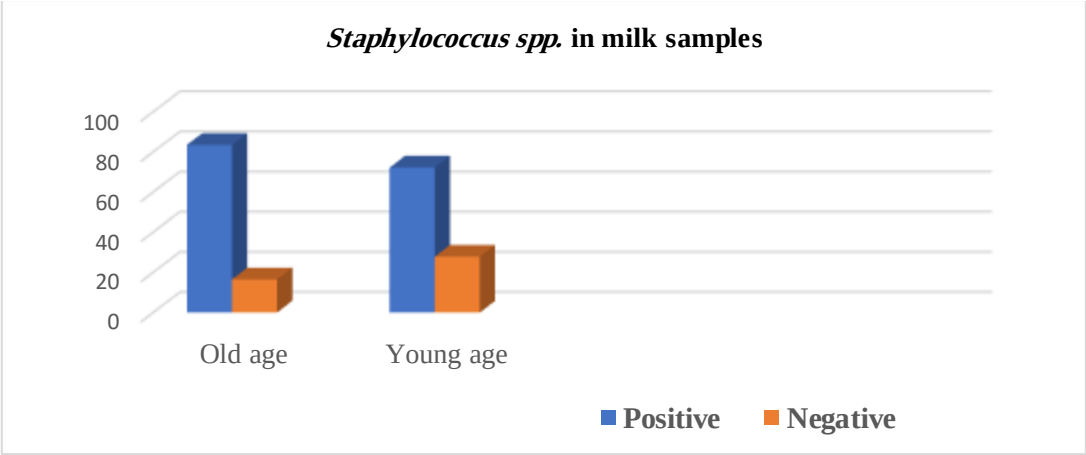

Fig. (1): The association between age of dairy cows and *Staphylococcus spp.* infection according to bacteriological and biochemical examination of milk samples.

Table (7): The association between age of dairy cows and Coliforms infection according to bacteriological and biochemical examination of milk samples:

Age	Results of Coliforms infection in milk samples			
	+ve	%	-ve	%
Old age (>4-8 years) (n=115)	98	85.22	17	14.78
Young age (2-<4 years) (n=72)	27	37.5	45	62.5
Chi-square	45.4908**			
P-value	<0.00001			

** (*Highly statistically significant*)

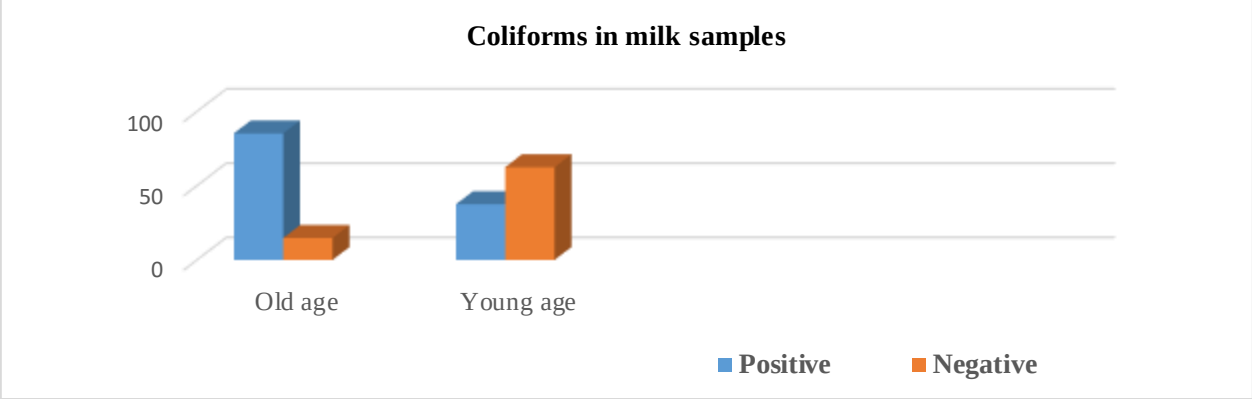

Fig. (2): The association between age of dairy cows and Coliforms infection according to bacteriological and biochemical examination of milk samples.

Table (8): The association between hygienic condition of examined farm (foot dipping, washing of udder before milking, post milking teat dipping and traffic control) and frequency of isolation of Coliforms from mastitic milk samples according to bacteriological and biochemical examination:

Hygienic condition	Results of Coliforms infection in milk samples				Chi-square	P-value
	+ve	%	-ve	%		
Good hygiene (n=62)	14	22.6	48	77.4	81.99**	<.00001
Poor hygiene (n=125)	111	88.8	14	11.2		

** *(Highly statistically significant)*

Table (9): The association between hygienic condition of examined farm (foot dipping, washing of udder before milking, post milking teat dipping and traffic control) and frequency of isolation of Coliforms from different swabs according to bacteriological and biochemical examination:

Hygienic condition	Results of Coliforms infection in different swabs				Chi-square	P-value
	+ve	%	-ve	%		
Good hygiene (n=70)	11	15.7	59	84.3	33.484**	<.00001
Poor hygiene (n=90)	55	61.1	35	38.9		

** *(Highly statistically significant)*

Table (10): The association between herd size of dairy cows and frequency of isolation of *Staphylococcus spp.* and Coliforms from milk samples according to bacteriological and biochemical examination:

Herd size	Results of *Staphylococcus spp.* infection in milk samples				Chi-square	P-value
	+ve	%	-ve	%		
Small herd (n=32)	28	87.5	4	12.5	1.633 NS	0.201
Large herd (n=155)	120	77.4	35	22.6		
Herd size	**Results of Coliforms in milk samples**					
	+ve	**%**	**-ve**	**%**		
Small herd (n=32)	2	6.25	30	93.75	63.96**	<.00001
Large herd (n=155)	123	79.4	32	20.6		

***(Highly statistically significant), NS** *(Non-significant)*

Table (11): The association between herd size of dairy cows and frequency of isolation of *Staphylococcus spp.* and Coliforms from different swabs according to bacteriological and biochemical examination:

Herd size	Results of *Staphylococcus spp.* infection in different examined swabs				Chi-square	P-value
	+ve	%	-ve	%		
Small herd (n=35)	6	17.1	29	82.9	89.91**	<.00001
Large herd (n=125)	117	93.4	8	6.4		
Herd size	**Results of Coliforms in different examined swabs**					
	+ve	**%**	**-ve**	**%**		
Small herd (n=35)	6	17.1	29	82.9	10.74*	0.001
Large herd (n=125)	60	48.0	65	52.0		

***(Highly statistically significant, *(Statistically significant)*

Table (12): Occurrence of some antibiotic resistance genes in *E. coli* isolates from mastitic milk and different environmental samples:

Type of sample	No. of isolates	No. (%) of positive isolates		
		blaTEM gene	blaSHV gene	BlaCMY-2 gene
Milk samples	6	5 (83.33)	6 (100.0)	0 (0.0)
Teat swabs	2	1 (50.0)	2 (100.0)	0 (0.0)
Milking machines swabs	2	1 (50.0)	2 (100.0)	0 (0.0)
worker's hand swabs	2	2 (100.0)	2 (100.0)	0 (0.0)
Bedding samples	2	0 (0.0)	1 (50.0)	0 (0.0)
Total	14	9 (64.3)	13(92.9)	0 (0.0)

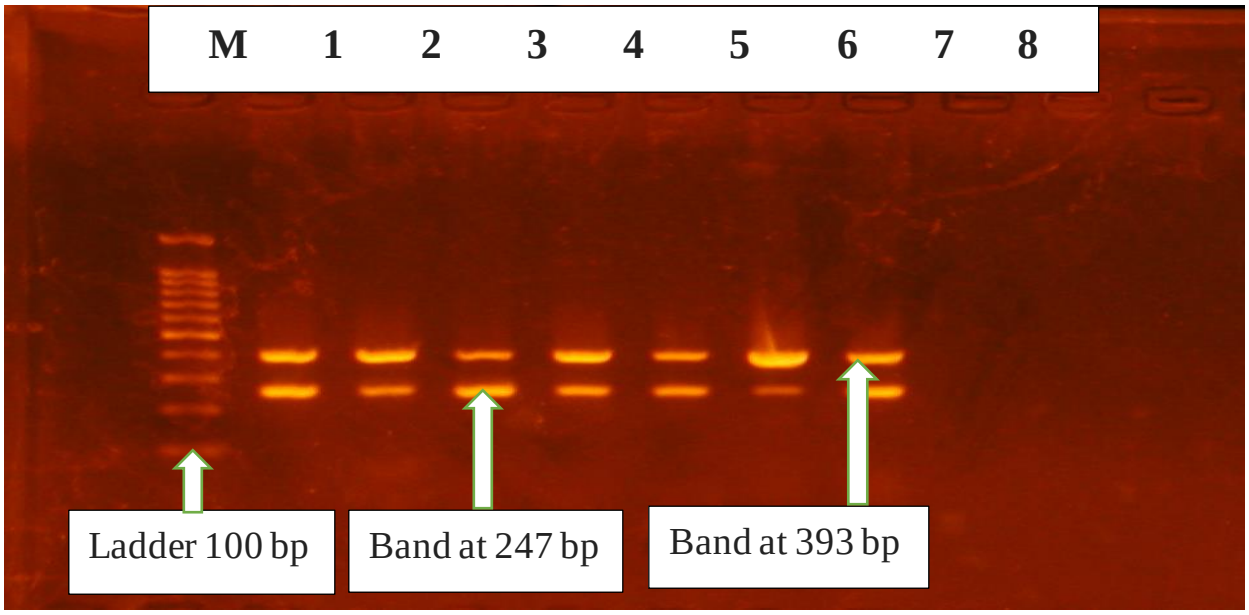

Fig. (3): Results of molecular detection resistance genes in *E.coli* isolates by using multiplex PCR where (M; is marker of 100 bp range, while lanes from (2 to 7) indicate positive isolates for blaTEM and blaSHV genes and results appear at 247 and 393 bp, respectively and negative isolates for blaCMY-2, moreover, lanes (1 and 8) represent control positive and control negative respectively.

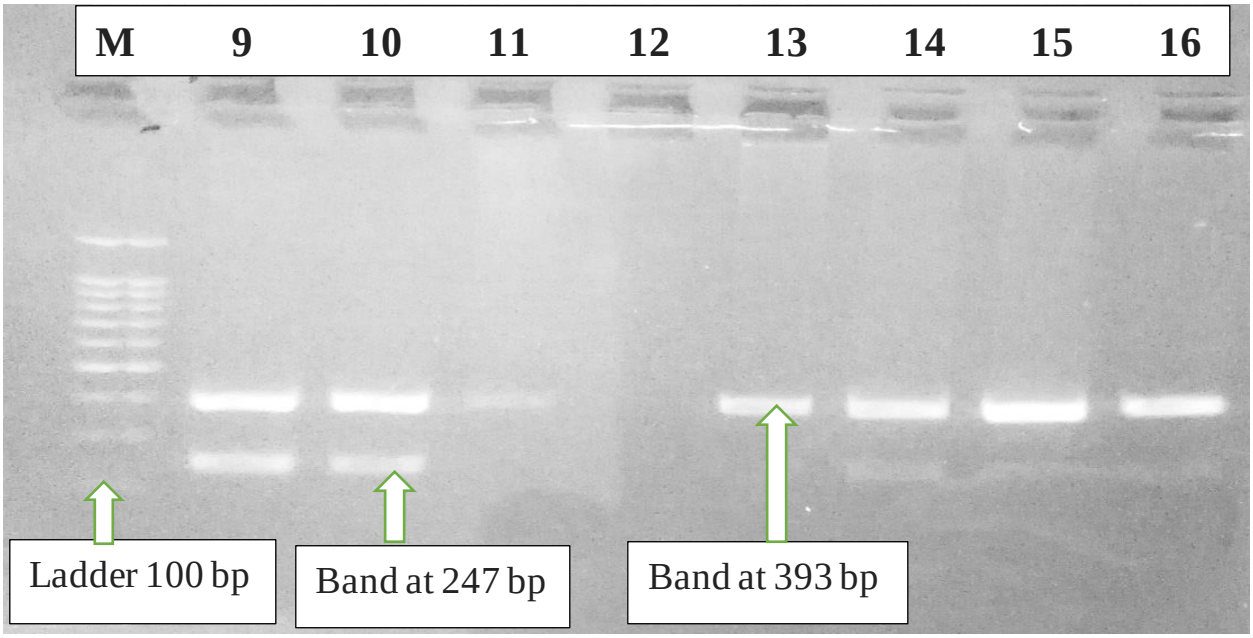

Fig. (4): Results of molecular detection resistance genes in *E.coli* isolates by using multiplex PCR where (M; is marker of 100 bp range, while lanes from (9,10 and 14) indicate positive isolates for blaTEM and blaSHV genes and results appear at 247 and 393 bp, respectively and negative isolates for blaCMY-2, lanes (11,13,15 and 16) indicate positive isolates for blaSHV genes and results appear 393 bp and negative for blaTEM and blaCMY-2, moreover, lane (12) indicate negative isolates for blaTEM, blaSHV and blaCMY-2

DISCUSSION

Staphylococcus is a genus in the family *Staphylococcaceae*, and facultative anaerobic pathogen. As shown in table (2), the frequency of isolation of *Staphylococcus spp*. from examined milk samples was 79.14 % (148/187); 65 % (39/60) in milk samples collected from clinically mastitic cows and 85.83 % (109/127) in milk samples collected from subclinically mastitic cows. These results were in close agreement with Tarabees and Bahlol (2018) who isolated 80 % (80/100) from clinically and subclinically mastitic milk and higher than results recorded by Pitkälä *et al.* (2004), Katsande *et al.* (2013), Lakshmi and Jayavardhanan (2006), Sumathi *et al.* (2008), Getahun *et al.* (2008), Workineh *et al.* (2002) who reported 10.2 %, 16.3 %, 36 %, 24 %, 42.6 % and 57 %, respectively.

Staphylococcus spp. may be of environmental origin as they were identified on many environmental samples (Tikofsky & Zadoks, 2005). The frequency of isolation of *Staphylococcus spp*. from various environmental samples was recorded in table (3), It was observed that the highest frequency of *Staphylococcus spp*. was recorded in examined worker's hand swabs (82%) followed by milking machines swabs and bedding samples (76%) and finally teat swabs (70%). These results indicated that surrounding environment of cows particularly worker's hands, bedding and teat cups of milking machines represent a potential source of mastitis.

The recorded data in table (4) showed the results of biochemical identification of *Staphylococcus spp*. isolated from different examined samples. It was observed that frequency of isolation of *Staph. aureus* from the total examined samples was (15.64%); the highest percentage was from milk samples collected from clinically mastitic cows (23.08%) followed by milk samples collected from subclinically mastitic cows (17.43%), worker's hand swabs (17.07%), teat swabs (11.9%), milking machines swabs (10.53%) and bedding samples (10.53%). These results agreed with Elbably *et al.* (2013) who stated that the frequency of isolation of *Staph. aureus* from milk samples, worker's hand swabs, teat swabs, milking machines swabs and bedding samples was 25.2%, 27.5%, 13.89%, 17.1% and 9.3%, respectively.

The frequency of isolation of CNS from the total examined samples was 84.36% (259/307); the highest percentage was from milking machines swabs (89.47%), bedding samples (89.47%) and

teat swabs (88.09%) followed by worker's hand swabs (82.93%), milk samples collected from subclinically mastitic cows (82.57%) and finally milk samples collected from clinically mastitic cows (76.92%).

Among several authors who studied the mastitis, Elbably *et al.* (2013) stated that the frequency of isolation of CNS from milk samples, teat swabs, milking machines swabs, worker's hand swabs and bedding samples was 37.8%, 37.3%, 46.34%, 45.45% and 23.3%, respectively.

Coliforms are considered environmental mastitis pathogens (Hogan *et al.*, 1999). According to table (2), the frequency of isolation of Coliforms from examined milk samples was 66.84 % (125/187); 55 % (33/60) in milk samples collected from clinically mastitic cows and 72.44% (92/127) in milk samples collected from subclinically mastitic cows. These results were in close agreement with Shpigel *et al.* (1998) who isolated 60.2% and higher than results recorded by Gonzalez *et al.* (1990) and Sargeant *et al.* (1998) who detected 37% and 17.2%, respectively.

The frequency of isolation of Coliforms from different environmental samples was recorded in table (3). The tabulated data showed that the frequency of isolation of Coliforms from bedding samples, milking machines swabs, teat swabs and worker's hand swabs was 60%, 54%, 40% and 30%, respectively.

A various Gram-negative bacterium have been isolated from different examined samples as shown in table (5) in which they are classified according to their biochemical identification to *E. coli* and other Coliforms. According to table (5), the frequency of isolation of *E. coli* from milk samples collected from clinically mastitic cows, milk samples collected from subclinically mastitic cows, milking machines swabs, teat swabs, bedding samples and worker's hand swabs was 33.33%, 21.74%, 18.52%, 16.67%, 13.33% and 13.33%, respectively.

Among several authors who studied the coliform mastitis Elbably *et al.* (2013) mentioned that the frequency of isolation of *E.coli* from mastitic milk samples, teat swabs, milking machines swabs, bedding samples and worker's hand swabs was 18.6%, 27.77%, 2.43%, 9.3% and 9.1%, respectively.

The higher percentage of *E.coli* in our study may be attributed to bad hygienic measures and high contamination of bedding materials, this lead to udder infection come from fecal contamination due to contaminated beddings (Liebisch *et al.*, 1994).

In studying the relationship between age of dairy cows and occurrence of mastitis we concluded that a significant relationship was found between age of dairy cows and coliform infection. According to table (7) and figure (2), The frequency of coliform mastitis in old (>4-8 years) and young (2-<4 years) cows was 85.22% and 37.5%, respectively. These results were similar to results recorded by Abera *et al.* (2010), Tolossa (1987), Bedane *et al.* (2012) and Biffa *et al.* (2005).

In accordance to the obtained results, Elbably *et al.* (2013) in Egypt, stated that adult cows were more affected by subclinical mastitis (33.83%) compared with clinical (9.45%). Furthermore, Zeryehun *et al.* (2013) declared that the prevalence of mastitis was 93.2% and 65% in adult and young cows, respectively, and this may be due to that old cows have large teats and sphincter muscles is more relaxed, which facilitate the invasion of infectious agent into the udder of cow.

On the other hand, Klibi *et al.* (2019) mentioned that the high prevalence of clinical mastitis in cattle aging between 7-10 years may be due to decreased immunity of cows and increased resistance of bacteria to antimicrobial agents that were widely used for the treatment of mastitis during previous infections.

Moreover, Radostits *et al.* (2007) stated that the prevalence of mastitis increases with age. This may be due to increased cellular reaction to infection or larger amount of permanent tissue damage of the udder. Older cows, especially after four lactations were submitted to more lactation, increasing the risk for mastitis and udder tissue damage (Pretorius, 2008).

According to table (8) and (9) a significant relationship was found between hygienic condition of farm (foot dipping, washing of udder before milking, post milking teat dipping and traffic control) and occurrence of coliform mastitis. In case of poor hygienic condition of the farm the frequency of Coliforms infection in milk samples and different examined swabs was 88.8% and 61.1%, respectively, while in case of good hygienic condition of the farm the frequency of Coliforms infection in milk samples

and different examined swabs was 22.6% and 15.7%, respectively. These results were in close agreement with Iraguha *et al.* (2015), Abrahmsén *et al.* (2014) and Mureithi and Njuguna (2016).

Moreover, Mureithi and Njuguna (2016) stated that there was a significant relationship between poor hygienic condition and increased risk of mastitis due to increase the exposure of teat to bacterial pathogens. Furthermore, Mekonnen *et al.* (2012) concluded that hygienic condition of the farm, every individuals and utensils used for milking practice are important things which aids in the prevention of mastitis.

On the other hand, Zeryehun *et al.* (2013) mentioned that the hygienic status of the farm was evaluated in relation to the prevalence of mastitis. Cow managed under bad hygienic condition had risk of 79.7% which had a significant association with prevalence of bovine mastitis.

In studying the relationship between herd size of dairy farms and the frequency of isolation of *Staphylococcus spp*. and Coliforms from milk samples and different swabs, we concluded that a significant relationship was detected. According to table (11), the frequency of isolation of *Staphylococcus spp*. from different swabs in small and large herds was 17.1% and 93.4%, respectively, and also the recorded data in table (10) and (11) showed that the frequency of isolation of Coliforms from milk samples and different examined swabs in small herds was 6.25% and 17.1%, respectively, compared with 79.4% and 48% in large herds in the same groups, respectively.

Our results were similar to results recorded by Abebe *et al.* (2016) who concluded that the presence of mastitis was significantly influenced by herd size. The result of positive California mastitis test was 3.1 times higher in large dairy herds than in small herds

Moreover, the higher incidence of mastitis occurrence in large herds may be associated with increased exposure of the cows for bacterial pathogens in their environment due to high stocking density, dirty ground, infected utensils, dirty ground and poor ventilation of house (Abebe *et al*., 2016).

Dairy cattle represent a major source for the transmission of antibiotic resistant genes to the human intestinal bacteria (Bandyopadhyay *et al.*, 2015), so, our study was directed to know the occurence of ESBL producing *E. coli* in mastitic milk of cattle. PCR was done on 14 isolates of *E.coli* for detection of some antibiotic resistance genes and according to table (12) and figure (3 and 4), the prevalence of blaTEM and blaSHV was 64.3% (9/14) and 92.9% (13/14), respectively, while none of the isolates carried blaCMY-2; the prevalence of blaTEM gene was 83.33% (5/6), 50% (1/2), 50% (1/2), 100% (2/2) and 0% (0/2) in milk samples, teat swabs, milking machines swabs, worker's hand swabs and bedding samples respectively, while the occurrence of blaSHV gene was 100% (6/6), 100% (2/2), 100% (2/2), 100% (2/2) and 50% (1/2) in the same groups, respectively.

The results of this study confirmed the occurrence of ESBL producing *E. coli* in milk samples and different environmental samples and the risk of the transmission of these bacteria from cows with mastitis to humans.

Our results were closely related to Das *et al.* (2017) who estimated that 48% (24/50) of Gram negative isolates were ESBL producing. Moreover, Freitag *et al.* (2017) in India, stated that the prevalence of ESBL producing Enterobacteriaceae isolated from mastitic milk was 13%. Furthermore, Ali *et al.* (2016) in china, mentioned that the prevalence extended-spectrum β-lactamase producing *E. coli* is rapidly increasing, becoming a global problem, and clarified that the prevalence of blaCTX-M, blaTEM and blaSHV was 77.78%, 55.56% and 16.67%, respectively.

CONCLUSION

Staphylococcus aureus and *E. coli* are considered two main pathogens of bovine mastitis. The prevalence of mastitis increases in old cows than young ones. Inadequate hygienic condition of the dairy environment, lack of washing of hand and udder before milking, absence of foot dipping, absence of teat dipping and traffic control are predisposing risk factors of mastitis, so, it is important that farmers should ensure good personal hygiene, better management practices in milking and housing animals and general sanitary condition of the farm should be improved. Careful and continuous surveillance of ESBL-producing *E. coli* in dairy cattle and subsequent application of preventive measures are needed to avoid a further spread of multidrug-resistant bacteria.

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