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Application of Specific Chromogenic Media and Api Technique for Rapid Confirmation of *Listeria monocytogenes* in Bulk Tank Milk and Dairy Farms Environment

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A B S T R A C T

The objectives of this study were to identify the prevalence and the sources of contamination of *Listeria monocytogenes* in BTM of examined 5 farms and to assess the used of the chromogenic media and API technique as rapid confirmation for the presence of this pathogen. The present study was carried on BTM and farm environmental samples collected from 5 dairy farms in Egypt. The samples were examined for the incidence of Listeria species using conventional isolation method and the identification of *L. monocytogenes* by the using of chromogenic media, API test and PCR technique. The detection method based on PCR amplification of the *hly*A gene revealed that the incidence of Listeria monocytogenes were 6.66%, 14%, 10%, 8%, 5.6%, 0% and 0% in BTM, feces, bedding, water troughs, teat skin, milking equipment and hand swabs, respectively. L. monocytogenes was isolated from 3 out of 5 farms investigated. Antimicrobial susceptibility was done for all identified strains isolated from BTM against 17 antimicrobial agents. All of the isolates were sensitive to Imipenem(IPM), Penicillin G(P), Ampicillin(AMP), Amoxycillin/clavulanic acid(AMC), Ampicillin/ sulbactam (SAM), Chloramphenicol(C), Levofloxacin(LEV), Cephradine, Ciprofloxacin (CIP), Cefquinome(CEQ), Ofloxacin(OFX) and Amikacin(AK).

Introduction

Food safety is a complex issue that has an impact on all segments of the society.

Diseases caused by foodborne pathogens constitute a worldwide public health problem. Listeriosis; a foodborne disease, has been considered to be an emerging zoonotic disease worldwide.

Listeria sp. is one of the most important zoonotic diseases which cause dangerous illness. It consists of six species *(Listeria monocytogenes, L. innocua, L. seeligeri, L. welshimeri, L. ivanovii* and *L. grayi*), but the most important one is *Listeria monocytogenes*. *L. monocytogenes* is a major concern for the food industry, as it

can cause *Listeriosis* in humans (Kathariou, 2002). Listeriosis is one of the most important infections in Europe (European Food Safety Authority-European Centre for Disease Prevention and Control, 2007) and in the United States, the Centers for Disease Control and Prevention estimated that approximately 1,600 persons become seriously ill with listeriosis each year, of whom 16% die (CDC, 2011).
Exposure to food borne *L. monocytogenes* may cause fever, muscle aches and gastroenteritis (Riedo *et al.,* 1994). In pregnant women, it may cause abortion (Linnan *et al.,* 1988 and Riedo *et al.,* 1994) or neonatal death (Linnan *et al.,* 1988), although there are rates of illnesses caused by *L. seeligeri*, *L. ivanovii* and *L. innocua* (Jeyaletchumi *et al.,* 2010).

Various species of Listeria are commonly found in soil, decaying vegetation, and water, and well as being part of the fecal flora of animals and humans. In dairy cattle, *L. monocytogenes* can result in several clinical presentations, including encephalitis, septicemia, abortion and mastitis (Radostitis *et al.,* 2007).

Mastitis caused by Listeria is infrequent, but infected mammary glands can shed this pathogen for periods as long as 12 months (Winter *et al.,* 2004). *L. monocytogenes* is present the dairy farm environment and can survive in the gastrointestinal tract of cows, thus constituting a source of contamination of bulk tank milk (BTM) (Latorre *et al.,* 2009). *Listeria monocytogenes* is capable of multiplying at temperatures ≤7°C and surviving in environments with a wide range of pH values (4.3 to 10) and high salt concentrations (Ryser, 2001).

The prevalence of *L. monocytogenes* in bulk tank milk has been reported to range from 0.2% to 20% (Table 1). Another important characteristic that makes *L. monocytogenes* *an* emerging concern to public health authorities is its ability to form biofilms and survive on materials commonly used in food processing equipment (Wong, 1998). Colonization of dairy processing equipment can result in cross-contamination of pasteurized milk in processing plants, which has been reported to be an important source of human Listeriosis. Introduction of *Listeria monocytogenes* into food processing plants results in reservoirs that are difficult to eradicate (Wong, 1998).

Sporadic outbreaks of Listeriosis linked to consumption of unpasteurized or cross-contaminated dairy products have occurred (Center for Science in the Public Interest, 2008). The high fatality rate, occurrence of outbreaks, and ability of *L. monocytogenes* to contaminate both unpasteurized and pasteurized foods has led the Microbiological specifications for food items including milk and dairy products often stipulate absence of *L. monocytogenes* in 25 g of food samples (Roberts, *et al.,* 1995) and FDA to adopt a "zero tolerance" policy for the presence of *L. monocytogenes* in ready-to-eat foods (FDA, 2003). In order to comply with this regulatory policy and minimize the risk of human listeriosis a reduction in the initial contamination of farm BTM has been an important objective of governmental agencies (Hassan, *et al.,* 2000).

Also, due to the increased multidrug resistance in the foodborne pathogen due to increase the use of antimicrobials in feeds for the control and treatment of diseases in animals, this work was carried out to study the prevalence and the antimicrobial susceptibility of *L. monocytogenes* isolated from milk and dairy farms environment.

Moreover, a rapid and simple diagnostic test that would reduce both the cost and time for identification of contaminated milk would

be useful for identification of this zoonotic pathogen on dairy farms. Current tests based on traditional microbiology or molecular methods are still expensive and time consuming, which may preclude their use in such programs.

Materials and Methods

Collection of Samples

BTM and environmental samples were collected from 5 dairy farms in Kafr-El sheikh Governorate and Alexandria road, Egypt, in between June, 2014 and June 2015. During each farm visit, a variety of environmental and BTM samples were collected (Table 2). Collected samples were transformed to the laboratory in an ice box as soon as possible for bacteriological examination.

Preparation of Samples

Environmental and BTM milk samples were processed as described by (Latorre *et al.*, 2009). Silage, bedding and fecal samples (50 grams) were mixed with 200 ml of peptone water in two-chamber filter bags.

Samples were stomached for one minute, after which five ml of the filtered sample were reserved for further incubation in Modified *Listeria* Enrichment Broth (Difco; BD Diagnostics, Sparks, MD).

Milking equipment swabs for each sampling location were composited in the laboratory by adding 4 ml of Neutralizing Buffer included in each tube to a sterile plastic vial. 5 ml of BTM, water, milking equipment swabs, and all other filtered samples were mixed with 5 ml of concentrated (two times the concentration recommended by the manufacturer) Modified *Listeria* Enrichment Broth and incubated at 30°C for 3 to 5 days.

Isolation of *L. monocytogenes* was done on the Basis Described by ISO 11290-1:1996 + A1:2004BS 5763-18:1997

Briefly, ten ml of the milk samples were added to 90 ml of Listeria primary enrichment of Half Fraser Broth (Oxoid, CM0895 + SR0166). Incubate for 24 hr ±2 hr at 30°C then transfer 0.1 ml of primary enrichment culture to 10 ml of Fraser Broth (Oxoid, CM0895 + SR0156) and incubate for 48 hr ±2 hr at 37°C.

A loopful of secondary enrichment broth was then streaked onto the surface of Palcam medium (Oxoid, CM0877 + SR0150) and Oxford medium (CM0856 + SR0140) and incubate for 24 hr ±3 hr at 37°C.

Colonies of presumptive Listeria spp from each selective agar plate were streaked on Tryptone Soya Yeast Extract Agar (TSYEA - Oxoid, CM0862 + 9–18g Agar) and Incubate for 24 hr at 37°C for purification.

Morphological and Biochemical Identification

All Purified presumptive Listeria isolates were examined for Gram's reaction, Motility, Blood haemolysis test (Blood agar base-Oxoid, CM854), CAMP test (CFSAN, 2001), sugars fermentation tests, Nitrate reduction and Catalase production.

Detection of *L. monocytogenes* on Chromogenic Media (ALOA) (ISO/ DIS11290-1/2004) Refer to Standards ISO 11290-1 & -2 Amendment 1(2004)

The positive biochemical samples for Listeria spp. in BTM and environmental samples were exposed to chromogenic media (ALOA) to quantify the result. Typically, this is done by preparing sample

dilutions in BPW, or enrichment broth without supplements, plating each dilution onto ALOA and incubated at 37°C for 24 hours ±3 hr. Typical *L. monocytogenes* were blue to blue-green colonies, round, regular, with opaque halo, diameter from one to 2 mm.

Identification of Listeria spp. using the API Test (Beumer, *et al.*, 1996)

10300 API Listeria (bioMerieux, Marcy-l'Etoile, France) consists of the following 10 tests: enzymatic substrate, hydrolysis of Aesculin, acid production from D-arabitol, D-xylose, L-rhamnose, *α*-methyl- D-glucose, α-methyl- D-mannose, D-ribose, glucose-1-phosphate and D-tagatose.

Suspected isolated Listeria spp. colonies were picked up and emulsified in an ampoule of API suspension medium (2 ml); turbidity of inoculated medium was adjusted to 1 McFarland. The incubation box was prepared (tray and lid) and about 3 ml of distilled water was distributed into the honeycombed wells of the tray to create a humid atmosphere. The strip was removed from its individual packaging, placed in the incubation box. After inoculation by the suspected colonies the strip box was closed and incubated for 18-24 hours at 37°C in aerobic conditions. Reaction results were determined according to color changes as an indicator as per manufacturer's instructions.

Molecular Detection of *L. Monocytogenes* using PCR Technique

Extraction of Genomic DNA from Cultures

Genomic DNA was extracted from the isolates of presumptive *L. monocytogenes* using QIAamp DNA mini kit instructions (Qiagen Pty Ltd, Australia), according to the manufacturer's protocol. Catalogue no.51304.

DNA Amplification by Polymerase Chain Reaction

Temperature and time conditions of the two primers during PCR are shown in Table (4) according to specific authors and Emerald Amp GT PCR mastermix (Takara) kit Code No. RR310A

The PCR Product Visualization

The amplicons of 553 bp(*16S Rrna gene)* and 174 bp(hlyA gene) of *listeria monocytogenes* were visualized by running in 2.5% agarose gel (Agarose gel was mixed in ethidium bromide) running by using horizontal gel electrophoresis, according to (Sambrook *et al.*, 1989) with modification.

The horizontal electrophoresis unit was connected by the power supply, which was 1-5 volts/cm of the tank length. The run was stopped after about 30 min and the gel was transferred to UV cabinet. The gel was photographed by a gel documentation system and the data was analyzed through computer software. The positive samples were detected by presence of amplified DNA fragment at expected size.

Antibiotic Sensitivity Test

Antimicrobial resistance of *Listeria monocytogenes* strains isolated from BTM were carried out against 17 antimicrobial agents. The standard antibiotic discs obtained from (Oxoid, Basingstoke, UK). The antimicrobial susceptibility testing of the isolates was performed by using the disc diffusion method according to recommendations of the national committee for clinical laboratory standard (NCCLS, 2002).

Result and Discussion

A total of 895 samples of which 75 were bulk tank milk of cattle origin, 250 feces samples, 50 samples from bedding material, 50 samples from water troughs, 250 teat skin swabs, 170 milking equipments' swabs (teat cups, pipelines, jars) and 50 hand swabs from dairy workers were collected from 5 dairy farms in Kafr-El-sheikh Governorate and Alexandria road, Egypt. The collected samples were examined for the prevalence of *Listeria monocytogenes* by conventional method and Presumptive positive *Listeria monocytogenes* isolates were further identified to species level by chromogenic media, API listeria strips and PCR to evaluate its accuracy.

The results show that the prevalence percentages of *Listeria monocytogenes* were 20, 20, 30, 14, 12, 0 and 0 using conventional method; 9.33, 15.2, 14, 10, 7.2, 0 and 0 using Chromogenic media; 6.66, 14.8, 10, 8, 6.4, 0 and 0 using API tests and 6.66, 14, 10, 8, 5.6, 0 and 0% using PCR technique in BTM, feces, bedding, Water troughs, teat skin swabs, milking equipments' swabs and hand workers swabs respectively (Table 5).

The isolation and identification of *L. monocytogenes* by different identification methods revealed that there was no significant difference in the incidence of *L. monocytogenes* in BTM and environmental farm samples by using API tests and PCR technique, similar results was recorded Srividya, *et al.*, (2013). However both Palcam and Oxford selective agar media could not compared to CHROMagar Listeria because the former two were generally used for detection of Listeria spp. while the latter enables direct detection of *L. monocytogenes*. The use of PALCAM agar and Listeria selective agar did not allow one-step detection of *L. monocytogenes* as further identification with biochemical test or PCR was needed to differentiate *L. monocytogenes* from other Listeria spp. Our results agree with, Jamali, *et al.*, 2013.

Therefore PCR result was used as final confirmation of the identity of *L. monocytogenes* colonies isolated in this current study.

Nearly Similar incidence of *L. monocytogenes* in BTM was recorded by Van Kessel, *et al.*, (2004), where the incidence of contamination was 6.5%. Abd ElAal & Atta (2009), Waak, *et al.*, (2002) and Rafie *et al.*, (2013) reported low incidence of 0.2%, 1.0% and 2.02% respectively, while higher incidence were reported by Amal (2014), Pantoja, *et al.*, (2012) and Hassan, *et al.*, (2000), 20, 16 and 12.6 respectively.

Currently, *Listeria monocytogenes* is considered one of the most important pathogens responsible for food-borne infection. It is often incriminated in outbreaks of human listeriosis (Ryser and Marth, 2007). Milk and dairy products contaminated with *Listeria monocytogenes* have been responsible for human listeriosis outbreaks (Dalton *et al.*, 1997). Pregnant women, infants, immune-compromised and the elderly people are at greatest risk for listeriosis (Gillespie *et al.*, 2010).

The serious consequences of listeriosis, such as a septicemia in elderly and immune compromised people, and abortion in pregnant women or death of their newborn constitute a serious threat to public health.

Another complication is that *Listeria* is able to grow well at law temperatures. Thus, refrigeration is not as effective in preventing growth of *Listeria* in food as it is for most

other bacteria that cause food-borne disease (Salyers and Whitt 2002).

Examination of farm environment samples show that the incidence of *L. monocytogenes* was highest in fecal samples, 14%, nearly Similar finding were reported by Husu, 1990 and Fedio *et al.*, 1992 16.1% and 14.5% respectively, lower incidence was reported by Hakan, 2003 was 1.53%. While our result was lowest in teat swabs, 5.6%, higher incidence were reported by Mohammed *et al.*, 2009 was 19%.

The incidence of *L. monocytogenes* in bedding samples were 10%, higher incidence were reported by ueno *et al.*, 1996 and mohammed *et al.*, 2009 were 22% and 30% respectively; while our incidence in water samples were 8%, lower incidence were reported by Pantoja *et al.*, (2012) and Atil *et al.*, (2011) were 6% and 4.5% respectively.

On the other hand *L. monocytogenes* failed to be isolated from milking equipments and hand swabs samples, similar results to our study were reported by pantoja *et al.*, 2012 and Atil *et al.*, 2011.

Raw milk was identified as a source of *L. monocytogenes*, common sources of *L. monocytogenes* in raw milk have been reported to be fecal (Husu, 2010) but environmental contaminations during milking have also been reported (Frece *et al.*, 2010). *Listeria monocytogenes* was isolated from BTM and different environmental samples obtained from 3 out of 5 farms examined. In farm A and B *L. monocytogenes* were isolated from BTM, feces, bedding, water troughs and teat skin swabs in percentages of 20, 34, 20, 20 & 18 and 13.33, 24, 20, 20 & 10 respectively, while on farm C, *L*isteria *monocytogenes* was only isolated from feces and bedding materials in percentages of 12 and 10, respectively (Table 6). High isolation rates of the organism in farm A and B may have been caused by shedding of the organism in the Feces of the cow from which farm environment were contaminated.

L. monocytogenes was isolated from feces and bedding material of farm E in percentages of 12 and 10 respectively, while farm C and D were free completely from *L. monocytogenes* (Table 6).

A similarity was seen in the distribution of the organisms at the two farms, A and B where the milking were manual and the hygienic conditions at the two dairy farms were poor. Further, it was observed that most of the milking animals are not regularly screened for diseases and as a result, there is a great danger of some diseases being transmitted to human beings.

The farm C, D and E were using milking machine. Despite the high cost of milking machine, it is highly effective and helpful to produce clean milk without any direct contact with the farmers and the surroundings. On the contrary Latorre *et al.*, 2009 concluded that the milking machine was the most likely source of contamination.

Regardless of the source of contamination, it is important to note that results of this and previous studies *(*Latorre, *et al.*, 2009 and Pantoja, *et al.*, 2012*)* demonstrate that farms can develop persistent sources of BTM contamination with *L. monocytogenes.* Therefore, longitudinal screening of BTM and dairy environment could be valuable for programs developed to improve the safety of milk. Identification of such contaminated farms could not only minimize the risk of listeriosis for consumers of unpasteurized dairy products but also prevent colonization of milk processing facilities and further cross contamination of pasteurized dairy products (Pantoja, *et al.*, 2012).

Table.1 Surveys on the Isolation of *L. monocytogenes* from BTM

Table.1 Surveys on the isolation of *L. monocytogenes* from BTM	
Isolation rate (%)	**Reference**
5.08 2.7 12.6 4.6 1.0 4.9 to 7.0 6.5 0.2 9.8% 16 2.02 8 10, 20 & 10	O'Donnell, (1995) Steele, *et al.*, (1997) Hassan, *et al.*, (2000) Jayarao & Henning (2001) Waak, *et al.*, (2002) Muraoka, et al. (2003) Van Kessel, *et al.*, (2004) Abd ElAal & Atta (2009) Ljupco, *et al.*, (2012) Pantoja, *et al.*, (2012) Rafie *et al.*, (2013) AL-Ashmawy, *et al.*, (2014) Amal (2014)

Table.2 The Collected Samples from the Dairy Farms

Table (2): The collected samples from the dairy farms.			
Source	**Size**	**References**	**No. of samples**
BTM	60-ml/sterile bottle	ISO 707: 2008	75
Feces	Full 1-L plastic bag	OIE, 2013	250
Bedding	Full 1-L plastic bag	Clegg et al, 1983	50
Water troughs	200 ml in sterile cup	APHA, 1995	50
Teat skin & orifice swabs	4 ml BPW (Oxoid, CM1049) /swab	Rendos *et al.*, 1975	250
Dairy equipments and utensils	4 ml sterile ¼ strength ringer's solution Oxoid: BR005/swab	ISO/FDIS 18593:2004	170
worker hands	4 ml BPW/swab.	ISO/FDIS 18593:2004	50

Table.3 List of Primers Used in the Identification of *Listeria monocytogenes*

Primer	Sequence	Amplified product	Reference
16S rRNA	CCT TTG ACC ACT CTG GAG ACA GAG C AAG GAG GTG ATC CAA CCG CAC CTT C	553 bp	Lantz *et al.*, 1994
hlyA	GCA-TCT-GCA-TTC-AAT-AAA-GA TGT-CAC-TGC-ATC-TCC-GTG-GT	174 bp	Deneer and Boychuk, 1991

Table.4 Cycling Conditions of the Different Primers During Cpcr

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
16S Rrna	94˚C 5 min.	94˚C 30 sec.	60˚C 45 sec	72˚C 45 sec	35	72˚C 10 min.
hlyA	94˚C 5 min.	94˚C 30 sec.	50˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.

Table.5 Incidence of *L. monocytogenes* in Btm and Environmental Farm Samples using Different Identification Methods

Sample type	Sample numbers	Positive samples for *Listeria monocytogenes*							
		Conventional Method		Chromogenic media		API Tests		PCR Technique	
		NO.	%	NO.	%	NO.	%	NO.	%
BTM	75	15	20	7	9.33	5	6.66	5	6.67
Feces	250	50	20	38	15.2	37	14.8	35	14
Bedding	50	15	30	7	14	5	10	5	10
Water troughs	50	7	14	5	10	4	8	4	8
• Teat swabs	250	30	12	18	7.2	16	6.4	14	5.6
*Milking equipments	170	0	0	0	0	0	0	0	0
Hand swabs	50	0	0	0	0	0	0	0	0

* Milking equipments' (tanks surfaces, clusters and teats cups of milking machines)
Teats skin and orifice swabs.

Table.6 The Incidence of *Listeria monocytogenes* in Different Farms Examined

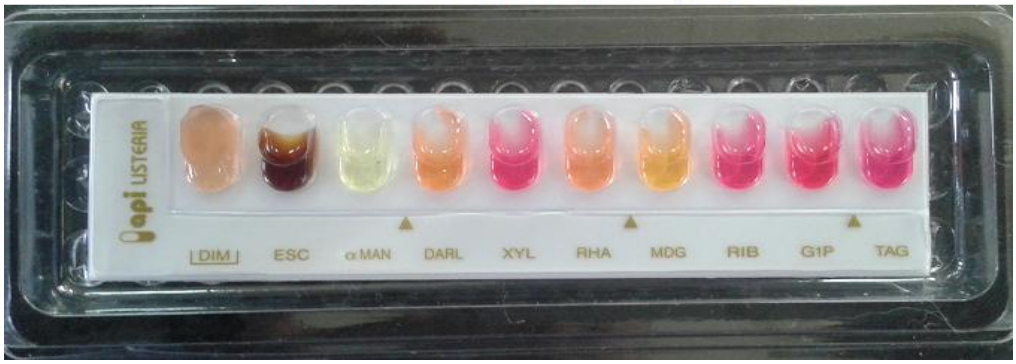
Farms	BTM (15)		Feces (50)		Bedding (10)		Water (10)		Teat swab (50)		Milking equipments (10)		Hands swabs (10)	
	Positive samples													
	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Farm A Manual	3	20	17	34	2	20	2	20	9	18	0	0	0	0
Farm B Manual	2	13.33	12	24	2	20	2	20	5	10	0	0	0	0
Farm C	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Farm D	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Farm E	0	0	6	12	1	10	0	0	0	0	0	0	0	0

The numbers in parentheses represent the numbers of samples taken from each farm

Table.7 Antimicrobial Susceptibility Profiles of *L. monocytogenes* isolated from bt**M**

Antimicrobial agent	Disk content level (µg)	Code (Oxoid manual)	Resistance		Sensitivity	
			NO.	%	NO.	%
Imipenem(IPM)	10 µg	CT0455B	0	0	5	100
Penicillin G(P)	10Unit	CT0043B	0	0	5	100
Ampicillin(AMP)	10 µg	CT0003B	0	0	5	100
Amoxycillin/ clavulanic acid(AMC)	30 µg	CT0223B	0	0	5	100
Ampicillin/sulbactam(SAM)	30 µg	CT1653B	0	0	5	100
Chloramphenicol(C)	30 µg	CT0013B	0	0	5	100
Levofloxacin(LEV)	5 µg	CT1587B	0	0	5	100
Cephradine	30 µg	CT0063B	0	0	5	100
Ciprofloxacin(CIP)	5 µg	CT0425B	0	0	5	100
Cefquinome(CEQ)	30 µg	----	0	0	5	100
Ofloxacin(OFX)	5 µg	CT0446B	0	0	5	100
Amikacin(AK)	30 µg	CT0107B	0	0	5	100
Gentamicin(CN)	10	CT0024B	1	20	4	80
Erythromycin(E)	15	CT0020B	1	20	4	80
Neomycin	10	CT0032B	2	40	3	60
Tetracycline(TE)	30	CT0054B	2	40	3	60
Streptomycin(S)	10	CT0047B	3	60	2	40

Fig.1: API-10300 *Listeria* showing positive results for identification of *Listeria monocytogenes*



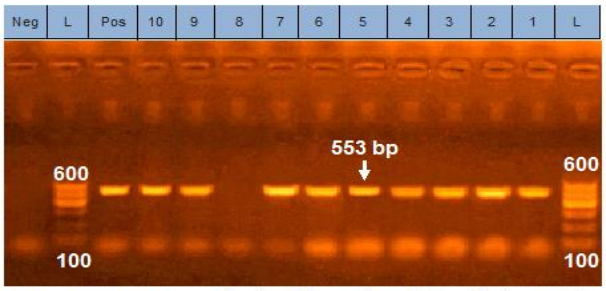


Fig. (2): Agarose gel showing polymerase chain reaction amplified product of 553 bp for *16S rRNA* gene in *L. monocytogenes* isolates, Lane 1, 2, 3, 4, 5, 6, 7, 9 and 10:Samples positive for *16S rRNA* gene, Lane 8: sample negative for Samples positive for *16S rRNA* gene, Lane (pos): Positive control, Lane neg: Negative control, Lane L: 100pb DNA ladder (DNA marker).

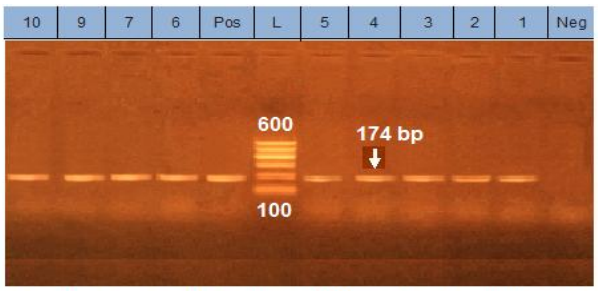
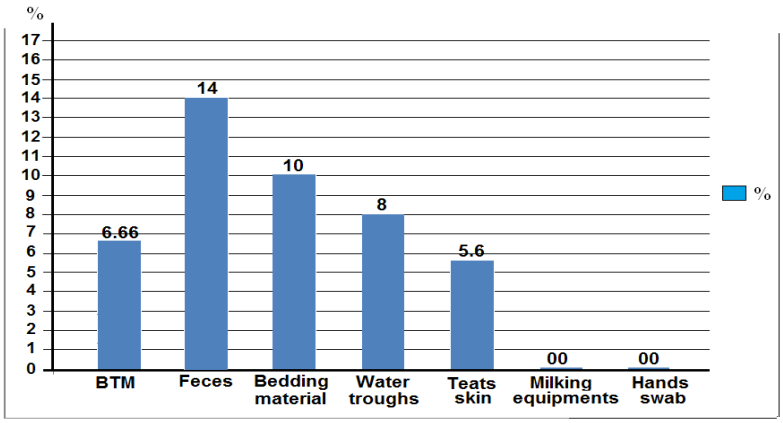


Fig. (3): Agarose gel showing PCR amplified product of 174 bp for *hly*A gene in *L. monocytogenes* isolates, Lane 1, 2, 3, 4, 5, 6, 7, 9 and 10: Samples positive for *hly*A, Lane (pos): Positive control, Lane neg: Negative control, Lane L: 100pb DNA ladder (DNA marker)

Fig.4 Incidence of *L. monocytogenes* in BTM and Environmental Farm Samples



The safety of milk is an important attribute of consumers of milk and dairy products. Milk pasteurization safeguards consumers from many potential food borne hazards. Despite the pasteurization process, the quality and safety of raw milk are important in reducing the risk of food borne diseases associated with milk because raw milk is the starting point of the milk production-consumption chain.

Antimicrobial resistance of *Listeria monocytogenes* strains (n=5) isolated from 5 BTM samples of farm A and B against 17 antimicrobial agents were evaluated, the results show that all of the isolates (100%) were sensitive to Imipenem(IPM), Penicillin G(P), Ampicillin(AMP), Amoxycillin/ clavulanic acid(AMC), Ampicillin/ sulbactam(SAM), Chloramphenicol (C), Levofloxacin(LEV), Cephradine, Ciprofloxacin(CIP), Cefquinome (CEQ), Ofloxacin (OFX) and Amikacin (AK). Four strains (80%) were sensitive to Gentamicin and Erythromycin, 3 strains (60%) were sensitive to Neomycin and Tetracycline, while two strains (40%) were sensitive to Streptomycin (Table 7).

Several studies have described differences in susceptibility of isolates obtained from farms with different histories of potential exposure to antimicrobials (Tikofsky *et al.*, 2003; Sato *et al.*, 2004; Rajala-Schultz *et al.*, 2004; Berghash *et al.*, 1983).

Nearly similar results were recorded by Farouk, *et al.,* (2015), who found that all isolates (100%) were sensitive to penicillin G, ampicillin, tetracyclin, amikacin and erythromycin. Also our results was nearly similar to Rota, *et al.,* (1996) and Slade and Collins-Thompson, (1990) they reported that Listeria is usually susceptible to a wide range of antibiotics especially ampicillin and ampicillin & erythromycin, respectively.

Bulk tank milk might be a potential source of *L. monocytogenes* which poses a significant clinical threat to consumers through excessive use of various antibiotics against this organism.

In conclusion, results of this study strongly suggest that the contamination of BTM with *L. monocytogenes* originated from inefficient cleaned and sanitized of dairy cows udder and stored water used for washing of equipment and drinking of animals. The results indicate that farm's environment can develop persistent sources of contamination.

Milk pasteurization safeguards consumers from many potential food borne hazards in milk and milk products. Despite the pasteurization process, the quality and safety of raw milk are important in reducing the risk of food borne diseases associated with milk because raw milk is the starting point of the milk production-consumption chain.

Results of this study demonstrated that *L. monocytogenes* isolated from the BTM of two dairy farms were susceptible to a wide range of antibiotics.

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