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Application of Specific Media, API Technique and PCR for Rapid Confirmation of *Listeria monocytogenes* in Foodstuffs and Water

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ABSTRACT

Listeria monocytogenes is responsible for causing the serious infectious disease listeriorosis which occurs due to food contaminated with *Listeria*. The study employed herein concerned with the isolation and identification of *L. monocytogenes* from foodstuffs and water. Hundred different food stuffs samples (milk, cheese and meat) and 50 fresh water samples were surveyed for presence of *L. monocytogenes*. These samples were inoculated into two enrichment media and cultivated on Oxford agar. Presumptively positive isolates were determined by morphology of colonies, gram stain, catalase test, haemolysis on sheep blood agar and motility test. The isolates were further identified to species level by 10300 API *Listeria* strips, PCR was done for all specimens to evaluate its accuracy. Out of 100 foodstuffs samples, 15 samples were showed positive results for *Listria* species. Out of 50 fresh water samples, 5 samples were showed positive results. Antibiotic susceptibility was done for all identified strains. All isolates were sensitive to penicillin G, ampicillin, tetracycline, amikacin and erythromycin.

Key words: Foodstuffs, water, *L. monocytogenes*, API strips, PCR

INTRODUCTION

The natural occurrence of *L. monocytogenes* in raw materials such as (milk and meat) causes contamination of some food processing environments. The ability of *L. monocytogenes* to grow at chilling temperature and to tolerate salt and low pH, make this pathogen particularly troubles to the food industry (Carminati *et al*., 2004). The *L. monocytogenes* is widely distributed in the environment and has been isolated from a variety of sources including water, sludge, soil, plants, vegetation, food, food processing plants and infected humans and animals (Dhama *et al*., 2013). *L. monocytogenes* exists in the environment as a saprophyte and can access the human food chain either directly or through infection or carriage in farm animals (Gahan and Colin, 2014).

Listeria monocytogenes is invasive bacteria associated with life-threatening food borne disease in human, following ingestion, bacteria cross the intestinal barrier by penetrating the intestinal cell epithelium, translocate into the liver, spleen, lymph node, brain and in pregnant woman cross the fetoplacental barrier leading to infection of the foetus (Pizarro-Cerda and Cossart, 2006).

The presence of any species of *Listeria* in food may be indicator of poor hygiene. *L. monocytogenes* is responsible for sever food-borne infections in human and causes 20-50% mortality in susceptible population such as newborn children, the elderly and immunocompromised persons (Huang *et al*., 2006). In the past, those individuals who develop listeriosis have usually

been treated with penicillin or ampicillin in conjunction with an amino glycoside (Jorgensen *et al*., 1999), although tetracycline, erythromycin or chloramphenicol alone or incombination, have also been used (Hof, 1991). Current therapy of all forms of listeriosis is a combination of ampicillin and gentamycin (Lorber, 1997).

The present study was carried out to isolate the *L. monocytogenes* from food and water resources and to evaluate the antibiotic susceptibility pattern. Presumptive *Listeria* spp. were isolated using a selective enrichment and isolation procedure and 3 *L. monocytogenes* isolates were identified based on colony morphology, hemolytic activity. Amplification of pathogenicity in lB gene was used. The product of the *inl*B gene is essential for the *L. monocytogenes* to enter the hepatocytes, which is a specific virulence factor for this bacterium (Goksoy *et al*., 2006). Moreover, the objectives of this study was to determine the level of *Listeria* spp. contamination in different food and water samples that were ready for human consumption in Egypt.

MATERIALS AND METHODS

Sample collection: Water and food stuffs samples were collected from different localities in Sharkia Governate, Egypt. Samples were collected under complete aseptic conditions using sterile containers and syringes. Milk samples: after discarding the first few drops of fore milk, about 50 mL of milk were poured into sterile screw capped bottle. The collected samples were transformed in an ice box for bacteriological examination. Cheese: Blocks of cheese of variable weights were collected. Water samples: One liter of surface water was collected from within a 0.5 m depth of the surface directly into sterile container, storage of samples was also carried out at refrigeration temperature.

Enrichment and isolation of *Listeria* spp. from water samples: The culture method was on the basis of international standard (ISO 11290-1-1997) as previously described by Narang (2004), 25 mL of each of the water samples were added into a sterile tube using sterile disposable pipettes and 225 mL of Fraser broth (Oxoid) were added to water samples to form 10^{-1} dilution. After 24 h incubation, 1.0 mL of the dilution was transferred to a tube containing 10 mL of *Listeria* enrichment broth (Oxoid). The tubes were incubated for 24-48 h at 37±2°C. One-two loops of *Listeria* broth culture were streaked onto *Listeria* selective agar (Oxford formulation, oxoid) and plates were incubated for 48 h at 37±2°C (Seelinger and Jones, 1986; Arora, 2004).

Enrichment and isolation of *Listeria* spp. from food samples: According to Goksoy *et al*. (2006) the following method was performed: Twenty five grams or mL of food samples were added to 225 mL *Listeria* Enrichment Broth (LEB) and mixed well by shaking. The enrichment culture was incubated for 2 days at 30°C then 0.1 mL of LEB culture was inoculated into 10 mL Fraser's secondary enrichment broth and incubated at 35°C for 24-48 h. After 24-48 h, the Fraser broth tubes showing black discoloration were streaked onto Oxford agar and incubated at 35°C for 48 h. Suspected colonies were cultured onto trypticase soya agar with 0.6% yeast extract (TSA-YE) and incubated at 30°C for 24 h, then maintained at 4°C for further investigation.

Morphological and biochemical characterization of *Listeria*: All separated colonies were subjected to stander biochemical characterization tests including gram staining, catalase, growth on sheep blood agar and motility testing. The motility was tested at both 25 and 37°C.

Identification of *Listeria* using the API test procedure: The API (10300 API *Listeria*) (Beumer *et al*., 1996) consists of the following 10 tests: enzymatic substrate, hydrolysis of esculin, 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.

Bacterial inocula were prepared by few well-isolated colonies were picked up and emulsified in an ampule 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 the strip box was closed and incubated for 18-24 h at 37°C in aerobic conditions.

Reaction results were determined according to color changes as an indicator as per manufacturers instructions (Table 1).

Identification of *Listeria* using PCR: Three major steps were performed, DNA extraction, PCR amplification and then the detection of the amplicons using agarose gel electrophoresis.

DNA extraction: DNA extraction was done using QI Aamp DNA mini kit (Qiagen GmbH, Hilden, Germany). The extraction was performed following the manufacturer's protocol for gram-positive bacteria. Cellular lysis was carried out by enzymatic treatment with lysozyme, RNase A and proteinase K in detergents and guanidium salts-containing buffers. After buffering condition adjustment, DNA was selectively bound to a silica-gel membrane. The yield of the DNA in each extract was examined by agarose gel electrophoresis. The appearance of a visible band under UV light was an indication of successful extraction.

PCR amplification: 1-PCR-gold master-mix beads (Bioron-Germany): 50 µL reaction contain (2.5 U taq DNA polymerase, 250 Mm each dNTP, 10 mM tris HCl (pH 9.0), 30 mM kCl, 1.5 Mm $MgCl_2$.

PCR primers (sigma): Oligonucleotide primers for the PCR assay were selected based on the published nucleotide sequence of the inlB gene. The product of the *inl*B gene is essential for the

Table 1: Identification of *Listeria* based on the API (10300 API *Listeria*)

Test	Active ingredients	QTY (mg cup^{-1})	Reactions	Results (ZYM B/<3 min) Negative	Positive
DIM	Enzymatic substrate	0.106	Differentiation *L. innocua*/*L. monocytogenes*	Pale orange Pink beige Grey beige	Orange
ESC	Esculin Ferric citrate	0.16 0.024	Hydrolysis (ESCulin)	Pale yellow	Black
αMAN	4-nitrophenyl-α D-mannopyranoside	0.045	α-MANnosidase	Colorless	Yellow
DARL	D-arabitoL	0.40	Acidification (D-ARabitoL)	Red/orange-red	Yellow/ yellow-orange
XYL	D-Xylose	0.40	Acidification (XYLose)		
RHA	L-Rhamnose	0.40	Acidification (RHAmnose)		
MDG	Methyl-αD-glucopyranoside	0.40	Acidification (Methyl-αD-Glucopyranoside)		
RIB	D-Ribose	0.40	Acidification (RIBose)		
G1P	Glucose-1-Phosphate	0.40	Acidification (Glucose-1-Phosphate)		
TAG	D-Tagatose	0.40	Acidification (TAGATOSE)		

L. monocytogenes to enter the hepatocytes, which is a specific virulence factor for this bacterium (Goksoy *et al*., 2006). Forward primer: Termed INB-L. Its sequence is 5´CTGGAAAGTTTGTATTTGGGAAA-3´. Reverse primer: Termed INB-R. Its sequence is 5´TTTCATAATCGCCATCATCACT-3´. These primers were designed to give a predicted product size of 343 bp. They were supplied in lyophilized form and were reconstituted according to the manufacturer's instructions using pyrogen free water. This was performed under laminar flow hood. Then, they were diluted to stock subworking solutions (appendix) in order to avoid repeated thawing and freezing of the original stock and kept at -20°C till their use.

Amplicon detection: This was done using conventional agarose gel electrophoresis (Robinson and Lafleche, 2000).

Interpretation: (1) The gel was then examined under the UV illuminator. The size of any resulting band could be detected using the molecular size marker which gave different bands ranging from 100-1000 bp. (2) The positive control gave a band at 343 bp, the negative control gave no band and positive cases gave bands at nearly the same level as the positive control, while negative cases gave no bands.

Antimicrobial susceptibility: The antibiotic susceptibility of the isolates was determined by the disk diffusion method on Muellar-Hinton Agar as previously described by Kirby Bauer method (Bauer *et al*., 1966). The antibiotic discs for testing the sensitivity of the isolated strains by disc diffusion technique were: Amikacin (30 μg), ampicillin (10 μg), penicillin G (10 U), vancomycin (15 μg), erythromycin (15 μg), ofloxacin (5 μg), tetracycline (30 μg) and trimethoprim-sulfamethoxazole (1.25 μg).

The inoculum was standardized by adjusting its density to equal the turbidity of barium sulphate which is the 0.5 McFarland turbidity and incubated at 37±2°C for 16-18 h. Diameters of inhibition zones were measured with a ruler on the under-surface of the Petri-dishes according to the Clinical and Laboratory Standards Institute (2006). The used antibiotic discs, the disc potency and the criteria for interpretation of inhibition zone diameters are shown according to Clinical and Laboratory Standards Institute (2006).

RESULTS

Isolation and identification of *Listeria* spp. from foodstuffs: Hundred food stuffs samples were collected from different places of Sharkia Governate. Samples were surveyed for presence of *L. monocytogenes* (Table 2 and Fig. 1-6). They were 15 *Listerial* isolates from all tested food types expect minced raw meat and beef. All *Listerial* isolates were gram positive short rod cells. Diplobacillis cells or occurring in short chains (Fig. 3). They were catalase positive, displayed a distinctive tumbling motility by light microscopy, umbrella like growth 2-5 mm below surface at soft agar (Fig. 4). These isolates were subcultured on sheep blood agar and incubated for 24 h at 37°C. Zone of beta hemolysis characteristic of *L. monocytogenes* was observed on sheep blood agar (Fig. 5). After recording the above preliminary characters which indicated that all isolates belonged to genus *Listeria*, biochemical testes were carried out by the 15 experimental *Listerial* isolates using the API (10300 API *Listeria*) strip according to the manufacturer's instructions (Fig. 6).

Table 2: Identification of the collected bacterial isolates from foodstuffs

No. of samples	Source	Fraser broth	Oxford agar	Gram stain	Catalase test	Motility	Sheep blood agar	API
1-4	Bufflo milk	-ve	-ve	-ve	-ve	-ve	-ve	-ve
5	Bufflo milk	+ve	+ve	+ve	+ve	motile	-ve	*Listeria welshimeri*
6-11	Bufflo milk	-ve	-ve	-ve	-ve	-ve	-ve	-ve
12	Bufflo milk	+ve	+ve	+ve	+ve	motile	-ve	*Listeria welshimeri*
13-14	Bufflo milk	-ve	-ve	-ve	-ve	-ve	-ve	-ve
15	Bufflo milk	+ve	+ve	+ve	+ve	motile	beta hemolysis	*Listeria monocytogenes*
16-19	Bufflo milk	-ve	-ve	-ve	-ve	-ve	-ve	-ve
20	Bufflo milk	+ve	+ve	+ve	+ve	motile	-ve	*Listeria welshimeri*
21-27	Bufflo milk	-ve	-ve	-ve	-ve	-ve	-ve	-ve
28	Bufflo milk	+ve	+ve	+ve	+ve	motile	-ve	*Listeria welshimeri*
29-30	Bufflo milk	-ve	-ve	-ve	-ve	-ve	-ve	-ve
31-35	Cow milk	-ve	-ve	-ve	-ve	-ve	-ve	-ve
36	Cow milk	+ve	+ve	+ve	+ve	motile	beta hemolysis	*Listeria monocytogenes*
37-40	Cow milk	-ve	-ve	-ve	-ve	-ve	-ve	-ve
41	Cow milk	+ve	+ve	+ve	+ve	motile	-ve	*Listeria gray*i
42-44	Cow milk	-ve	-ve	-ve	-ve	-ve	-ve	-ve
45	Cow milk	+ve	+ve	+ve	+ve	motile	-ve	*Listeria grayi*
46-52	Cow milk	-ve	-ve	-ve	-ve	-ve	-ve	-ve
53	Cow milk	+ve	+ve	+ve	+ve	motile	-ve	*Listeria grayi*
54-58	Cow milk	-ve	-ve	-ve	-ve	-ve	-ve	-ve
59	Cow milk	+ve	+ve	+ve	+ve	motile	-ve	*Listeria seeligeri*
60	Cow milk	-ve	-ve	-ve	-ve	-ve	-ve	-ve
61-63	Sheep milk	-ve	-ve	-ve	-ve	-ve	-ve	-ve
64	Sheep milk	+ve	+ve	+ve	+ve	motile	-ve	*Listeria grayi*
65-69	Sheep milk	-ve	-ve	-ve	-ve	-ve	-ve	-ve
70	Sheep milk	+ve	+ve	+ve	+ve	motile	-ve	*Listeria welshimeri*
71-73	Cheese	-ve	-ve	-ve	-ve	-ve	-ve	-ve
74	Cheese	+ve	+ve	+ve	+ve	motile	-ve	*Listeria seeligeri*
75-76	Cheese	-ve	-ve	-ve	-ve	-ve	-ve	-ve
77	Cheese	+ve	+ve	+ve	+ve	motile	-ve	*Listeria innocua*
78-80	Cheese	-ve	-ve	-ve	-ve	-ve	-ve	-ve
81	Luncheon	+ve	+ve	+ve	+ve	motile	-ve	*Listeria welshimeri*
82-84	Luncheon	-ve	-ve	-ve	-ve	-ve	-ve	-ve
85	Luncheon	-ve	+ve	+ve cocci	-ve	-ve	-ve	*S. aureus*
86-90	Luncheon	-ve	-ve	-ve	-ve	-ve	-ve	-ve
91	Minced raw meat	-ve	-ve	-ve	-ve	-ve	-ve	-ve
92	Minced raw meat	-ve	+ve	+ve cocci	-ve	-ve	-ve	*S. aureus*
93-95	Minced raw meat	-ve	-ve	-ve	-ve	-ve	-ve	-ve
96-100	Beef*	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Beef*: Sodium nitrite 0.005%

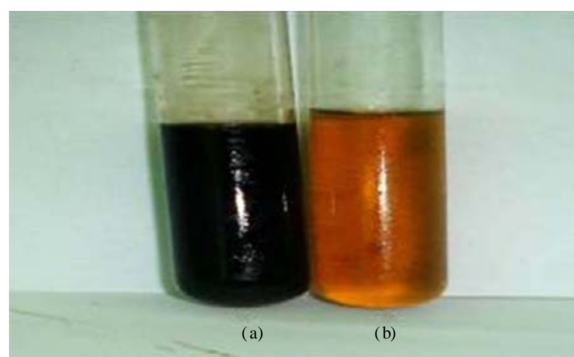

Fig. 1(a-b): Fraser enrichment broth, (a) Black tube shows presumptively diagnosed *Listeria* and (b) Tube negative for *Listeria*

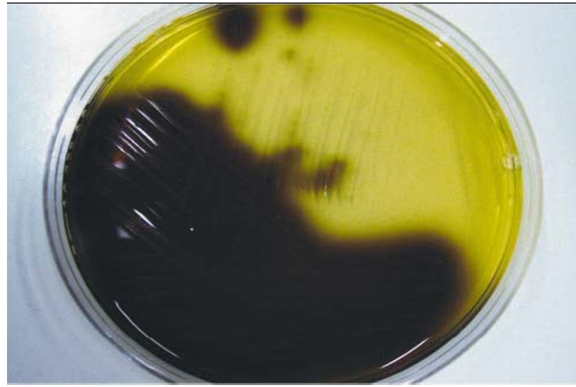

Fig. 2: Oxford agar medium. It shows brown green colonies with black halo appearance characteristic of *Listeria* spp.

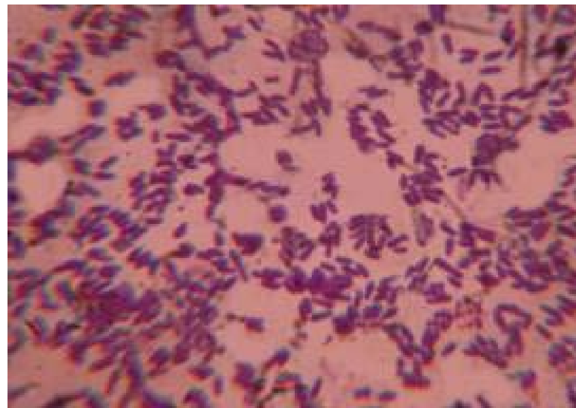

Fig. 3: Gram positive bacilli of suspected *Listeria* isolates

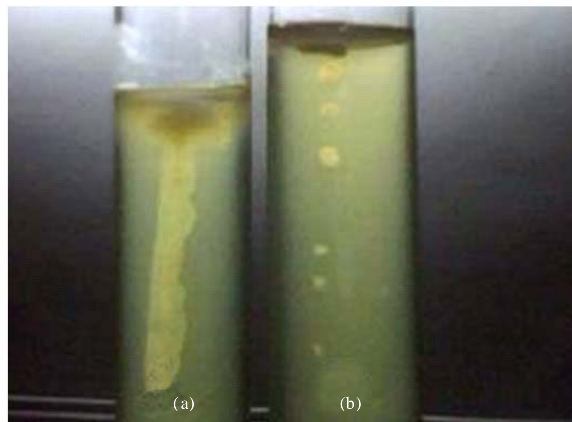

Fig. 4(a-b): Soft agar medium, (a) Tube showing umbrella like motility of *Listeria monocytogenes* and (b) Tube negative for *Listeria*

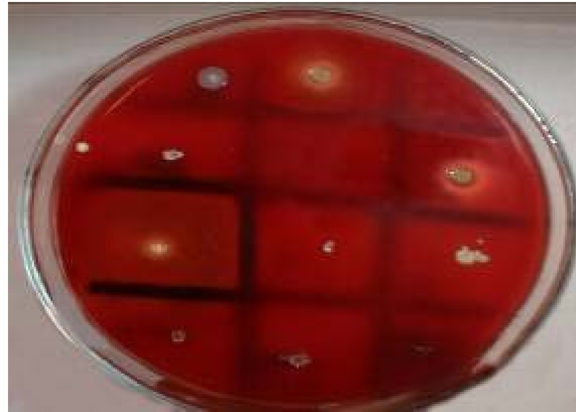

Fig. 5: Sheep blood agar, stab grid method. Each isolate was surrounded by zone of beta hemolysis characteristic of *Listeria*

Fig. 6: API-10300 *Listeria* showing positive results for identification of *Listeria monocytogenes*

Distribution of *Listeria* spp. in foodstuffs samples: One hundred food samples were studied including 30 (30%) bufflo milk, 30 (30%) cow milk, 10 (10%) sheep milk,10 (10%) cheese, 10 (10%) luncheon, 5 (5%) minced raw meat and 5 (5%) beef. Fifteen isolates were diagnosed as *Listeria* spp. (Fig. 7).

Isolation and identification of *Listeria* spp. from fresh water: A total of fifty water samples were surveyed for presence of *L. monocytogenes* (Table 3), only five samples were showed positive results of *Listeria* spp.

Distribution of *Listeria* spp. in fresh water samples: The raw water samples showing *Listeria* spp. were recorded. Results in Table 4 showed that fifty samples were studied including 25 (50%) raw water and 25 (50%) treated water. Only five isolates from raw water were diagnosed as *Listeria* spp. The treated water has not any *Listeria* spp.

Detection of *L. monocytogenes by* Polymerase Chain Reaction (PCR) in different food stuffs and water samples: The PCR test was specific for *L. monocytogenes* strains. The

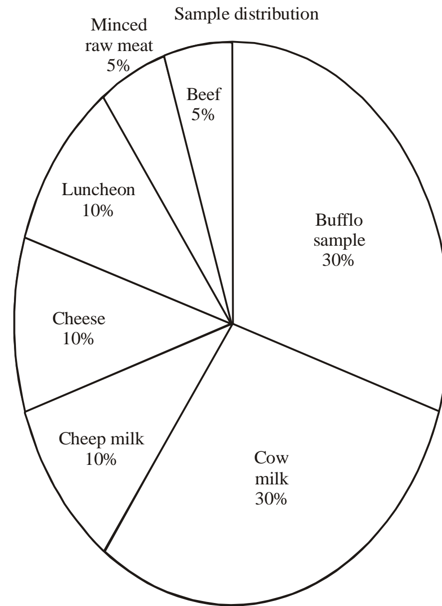

Fig. 7: Food stuffs samples distribution

Table 3: Identification of the collected bacterial isolates from water

No. of samples	Source	Fraser broth	Oxford agar	Gram stain	Catalase test	Motility	Sheep blood agar	API
1-5	Raw water	-ve	-ve	-ve	-ve	-ve	-ve	-ve
6	Raw water	+ve	+ve	+ve	+ve	motile	-ve	*Listeria grayi*
7	Raw water	-ve	-ve	-ve	-ve	-ve	-ve	-ve
8	Raw water	+ve	+ve	+ve	+ve	motile	-ve	*Listeria grayi*
9-11	Raw water	-ve	-ve	-ve	-ve	-ve	-ve	-ve
12	Raw water	+ve	+ve	+ve	+ve	motile	Beta hemolysis	*Listeria monocytogenes*
13	Raw water	-ve	-ve	-ve	-ve	-ve	-ve	-ve
14	Raw water	+ve	+ve	+ve	+ve	motile	-ve	*Listeria seeligeri*
15-19	Raw water	-ve	-ve	-ve	-ve	-ve	-ve	-ve
20	Raw water	+ve	+ve	+ve	+ve	motile	-ve	*Listeria welshimeri*
21-25	Raw water	-ve	-ve	-ve	-ve	-ve	-ve	-ve
26-50	Treated water	-ve	-ve	-ve	-ve	-ve	-ve	-ve

**Raw water: Raw Nile River water without any treatment. *Treated water: Water after clarification and disinfection in conventional water treatment by using chemical coagulants and chlorine

Table 4: Percentage value of bacterial isolates in collected water samples

Sample source	No. of collected sample	Distribution (%)	No. of *Listeria* spp.	*Listeria* spp.
Raw water	25	50	1	*Listeria monocytogenes*
			2	*Listeria grayi*
			1	*Listeria seeligeri*
			1	*Listeria welshimeri*
Treated water	25	0	0	No growth of *Listeria* spp.

amplification of *L. monocytogenes* yielded the expected amplicon at approximately 343 bp. Among the target gene for PCR detection of *L. monocytogenes* is *inl*B (encoding internalin B). Three samples were detected as positive PCR amplification revealed a band at approximately 343 bp (Fig. 8).

Rate of detection of different *Listeria* species by API from dairy products: Result summarized in Table 5, indicate that Percentage of incidence of *Listeria* spp. in the studied dairy

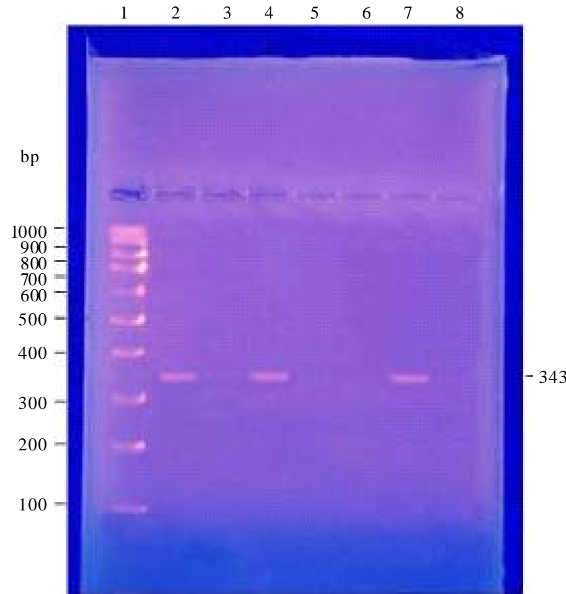

Fig. 8: Ethidium bromide stained agarose gel showing results of PCR technique of different samples: Lane 1: DNA ladder, Lanes 2, 4, 7: *Listeria monocytogenes* and Lanes 3, 5, 6, 8: Negative specimens

Table 5: Rate of detection of different *Listeria* species by API from milk and cheese

Type of food sample	No. of samples tested	*Listeria* species No.	*L. seeligeri* No.	%	*L. grayi* No.	%	*L. welshimeri* No.	%	*L. innocua* No.	%	*L. monocytogenes* No.	%
Buffalo milk	30	5	0	0.0	0	0.0	4	13.3	0	0.00	1	3.3
Cow milk	20	4	0	0.0	3	15.0	0	0.0	0	0.00	1	3.3
Sheep milk	10	2	1	10.0	1	10.0	0	0.0	0	0.00	0	0.0
Cheese	10	3	1	10.0	0	0.0	0	0.0	1	10.00	1	10.0
Pasteurized milk	10	0	0	0.0	0	0.0	0	0.0	0	0.00	0	0.0
Total	80	14	2	2.5	4	5.0	4	5.0	1	1.25	3	2.5

Different *Listeria* species identified by API. Percentage value was calculated in relation to total number of *Listeria* from each specimen, %: Number of positive samples/Total samples×100

products: Milk and cheese were calculated. The presumptively diagnosed strains on Oxford agar were further identified by API to determine species level. As shown in Table 5, the detection rates of different *Listeria* species in milk and cheese were (2.5, 5.0, 5.0, 1.25 and 2.5%, respectively for *L. seeligeri*, *L. grayi*, *L. welshimeri*, *L. innocua* and finally *L. monocytogenes*, respectively.

Detection rates of different *Listeria* species in meat samples: Percentage of incidence of *Listeria* spp. in the studied meat was calculated. Results are given in Table 6. Only *L. welshimeri* was isolated from 10 samples of Luncheon. Its incidence percent was 10.0%.

Water samples showing *Listeria* spp. The results are given in Table 7, the detection rates of different *Listeria* species in water samples were 2.0, 4.0, 2.0, 0.0 and 2.0%, respectively for *L. seeligeri*, *L. grayi*, *L. welshimeri*, *L. innocua* and *L. monocytogenes*, respectively.

Table 6: Rate of detection of different *Listeria* species by API from meat samples

Type of food sample	No. of samples tested	*Listeria* species No.	*L. seeligeri* No.	%	*L. grayi* No.	%	*L. welshimeri* No.	%	*L. innocua* No.	%	*L. monocytogenes* No.	%
Luncheon	10	1	0	0	0	0	1	13.3	0	0	0	0
Beef	5	0	0	0	0	0	0	0.0	0	0	0	0
Minced	5	0	0	0	0	0	0	0.0	0	0	0	0
Total	20	1	0	0	0	0	1	5.0	0	0	0	0

*Percentage value was calculated in relation to total number of *Listeria* from each specimen, %: Number of positive samples//Total samples×100

Table 7: Rate of detection of different *Listeria* species by API from water samples

Type of water sample	No. of samples tested	*Listeria* species No.	*L. seeligeri* No.	%	*L. grayi* No.	%	*L. welshimeri* No.	%	*L. innocua* No.	%	*L. monocytogenes* No.	%
Raw water	25	5	1	4.0	2	8.0	1	4.0	0	0	1	4.0
Treated water	25	0	0	0.0	0	0.0	0	0.0	0	0	0	0.0
Total	50	5	1	2.0	2	4.0	1	2.0	0	0	1	2.0

*Raw water: Raw Nile River water without any treatment, **Treated water: Water after clarification and disinfection in conventional water treatment by use. chemical coagulants and chlorine. *Percentage value was calculated in relation to total number of *Listeria* from each specimen, %: Number of positive samples/Total samples×100

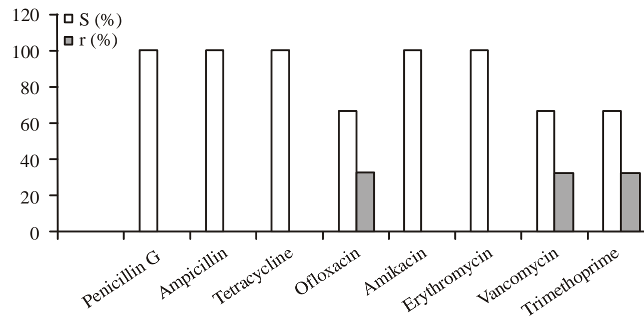

Fig. 9: Antimicrobial sensitivity of *Listeria monocytogenes*

Antimicrobial susceptibility of *Listeria monocytogenes*: All isolates (100%) were sensitive to penicillin G and ampicillin, tetracycline, amikacin and erythromycin. Only 1 (33.3%) of isolates were resistant to ofloxacin, vancomycin and trimethoprim-sulfamethoxazole (Fig. 9 and 10).

DISCUSSION

Listeria monocytogenes is a gram positive foodborne pathogen capable of causing infection of the fetus in pregnant women and meningitis, meningoencephalitis or febrile gastroenteritis in non-pregnant individuals. The pathogen continues to cause large common-source outbreaks from ready-to-eat food sources and represents a significant cause of food-related mortality (McCollum *et al*., 2013). Listeriosis is a serious infections disease caused by *Listeria monocytogenes* which has been recognized as a significant pathogen, occurring worldwide, capable of causing animal and human infections (Abram *et al*., 2003) leading to sever economic losses.

From this point of view, this work was designed to isolate and identify *L. monocytogenes* from different water samples and food stuffs. During the study period, three *L. monocytogenes* isolates were collected from 100 foodstuffs and 50 water samples. A total of 100 food samples including meat and dairy products, also 50 samples from water were analyzed for incidence of *Listeria* spp.

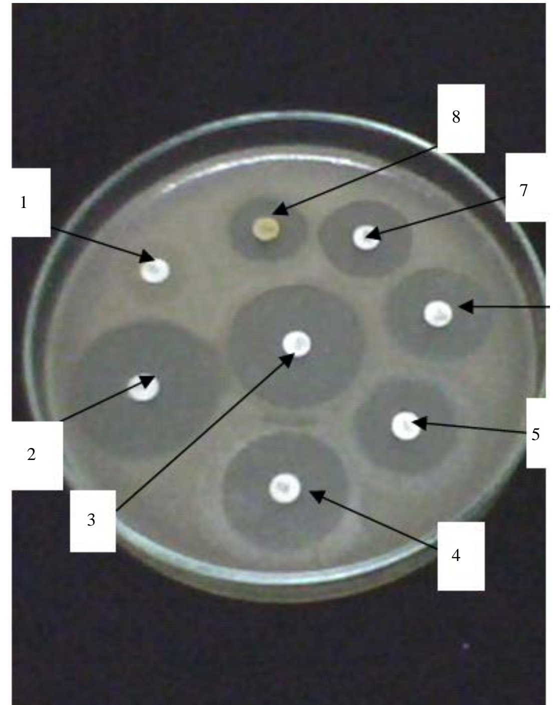

Fig. 10: Disc diffusion method showing antimicrobial susceptibility of *L. monocytogenes*. Sensitive to (2, 3, 4, 5, 6) but resistant to (1, 7, 8). 1: Ofloxacin, 2: Penicillin G, 3: Ampicillin, 4: Amikacin, 5: Erythromycin, 6: Tetracycline, 7: Vancomycin and 8: Trimethoprim-sulfamethoxazole

Due to the acute pathogenicity and toxicity of *Lisetria monocytogenes* to humans, search regarding isolation and identification of this pathogens in foods and water to find out mode of virulence and pathogenesis. It is also necessary to be able to protect and preserve food products to avoid *Listeria* contamination.

However, all *Listeria* isolates colonies were very easy to identify on Oxford medium due to its characteristic dark brown or green colonies with black halo. New methods and improvement of classical methods have been done for the detection and isolation of *Lisetria monocytogenes*. Among them the Oxford medium (Curtis *et al*., 1989).

Listeria monocytogenes isolates were heamolytic (β-haemolysis). Production of β-haemolysin and fermentation of D-xylose and L-rhamnose are essential biochemical tests for confirmation of *Listeria* isolation (Notermans *et al*., 1991). The fact that *Listeria* produces haemolysis on blood agar was observed for the first time in 1934 (Anton, 1934). β-haemolysis has been considered as an important diagnostic feature (Ralovich, 1989).

In this study, 20 isolates of *Listeria* were recovered. All *Listeria* isolates were gram-positive, catalase positive, under oil immersion using light microscope, cell were short rods, diplobacillis or short chain and showed tumbling motility. Hence, all the 20 isolates can be distinguished from *streptococci* by cell morphology, positive motility, producing of catalase and growth on Oxford selective agar (Seeliger and Jones, 1986).

One of these recent techniques adapted for identification of listeria isolates belonging to genus *Listeria* is the API-*Listeria* system ((10300 API *Listeria*). The strip contained 10 wells. The

reactions were as follows: Enzymatic substrate, hydrolysis of esculin and 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. According to the manufacturer's instructions, the following known species of *Listeria* can be identified using the (10300 API Listeria): *L. monocytogenes*, *L. innocua*, *L. seeligeri*, *L. ivanovii*, *L. welshimeri* and *L. grayi*. Results of the API-Listeria kit showed that three biochemical characteristics were positive with all *Listeria* isolates. Hydrolysis of esculin and acid production from D-arabitol. The presence of acid production from D-xylose, L-rhamnose, D-ribose, glucose-1-phosphate and D-tagatose were used for species identification. The same findings were also reported previously (Bille *et al*., 1992) upon using the API commercial system for identification among Wspp.

The detection rates of different *Listeria* species in milk and cheese were 17.5% and this result was in agreement with Jamali *et al*. (2013) isolated *Listeria* spp. from about (18.6%) of raw milk samples. As regards, isolation of *Listeria* spp. from different food samples. In our study, all specimens were submitted to 2 step of enrichment followed by culture on Oxford agar medium where 14 (17.5%) isolate were from milk cheese and one (5%) from different types of meat were presumptively diagnosed as *Listeria* species. Previous studies also reported that isolated *Listeria* at a rate of 17 and 3.3%, respectively from milk and meat. *L. monocytogenes* isolates 2 (2.5%) were from raw milk and this result was in agreement with Mahmoodi (2010) isolated *L. monocytogenes* from raw milk samples (2.5%) collected from two traditional dairy manufactures. The absence of *L. monocytogenes* in cheese was explained by many authors due to decrease in pH, presence of lactic acid and pasteurization (Kalorey *et al*., 2006).

Listeria spp. can be widely found in nature in soil, on plants, in waters, on animal hair and birds bodies and so forth thus they can easily get into the feed for dairy cows (Konosonoka *et al*., 2012). PCR is deemed to be more reliable than conventional identification since it is based on stable genotypic characteristics rather than relying on biochemical or physiological traits, which can be genetically unstable (Malorny *et al*., 2003). As regards studying the antimicrobial susceptibility pattern of isolated *L. monocytogenes*. They were tested for sensitivity of the following antibiotics: Amikacin, ampicillin, penicillin G, vancomycin, erythromycin, ofloxacin, tetracycline and trimethoprim-sulfamethoxazole. Wiggins *et al*. (1978) indicated that *Listeria* organisms were sensitive to ampicillin, erythromycin and tetracycline.

Rota *et al*. (1996) reported that, ampicillin was the most effective drugs against *Listeria*. Slade and Collins-Thompson (1990) recorded that *Listeria* is usually susceptible to a wide range of antibiotics including ampicillin and erythromycin. All isolates (100%) were sensitive to penicillin G, ampicillin, tetracycline, amikacin and erythromycin. only 1 (33.3%) of isolates were resistant to ofloxacin, vancomycin and trimethoprim-sulfamethoxazole.

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