Rapid detection of pathogenic bacteria in vegetables and fruits in Egyptian Farms

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Abstract: *Escherichia coli* 0157 H:7, *Salmonella typhimurium* and *Listeria monocytogenes* are risky members of food-poisoning pathogens. In the present work, PCR assays were validated in artificially contaminated samples that covering nine types of products. Soil, compost, irrigation water, wash water, fresh cut lettuce, carrots, Herbs, ready-to-eat salads and strawberry. Samples of food products were stomached in stomacher, followed by 18 hour enrichment in LB media at 37°C. DNA templates were prepared by boiling of 100 ul from the enrichment cultures. The obtained results by PCR were confirmed by that obtained by colony identification (microbiological methods). The data showed that plate count, streaking on selective media and confirmation tests take from 5-7 days. On the other hand, the PCR technique tacks few hours to give very accurate and sensitive detection. Moreover, PCR could detect down to ≤ 10 cfu/g^{-1} while plate streaking could not.
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Key wards: *Escherichia coli* 0157 H:7, *Salmonella typhimurium*, *Listeria monocytogenes*, food-poisoning pathogens, PCR

1. Introduction

E. coli 0157:H7, L. monocytogenes and *S. typhimurium* have been identified as major food-borne pathogens worldwide (Nam *et al*., 2004). It was isolated from ready-to eat vegetables and fruits (Shearer *et al*., 2001).

Shiga Toxigenic *E. coli* (STEC) is an important cause of gastrointestinal disease in humans. STEC belonging to sero-group 0157 and there is STEC that belonging to 0111 sero-group (Caprioli *et al*., 1994; Minami, 1997). Several *E. coli* 0157:H7 virulence involved in pathogenicity include: production of stx1 (Jackson *et al*., 1987 a) and/ or stx2 (Jackson *et al*., 1987 b) which inhibit protein synthesis in host cells and *eae* genes which are involved in the formation of attaching and effacing lesions on intestinal cells (Yu and Kaper, 1992).

The capacity to rapidly determine whether the food is contaminated with STEC belonging to serogroup 0111 or 0157 or whether the STEC produces virulence factors associated with more serious disease, so, there is an increasing demand for improved diagnostic procedures (Adrienne and James, 1998). Production of one or more toxins (the shiga-like toxins) by *E. coli* 0157: H7 may lead to haemorrhagic colitis up to haemolyticuraemic syndrome (HUS) (Jones, 1999). In humans ingestion of as few as 10 organisms be sufficient to cause infection (Willshaw *et al*. 1994). *E. coli* 0157:H7 is readily shed by cattle (Zhao *et al*., 1995). Source of this pathogen in the environment (specially the terrestrial environment). Thus, a potential risks of contamination to food crops will be found. Traditional methods depending on a preliminary enrichment followed by plating on selective media, such as sorbitol MacConkey agar (SMAC). Then, selectivity for the 0157: H7 will need additional methods (Wright *et al*. 1994). These methods are time required for positive identification of the 0157: H7 serotype. Further characterization of the potential of isolates. Shiga-like toxin adds to the time expense of culture-based assays.

Listeria monocytogenes is a pathogen that cause severe illness, listeriosis (Jofre *et al*., 2005). Salmonellosis is as a result of *Salmonella*. *Salmonella* can be extremely tolerant to acidic environment and disease outbreaks have been associated with consumption of unpasteurized orange juice. The infectious dose of both organisms is relatively low.

Salmonellosis and listeriosis are two of the most commn foodborne diseases (Mead *et al*. 1999; anonymous 2001). *Listeria monocytogenes* is a pathogen that causes severe illness, 100 cfu/g^{-1} food of *L. monocytogenes* at the time of consumption is steel non-risky (Roberts *et al*. 1996), salmonellosis is less severe.

Calssical microbiological culture plating techniques for discovering the pathogens are not sensitive and time consuming. Bacterial cells may face abnormal conditions as starvation or physical stress which explain why sometimes could not isolate

the microorganisms from contaminated samples (Rollins and Colwell, 1986; Besnard *et al*., 2000). In other ward, Bacterial cells may enter a viable-but-nonculturable (VBNC) state, according to starvation and physical stress. That explain why in sometimes, the culture techniques fail to isolate the organisms from contaminated samples.

The process of isolation and final identification of *salmonella* spp and *L. monocytogenes* with biochemical standard methods are laborious and time – consuming and can tack up to 7-10 days (Anonymous, 1998; Anonymous, 2002). So PCR-based methods have been reported as a rapid, specific and sensitive (Hoorfar and Cook, 2003)

Polymerase chain reaction (PCR) assays have been used in specific detection of food-borne bacterial pathogens for more than 20 years ago (Holko *et al.,* 2002; Grant, 2003). As part of quality-control system of food safety (Wang *et al*., 1999; Waller and Ogata, 2000).

The main target of this work was to replace the classical microbiological methods with a more sensitive, reliable, rapid and cost effective methods for the detection of the most critical food-borne pathogens, *E. coli* 0157:H7, *S. typhemerum* and *L. monocytogenes* at some Agricultural in Egypt.

2. Material and Methods

Bacterial strains and growth media:

Bacterial strains *E. coli*, *E. coli* organism Migula and serotype 0157:H7 (doesn't produce either shiga-like- toxins I or II which doesn't possess the genes for these toxins), *L. monocytogenes* and *S. typhimurium* were purchased from ATCC. The bacterial strains were grown separately in LB media (Trypton 10 g/L, yeast 5 g/L, sodium chloride 5 g/L) for 18-20 hours at 37°C. Serial dilution were done and each dilution was counted by plate count and the bacterial cells have shown in cfu/ml culture (colony forming unit)

Microbiological methods: All microbiological methods were done according the Manual of microbiological methods for the food and drinks industry (Baylis, 2007) (CCFRA 2007).

- Standard plate count (Total viable count)

This method based on a non selective nutrient medium (PCA) is used to culture a wide range of micro-organisms (method 1.1.1:2007, CCFRA 2007). It is important to recognize that only these micro-organisms capable of form colonies under the condition of that test.

- Enumeration of *E. Coli* alternative colony count technique using chromogenic medium without membranes (method 2.4.2:2003, CCFRA 2007). This technique employs chromogenic medium tryptone bile agar containing 5-bromo-4-chloro-3-indolyl-B-D-glucuronic acid (BCIG) (tryptone bile x glucuronide agar). The medium relies on the use of bile salts and elevated incubation at 44°C to suppress competitor organisms. The chromogenic TBX is used to indicate the presence of B-glucuronidase activity which is common in 95% of *E. coli* strains. In this test *E. coli* 0157 may be not detected.

- Detection of *E. coli* 0157:H, Cultural technique incorporating immunomagnetic separation (IMS) (method 3.4.1:2007, CCFRA 2007). This technique relies on a selective enrichment and immuno-magnetic separation on antibody-coated magnetic particles which are plated onto a selective agar; such as sorbitol MacConkey (SMAC) agar

- Detection of *Salmonella* (method 3.1.1:2007, CCFRA 2007), this method is based on primary enrichment (buffer peptone water, BPW), secondary selective enrichment (Rappoport-Vassiliadis Soy, RVS) and selective plating (XLD agar, Xylose lysine deoxy cholate agar). Then, followed by confirmation method (3.1.5:2007, CCFRA 2007), using a range of biochemical tests. suspect *salmonella* isolates can be confirmed to genus, it is common practice to use commercial identification kits, which provide a range of tests in a convenient ready-to-use format.

- For *listeria monocytogenes*, (methods 3.2.2:2007, CCFRA 2007), this technique relies on the enrichment of *Listeria* spp in selective media containing selective agent including nalidixic acid, acriflavine and lithium chloride to suppress most competitors. The use of chromogenic medium (ALOA, Agar Listeria according to Ottaviani and Agosti) that relies on B-glucosidase activity of *Listeria* spp. And phospholipase activity of *L. monocytogenes*. The method (3.2.7:2007, CCFRA, 2007) is for confirmation of *Listeria* species.

Artificial contamination of samples: The different samples were artificially contaminated with bacterial culture the same as (Wang and Slavik, 2005)

Molecular methods:

DNA isolation: After collection of the bacterial cells from the original bacterial strains and from both the natural and the artificially contaminated enrichment samples. DNA extraction and purification was performed using QiaAmp DNA mini kit (Qiagene, Germany) according to the manual of manufacturer and then were subjected to the PCR.

Crude DNA also was prepared by boiling method: 20 ul were withdrawn with a sterile syringe from the sealed, sterile package of the enrichment culture of the different samples or from the culture of the original strains. the 20 ul were added to 80 ul dH2O and then boiling for 10 Minutes. This aliquot was used as DNA template and subjected to PCR

PCR: Specific primers used:

FL & RL for *L. monocytogenes*, FL 5'AGCTCTTAGCTCCATGAGTT3' and RL 5' ACATTGTAGCTAAGGCGACT3' that give 450 bp PCR product (Golsteyn *et al*., 1991)

UidA&Uidb for *E. coli* (Lang *et al*., 1994) specific for uid A gene of 0157:H7/H-(Cebula *et al.*, 1995)

VT1 F&R for *E. coli* 0157:H that give 614 bp PCR product (Gannon *et al*., 1992) VT1F, 5' ACACTGGATGATCTCAGTGG 3' and VT1R, 5' CTG AAT CCC CCT CCA TTA TG 3'

16sF & 16sR for *E. coli* 0157:H7, 16s F, 5' AGAGTTTGATCATGGCTCAG 3'. 16sR, 5' GGACTACCAGGGTATCTAAT 3' that give 798 bp PCR product (Ehresmann *et al*., 1972)

S29 & S30 for *S. typhimurium*, S29 5' CAGTATCAGGGCAAAAACGGC 3' and S30 5' TTCAAAGTTCTGCGCTTTGTT 3' that give 360 bp PCR products (Mahon *et al*., 1994)

Professional tests: Internal professional tests were made to make sure that the work is going well. for example: the serial dilutions of *S. typhimurium* bacterial strain were from 10^6 to 10^1 cfu/ml, then, it was evaluated on selective agar medium plates and simultaneously on PCR thermo-cycler.

3. Results

Conventional microbiological techniques for the detection of bacterial pathogens in food, including isolation on selective media and biochemical identification of the bacteria. It takes from 7 to 10 days. Thus, developing an efficient system for detection of that food borne pathogens in the Egyptian Agricultures by using PCR-based techniques that could decrease the number of reactions to be performed in food samples.

The total plate count (TPC) of the freshly cut lettuce plants (without any treatment), showed significant difference from the samples that undergone outer sterilization and there were no significant difference between the samples in the washing stage until the final stage of being the product in the cold room (refrigerator) (Fig 1).

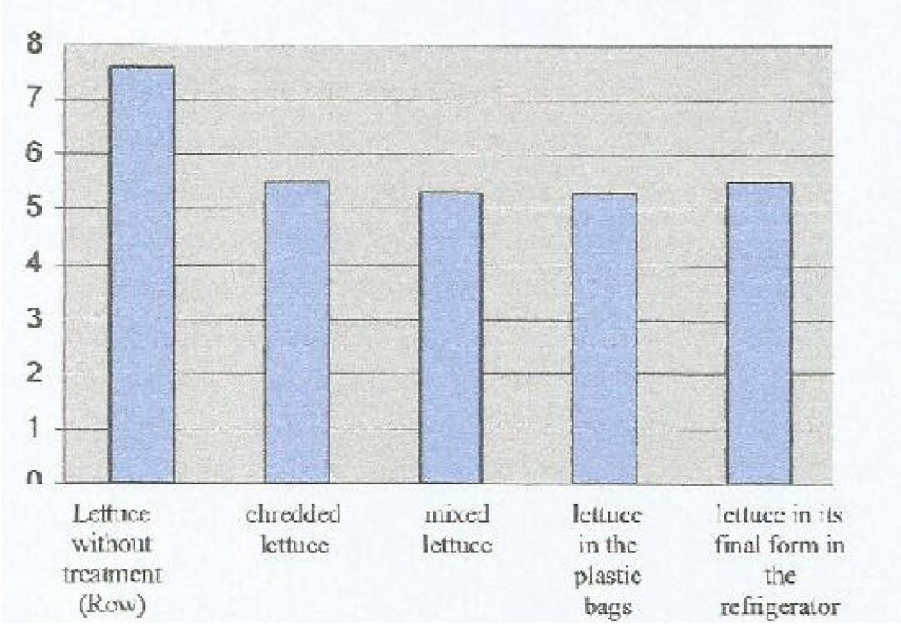


Fig 1: shows the chart of total plat count in cfu/gm in lettuce sample started from fresh cut until the final form of lettuce sample stored in the refrigerator

The carrot products showed the same results. The carrot samples were followed up from the beginning of freshly cut until the last stage of the final product in the refrigerator. The TBC were very high in the fresh untreated carrots and become to decline down after washing and outer sterilization which showed significant differences between the row carrots and the treated ones. Fig (2) showed these data.

Detection of pathogenic bacteria: *E. coli*, *E. coli* 0157:H7, *Salmonella typhimurium* and *Listeria monocytogenes* by microbiological methods: The illustrated figures showed the typical colonies of the mentioned microbes on plates and the experiments have taken from 7 to 10 days.

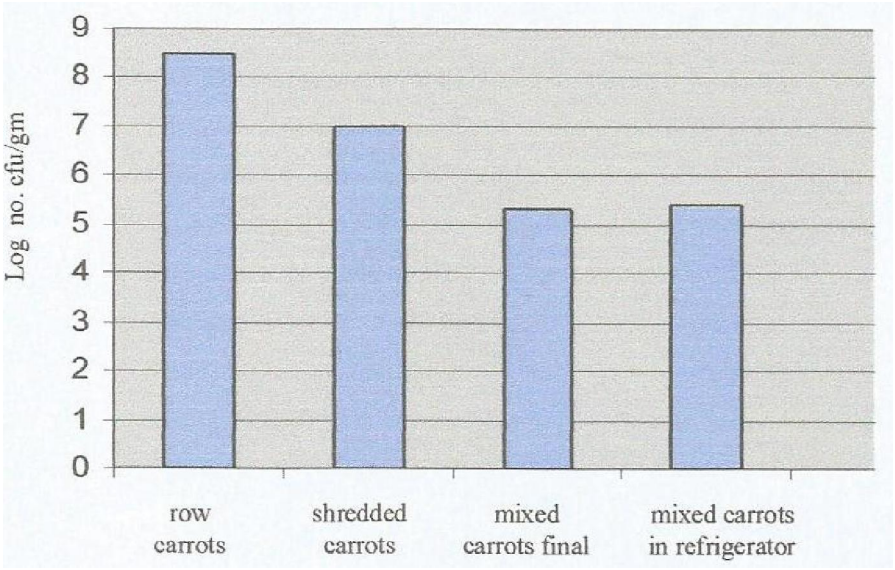
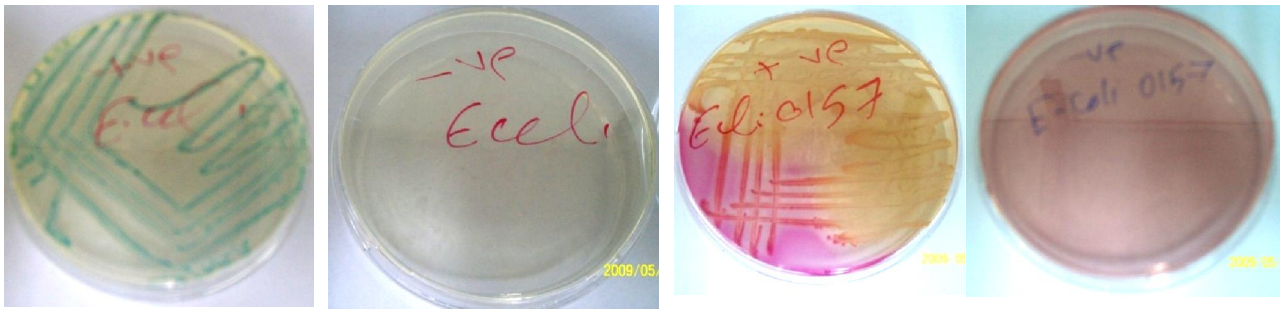
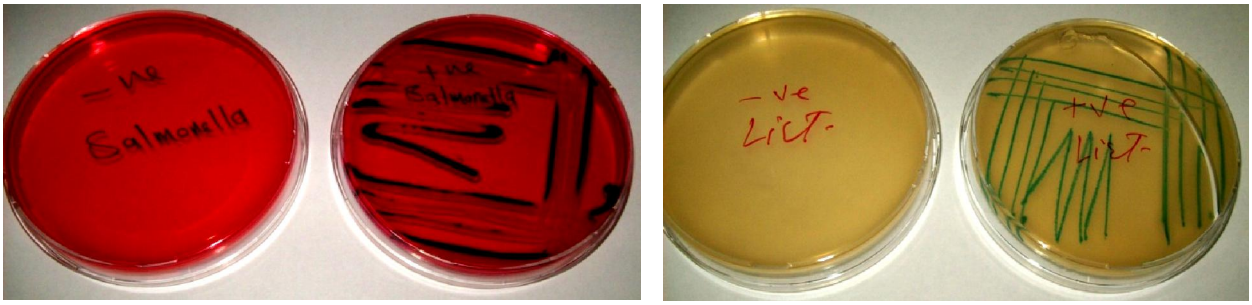


Fig 2: shows the chart of total plat count in cfu/gm in carrots sample started from fresh cut until the final form of carrots sample stored in the refrigerator



A **B**



C **D**

Fig 3: Shows the typical pathogenic bacterial colonies on selective plates

Fig 3: A: showed the typical colonies of *E. coli* on chromogenic TBX medium. the presence of the colonies indicated the presence of B-glucuronidase activity of *E. coli* strains (+ve plate).

Fig 3: B: the figure showed the typical colonies of *E. coli* 0157 on SMAC agar which are small, round, smooth and colorless (+ve plate).

Fig 3: C: The figure showed the typical *salmonella* colonies which are black with red surrounded on selective XLD agar (+ve plate).

Fig 3: D: The figure showed the typical colonies of *listeria* spp on ALOA medium which appear as blue green colonies surrounded by opaque halos (+ve plate). Chromogenic medium (ALOA) is based on B-glucosidase activity of *Listeria* spp and phospholipase activity of *l. monocytogenes*

PCR detection of *Salmonella typhimurium* in pure bacterial culture: The specific primers S29 & S30 gave the expected 360 bp PCR product (Fig. 4) and detection of salmonella in infected lettuce plants using the same primers pair S29 & S30 has been succeeded as shown in fig 8. Fig 4 showed the accuracy of the primers that used in detection of the *salmonella* in the pure culture as well as in the mixed culture (*Salmonella* with *E. coli*). PCR detection of *L. monocytogene*s (Fig. 5) showed the sensitivity of PCR test when using the specific primers PF & R in both pure culture and mixed one giving the expected PCR product 450 bp while one cannot observe the product in the non Listeria culture (lane 3, Fig. 5). Fig (6) showed the resulted PCR products from infected salad with both *S. typhimurium* and *L. monocytogens* at concentration 10^7 & 10 cfu/ml respectively and enrichment for 16 h at 37°C (when homogenized salad and lettuce samples have been infected with 1 ml of bacterial cultures containing 10 cfu/ml and other time with 10^7 and DNA was extracted from the enriched homogenized samples. Detection of *E. coli* 0157:H7 in artificially infected lettuce plants: detection has been done on directly infected lettuce and on that has been enrichment for 4 hours shacking at 37°C, fig (7) shows the PCR product from using the primers pair VTF & VTR that give 614 bp PCR product with *E. coli* 0157:H7.

PCR assays were validated in 14 type of samples covering nine types of products to check for contamination. This work has been done for a long of two years in the farm. Results obtained by colony identification (as shown in fig(s) 9, 10 and 11) were confirmed all the time with PCR detection. Thus, a multiplex PCR combining the three pair of specific primers (16S F & 16S R, S29 & S30 and PF & P R) for detection of *E. coli* 0157:H7 (PCR product 798 bp), *S. typhimurium* (PCR product 360 bp) and *L. monocytogenes* (PCR product 450 bp) respectively, were done for detection of the mentioned food pathogens as shown in the fig(s) 12, 13 and 14.

DNA were isolated from all samples, then they were subjected to PCR. In parallel, DNA template was prepared from the samples by boiling the samples for 10 minutes which gave the same results. Thus, the step of DNA isolation was replaced by direct boiling the samples. So, saving time, effort and the price of kit.

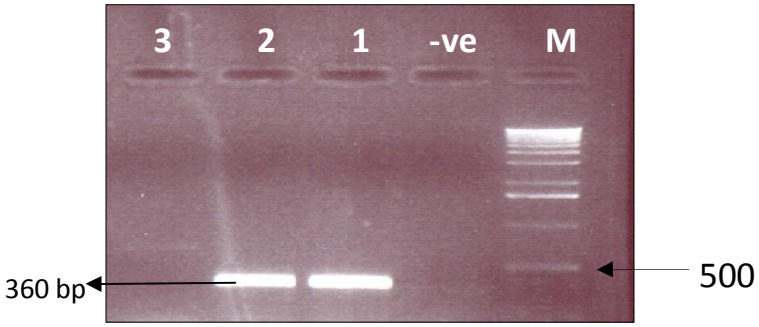


Fig 4: Agarose gel electrophoresis of PCR amplified DNA from pure culture of *salmonella* using *S. typhimurium* primers pair (S29 & S30). Lane 1: PCR product from the isolated DNA from pure salmonella culture, Lane 2: DNA isolated from mixed pure cultures, Lane 3: a none salmonella culture, M: 1kb DNA ladder (Fermentas, Canada), Lane –ve: no template control test.

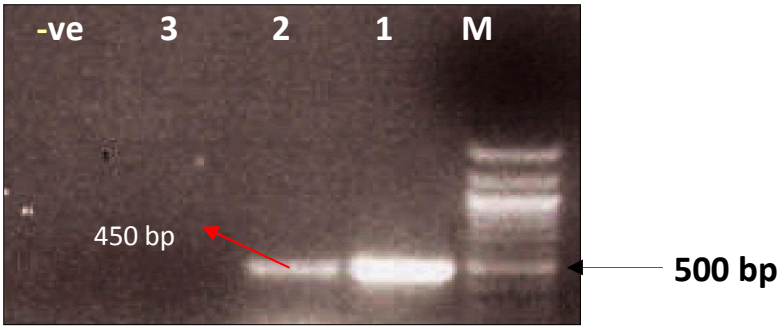


Fig 5: Agarose gel electrophoresis of PCR amplified DNA from pure culture of of *Listeria monocytogenes* using the primers pair (PF & PR), Lane 1: PCR product from pure culture of *L. monocytogenes*, lane 2: PCR product for DNA isolated from mixed culture (*listeria* and non-*listeria* microorganisms), lane 3: none- *listeria* strain PCR test. M is a 100 bp DNA ladder (Fermentas, Canada), Lane –ve: for no template control.

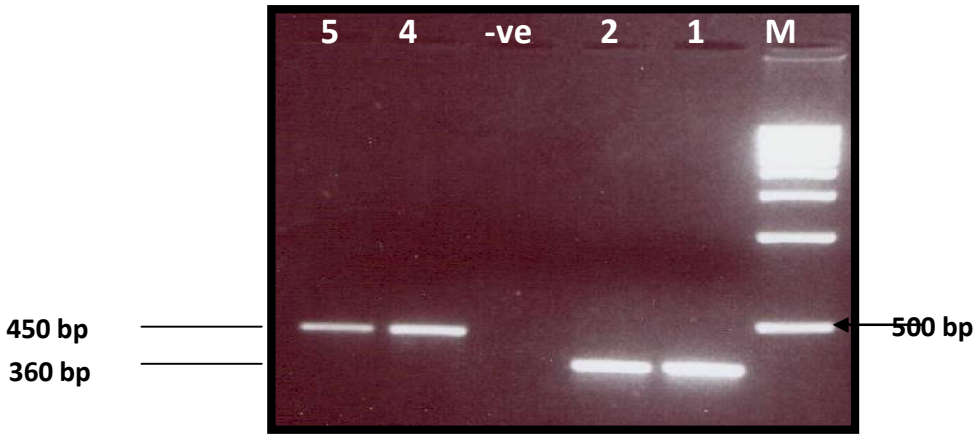


Fig 6: Agarose gel electrophoresis of PCR amplified DNA from PCR detection of equal concentrations of isolated DNA (< 1 ug) of salad samples that infected with 10 and 10^7 dilution of both *salmonella* and *L. monocytogenes*, Lanes 1 & 2 represent PCR products of *salmonella* at Mwt 360 bp for both dilutions 10^7 and 10 respectively, Lanes 4 & 5 represent PCR products from *L. monocytogenes* at Mwt of 450 bp for both dilutions, Lane –ve: no template negative sample, M: 1 kb DNA ladder (Fermentas, Canada).

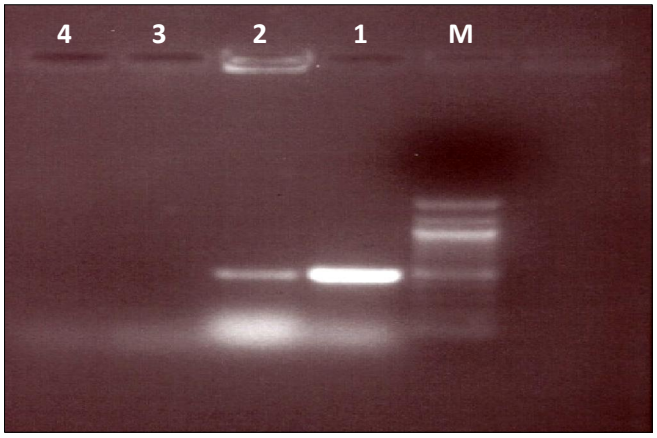


Fig 7: Agarose gel electrophoresis of PCR amplified DNA from PCR detection of *E. coli* 0157:H7 in artificially infected lettuce plants using the primers pair VT F & R that give 614 bp. Lane 1: *E. coli* O157:H7 bacterial cells (+ve control), Lane 2: infected plants (after 4 hours enrichment) . Lane 3: infected plants without enrichments. 4: healthy plants. M: 100 bp DNA ladder (Fermentas).

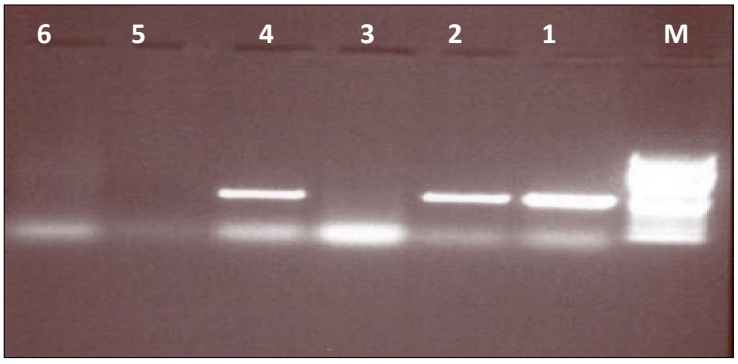
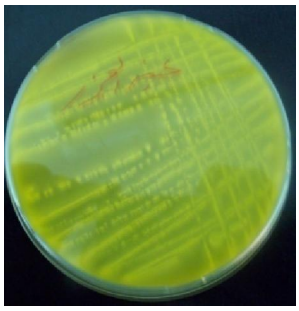
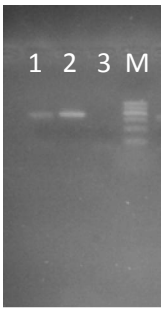


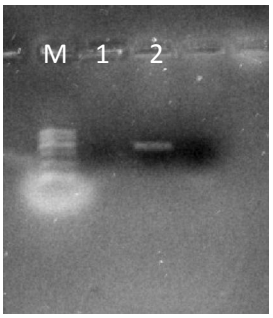
Fig 8: Agarose gel electrophoresis of PCR amplified DNA from PCR detection of *salmonella* in infected lettuce plants using *S. typhimurium* primers pair (S29 & S30), Lane 1: *Salmonella* as bacterial cells (+ve control), the DNA was prepared by the kit, Lane 2: *Salmonella* as bacterial cells (+ve control), the crude DNA was prepared by the boiling method, Lane 3: Healthy non infected plants, Lane 4: Infected lettuce plants (concentrated), Lane 5: -ve control for the PCR test, Lane 6: Non *salmonella* bacterial cells, M: 100 bp DNA ladder (Fermentas).





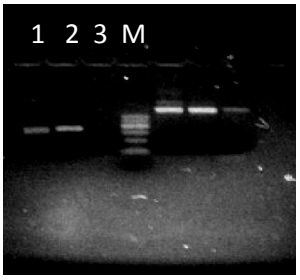
A B

Fig 9: showing the plate streaking and the PCR of carrots sample. **Fig 9 A:** plate streaking of bacterial culture that coming from enrichment of homogenized carrots sample on *salmonella* selective agar . The plate showed no *salmonella* (- ve result), **Fig 9 B:** showed the PCR from the same bacterial culture using the primers pair S29 & S30 for detection of *S. typhimurium* that give 360 bp PCR product. Lane 1: +ve control of original bacteria of *S. typhimurium*, lane 2: carrots sample, lane 3: -ve control (which no DNA used), M: 100 bp DNA ladder (Fermentas).



A B

Fig 10: The figure shows the test for detection of salmonella in strawberry. **Fig 10 A**: showed the PCR from the bacterial culture that coming from enrichment of homogenized strawberry sample using the primererrs pair S29 & S30 for detection of *S. typhimurium* that give 360 bp PCR product, Lane 1: strawberry sample using S29 & S30 primers pair, lane 2: + ve control (original bacteria of *S. typhimurium*) using S29 & S30 primers pair, M: 100 bp DNA ladder (Fermentas). **Fig 10 B:** plate streaking of bacterial culture that coming from the same enrichment of homogenized strawberry sample on *salmonella* selective agar (here the detection of PCR gave the same results as the plate streaking which no contamination with *S. typhimurium*)



A **B**

Fig 11: The figure shows the check for *samonella* in carrots sample by plate streak and PCR. **Fig 11 A**: Lane 1: carrots sample coming from enrichment of homogenized carrots sample with primers pair S29 & S30 giving 360 bp, Lane 2: The + ve control (original bacteria of *S. typhimurium* using S29 & S30 primers pair). Lane 3: - Ve control (no DNA in the reaction). M: 100 bp DNA ladder. **Fig 11 B:** plate streaking of bacterial culture that coming from enrichment of homogenized carrots sample on *salmonella* selective agar (here we see that the PCR detected the *salmonella* while the plate streaking couldn't detect the *salmonella*)

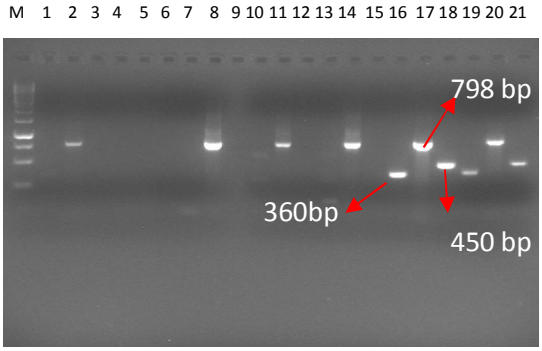


Fig 12: Agarose gel electrophoresis for PCR products from multiplex PCR reaction using 3 pair of primers for detection of *E. coli* 0157:H7, *S. typhimurium* and *L. monocytogenes* in lettuce, compost, water, soil grown on Bion media, soil grown on nutrient broth samples that collected from the farm. **Lanes 1,2,3 :** lettuce sample with primers of *salmonella*, *E. coli* 0157:H7 and *L. monocytogenes* respectivily. The gel showed that the sample is free from any of the three pathogens. **Lanes 4,5,6 :** compost sample with primers of *salmonella, E. coli* 0157:H7 and *L. monocytogenes* respectivily. The gel showed that the sample is free from any of the three pathogens. **Lanes 7,8,9 :** Water sample with primers of *salmonella, E. coli* 0157:H7 and *L. monocytogenes* respectivily. The gel showed that water sample (Lane: 8) contaminated with *E. coli* 0157:H7. **Lanes 10,11,12:** soil sample (grown on bion broth) with primers of *salmonella, E. coli* 0157:H7 and *L. monocytogenes* respectivily. The gel showed that soil samples are free from any of the three pathogenes. **Lanes 13, 14, 15:** Soil samples (grown on N.B) with primers of *salmonella*, *E. coli* 0157:H7 and *L. monocytogenes* respectivily. The gel showed the contamination of sample 14 with *E. coli* 0157:H7. **Lanes 16, 17, 18:** positive control of bacterial culture *salmonella, E. coli* 0157:H7 and *L. monocytogenes* respectivily with the primers of *salmonella, E. coli* 0157:H7 and *L. monocytogenes* respectivily that give **360 bp**, **798 bp** and **450 bp** respectivily.

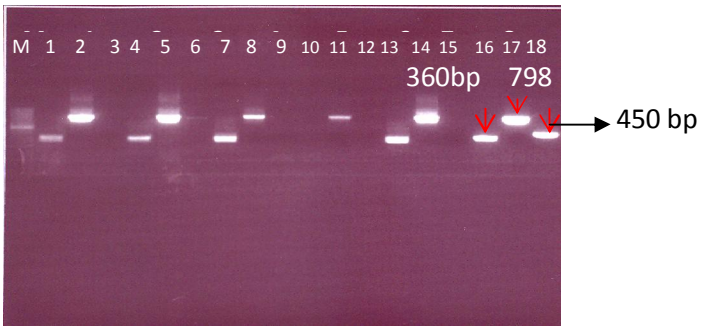


Fig 13: Agarose gel electrophoresis for PCR products from multiplex PCR reaction using 3 pair of primers for detection of *E. coli* 0157:H7, *S. typhimurium* and *L. monocytogenes* in salad, carrots shredder, lettuce shredder, wash water samples. **Lanes 1,2,3 :** salad sample with primers of *salmonella*, *E. coli* 0157:H7 and *L. monocytogenes* respectivily. **Lanes 4,5,6 :** carrots shredder sample with primers of *salmonella, E. coli* 0157:H7 and *L. monocytogenes* respectivily. **Lanes 7,8,9 :** lettuce shredder sample with primers of *salmonella, E. coli* 0157:H7 and *L. monocytogenes* respectivily. **10,11,12:** wash water sample with primers of *salmonella, E. coli* 0157:H7 and *L. monocytogenes* respectivily. **Lanes 16, 17, 18:** positive control of bacterial culture *salmonella, E. coli* 0157:H7 and *L. monocytogenes* respectivily with the primers of *salmonella, E. coli* 0157:H7 and *L. monocytogenes* respectivily. The gel showed the contamination of salad, carrots shredder and lettuce shredder with *salmonella* and *E. coli* 0157:H7 lanes: 1,2,4,5,7,8 respectivily as compared with the positive control lanes 16,17. On the other hand, the gel showed that the samples were free from *Listeria*, also, the wash water was free from all the microbes lanes 10, 11, 12

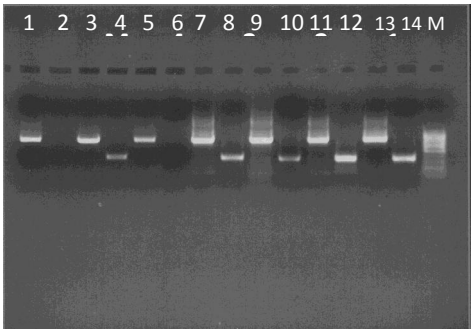


Fig 14: The figure shows the PCR detection of *S. typhimurium* and *E. coli* 0157 in shredded lettuce, carrots, salads, wash water. The gel showed that the samples of shredded lettuce, carrots, salads and lettuce were contaminated with *salmonella* Lanes: 14, 12, 10 and 8 as compared with + ve control (lane 4). The gel also showed that the wash water was free from *salmonella* & *E. coli* 0157 (lane 6 and 5) as compared with - ve control (lanes 2 and 1). The gel showed that the samples were contaminated with *E. coli* 0157 (lanes 13, 11, 9, 7) as copared with +ve control (lane3).

4. Discussion

The aim of our study was to replace the classical microbiological methods with a rapid, sensitive and cost effective method in the Egyptian farms. conventional microbiological techniques for the detection of bacterial pathogens in food including isolation of bacteria on selective media, and then biochemical identification of the bacteria. It takes from 7 to 10 days. On the other hand, PCR techniques decreasing the number of reactions that should be performed in a food samples and will develop an efficient system for detection of food borne pathogens in the Egyptian Agricultures. Farms that export its fresh produce abroad and distribute it in the local market, should have quick, accurate and sensitive pathogen detection system. This give one more important reason for using PCR technology. Food borne bacterial pathogens as *E. coli*, *S. typhimurium*, *L. monocytogens* are the most dangerous to be find within fresh product. *E. coli* 0157:H7 that produce shiga like toxins, ingestion of as few as 10 organisms may be sufficient to cause infection (Willshow *et al*., 1994). Thus, non toxigenic *E. coli* 0157 are available for use as positive control in the laboratory, and that is because all handling of known cultures of verotoxin producing (+ VT) strains of *E. coli* must be conducted in a laboratory with the appropriate containment facilities and by staff suitably trained to handle these organisms in accordance with the relevant safety guidelines. Strains of *E. coli* 0157 verocytotoxin producing are now classified as hazard group 3 pathogens. Laboratories working with toxin producing strains have containment level 3 facilities. Health and safety executive (HSE) and the advisory committee on dangerous pathogens (ACDP) must be followed. All of these cautions may be not found in our farms that reinforced our study in using the PCR technology.

DNA of the original bacterial cultures and that of the enrichment cultures of samples that artificially contaminated or naturally contaminated were isolated and purified by kits as in fig (8). At the same time crude DNA were extracted by boiling the bacterial culture (Fig. 8) which gave the same results as the purified one. Thus, it was established in our work to prepare the DNA as crude for saving time of steps of reactions and consequently the cost. (Adrienne and James, 1998; Xuan *et al*., 2000) reported that both the crude DNA (which obtained after boiling the bacterial cells) and the purified DNA worked the same in the PCR. Thus, they used the crude DNA in detection of *salmonella* and STEC in fresh produce. Pathogenic bacterial cells *E. coli* 0157:H7, *salmonella* sp. are not considered pathogenic for plants, thus, these bacteria are not proliferate between plant cells, so, the numbers of these bacteria are not found in huge numbers within plant tissues. **Then, Enrichment** and highly sensitive detection methods are very important. These are agree with our results, that showed that the enrichment is limiting factor(6-18 hours) in detection the food borne pathogens (Fig 7). Xuan *et al*., (2000), Campbell *et al.* (2001) did 8 hours enrichment for the culture samples which enabled detection of as few as 6 cfu / g^{-1} soil and 10^4 cfu / g-1 with 6 hours enrichment. Adrienne and James, (1998) used PCR as a most sensitive technique to detect STEC in food samples. Patricia *et al*., (2004) reported that the conventional microbiological techniques, plate count agar and coliform tests are laborious and time consuming and replaced these methods with biophysical methods to discover the microbiological quality of water. Jofr *et al*. (2005) used PCR methods for detection of *E. coli* 0157, *L. monocytogenes* and *Salmonella* in food samples. Lynette *et al*., 2005 used PCR for detection of *salmonella* and *E. coli* 0157:H7 in sprouts, spent irrigation water and final product. Campbell *et al*., 2001, used multiplex PCR technique in combination with an enrichment for sensitive, and speed detection of *E. coli* 0157:H7 in high risk of *E. coli* 0157:H7 contamination of rural drinking water, soil and water within one working day. Fratamico and Strobaugh, (1998) also used PCR for detection of *E. coli* and *salmonella* spp in food samples as rapid and sensitive method. Beili *et al*. (2013) developed a novel application GeXp analyzer for simultaneous detection of six pathogens of food poisoning outbreaks based on PCR technology, in an attempts to get a more rapid, sensitive and no time consuming. Oliver *et al*. (2014) also, relying on PCR technology, have presented an implementation of parallel, real time PCR based detection of up to 6 different food-borne pathogens on a centrifugal microfluidic LabDisk platform . Thus, all of these mentioned references and others agree with the present study in that; they are searching and discovering for a rapid, sensitive, cost effective and non time consuming methods for food pathogen detection. **All the PCR detection** of the pathogens were confirmed with the established microbiological methods as in Figs. 9,10 and 11. the obtained data showed that the PCR detections were more sensitive because all the positive detections of the pathogens by plating were agreed with PCR detections. On the other hand, there were positive detections by PCR but the plating methods were negative as in fig 9. PCR assays could detect 10^1 and 10 cfu / ml after enrichment (Xuan *et al*., 2000).

The work by Jofre *et al*., (2005) multiplex PCR based assay for simultaneous detection of *L. monocytogenes* and *salmonella* gave final results in 48 hours in comparison with microbiological methods that take up to 7-10 days also can identify

colonies from agar plates without nucleic acid isolation. This work also agree with our conclusion, that PCR methods avoids plating steps and inherent problems relating to the isolation of the pathogen. PCR technology also use a more flexible approach in either identifying bacterial isolates, or screening large numbers of environmental samples.

In Summary: The PCR technology showed an ability of detection of food-borne pathogens more over of the classical microbiological methods. all the agriculture sources of the farm, as irrigation water, spent irrigation water, water, wash water, compost, soil, fresh produce of vegetables and fruits and salad were subjected to PCR for detection of the three food-borne microbes; *E. coli* 0157:H7, S. *typhimurium* and *L. monocytogenes* which are three of the most serious food contamination. In this study, DNA templates were prepared by direct boiling of the enriched cultures (6-18 h) of the different samples. Three pair of specific primers were used to detect simultaneously the three mentioned pathogens in single plex. Thus, highly sensitive test, saving time & cost and taking quick decision for exportation and local distribution of the fresh produce of the farm were the outcome of our study.

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