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Real Time PCR versus Conventional Methods For Detection of Viable But Non-Culturable *E. Coli O157* Isolated From Food of Animal Origin

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ABSTRACT

Escherichia coliO157:H7 consider an important zoonotic food borne pathogen that cause bloody diarrhea, hemorrhagic colitis (HC), hemolytic-uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP) in human. *E. coli O157:H7* use a viable but non culturable (VBNC) state as a survival strategy which regarded as a threat to food security and the health of society. In this dormant stage, pathogens can evade identification by standard techniques. So, PCR methods are used for identifying all viable (culturable and non-culturable). This study was performed on 500 samples (300 raw meat and 200 raw milk samples) collected from different supermarkets allover Menoufia, Egypt. Samples were subjected to conventional culture method then positive-culture samples were confirmed by biochemical and serological identification while negative-culture sample subjected to conventional PCR and PMA in combination with SYBR green real-time PCR for VBNC *E. coli O157:H7* detection. The obtained data showed that the incidence of *E. coli O157:H7* by the conventional method was 3% while the incidence of VBNC *E. coli O157:H7* by PMA with SYBR green real-time PCR together was 10% for ten tested samples and 0.21% of 475 negative-culture samples. This study revealed that conventional and immunoassay methods were unable to identify VBNC case, also conventional PCR is unable to distinguish between dead and live bacterial cells. However, employing a real-time PCR technique alongside PMA has the necessary efficiency and sensitivity for identifying all viable (culturable and non-culturable) *E. coli O157:H7*.

Keywords: *E. coli O157:H7*, Food, VBNC, *pho*A gene and SYBR green real-time PCR.

INTRODUCTION

The foodborne pathogen *Escherichia coli O157:H7* is hazardous with a minuscule infectious dose (Rani et al., 2021). *E. coli O157:H7* infections have all been associated with hemolytic uremic syndrome, non-specific diarrhea, and hemorrhagic colitis (Lin et al., 2019). Food contamination from *E. coli O157:H7* epidemics are associated with bacterial outbreaks in beef, dairy, juice, tomatoes, eggs, poultry, seafood, and lettuce (Chen et al., 2021). Since this strain's discovery in 1982, the USA has experienced over 30 major *E. coli O157:H7*outbreaks. A particularly catastrophic epidemic occurred in 1993, when over 730 people were infected, 55 of them were diagnosed with HUS, and four kids passed away (Acheson and Keusch, 1996). According to the Centers for Disease Control and Prevention (CDC), *E. coli O157:H7* causes 73480 infections, 60 fatalities, and more than 2000 cases annually (Mead et al., 1999). In the USA, romaine lettuce

consumption-related epidemics of *E. coli O157:H7* in 2018 (CDC, 2018); 210 people were sick, 96 were hospitalized, there were 27 hemolytic uremic syndrome cases, and there were five death cases. According to estimates, the cost of *E. coli O157:H7* infections between 1996 and 2004 was $405 million per year, $370 million for mortality, $30 million for medical care, and $5 million for lost productivity (Frenzen et al., 2005). A state of dormancy known as "Viable but Non-Culture" (VBNC) is brought on by unfavorable conditions such as famine, an unsuitable temperature, high osmotic concentrations, oxygen availability, light exposure, UV radiation, decontamination treatments, the modified atmosphere during packaging, food preservation, heavy metals, and pasteurization of milk (Oliver, 2010; Maertens et al., 2021). It is extremely difficult to detect foodborne pathogens using the traditional culture approach since VBNC bacteria, in contrast to viable bacteria, do not thrive there (Oliver, 2005). One of the microorganisms involved was *E. coli O157:H7* that employed the VBNC condition as a survival tactic (Li et al., 2014). Although VBNC cannot be detected using standard methods, they are still capable of reviving under favorable conditions, which is a concern for both public health and the safety of food (Pinto et al., 2015). Hence, sensitive and precise *E. coli O157:H7* detection techniques are necessary to safeguard the general populace's health and food safety. Conventional culture methods are frequently employed because of their sensitivity, accessibility, convenience of use, and capacity to maintain and monitor cell viability (Murakami, 2012); yet, these procedures are still time- and labor-consuming (Ma et al., 2021), and they are unable to recognize the viable but uncultivable stage of bacteria that still have virulence or resuscitate under favorable conditions and recover virulence (Li et al., 2014). Nevertheless, compared to traditional culture methods, the target microorganism can be quantified in samples using the sensitive, rapid, and accurate polymerase chain reaction (PCR) method in just three hours (Li et al., 2021). However, PCR is unable to distinguish between viable cells, VBNC cells, and dead cells, which can result in false-positive results (Li et al., 2017); and Real-time PCR is limited by the chemicals present in ambient and food samples that naturally arise and hinder the target DNA from being amplified. To solve these problems, A real-time PCR process included an internal amplification control (IAC) to control, enhance, and avoid false-negative outcomes (Murphy et al., 2007). Propidium monoazide and real-time PCR together, provide differentiation between live and dead cells and form a covalent link of dead cell's DNA with PMA, will render DNA from dead cells or cells with damaged membranes to be amplified by PCR (Zhong and Zhao, 2018). PMA has been employed in conjunction with real-time PCR for the detection of such organisms as *Campylobacter* (Ruiling et al., 2019), *Salmonella* (Zhai et al., 2019; and Ramírez et al., 2023), *E. coli* (Ramírez et al., 2023), *Vibrio parahaemolyticus* (Liu Y et al., 2018), and *E. coli O157:H7* (Zhong and Zhao, 2018; and María et al., 2022) in environmental and food samples.

This study aimed to compare between conventional culture method, conventional PCR method, and PMA together with the SYBR green real-time PCR technique to identify all living *E. coli O157:H7* (viable-culturable, and VBNC) to suggest the most rapid, sensitive, suitable, and accurate method.

MATERIALS AND METHODS

Collection and processing of samples

500 samples (300 samples of raw meat and 200 samples of raw milk) were aseptically collected during the period of 12th march to 28th June 2018 at random manner from various supermarkets in the Menoufia

governorate, Egypt. The samples were aseptically put into sterile containers in ice box, labeled and transferred as soon as possible to the bacteriology lab at the Faculty of Veterinary Medicine, University of Sadat City, Menoufia, Egypt, for bacteriological analysis according to Ahmed et al., (2007) within 2 hours. For each sample 25 g was taken and stomached in a sterile stomacher bag, mixed with 225 ml buffered peptone water (BPW) (Oxoid) to be prepared for further examination.

E. coli O157:H7 isolation and identification

E. coli O157:H7 was isolated and identified in accordance with ISO 16654:2001. Briefly, samples were cultivated in enrichment broth, buffered peptone water (Oxoid, UK), then incubated at 37°C for 24 hours. A loop of incubated enrichment broth was streaked on Eosin Methylene Blue Agar plates (Levine) (Oxoid, UK); a loop of *E. coli* colonies was streaked on Sorbitol MacConkey Agar plates (Oxoid, UK); and CHROM agar^{TM} O157 (CHROM agar^{TM}, France) for isolation of *E. coli O157:H7*. At 37 °C, all culture media plates were grown for 24 hours. According to (ISO 16654:2001), via the conventional method, based on IMVIC reactions, gas production, and sugar fermentation, biochemical reactions and microscopic examination validated the suspicious colonies. Serological identification according to (ISO 16654:2001) by using quick diagnosis sets of *E. coli O157:H7* antibodies (DENKA SEIKEN Co., LTD).

Molecular identification of E. coli O157:H7 in VBNC state

Extraction of DNA and PMA assay

Propidium iodide staining is the first step in the preparation of propidium monoazide (Taskin et al., 2011) for all methods and (Deng et al., 2016) for 500W lamps. To make a stock solution of 10 mM of PMA, 20% dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO) was used to dissolve it, and it was stored at -20°C. Second, using a QIAamp DNA micro kit from QIAGEN in Germany and following the manufacturer's instructions, DNA was extracted. Samples were separated into two groups: one for real-time PCR with PMA treatment and the other for conventional PCR without PMA treatment. Briefly, 25g of each sample was mixed with brain-heart infusion broth (225 milliliters), stomached for two minutes in a stomacher, then at 37 °C overnight cultivation (Jones et al., 2002). Samples were subjected to PMA treatment before SYBR green real-time by transferring 500 µl of culture mixes with a final PMA stock solution concentration of 100 µM. To avoid any potential chemical changes to the PMA structure, all PMA solution manipulations were carried out in low light. After that, samples were exposed for 5 min to a 500-W halogen light source at a distance of 15 to 20 cm from the light source after being incubated in the dark for 10 min. To prevent overheating during the light exposure, tubes were put on the ice. Then the DNA was extracted.

Conventional PCR for Detection of VBNC E. coli O157:H7

By using a developed primer for *pho*A gene, a total of 15 culture-negative samples (9 meat samples and 6 milk samples) were examined for *E. coli O157:H7* presence.

The study's target gene, the *pho*A gene and the gene's primer of *E. coli O157:H7*were selected according to (Hu et al., 2011). The primer sequences for the *pho*A gene were:

CGTGATCAGCGGTGACTATGAC and CGTGATCAGCGGTGACTATGAC

(metabion, Germany), and their product size is 720 bp. The Emerald Amp GT PCR master mix was used to create the PCR master mix (Takara Bio, USA) (template DNA (6 µl), 1 µl forward primer (20 pmol), 1 µl reverse primer (20 pmol), 4.5 µl PCR grade water, and 12.5 µl Emerald

Amp GT PCR master mix (2x premix)) to get the final master-mix amount of 25 µl. The primer cycling conditions during conventional PCR were based on the individual author's findings and master-mix kit of the Takara Emerald Amp GT PCR (Takara Bio, USA) with a few changes. Briefly, the phoA gene was amplified through5 minutes at 94°C for initial denaturation, then 35 cycles of (at 94°C for 30 seconds of denaturation, at 55°C for 40 seconds of annealing, and at 72°C for 45 seconds of extension). A 10-minute of final extension at 72°C for put an end to the thermal cycles. Electrophoresis was used to separate the PCR products on a 1.5% agarose gel with modifications to ascertain the fragment sizes (Sambrook et al., 1989). The gel was relocated to the UV cabinet after 30 minutes of operation at 1–5 volts/cm of tank length. A gel documentation system took photos of the gel, and computer software was used to interpret the data.

Real-time PCR for the identification of E. coli O157:H7 in VBNC

Ten culture-negative samples (positive in the *pho*A gene by conventional PCR) were subjected to genotypic identification of viable but unculturable *E. coli O157:H7* by real-time PCR using designed primer of *pho*A gene, which was chosen as the target gene in this study, and the primer for it was chosen according to Hu et al. (2011). The primer sequence of the *pho*A gene were: CGATTCTGGAAATGGCAAAAG and CGTGATCAGCGGTGACTATGAC (metabion, Germany), and the size of the product is 720 bp.The PCR Master Mix was made using SYBR Green PCR Kit (Quantitect) from QIAGEN (Germany), which had 25 µl as final amount that contained SYBR Green PCR Master Mix (2x QuantiTect) (12.5 µl), Forward Primer (20 pmol) (0.5 µl), Reverse Primer (20 pmol) (0.5 µl), RNase Free Water (8.5 µl), and Template DNA (3 µl). Cycling conditions for SYBR green real-time PCR were carried out at 94°C for 5 minutes of initial denaturation, then 40 cycles of (30 seconds at 94°Cof denaturation, 30 seconds at 55°Cof annealing, and 1 minute at 72°Cof extension). Finishing of thermal cycles was achieved by dissociation by (denaturation at 95°C for 1 minute, annealing at 95°C for 1 minute, and final denaturation at 95°C for 30 seconds).

Statistical analysis

Data were presented as the mean ± standard deviation. Means were compared using ANOVA using statistical package for Social Sciences (SPSS) version 23.0 (IBM Crop., Armonk, NY), and analysis of variance at 95% significance (P = 0.05).

RESULTS

E. coli O157:H7 frequency in examined samples

Bacteriological evaluation of 500 samples, of which 300 raw meat samples and 200 dairy samples, were gathered from various supermarkets in Egypt's Menoufia Governorate, revealed 15 different *E. coli O157:H7* isolates in total with a percentage of 3% of collected samples and the greatest frequency in Shohda district with prevalence 3.63%. First, isolation of *E. coli* from all collected samples: we found 206 samples with a percentage of 41.2%, 162 meat samples with a percentage of 54% of collected meat samples and 32.4% of all collected samples, and 44 milk samples with a percentage of 22% of collected milk samples and 8.8% of all collected samples. Second, *E. coli O157:H7*isolation from all analyzed specimens: we found that 15 samples with a percentage of 3% were collected from 10 meat samples with a percentage of 3.33% of collected meat samples and 2% of all collected samples, and 5 milk samples with a percentage of 2.5% of collected meat samples and 1% of all collected samples, as Figures (1, 2, and 3) and Tables (1, 2, and 3) show these.

Table 1: Overall incidence in Meat samples of *E. coli O157:H7*.

District	Type of sample	Number of samples	Incidence			
			Number of Positive Sample	%*	%**	%***
Shohda	Meat	**50**	**2**	**1.81**	**13.33**	**0.4**
Sadat	Meat	**70**	3	**1.76**	**20**	**0.6**
Shibin	Meat	**30**	1	**2.5**	**6.66**	**0.2**
Ashmoun	Meat	**60**	1	**1.43**	**6.66**	**0.2**
Menouf	Meat	**40**	**1**	**2.0**	**6.66**	**0.2**
Tala	Meat	**50**	**2**	**3.33**	**13.33**	**0.4**
Total		300	10	**12.83**	**1**	**3.33**

*Percentage was calculated according to examined samples of each city.
**The percentage was determined using all the positive sample data. (n=10).
*** The percentage was determined using all sample data (n=300).

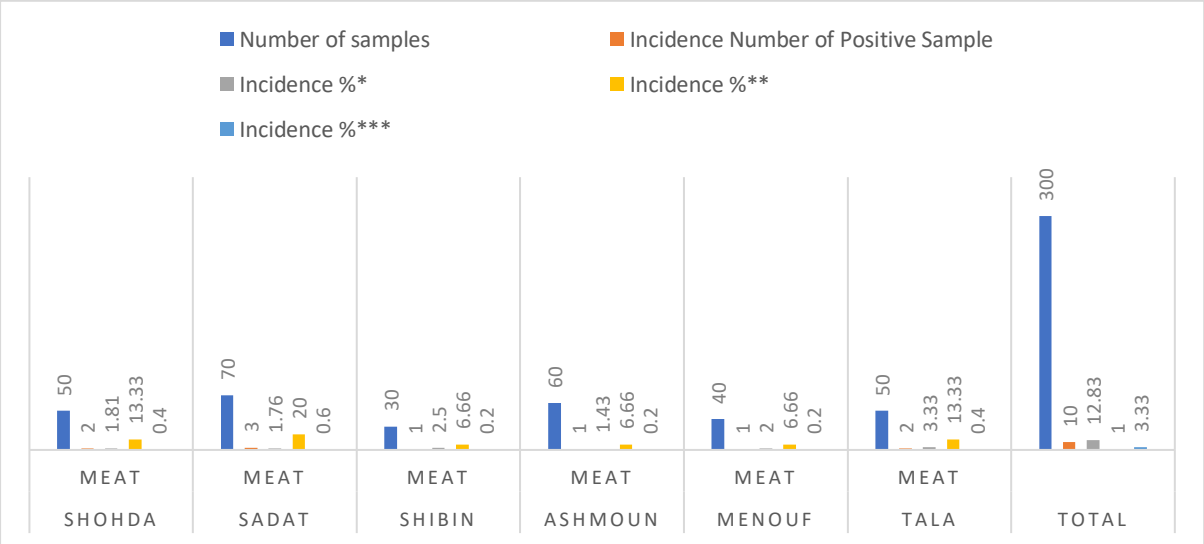

Fig. 1: *E. coli O157:H7* frequency across meat samples under investigation.

Table 2: Overall incidence in Milk samples of *E. coli O157:H7*.

District	Type of sample	Number of samples	Incidence			
			Number of Positive Sample	%*	%**	%***
Shohda	Milk	60	2	**1.81**	**13.33**	**0.4**
Sadat	Milk	100	2	**1.17**	**13.33**	**0.4**
Shibin	Milk	10	0	**0**	**0**	**0**
Ashmoun	Milk	10	1	**1.43**	**6.66**	**0.2**
Menouf	Milk	10	0	0	0	0
Tala	Milk	10	0	0	0	0
Total		200	5	**4.41**	**1**	**2.5**

*Percentage was calculated according to examined samples of each city.
**The percentage was determined using all the positive sample data. (n=5).
*** The percentage was determined using all sample data (n=200).

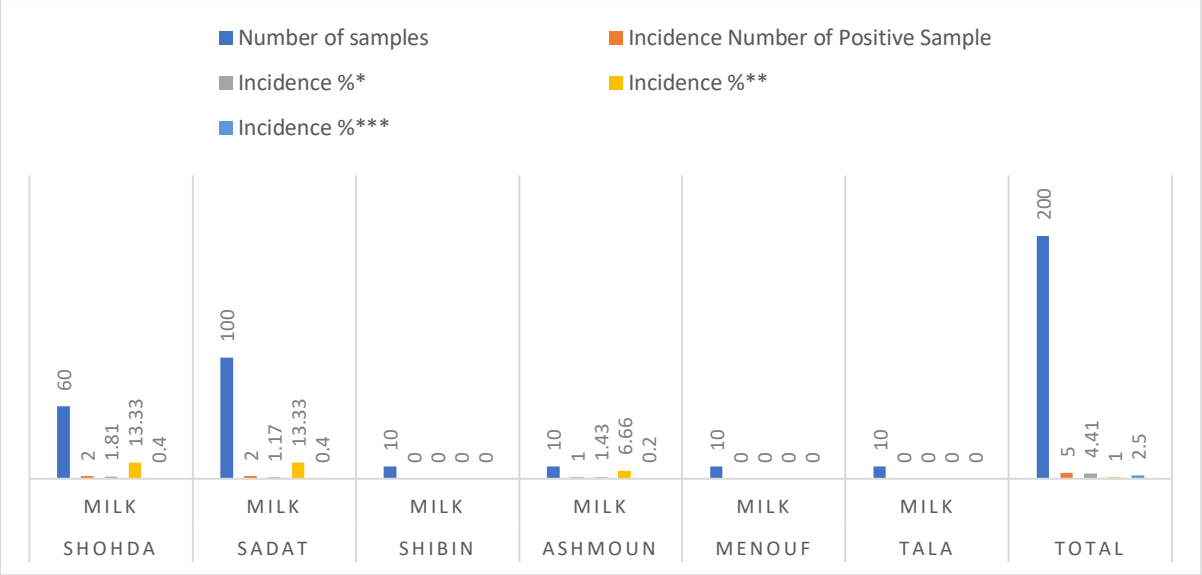

Fig. 2: *E. coli O157:H7* frequency across milk samples under investigation.

Table 3: Overall incidence in all tested samples of *E. coli O157:H7*.

City	No of samples	Incidence			
		No of Positive Sample	%*	%**	%***
Shohda	110	4	**3.63**	**26.66**	**0.8**
Sadat	170	5	**2.35**	**26.66**	**0.8**
Shibin	40	1	**2.5**	**6.66**	**0.2**
Ashmoun	70	2	**2.85**	**13.33**	**0.4**
Menouf	50	1	**2.5**	**6.66**	**0.2**
Tala	60	2	**3.33**	**13.33**	**0.4**
Total	500	15	**17.16**	**100**	**3**

*Percentage was calculated according to examined samples of each city.
**The percentage was determined using all the positive sample data. (n=15).
*** The percentage was determined using all sample data (n=500).

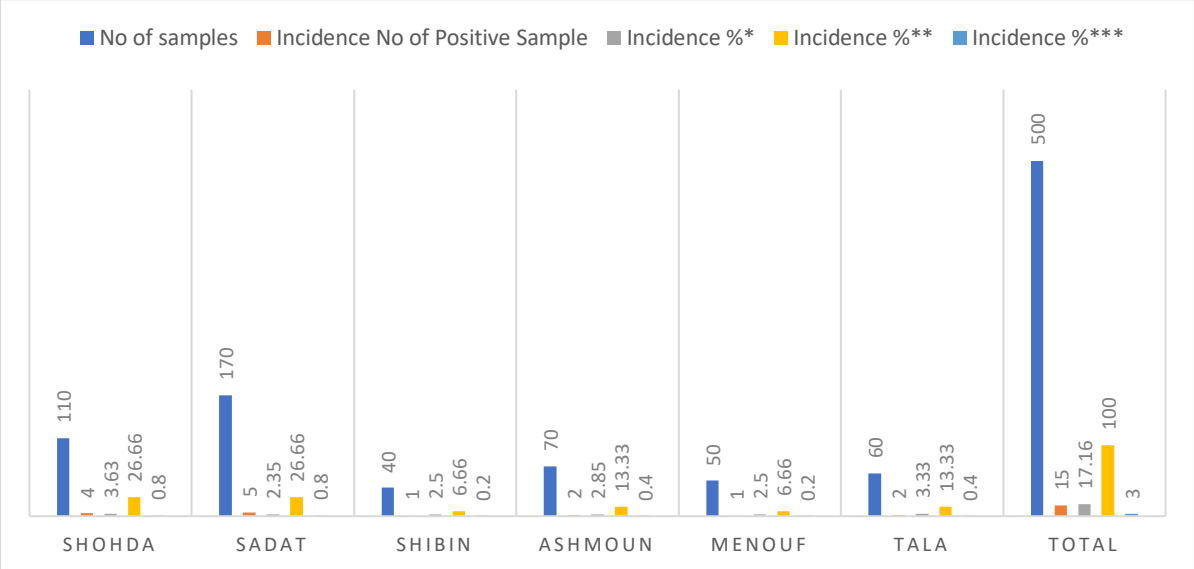

Fig.3: *E. coli O157:H7*frequency across all specimens under investigation.

***E. coli O157:H7* Identification**

E. coli O157:H7 strains were identified using the developing characteristics on Sorbitol MacConkey agar medium (Oxoid, UK) appeared colorless or pale, and CHROMagarTMO157 appeared mauve color. Gram staining, which revealed suspected colonies to be Gram-negative short rods, motile, flagellated, and spore-free, was used to identify suspected colonies. All tested isolates were then verified using several biochemical tests, as shown in Table (4).

Table 4: Result of *E. coli O157:H7's* biochemical characterization.

Biochemical test	**Result**
Indole	Positive
MR	Positive
VP	Negative
Citrate	Negative
Triple sugar iron agar medium test	Acid/acid/gas (A/A/Gas)
Urease	Negative
Oxidase	Negative

Serotyping of *E. coli O157:H7*

E. coli O157:H7 isolates were confirmed by serological testing, as shown in Table (5).

Table 5: Result of serotyping of *E. coli O157:H7*.

No of Samples	District	Type of samples	Polyvalent	Monovalent
1	Shohda	Meat	3	O157:H7
2		Meat	3	O157:H7
3		Milk	3	O157:H7
4		Milk	3	O157:H7
5	Sadat	Meat	3	O157:H7
6		Meat	3	O157:H7
7		Meat	3	O157:H7
8		Milk	3	O157:H7
9		Milk	3	O157:H7
10	Shibin	Meat	3	O157:H7
11	Ashmoun	Meat	3	O157:H7
12		Milk	3	O157:H7
13	Menouf	Meat	3	O157:H7
14	Tala	Meat	3	O157:H7
15		Meat	3	O157:H7

Molecular Characterization of E. coli O157in VBNC state

Molecular Identification of VBNC E. coli O157 phoA gene in culture-negative samples by using conventional PCR

*pho*A gene designed primer was used in conventional PCR to genotypically identify *E. coli O157:H7* from fifteen samples that had negative results from culture. The outcome demonstrated that *E. coli O157*occurrence by *pho*A gene was 100 percent in all 15 negative-culture samples (9 meat samples and 6 milk samples), and the incidence is 3.15% of 475 culture-negative samples, as shown in Table (6).

Table 6: The result of a conventional PCR used to amplify the *pho*A gene in tested samples.

District	Type of sample	phoA gene result
Shohda	Meat	+VE
	Meat	+VE
	Milk	+VE
sadat	Meat	+VE
	Meat	+VE
	Milk	+VE
Shibin	Meat	+VE
	Milk	+VE
Ashmoun	Meat	+VE
	Milk	+VE
Menouf	Meat	+VE
	Milk	+VE
Tala	Meat	+VE
	Meat	+VE
	Milk	+VE

All tested samples have *pho*A gene were identified by PCR. *pho*A gene's 720 base-pair presence in all samples revealed a positive result, establishing their status as *E. coli O157*, as shown in figures (4).

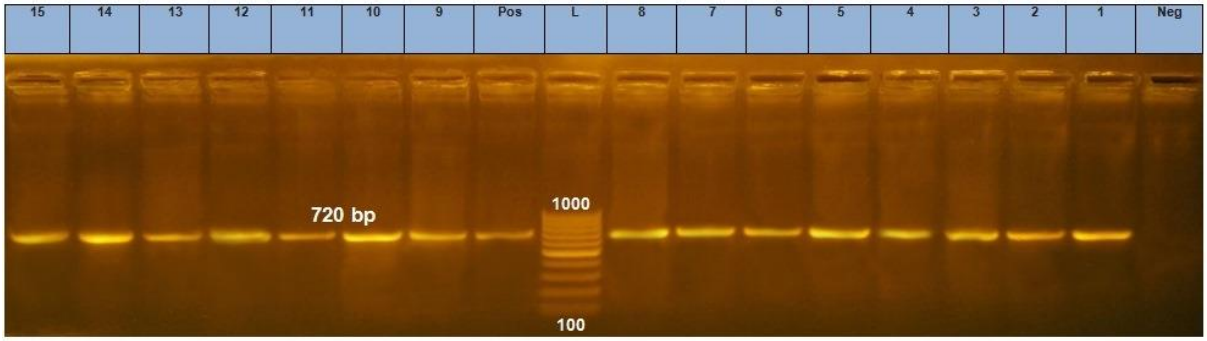

Fig. 4. 1.5% Agarose gel electrophoresis showing specific PCR of *E. coli O157* in all tested samples that were culture-negative through usage of a primer set for the *pho*A gene. In this image, the letters (pos) and (neg) stand for positive and negative controls, respectively, and the numbers denote the lanes of samples, all lanes are positive: lanes (1-9) positive *pho*A gene for meat samples, and lanes (10-15) positive *pho*A gene for milk samples. All samples display the specific band at 720 bps in Lane L (100-1000 bps marker).

E. coli O157:H7:H7 molecular detection in samples employing SYBR green real-time PCR with propidium monoazide

Ten culture-negative samples (positive in the *pho*A gene by conventional PCR) were subjected to VBNC *E. coli O157*genotypic identification of using designed primer of *pho*A gene for SYBR green real-time PCR, after utilizing PMA treatment to the examined samples, the results showed that one sample had an incidence level (of 10%) of ten negative-culture samples that housed VBNC state, and the incidence is 0.21% of 475 negative-culture samples, as shown in Table (7) and Figures (5).

Table 7: Results of amplification of the *pho*A gene in *E. coli O157:H7* using SYBR green real-time PCR.

District	Sample's Type	*pho*A gene result
Shohda	Meat	-
	Milk	-
Sadat	Meat	-
	Milk	-
Shibin	Meat	-
Ashmoun	Meat	-
	Milk	-
Menouf	Meat	-
Tala	Meat	-
	Meat	+

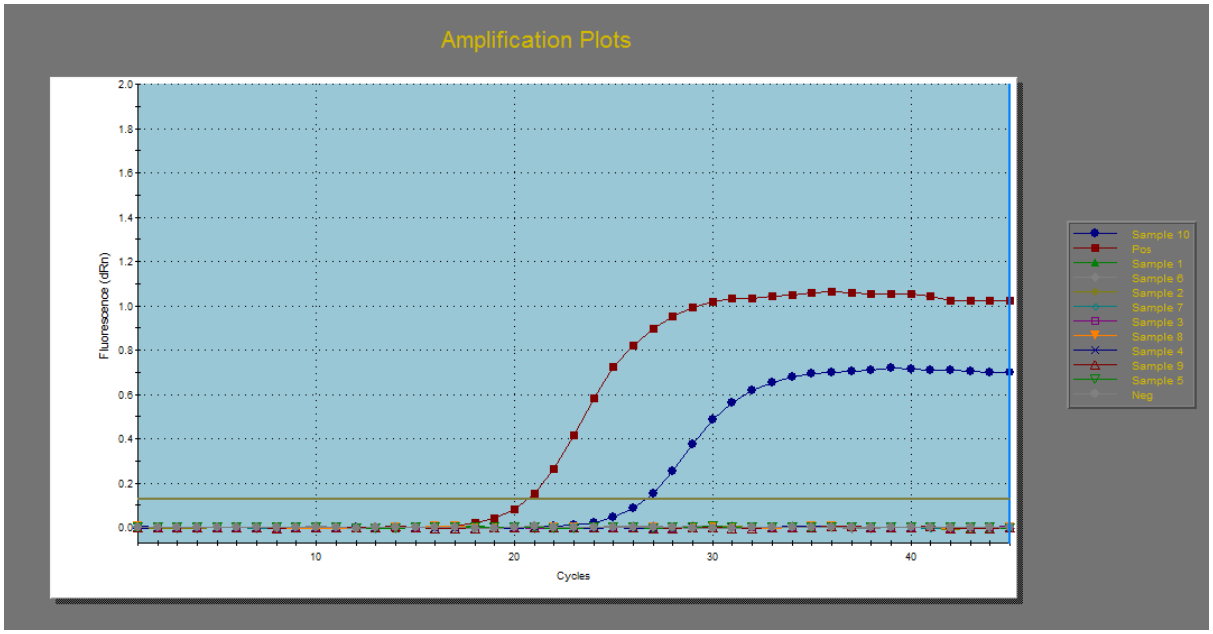

Fig. 5. *E. coli O157* quantitative real time PCR amplification plots in analyzed samples. Positive control: the curve became visible at Ct 21 in the datasheet. Negative control: It was visible below the threshold line. Only one positive sample for *pho*A gene appeared at Ct 26.5.

DISCUSSION

*Escherichia coli O157:*H7 is a harmful food-borne bacteria that can seriously illness both humans and animals (Rani et al., 2021a). It is typically found in the intestines of cattle and can contaminate food items, like beef, and dairy stuffs, through fecal contamination (Chen et al., 2021). Rapid and precise *E. coli O157:H7* detection in such food products is necessary to ensure the safety of the food supply, prevent food-borne diseases, and assure food safety. Because it is challenging to identify using traditional techniques, Viable but non-culturable (VBNC) state poses a major risk to public health and food safety. The danger comes from pathogenic bacteria that may still be virulent and still produce their toxins to cause disease (Liu et al., 2018b) or that may be able to resuscitate under the right circumstances. Identification and detection of VBNC bacteria are therefore crucial (Zhao et al., 2018). It has been discovered that more than 100 different types of microorganisms reach the VBNC phase (e.g., *Listeria, E. coli O157:H7, Vibrio, Campylobacter, Yersinia, and Salmonella*) (Zhao et al., 2017), by contaminated eating items, as beef products (Reissbrodt et al., 2002), Milk (Barron and Forsythe, 2007), Chicken (Chen et al., 2019), fish (Cao et

al., 2019), and fruits and vegetables (Dinu and Bach, 2013). If culture produces negative results, food, environmental, and clinical samples can no longer be assumed to be pathogen-free. The general public's susceptibility to aseptic infections could have been impacted by food that contains VBNC (Colwell and Grimes, 2000).

Screening of E. coli O157:H7 by conventional methods

500 samples (300 meat samples and 200 raw milk samples) were collected and examined by conventional methods (culture method, biochemical identification, and serological identification), 15 samples with a percentage of 3% from 10 meat samples with percentage 3.33% of collected meat samples and 2% of all collected samples, and 5 milk samples with a percentage of 2.5% of collected raw milk and 1% of all examined specimens. The detection of *E. coli O157:H7* in this present investigation following pre-enrichment on BPW for 24 hours at 41°C. After incubation, it was inoculated on EMB agar medium for the isolation of *E. coli*. In order to isolate *E. coli O157*, positive colonies of *E. coli* were inoculated on Sorbitol MacConkey agar medium and CHROM agar™ O157 agar media: H7. This agrees with other studies (Tahamtam et al., 2011). For additional morphological, biochemical, and serological identification, typical colonies on TSA were employed, as done by (Santaniello et al., 2006). The obtained result by the conventional culture method agrees with or is close to the study in Saudi Arabia made by (Ashgan et al., 2015) on 370 meat samples collected during the period of January 25, 2013, to March 25, 2014, from abattoirs and markets, they found 11 positive meat samples (2.97%). This result is lower than other previous studies: a study in Egypt conducted by (Ashraf et al., 2014) with an incidence of (3.4%); and in Central Ethiopia (Besada et al., 2018) with a frequency of (3.5%). In the other hand, our result is higher than other studies as, (Khalid et al., 2013) conduct a study in Egypt with an incidence of 17.2%; and A study in Zaria, Nigeria by (samole et al., 2014) with an incidence (2.2%).

Screening of culture-negative samples for VBNC *E. coli O157:H7* by conventional PCR

Employing a specially created primer for the *pho*A gene by conventional PCR. In order to genotype and identify *E. coli O157:H7*, 15 samples of negative-culture samples were examined. The results showed that a 100% incidence of the *pho*A gene was discovered in all 15 samples. Conventional PCR has been employed to identify *E. coli O157:H7*in previous studies as (Maria et al., 2011; Khalid et al., 2013; and Salome et al., 2014).

Screening by PMA SYBR green real-time PCR for VBNC E. coli O157 in culture-negative specimens

Propidium monoazide is employed in quantitative PCR for the detection of live bacteria. The creation of a covalent connection between PMA and the dsDNA is induced by visible light (high-power halogen lamps or LED devices), which prevents the DNA from being amplified by PCR. PMA can only penetrate compromised cells and be induced to form a covalent bond with their DNA, preventing qPCR from amplifying them. Only the DNA from living bacteria was able to be amplified by qPCR after being exposed to the chemical. This aids in identifying infections that are viable in particular samples (El-Aziz et al., 2018). In this study, 10 culture-negative samples (positive *pho*A gene by conventional PCR) were used to identify the genotype of VBNC *E. coli O157:H7* using primer for the *pho*A gene by combined real-time PCR with PMA. The result revealed that one culture-negative sample harbored a VBNC state when examined by SYBR green real-time PCR after PMA treatment with an incidence level of 10%. DNA

amplification by PCR using dead cells and the erroneous positive results that arise can be successfully reduced by including the PMA dye step before DNA extraction, as revealed in this study. The recent study unequivocally agrees with Martin et al.'s (2010) contention that conventional methods for identifying bacteria in food need a lot of time and labor. The real-time PCR-based technique that has shown tremendous promise because of its specificity, sensitivity, speed, and capacity to measure the target. PMA can penetrate dead cells, preventing false-positive findings which come from amplification of dead cells. Given that slow-growing or picky bacteria require long durations of culture and that it is difficult to calculate or estimate cell survival using this method, its application is of utmost significance in certain situations comes in relation to (Deng et al., 2016; Liu et al., 2018; and Zhong and Zhao., 2018). The obtained data are consonant with other studies of VBNC *E. coli O157:H7* such as (Mahmoud et al., 2017; Zhong and Zhao., 2018; Dan et al., 2021; and Maria et al., 2022).

CONCLUSIONS

From the obtained data, the most accurate way to find every viable *E. coli O157:H7* is to use PMA together with SYBR green real-time PCR(viable culturable and viable but non-culturable) as PMA permits the differentiation of live from dead cells by selective permeability to dead thus DNA amplification for cells with the intact cell membrane to overcome false-positive results in food samples by conventional PCR and false-negative results in food samples by conventional culture methods which cannot detect VBNC state, supplying the approach that PMA together with SYBR green real-time PCR is the most precise, sensitive, quick, and specific way possible to find each viable *E. coli O157:H7*.

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