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MOLECULAR DETECTION OF *SALMONELLA* AND *E. COLI* MICROORGANISMS AMONG DAIRY FARMS WITH DETECTION OF VIRULENCE AND ANTIBIOTICS RESISTANCE GENES

GIHAN MOHAMED OMER MOHAMED HASSAN [1]
and HASSAN EL-SAYED MOHAMED FARAG [2]

[1] Assistance Researcher of Microbiology Department, Port-Said Lab Animal Health Research Institute Dokki –Giza.
[2] Chief Researcher of Food Hygiene Department, Port-Said Lab Animal Health Research Institute Dokki –Giza.

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ABSTRACT

A total of 500 samples, 100 of each milk, feed, swabs from milking equipment (milk tanks), drinking tanks swabs and dairy cows fecal swabs samples were collected from different small herds of apparently or subclinical dairy cattle in El-Kabotti and Bahr El-Baker zone at Port-Said Governorates during the period from September to December 2018. The samples were examined for isolation and identification of *Salmonella* species and *E. coli* with studied of their virulence and resistance gens and sequence of some genes. The results revealed that *Salmonella* species and *E. coli* could be detected in a percentage of 1.8% and 2.8% respectively from the examined samples. *Salmonella* isolates from the examined samples were identified biochemically and serological as *S.* Typhimurium *S. Entiriditis* and *S. saintipaul* with a percentage of 66.67% (6/9), 2.22% (2/9) and 11.11% (1/9) respectively, while that of *E. coli* were O26 (5/14), O119 (2/14), O125 (4/14), O126 (1/14) and O127 (2/14) with a percentage of 35.71%, 14.28%, 28.60%, 7.14% and 14.28% respectively. The isolated strains of *Salmonella* species (n=9) and *E. coli* stains (N=14) were investigated for antibiotic susceptibility profile to 10 antibacterial agents by disc diffusion method. The resistances of the isolated *Salmonella* and *E. coli* strains were ranged from a various degree of resistances to complete resistances (100%). By using conventional PCR, all *Salmonella* were harbored *InvA, stn* and *bcfC* genes while *E. coli* were harbored *PhoA, TraT* and *fimH* genes. The resistance genes that detected in *Salmonella* strains were *ampC, mphA* and *aacC* while that of *E. coli* were *bltEm, ampC, mphA, Aada1* and *aacC.* The prevalence of the resistance genes were discussed. DNA sequencing of *stn* and *bcfC* genes for *Salmonella* and *TraT* and *fimH* genes for *E. coli* were discussed and compared with other strains in Gen Bank. The mutations in quinolone-resistance gene were studied by determining regions of the *gyrA* gene for *Salmonella* and *E. coli.* The public health hazards of these microorganisms as well as recommended measures to improve hygiene measures in dairy farms were discussed.

Key words: *Salmonella* species, *E. coli*, milk, fecal, swabs of milking equipment's, swabs of drinking equipment, feed, PCR, virulence genes, resistance genes, sequence of genes, public health.

INTRODUCTION

Although there have been increases in the modern and advanced methods of care for livestock, small herds in different localities were still found especially in the developing and underdeveloped country whereas the growth of animals in conditions of overcrowding often enhanced the appearance of bacterial and others infectious disease (Godinho and Carvalho, 2013) that affect the animals health and their productivity, resulting in large economic losses. Bacteria can occur in milk through, colonization in the teat canal or infected udder (clinical and subclinical mastitis), milker (manual as well as automated), extraneous dirt, milk utensils and unclean processing water (Hayes *et al.,* 2001). *Salmonellae* and *E. coli* are the most economically important pathogens (Achá *et al.,* 2004) affecting dairy cattle and calf.

Salmonella is an enteric pathogen found in the intestinal tract of animals and excreted in feces and spread in water, soil, plant surface, animal feces and dairy farms (Halimi *et al.,* 2014). The severity of infection and symptoms varies depending on the host species and serovars and ranging from severe disease to asymptomatic (Coburn *et al.,* 2007). Although cattle are considered a major reservoir for infections with *S.* Typhimurium (Nastasi *et al.,* 1993) where *Salmonella* have been isolated from the feces of healthy cattle and considered a normal or transient member of the gastrointestinal microbial population (Callaway *et al.,* 2005). Salmonellosis manifestations

Corresponding author: Dr. Gihan Mohamed Omer Mohamed
E-mail address: dr.gehanomer@yahoo.com
Present address: Assistance Researcher of Microbiology Department, Port-Said Lab Animal Health Research Institute Dokki –Giza.

include fever, anorexia, diarrhea, dehydration, abortion, decreased milk production, depressed mentation, pneumonia, septic arthritis, meningitis, gangrene of distal extremities and sudden death (Mohler and House, 2009).

Salmonella produce a variety of putative virulence determinants including haemaglutinins, adhesion, invasions, fimbriae exotoxin and endotoxins (Lee *et al.,* 1996). The *invA* gene of *Salmonella* contains sequence unique and recognized as an international standard for detection of *Salmonella* genus (Malorny *et al.,* 2003) and considered a potential diagnostic for all known serovars of *Salmonella* (Jamshidi *et al.,* 2008). While *Salmonella* enterotoxin (*stn*) is a putative virulence factor responsible for enterotoxic activity (Chopra *et al.,* 1999) and *bcfC* coding for bacterial fimbriae, involved in surface adhesion and gut colonization (Barrow *et al.,* 2010).

On the other hand, *E. coli* are a large and diverse group of bacteria of the family *Enterobacteriaceae* commonly found in the lower intestine of a variety of warm-blooded animals including cattle and humans (CDC, 2011).

E. coli is an ideal indicator organism for fecal contamination in water (well water, river water, other contaminated surface waters, soil and plants) or in food (milk, meat, vegetables ect.) (Kaper *et al.,* 2004) and this increase the possibility for presence of enteropathogenic or toxigenic *E. coli* (Pamela *et al.,* 2008).

The pathogenicity of *E. coli* is dependent on the regulation and interaction between a number of virulence factors, and it is affected by environmental conditions such as host species, host health status, interaction with other bacteria species (Clermont *et al.,* 2011).

E. coli pathovars, such as enteropathogenic *E. coli* (EPEC), Shiga-toxigenic *E. coli* (STEC), and enterohemorrhagic *E. coli* (EHEC), have been observed in dairy herds (Farrokh *et al.,* 2013), milk (Van Kessel *et al.,* 2011) and other dairy products (Solomakos *et al.,* 2009), with a unique set of virulence and colonization factors encoded in the chromosome or in episomal structures (Rúgeles *et al.,* 2010).

Enteropathogenic *E. coli* (EPEC) strains belonged to a series of O antigenic groups including 12 serogroups such as O26, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142, and O158 (Hernandes *et al.,* 2009).

Conformation of *E. coli* from other bacteria can detect by the housekeeping gene, *phoA* (The alkaline phosphastase gene) which present in all *E. coli* strains (Kong *et al.,* 1995) and encodes for a hydrolase enzyme, responsible for removing phosphate groups from molecule (Chang *et al.,* 1986).

TraT gene is one of the virulent factors of *E. coli* that have been shown to be located on conjugative plasmids. The *TraT* (conjugal transfer surface exclusive protein) gene is a major outer membrane protein (Moll *et al.,* 1980) which reduces the susceptibility of bacteria to phagocytosis (Agüero *et al.,* 1984).

FimH is a mannose-specific adhesion located on the tip of type 1 fimbriae of *E. coli* that is responsible for mediating shear-enhanced bacterial adhesion and invasive properties of *E. coli* (Chassaing *et al.,* 2011).

Antimicrobial resisitance has emerged in the past few years as a major problem in human and vetrinary medicine (Lanz *et al.,* 2003) due to the wide spread use and misuse of antimicrobials in farms animals (Suojala *et al.,* 2011). Also uses of antibiotic as growth promotion give raise to antimicrobial resistance in farms animals (Philips *et al.,* 2004). The resistance can occur between and within bacteria through mutation of genes and horizontal gene transfer (Buller *et al.,* 2014). Thus antimicribial resistance strains can increase the treatment cost and period of treatment (Sawant *et al.,* 2007). Therefore, identification of resistance genes of bacteria seems to be so essential in reduction of treatment costs (Suojala *et al.,* 2011).

GyrA (A subunit) is essential for epithelial invasion (Galan and Curtis, 1989), found predominantly in bacteria and composed of a single polypeptide, as in most eukaryotes. *GyrA* has two functional domains: N-terminal responsible for the breaking- and rejoining function and C-terminal that can bind DNA non-specifically (Huang, 1996).

Thus the aim of the current study was carried out for molecular detection of *Salmonella* and *E. coli* in different types of samples in dairy farms with detection of some virulence and resistance genes of the isolated strain. Also genes sequences of some strains were determined.

MATERIALS AND METHODS

1-Sample collection:

A total of 500 samples, 100 of each milk (pooling from 1000 lactating cows), feed, swabs from milk tanks, drinking tanks swabs and dairy cows fecal swabs samples (pooling from 1000 lactating cows) were collected from small herds of apparently healthy or subclinical dairy cattle in El-Kabotti and Bahr El-Baker zone at Port-Said Governorates during the period from September to December 2018. Each positive pooling samples were re-examined one by one.

2-Samples preparation, homogenation and pre-enrichment:

2-1: Milk samples:

Preparation of teats and udder for milk collection was done according to Cabral *et al.,* 2015. Each milk sample was collected aseptically in clean, sterilized, marked and identified sterilized bottle and Keep in the refrigerator or on ice at 4^{o}C until microbiological examination. Under aseptic condition homogenation of milk samples with sterile buffered peptone water (BPW) and incubated at 34^{o}C- 38^{o}C for 18 h ± 3 h according to ISO 6887-1:2017 and ISO 6887-5:2017.

2-2: Feed samples:

Aseptically collection of feed samples and kept in refrigerator until bacteriological examination. Homogenation of grinding feed with sterile BPW and incubation was done according to ISO 6887-1:2017 and ISO 6887-4:2017.

2-3: Swabs from milking equipment (milk tanks):

According to WHO/FAO, 1994 milk tanks swabs were taken under aseptic condition and kept at 4^{o}C until bacteriological examination. Preparation of 1:10 and incubation at 34^{o}C- 38^{o}C for 18 h ± 3 h was done according to ISO 6887-1:(2017).

2-4: Drinking tanks swabs:

Under aseptic condition drinking tanks swabs were collected and kept at 4^{o}C until bacteriological examination according to WHO/FAO, 1994. Preparation of 1:10, homogenation and incubation at 34^{o}C- 38^{o}C for 18 h ± 3 h (ISO 6887-1:2017).

2-5: Dairy cows fecal swabs:

Fecal swabs were collected and kept at 4^{o}C until bacteriological examination according to WHO/FAO, (1994). Prepare a 1:10 dilution, homogenate and incubation at 34^{o}**C**- 38^{o}**C** for 18 h ± 3 h (ISO 6887-1:2017.

3- Isolation of microorganisms:

3-1: Isolation *Salmonella* species:

From each culture, 0.1 ml of pre-enrichment broth was added to 10 ml Rappaport-Vassiliadis broth with soya then incubated at 41.5^{o}C ± 1 ^{o} C for 24 hr ± 3h. and 1 ml from the culture of the same sample was added to 10 ml Muller-Kauffmann Tetrathionate/ novobiocin broth and incubated at 37 ^{o} C ± 1 ^{o} C for 24 hr ± 3 h. Then a loopful from the enriched broth was streaked onto the each surface of Xylose Lysine Deoxycholate agar plates and Brilliant Green agar plates then incubated at 37^{o}C±1^{o}C for 24 h ± 3 h according to ISO 6887-1:(2017).

3-2: Isolation *E. coli*:

A loopful of the homogenate (pre-enriched culture were added to Lauryl sulphate tryptose broth (LST) test tube and incubated at 35°C ± 0.5°C. A loopful of each positive cultured tube (turbid and gas production) was transferred to tube of *E. coli* medium, (EC) and incubated at 44.5°C for 48 ± 3 h examined each 24 ± 2 h for gas production. A loopful from positive culture of EC broth was streak on L-EMB agar plate and incubates for 18-24 h at 35°C ± 0.5°C according to FDA's, (2017).

4- Identification of microorganisms:

4-1: Biochemical identification:

4-1-1: Biochemical identification of *Salmonella* species:

Presumptive colony with a characteristic morphology of typical *Salmonella* species were subjected to biochemical identification according to ISO 6579-1: (2017).

4-1-2: Biochemical identification of *E. coli:*

The suspected typical colonies of *E. coli* on L-EMB media was conducted to Gram's staining, oxidase and catalase tests. Then the colonies were subjected to various biochemical tests (Hitchins *et al.,* 2001).

4-2: Serological identification of the isolates:

All biochemically identified *Salmonella* species and *E. coli* isolates were subjected to serologically identification.

4-2-1: Serological identification of *Salmonella* isolates:

Pure and primary culture plate of *Salmonella* species isolates were serotyped by slide agglutination test depending upon white-Kauffman-Le Minor scheme according to Grimont and Weill, 2007.

4-2-2: Serological identification of *E. coli* isolates:

Pure and primary culture plate of *E. coli* was agglutinated by slide agglutination test based on the presence of three principal surface antigens, O-antigens, flagellar H-antigens, and capsular K-antigens according to Ørskov and Ørskov (1984).

5-Antibiotic susceptibility testing:

All confirmed *Salmonella* serovars and *E. coli* serotypes were conducted to the antimicrobial susceptibility testing using the agar disk diffusion method and the interpretation of the results according to CLSI, (2013). All isolates were tested for susceptibility to 10 different antimicrobials agents as follows: ceftroiaxon (CRO) 30 µg; erythromycin (E) 15 µg; gentamicin (CN) 10µg; lioncomycin (MY) 10 µg; oxolinic acid (OA) 2 µg; oxytetracycline (OT) 30µg; penicillin G (P) 10 I.U; streptomycin (S) 10 µg; trimethoprim + sulphamethoxazole (SXT) (1.25 + 23.75) µg and vancomycin (VA) 30 µg.

6-Molecular study:

6-1: Conformation of *Salmonella* spp. and *E. coli* and their virulence and antibiotics resistance genes:

6-1-1: DNA extraction:
DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56°C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer.

6-1-2: Oligonucleotide Primer:
Primers used were supplied from Metabion (Germany) are listed in table (1).

Table 1: Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)				
				Secondary denaturation	Annealing	Extension	Final extension	Reference
E. coli phoA	CGATTCTGGAAAT GGCAAAAG CGTGATCAGCGGT GACTATGAC	720	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Hu *et al.*, (2011)**
Salmonella invA	GTGAAATTATCGC CACGTTCGGGCAA TCATCGCACCGTC AAAGGAACC	284	94˚C 5 min.	94˚C 30 sec.	55˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	**Oliveira *et al.*, (2003)**
blaTEM	ATCAGCAATAAAC CAGC CCCCGAAGAACGT TTTC	516	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Colom *et al.*, (2003)**
Aada1	TATCAGAGGTAGT TGGCGTCAT GTTCCATAGCGTT AAGGTTTCATT	484	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Randall *et al.*, (2004)**
ampC	TTCTATCAAMACT GGCARCC CCYTTTTATGTAC CCAYGA	550	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Lynne *et al.*, (2008)**
aacC	GGCGCGATCAAC GAATTTATCCGA CCATTCGATGCCG AAGGAAACGAT	48	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Lynne *et al.*, (2008)**
fimH	TGCAGAACGGAT AAGCCGTGG GCAGTCACCTGCC CTCCGGTA	508	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Ghanbar pour and Salehi, (2010)**
TraT	GATGGCTGAACCG TGGTTATG CACACGGGTCTGG TATTTATGC	307	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	**Kaipainen *et al.*, (2002)**
stn	TTG TGT CGC TAT CAC TGG CAA CC ATT CGT AAC CCG CTC TCG TCC	617	94˚C 5 min.	94˚C 30 sec.	59˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	**Murugkar *et al.*, (2003)**
bcfC	ACC AGA GAC ATT GCC TTC C TTC TGC TCG CCG CTA TTC G	467	94˚C 5 min.	94˚C 30 sec.	53˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Huehn *et al.*, (2010)**
mphA	GTGAGGAGGAGC TTCGCGAG TGCCGCAGGACTC GGAGGTC	403	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Nguyen *et al.*, (2009)**
gyrA	AAATCTGCCCGTG TCGTTGGT GCCATACCTACTG CGATACC	344	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	**Fàbrega *et al.*, (2009)**

6-1-3: PCR amplification:
Primers were utilized in 25 µl reactions containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentrations, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an applied biosystem 2720 thermal cycler.

6-1-4: Analysis of the PCR Products:
The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the products was loaded in each gel slot. Gelpilot 100 bp (Qiagen, Germany, GmbH) and gene ruler 100 bp ladder (Fermentas, Germany) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

6-1-5: DNA Sequence:
PCR products were purified using QIAquick PCR Product extraction kit. (Qiagen, Valencia). Big dye Terminator V3.1 cycle sequencing kit (Perkin-Elmer) was used for the sequence reaction and then it was purified using Centrisep spin column. DNA sequences were obtained by Applied Biosystems 3130 genetic analyzer (HITACHI, Japan), A BLAST® analysis (Basic Local Alignment Search Tool) (Altschul *et al.*, 1990) was initially performed to establish sequence identity to Gene Bank accessions.

6-1-6: Phylogenetic analysis:
The phylogenetic tree was created by the Meg Align module of Laser gene DNA Star (Thompson *et al.*, 1994) and Phylogenetic analyses was done using maximum likelihood, neighbor joining and maximum parsimony in MEGA6 (Tamura *et al.*, 2013).

RESULTS

Table 2: Prevalence of the isolated *Salmonella* species and *E. coli* isolated from the examined samples.

Types of samples	Samples number	Positive samples for *Salmonella* spp.		Positive samples for *E. coli*	
	No.	No.	%	No.	%
Milk	100	1	1	2	2
Feed	100	1	1	2	2
Milking equipment's swabs (milk tanks)	100	1	1	1	1
Drinking tanks Swabs	100	1	1	1	1
Fecal swabs	100	5	5	8	8
Total	500	9	1.8	14	2.8

Table 3: Prevalence of *Salmonella* serotyping (n=9) isolated from the examined samples.

Salmonella species	Total strain (9 in No.)	Examined samples									
		Milk		Feed		Milking equipment's swabs (milk tanks)		Drinking tanks swabs		Fecal swabs	
	No. (%)	No.	%	No.	%	No.	%	No.	%	No.	%
S. Typhimurium 1, 4, [5], 12:i: 1,2	6/9 (66.67)	1/6	16.67	1/6	16.67	1/6	16.67	0	0	3/6	50
S. Enteritidis 1, 9, 12: g, m :-	2/9 (22.22)	0	0	0	0	0	0	1/2	50	1/2	50
S. Saintipaul 1,4,[5],12:e,h: 1,2	1/9 (11.11)	0	0	0	0	0	0	0	0	1/1	100

Table 4: Prevalence of *E. coli* serotyping (n=14) isolated from the examined samples.

E. coli	Total strain (14 in No.)	Examined samples									
		Milk		Feed		Milking equipment's swabs (milk tanks)		Drinking tanks swabs		Fecal swabs	
	No. (%)	No.	%	No.	%	No.	%	No.	%	No.	%
O26	5/14 (35.71)	0	0	1/5	20.00	0	0	0	0	4/5	80.00
O119	2/14 (14.28)	0	0	0	0	1/2	50	0	0	1/2	50
O125	4/14 (28.60)	1/4	25.00	1/4	25.00	0	0	1/4	25.00	1/4	25.00
O126	1/14 (7.14)	0	0	0	0	0	0	0	0	1/1	100
O127	2/14 (14.28)	0	0	1/2	50	0	0	0	0	1/2	50

Table 5: Antimicrobial susceptibility pattern of *Salmonella* serovar (n=9) and *E. coli* (n= 14) recovered from the examined samples.

Antibiotic agents	Symbol	Concentration	*Salmonella* isolates (n=9)						*E. coli* isolates (n= 14)					
			Resistant		Intermediate		Sensitive		Resistant		Intermediate		Sensitive	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Ceftroiaxon	CRO	30 µg	9	100	0	0	0	0	14	100	0	0	0	0
Erythromycin	E	15 µg	5	55.56	0	0	4	44.44	3	21.42	1	7.14	10	71.42
Gentamicin	CN	10 µg	9	100	0	0	0	0	3	21.42	3	21.42	8	57.14
Lioncomycin	MY	10 µg	9	100	0	0	0	0	14	100	0	0	0	0
Oxolinic acid	OA	2 µg	8	88.89	1	11.11	0	0	14	100	0	0	0	0
Oxytetracycline	OT	30 µg	3	33.33	5	55.56	1	11.11	5	35.71	9	64.29	0	0
Penicillin G	P	10 I.U	7	77.78	2	22.22	0	0	14	100	0	0	0	0
Streptomycin	S	10 µg	8	88.89	1	11.11	0	0	7	50.00	0	0	7	50.00
Trimethoprim + Sulphamethoxazole	SXT	1.25+ 23.75 µg	2	22.22	6	66.67	1	11.11	2	14.30	11	78.57	1	7.14
Vancomycin	VA	30 µg	9	100	0	0	0	0	12	85.71	2	14.30	0	0

Table 6: Prevalence of confirmatory genes among *Salmonella* (n=9) and *E. coli* (n= 14) strains isolated from the examined samples

	Salmonella serovars (n=9)	*E. coli* serovars (n= 14)
Confirmatory genes	*invA* gene	*PhoA* gene
No. of detected genes	9/9	14/14
%	100	100

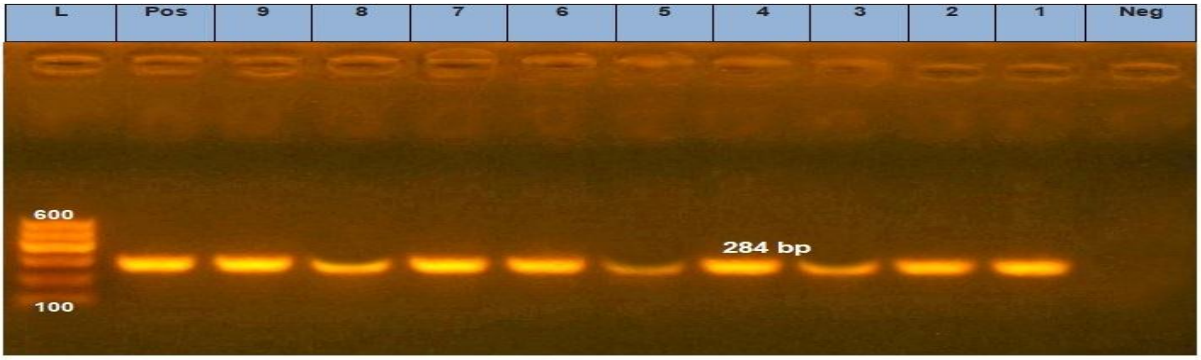

Figure (1): Agarose gel electrophoresis of PCR products after amplification of: 1- *invA* gene for *Salmonella* strains, MWM-molecular weight marker (100 – 600 bp DNA ladder), control (Positive, Negative) and different strains of *Salmonella* species. (*invA* gene products at 284 bp).

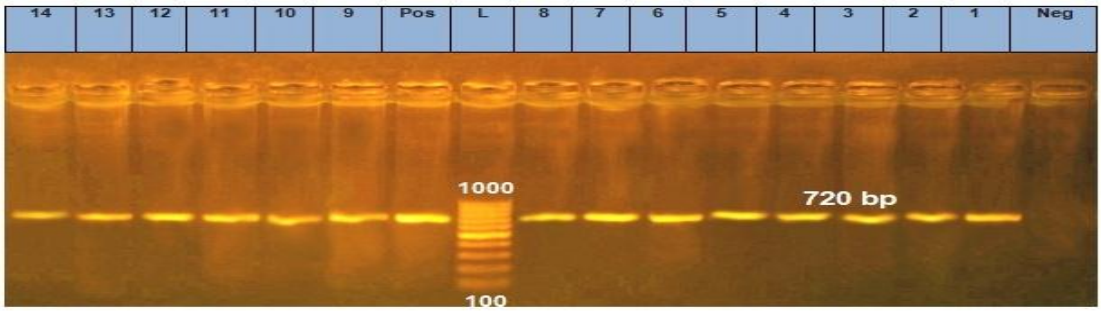

Figure (2): Agarose gel electrophoresis of PCR products after amplification of: 1- *phoA* gene for *E. coli* strains, MWM-molecular weight marker (100 – 1000 bp DNA ladder), control (Positive, Negative) and different strains of *E. coli*. (*phoA* gene products at 720 bp).

Table 7: Prevalence of some virulence genes among *Salmonella* strains (n=9) isolated from the examined samples.

Salmonella strains	Sample No.	Total strains	No. of detected genes		
			invA	*stn*	*bcfC*
***S.* Typhimurium 1, 4, [5], 12:i: 1,2**	**2-3-4-5-6-9**	**6/9 (66.67%)**	**6/6 (100%)**	**6/6 (100%)**	**6/6 (100%)**
***S. Enteritidis* 1, 9, 12: g, m :-**	**7-8**	**2/9 (22.22%)**	**2/2 (100%)**	**2/2 (100%)**	**2/2 (100%)**
***S. Saintipaul* 1,4,[5],12:e,h: 1,2**	**1**	**1/9 (11.11%)**	**1/1 (100%)**	**1/1 (100%)**	**1/1 (100%)**
Total detected gene	**No.**	**9/9**	**9/9**	**9/9**	**9/9**
	%	**(100%)**	**(100%)**	**(100%)**	**(100%)**

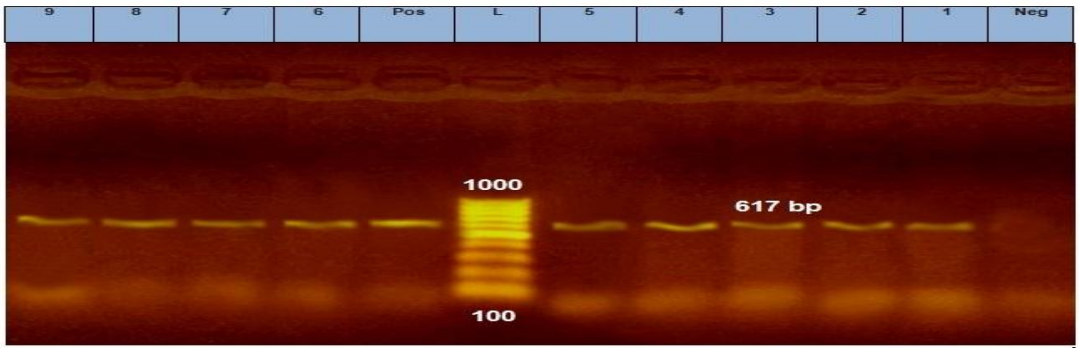

Figure (3): Agarose gel electrophoresis of PCR products after amplification of: 1- *stn* gene for *salmonella strain*s, MWM-molecular weight marker (100 – 1000 bp DNA ladder), control (Positive, Negative) and different strains of *Salmonella* (*stn* gene products at 617 bp).

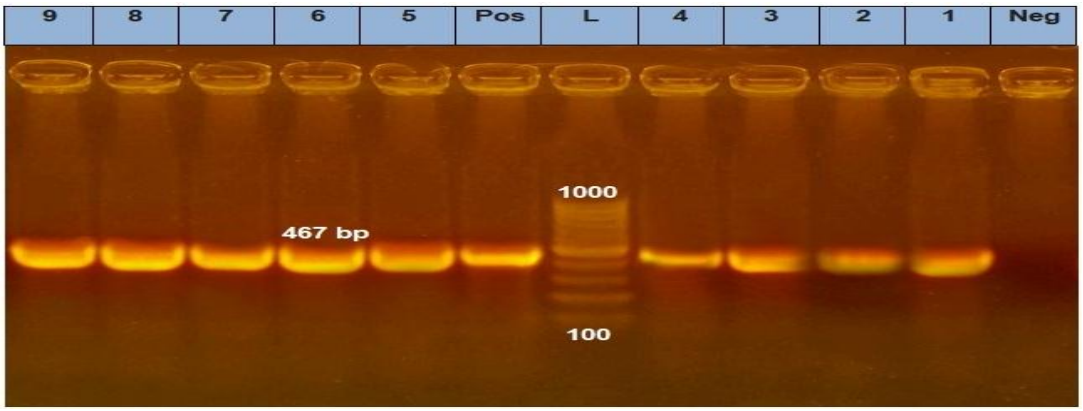

Figure (4): Agarose gel electrophoresis of PCR products after amplification of: 1- *bcfC* gene for *salmonella strain*s, MWM-molecular weight marker (100 – 1000 bp DNA ladder), control (Positive, Negative) and different strains of *Salmonella* (*bcfC* gene products at 467 bp).

Table 8: Prevalence of some virulence genes among *E. coli* strains (n=14) isolated from the examined samples.

E. coli strain	Sample No.	Total strains	No. of detected genes		
			phoA	*TraT*	*fimH*
O26	3-6-11-12-13	5/14 (35.71%)	5/5 (100%)	5/5 (100%)	5/5 (100%)
O119	7-14	2/14 (14.28%)	2/2 (100%)	2/2 (100%)	2/2 (100%)
O125	4-5-9-10	4/14 (28.57%)	4/4 (100%)	4/4 (100%)	4/4 (28.57%)
O126	2	1/14 (7.14%)	1/1 (100%)	1/1 (100%)	1/1 (7.14%)
O127	1-8	2/14 (14.28%)	2/2 (100%)	2/2 (100%)	2/2 (14.28%)
Total detected gene	No.	14/14	14/14	14/14	14/14
	%	(100%)	(100%)	(100%)	(100%)

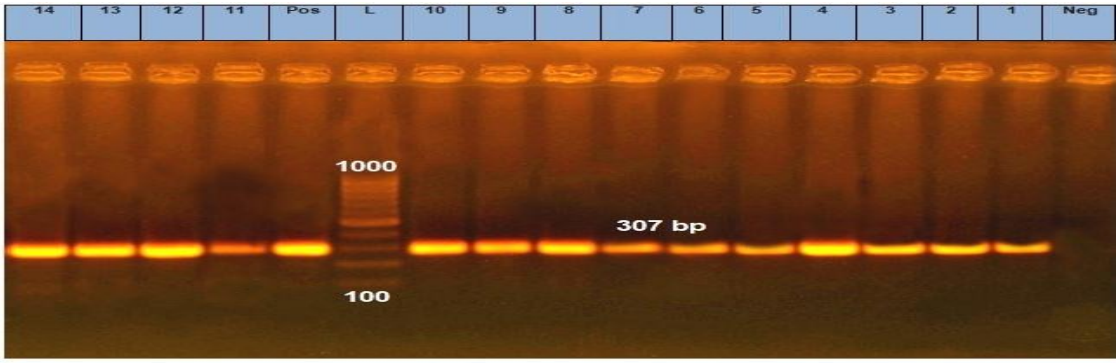

Figure (5): Agarose gel electrophoresis of PCR products after amplification of: 1- *TraT* gene for *E. coli* strains, MWM-molecular weight marker (100–1000 bp DNA ladder), control (Positive, Negative) and different strains of *E. coli*. (*TraT* gene products at 307 bp).

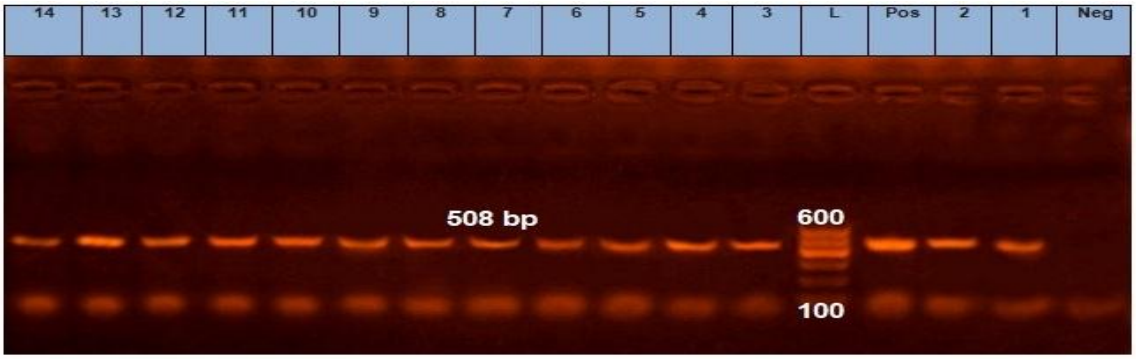

Figure (6): Agarose gel electrophoresis of PCR products after amplification of: 1- *fimH* gene for *E. coli* strains, MWM-molecular weight marker (100 – 600 bp DNA ladder), control (Positive, Negative) and different strains of *E. coli*. (*fimH* gene products at 508 bp).

Table 9: Prevalence of resistance genes among *Salmonella* strains (n=9) isolated from the examined samples.

Salmonella strains	Sample No.	Total strains	No. of detected genes		
			ampC	*mphA*	*aacC*
S. Typhimurium 1, 4, [5], 12:i: 1,2	2-3-4-5-6-9	6/9 (66.67%)	6/6 (100%)	3/6 (50%)	6/6 (100%)
S. Enteritidis 1, 9, 12: g, m :-	7-8	2/9 (22.22%)	2/2 (100%)	1/2 (50%)	2/2 (100%)
S. Saintipaul 1,4,[5],12:e,h: 1,2	1	1/9 (11.11%)	1/1 (100%)	1/1 (100%)	1/1 (100%)
Total detected gene	No.	9/9	9/9	5/9	9/9
	%	(100%)	(100%)	(55.56%)	(100%)

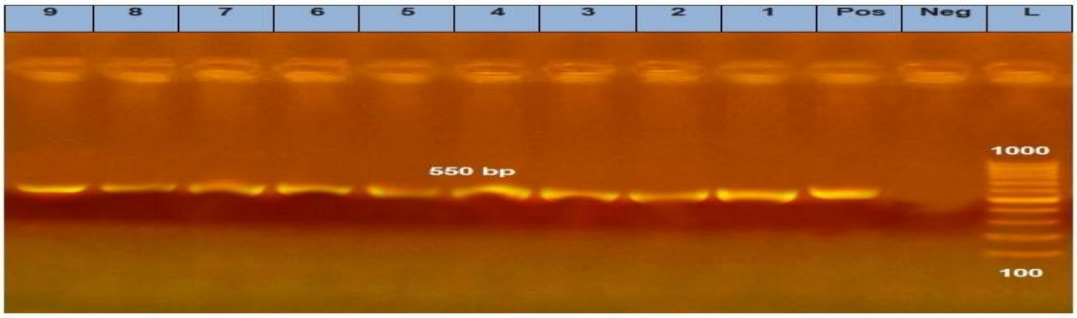

Figure (7): Agarose gel electrophoresis of PCR products after amplification of: 1- *ampC* gene for *salmonella strains*, MWM-molecular weight marker (100 – 1000 bp DNA ladder), control (Positive, Negative) and different strains of *Salmonella* (*ampC* gene products at 550 bp)

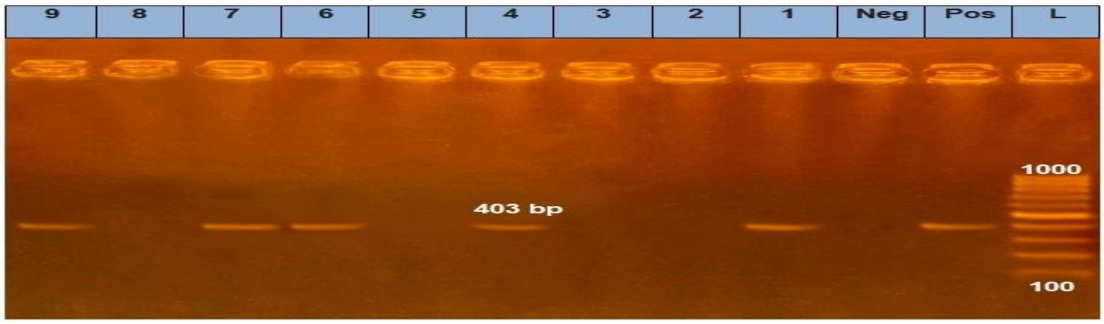

Figure (8): Agarose gel electrophoresis of PCR products after amplification of: 1- *mphA* gene for *salmonella strains*, MWM-molecular weight marker (100 – 1000 bp DNA ladder), control (Positive, Negative) and different strains of *Salmonella* (*mphA* gene products at 403 bp).

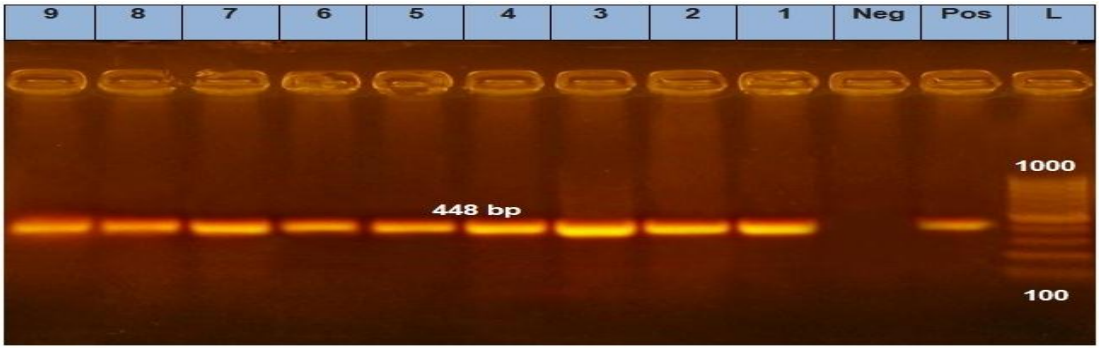

Figure (9): Agarose gel electrophoresis of PCR products after amplification of: 1- *aacC* gene for *salmonella strains*, MWM-molecular weight marker (100 – 1000 bp DNA ladder), control (Positive, Negative) and different strains of *Salmonella* (*aacC* gene products at 448 bp).

Table 10: Prevalence of some resistance genes among *E. coli* strains (n=14) isolated from the examined samples.

E. coli serovars	Sample No.	Total serovar	No. of detected genes				
			blaTEm	*ampC*	*mphA*	*Aada1*	*aacC*
O26	3-6-11-12-13	5/14 (35.71%)	5/5 (100%)	5/5 (100%)	0/5 (0.00%)	3/5 (60%)	1/5 (20%)
O119	7-14	2/14 (14.28%)	2/2 (100%)	2/2 (100%)	0/2 (0.00%)	1/2 (50%)	1/2 (50%)
O125	4-5-9-10	4/14 (28.57%)	4/4 (100%)	4/4 (100%)	3/4 (75%)	2/4 (50%)	0/4 (0.00%)
O126	2	1/14 (7.14%)	1/1 (100%)	1/1 (100%)	0/1 (0.00%)	0/1 (0.00%)	0/1 (0.00%)
O127	1-8	2/14 (14.28%)	2/2 (100%)	2/2 (100%)	0/2 (0.00%)	1/2 (50%)	1/2 (50%)
Total detected genes	No.	14/14 (100%)	14/14 (100%)	14/14 (100%)	3/14 (21.43%)	7/14 (50%)	3/14 (21.43%)
	%						

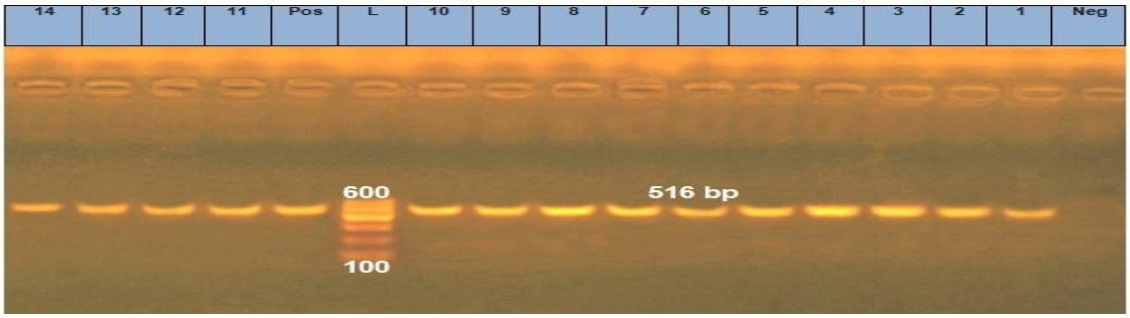

Figure (10): Agarose gel electrophoresis of PCR products after amplification of: 1- *blaTEm* gene for *E. coli* strains, MWM-molecular weight marker (100 – 600 bp DNA ladder), control (Positive, Negative) and different strains of *E. coli* (*blaTEm* gene products at 516 bp).

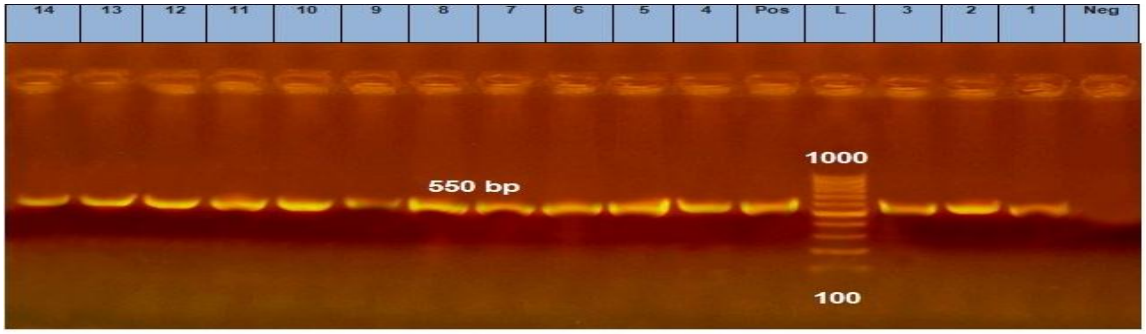

Figure (11): Agarose gel electrophoresis of PCR products after amplification of: 1- *ampC* gene for *E. coli* strains, MWM-molecular weight marker (100 – 1000 bp DNA ladder), control (Positive, Negative) and different strains of *E. coli* (*ampC* gene products at 550 bp).

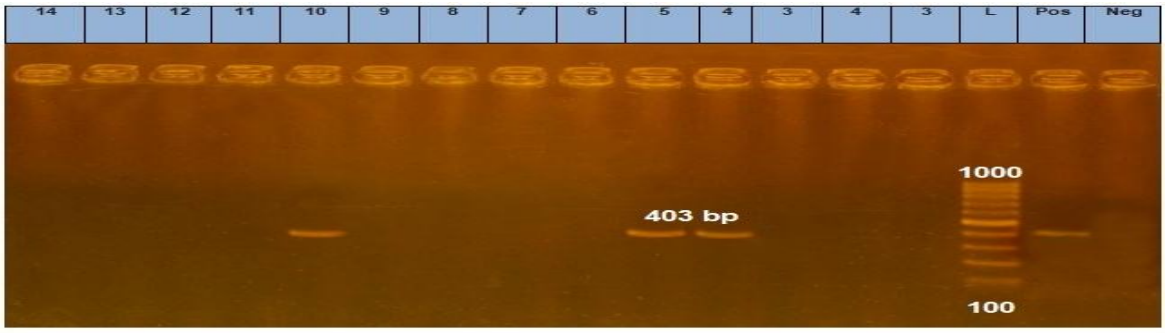

Figure (12): Agarose gel electrophoresis of PCR products after amplification of: 1- *mphA* gene for *E. coli* strains, MWM-molecular weight marker (100 – 1000 bp DNA ladder), control (Positive, Negative) and different strains of *E. coli* (*mphA* gene products at 403 bp).

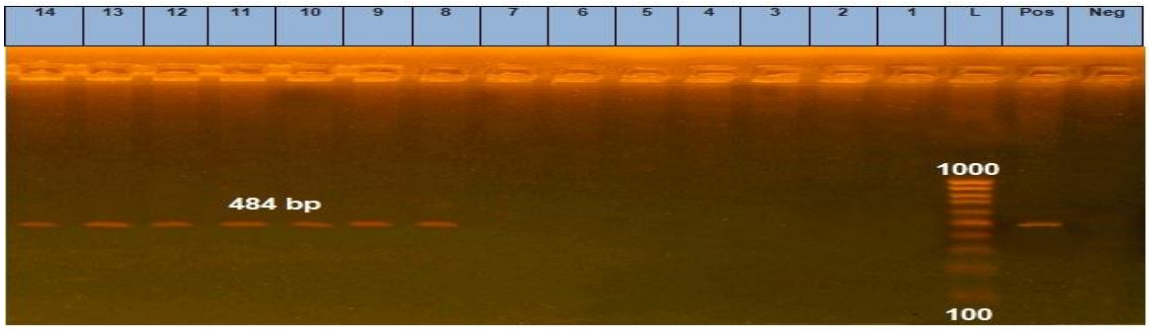

Figure (13): Agarose gel electrophoresis of PCR products after amplification of: 1- *Aada1* gene for *E. coli* strains, MWM-molecular weight marker (100 – 1000 bp DNA ladder), control (Positive, Negative) and different strains of *E. coli* (*Aada1* gene products at 484 bp).

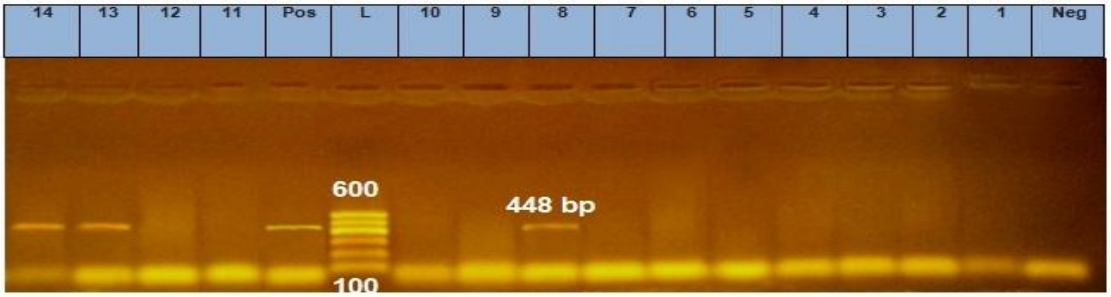

Figure (14): Agarose gel electrophoresis of PCR products after amplification of: 1- *aacC* gene for *E. coli* strains, MWM-molecular weight marker (100 – 600 bp DNA ladder), control (Positive, Negative) and different strains of *E. coli* (*aacC* gene products at 448 bp).

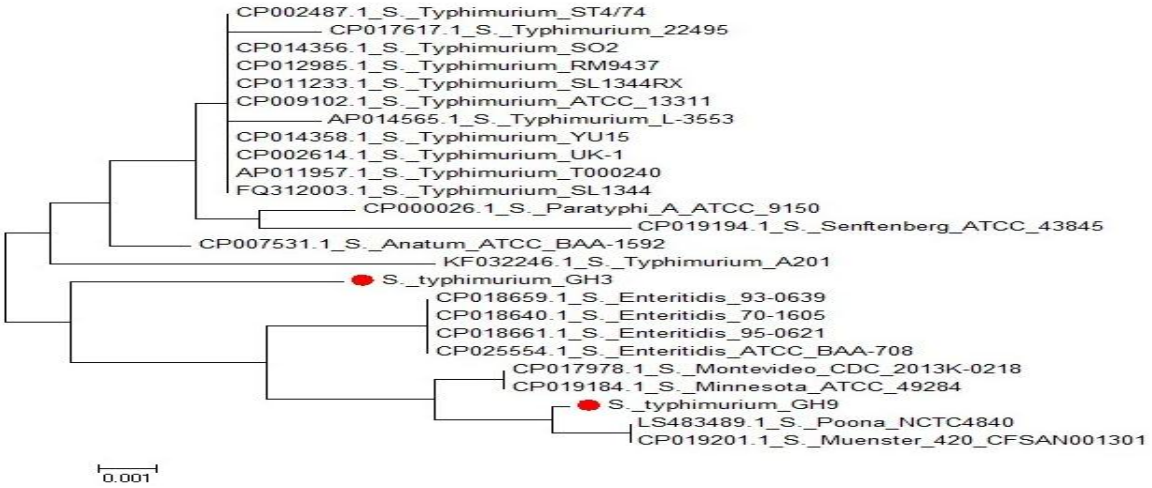

Figure (15): Phylogenic diversity tree for *stn* gene amino acids sequence of *S.* Typhimurium GH3 (sample 3) isolated from milk of cattle and *S.* Typhimurium GH9 (sample 9) isolated from fecal swabs of cattle with 23 of the most similar *stn* gene amino acid sequences from Gene bank.

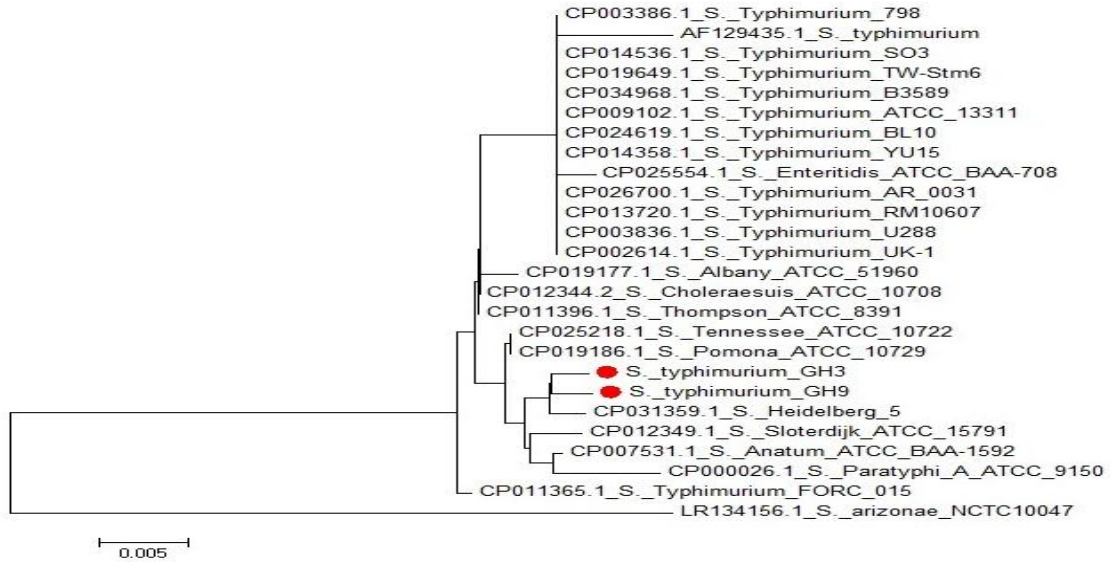

Figure (16): Phylogenic diversity tree for *bcfC* gene amino acids sequence of *S.* Typhimurium GH3 sample 3) isolated from milk of cattle and *S.* Typhimurium GH9 (sample 9) isolated from fecal swabs of cattle with 24 of the most similar *bcfC* gene amino acid sequences from Gene bank.

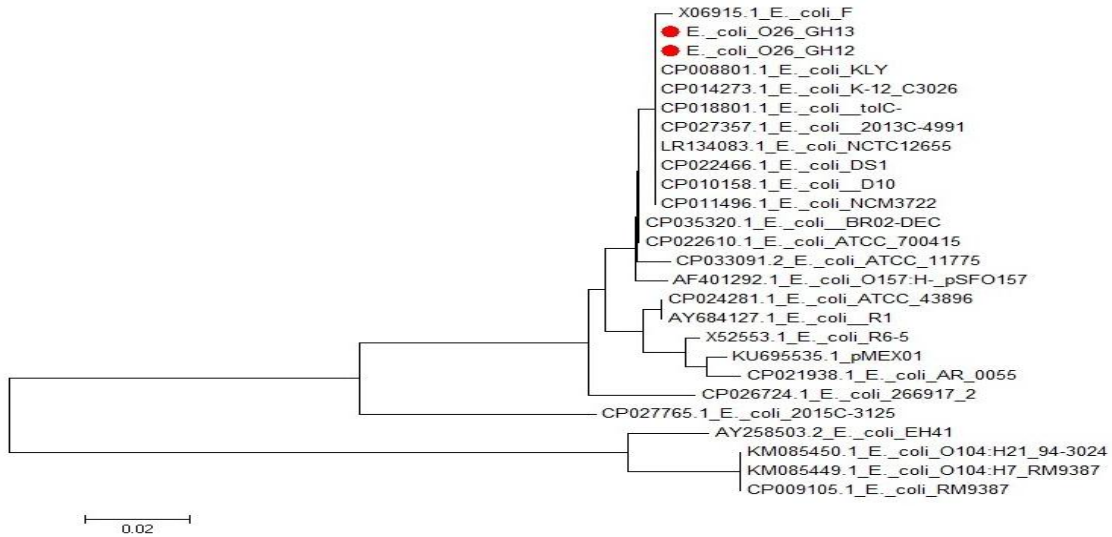

Figure (17): Phylogenic diversity tree for *TraT* gene amino acids sequence of *E. coli* O26 GH12 (sample 12) isolated from fecal swab of cattle and *E. coli* O26 GH13 (sample 13) isolated from fecal swab of cattle with 24 of the most similar *TraT* gene amino acid sequences from Gene bank.

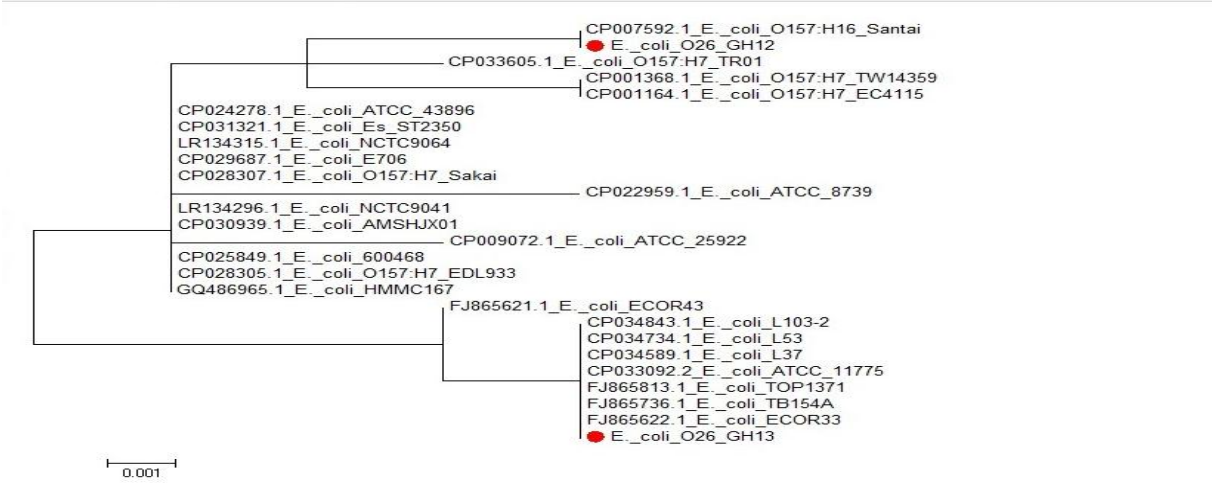

Figure (18): Phylogenic diversity tree for *fimH* gene amino acids sequence of *E. coli* O26 GH12 (sample 12) isolated from fecal swab of cattle and *E. coli* O26 GH13 (sample 13) isolated from fecal swab of cattle with 24 of the most similar *fimH* gene amino acid sequences from Gene bank.

Table 11: Nucleotide change in *gyrA* gene of two isolates of *S.* Typhimurium.

Strain no.	Serovar	Source	Nucleotide change at *gyrA* Positions (Mutation point)		
			83 TCC [Ser]	87 GAC [Asp]	179 TCC [Alar]
2	***S.* Typhimurium GH2**	**Feed**	TTC [Phe]	None	None
4	***S.* Typhimurium GH4**	**Fecal**	TTC [Phe]	None	None

Table (12): Nucleotide change in *gyrA* gene of two isolates of *E. coli.*

Strain no.	Serovar	Source	Nucleotide change at *gyrA* Positions (Mutation point)		
			83 TCG [Ser]	87 GAC [Asp]	179 TCC [Alar]
3	***E. coli* O26 GH3**	**Feed**	TTC [Leu]	None	None
11	***E. coli* O26 GH11**	**Fecal**	TTC [Leu]	None	None

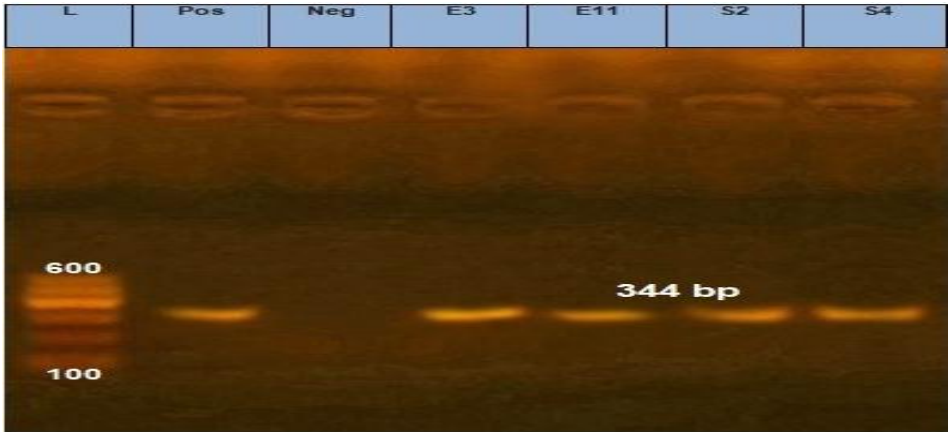

Figure (19): Agarose gel electrophoresis of PCR products after amplification of: 1- *gyrA* gene for *S.* Typhimurium (No. 2 and 4) *and E. coli* O26 (No. 3 and 11) strains, MWM-molecular weight marker (100 – 600 bp DNA ladder), control (Positive, Negative) and *gyrA* gene products at 344 bp).

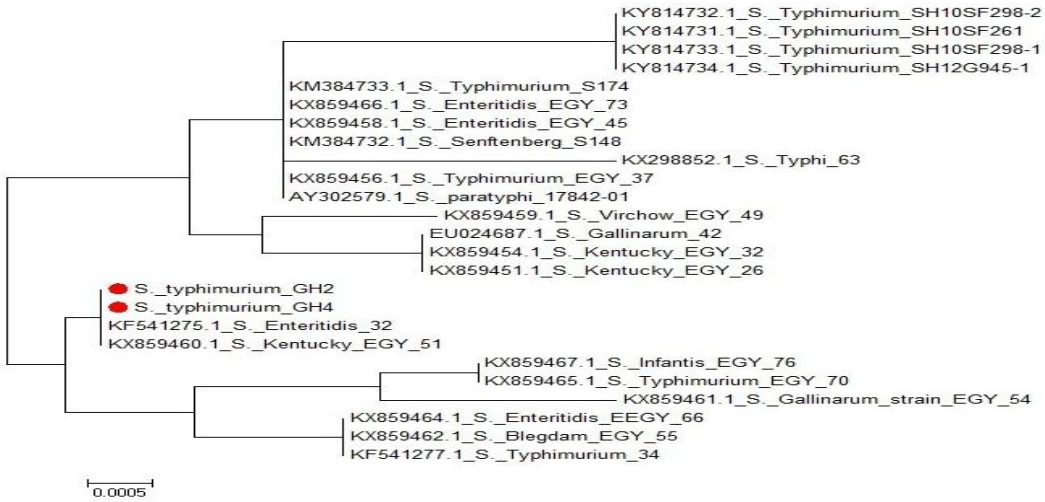

Figure (20): Phylogenic diversity tree for *gyrA* gene amino acids sequence of *S.* Typhimurium GH2 (sample 2) isolated from feed sample and *S.* Typhimurium GH4 (sample 4) isolated from fecal swab of cattle with 23 of the most similar *gyrA* gene amino acid sequences from Gene bank.

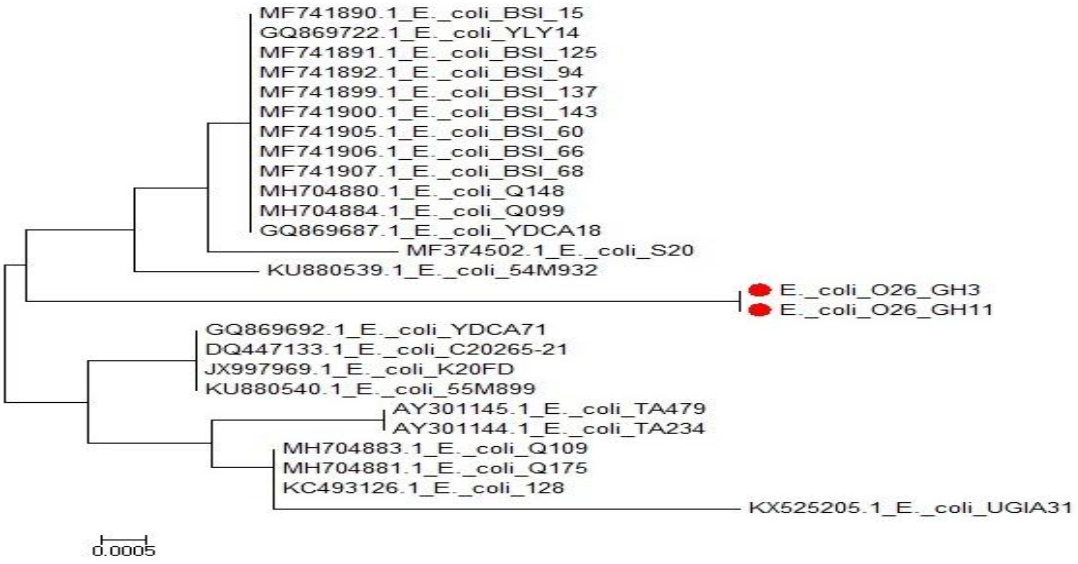

Figure (21): Phylogenic diversity tree for *gyrA* gene amino acids sequence of *E. coli* O26GH3 (sample 3) isolated from fecal swab of cattle and *E. coli* O26 GH11 (sample 11) isolated from feed sample with 23 of the most similar *gyrA* gene amino acid sequences from Gene bank.

DISCUSSION

For long term milk production with hygienic measures**,** dairy cattle should be in a good health condition (Godinho and Carvalho, 2013).

The results in Table (2) showed that the isolated *Salmonella* species and *E. coli* were found with a percentage of 1.8% and 2.8% in the total examined samples respectively. On the other hand *Salmonella* species in each of the examined milk, feed, milk tanks swabs, drinking tanks swabs and fecal swabs samples were found with an incidence of 1%, 1%, 1%, 1% and 5%, respectively, while that of *E. coli* were 2%, 2%, 1%, 1% and 8%, respectively. These results were approximately agreed with the result recorded by Halimi *et al.* (2014) and Warnick *et al.* (2003) who found the incidence of *Salmonella* spp. was 1.5% and 1.1%, respectively, meanwhile lower than that recorded by each of Wells *et al.* (2001) who found that *salmonella* species were isolated from fecal and milk samples with an incidence 5.4% and 21.1%, respectively, that of El-Gedawy *et al.* (2014) who found that the incidence of *Salmonella* spp. in bulk tank milk and milking equipment were 9% and 6%, respectively and that of Sotohy and Khalifa (2018) who found that the incidence of the isolated *Salmonella* species from dairy farms was 3.2%. The lower incidence may be attributed to the sample may contain other organisms that may compete with *Salmonella* (Karns *et al.*, 2005). On the other hand the results were higher than that recorded by Halimi *et al.* (2014) who found that no *Salmonella* species recovered from water, feed, milk filers, and milk fed to calves. The variation between our results and that of other author's may be referred to the differences in the survival of *Salmonella* spp. in water, soil and pasture depending up on the differing serovars, dose rates and environmental conditions whereas *Salmonella* spp. can survive for up to 20 weeks in soil and water (Guan and Holley, 2003). In case of *E. coli*, our results were lower than that recorded by Abd El- Tawab *et al.* (2017) who found that the incidence of *E. coli* in milk collected from different localities in Egypt was 6.2% and this may be attributed to the variation in samples types whereas our samples from apparently healthy while the other sample from mastitic milk and that recorded by Maity *et al.* (2010) who found the incidence of *E. coli* in fecal sample was 27.91% and the potential EPEC was 21.66%.

Serological results showed in Table (3 and 4) revealed that *Salmonella* species were serotyped as *S.* Typhimurium, *S. Enteritidis* and *S. Saintipaul* and found with a percentage of 66.67%, 22.22% and 11.11% from the total isolated *Salmonella* species, respectively. *S.* Typhimurium was the predominant serotype and found in milk, feed, milk tanks swabs and the fecal swabs samples with an incidence of 16.67%, 16.67% and 50% respectively. The serotyped *S. Enteritidis* was found in drinking tanks and fecal samples with an incidence 50% and 50% respectively, while *S. Saintipaul* was found with an incidence of 100% in the fecal samples only. The results of the isolated *S. Saintpaul* in our results were lower than that recorded by Sotohy and Khalifa (2018) who isolates 2 strain of *S. Saintpaul*, one from air 4% and one from manure 2.9%. *S.* Typhimurium was the predominates serotypes found with an incidence 66.67% and this attributed to the ability of *S.* Typhimurium can survive up to 28 weeks on pasture (Josland, 1951). *E. coli* isolates were serotyped to O26, O119, O125, O126 and O127 with an incidence 35.71%, 14.28%, 28.60%, 7.14% and 14.28%, respectively. This serotyped *E. coli* considered members of the enteropathogenic *E. coli* and this agree with the classification performed by WHO, (1987). The most predominant serotyped were O26 which found with an incidence of 80% and 20% in the fecal and feed samples respectively, followed by O125 which present with a percentage 25% for each of milk, feed, drinking tanks and fecal samples. The incidence of O119 was 50% for each of the examined milk tanks and fecal samples, while O127 was typed with an incidence of 50% for each of feed and fecal samples. The lowest incidence was O126 which present in the fecal samples only with an incidence of 100%. Our results of *E. coli* serotyping were differed than results recorded by Sayed, (2014) who found that *E. coli* isolates were 18 strains (17.82%) from 101 clinical mastitic milk samples of cows and serotyped to nine different serogroups; O111:H4 (3), O127:H6 (3), O26 (2), O126 (2), O119:H6 (1), O114:H21 (1), O55:H7 (1), O44:H18 (1), O124 (1) and (3) untyped. Also differed than that of Abd El-Tawab *et al.* (2017) who found that *E. coli* serotypes were 15 typed as O27, O146, O125, O126, O111, O20 and O157 and 2 untyped. This variation was attributed the difference in in their natural reservoir (Foley *et al.*, 2008).

By agar disk diffusion method, antibiotic sensitivity test were applied against the isolated *Salmonella* (n=9) and *E. coli* (n= 14) strains and recorded in Table (5). The results revealed that *Salmonella* and *E. coli* strains have led to development of resistance to antimicrobial agents which originally effective against the examined microbes. *Salmonella* strains showed 100% resistance against ceftroiaxon; gentamicin; lioncomycin and vancomycin. Also *Salmonella* strains showed a resistance against oxolinic acid; penicillin G and streptomycin; with an incidence 88.89%, 77.78% and 88.89%, respectively. While the incidence of resistance *Salmonella* strains against erythromycin; oxytetracycline; trimethoprim + sulphamethoxazole were 55.56%, 33.33% and 22.22%, respectively. Our results showed multi-drugs resistance as recorded by Halimi *et al.* (2014) and agree with the results recorded by Tamba *et al.* (2016) who found

that all isolates of *Salmonella* showed 100% resistance to lincomycin. Also our resistance results of *Salmonella* species against oxytetracycline were lower than that recorded by Halimi *et al.* (2014) who found that 94.74% of *salmonella* species were resistance to oxytetracycline and that of Tamba *et al.* (2016) who recorded that 85.71% of *Salmonella* species were resistance to erythromycin. On the other hand, our results were higher than that of Mohamed *et al.* (2011) who found that 14.3% of *Salmonella* strains were resistant to gentamycin, that of Wells *et al.* (2001) who recorded that 0.1% of *Salmonella* strains were resistant to gentamycin. The difference between our results and the results recorded by Halimi *et al.* (2014) come back to the differences between farms in the frequency of usages, widespread and inappropriate usage of oxytetracycline in dairy operations and dairy farms and association with fecal shedding.

Also the resistance of *E. coli* strain against ceftroiaxon; lioncomycin; oxolinic acid; penicillin G and vancomycin were highly resistance and found with incidence 100 %, 100 %, 100 %, 100 % and 85.71%, respectively, while the resistance against erythromycin; gentamicin; oxytetracycline; streptomycin and trimethoprim + sulphamethoxazole were 21.42 %, 21.42 %, 35.71%, 50.00% and 14.30 %, respectively. Our results showed multi-drugs resistance as recorded by Abd El- Tawab *et al.* (2017) and Yassin *et al.* (2017). On the other hand our results varied with the results recorded by other authors whereas my results were lower than that of Abd El- Tawab *et al.* (2017) who found that the incidence of resistance against gentamicin; oxytetracycline and streptomycin were 30%, 70% and 100%, respectively, while a higher than the resistance against penicillin G (80%) recorded by Abd El- Tawab *et al.* (2017) and also higher than that recorded by Yassin *et al.* (2017) who found that the incidence of resistance against gentamicin; streptomycin; ceftroiaxon and trimethoprim + sulphamethoxazole were 8.2%, 18.0%, 4.9% and 18.0%, respectively. The variation in the incidence of resistance against antibacterial between our results and the results of other authors were referred to the variation between the use and misuse of antimicrobials in farm animals (Sawant *et al.*, 2007).

All serotyped *Salmonella* (No. =9) and *E. coli* (No. =14) were conducted for molecular characterization by conventional PCR Table (6) and Fig. (1and2). Firstly, confirmation of *Salmonella* and *E. coli* applied by detection of *invA* gene (at 284 bp) and *phoA* gene (at 720 bp) which were found with an incidence 100% and 100%, respectively. These results agree with results reported by Sotohy and Khalifa (2018) who found that *invA* (at284 bp) virulence and conformity gene was found in all isolated *Salmonella* strains. In this study *invA* gene was used as confirmatory genes for genus *Salmonella* due to the *invA* gene of *Salmonella* species contains unique sequences to this genus and has been proved to be a suitable PCR target with potential diagnostic application (Jamshidi *et al.*, 2008). *invA* gene is recognized as an international standard for detection of *Salmonella* genus (Malorny *et al.*, 2003). On the other hand *phoA* genes was selected to confirm the detection of *E. coli* strains and this results agree with result recorded by Kong *et al.* (1995); Kong *et al.* (1999) and Yu and Thong, (2009) who performed the confirmation of *E. coli* by detection of *phoA* gene at 720 bp. which present in all *E. coli* strains.

The results in Table (7) and Fig. (1, 3 and 4) showed that the incidences of each of the studied *invA* (at 284 bp), *stn* (at 617 bp) and *bcfC* (at 467 bp) virulence genes were detected in 100% of each of the isolated *S.* Typhimurium, *S. Enteritidis and* S. *Saintipaul*). The incidence of each of *invA* gene, *stn* gene and *bcfC* gene in all isolated *Salmonella* species were 66.67%, 22.22% and 11.11%, for *S.* Typhimurium, *S. Enteritidis* and S. *Saintipaul* respectively. Our results showed that the virulence *invA*, *stn* gene and *bcfC* gene were detected in all of the isolated *salmonella* strains and this was disagree with results recorded by Sotohy and Khalifa (2018) who found only *invA* (at 284 bp) virulent gene was detected in *S. Saintipaul*. This variation was attributed the difference in their natural reservoir (Foley *et al.*, 2008).

Our results of *stn* gene were higher than that of Maysa and Abd-Elall (2015) who found that *stn* were detected in *S.* Typhimurium and *S. Enteritidis* with incidence of 78.9% and 75%, respectively. Our results of *bcfC* were agree with the results recorded by Maysa and Abd-Elall (2015) who found that *bcfC* was detected in 100% of *S. Enteritidis*, while higher than that of Maysa and Abd-Elall (2015) who found *bcfC* in 88.9% of the isolated *S.* Typhimurium. The variation between our results and results recorded by other authors were regarded to widely distribution of the microorganisms among animals, humans and environment and some diversity in distribution could be explained by serovar specificity of virulence plasmid (Heithoff *et al.*, 1997 and Rotger and Casadesus, 1999).

The studied *phoA* (at 720 bp), *TraT* (at 307 bp) and *fimH* (at 508 bp) virulence genes of the isolated *E. coli* present in Table (2) and Fig. (2, 5 and 6) were detected in 100% of each the isolated *E. coli* serotypes. Each of *phoA* gene, *TraT* gene and *fimH* gene were detected in all serotyped *E. coli* with incidences 35.71%, 14.28%, 28.57%, 7.14% and 14.28%, for O26, O119, O125, O126 and O127 respectively. The *phoA* gene (at 720 bp) was detected in *E. coli* strains and this result agrees with result recorded by Hu *et al.* (2011) and Alnahass *et al.* (2016).

Our results of the detection of *TraT* gene were higher than that recorded by each of Ashraf *et al.* (2018) who found that the incidence of *TraT* in the isolated *E. coli* was 66%, that of Nemeth *et al.* (1991) who found that the incidence of *TraT* in the isolated *E. coli* from mastitic milk and milk filler samples were 43% and 40%, respectively and that recorded by Mahmoud *et al.* (2015) who found that the incidence of *TraT* gene was 25%. Our results of *fimH* gene agree with the results recorded by Fernandes *et al.* (2011) and Abd El-Tawab *et al.* (2017) who found that the incidence of *fimH* in all strains of the isolated *E. coli* were 100%. The incidence of *fimH* in O125 and O126 were agreed with the results recorded by Abd El- Tawab *et al.* (2017). Our results were higher than the results recorded by Bronzato *et al.* (2017) who detected *fimH* with incidence 77.7% in isolated *E. coli* strain. This variation may be due to difference in the percentage of dispersion of microorganism in the dairy farm environment and horizontal gene transfer (Madsen *et al.*, 2012).

The detected resistance genes in Table (9) and Fig. (7, 8 and 9) showed that each *ampC* gene (at 550 bp) and *aacC* gene (at 448 bp) were detected in 100% of each of the isolated *S.* Typhimurium, *S. Enteritidis* and S. *Saintipaul*, while *mphA* gene (at 403 bp) was detected in each of *S.* Typhimurium, *S. Enteritidis* and S. *Saintipaul*, with an incidence 50%, 50% and 100% respectively. The incidences of each *ampC* gene and *aacC* gene in all isolated *Salmonella* species were 66.67%, 22.22% and 11.11%, for *S.* Typhimurium, *S. Enteritidis* and S. *Saintipaul* respectively, while *mphA* gene was detected in all *Salmonella* strain with an incidence 55.56%. Our result for resistance *mphA* gene (at 403 bp) for the isolated *Salmonella* strain were higher than that recorded by Wang *et al.* (2017) who detected the *mphA* gene with a percentage of 48.39% of resistant *Salmonella* isolates and that of Abdel Aziz *et al.* (2018) who found that the *mphA* resistance gene cassette was detected in 41.7% of isolated *salmonella* showed multidrug resistance. Also our results of *ampC* gene (at 550 bp) was higher than that recorded by Zhao *et al.* (2008) who found that *ampC* resistance gene was detected in *Salmonella* species isolated from ground turkey meat and chicken breast with a percentage of 46.67% and 11.11%, respectively. Also higher than that cited by Public Health Agency of Canada (2007) whereas *ampC* resistance gene was detected in approximately 30% of *salmonella* isolates in 2003 and the prevalence was gradually increased to approximately 48% in the second quarter of 2005. The results of *aacC* gene (at 448 bp) was higher than that of Randall *et al.* (2004) and Lynne *et al.* (2008) who recorded that *aacC* was detected in 71.43 % and 42.90% (3) isolates, respectively.

While the detected resistance genes of *E. coli* in Table (10) and Fig. (10, 11, 12, 13 and 14) showed that *blaTEm* gene (at 516 bp) and *ampC* gene (at 550 bp) resistance genes were found in 100% of each the isolated *E. coli* serotypes while that of *mphA* gene (at 403 bp), *Aada1* gene (at 484 bp) and *aacC* gene (at 448 bp) resistance genes were found in 21.43%, 50.00% and 21.43%, respectively. Each of *blaTEm* gene and *ampC* gene resistance genes were detected in each of O26, O119, O125, O126 and O127 with an incidence 35.71%, 14.28%, 28.57%, 7.14% and 14.28%, respectively, while *mphA* gene was detected only in O125 with incidence of 75%. Also the results revealed that *Aada1* resistance gene was detected in O26, O119, O125 and O127 with an incidence of 60%, 50%, 50% and 50%, respectively. Meanwhile *aacC* gene was found with incidence of 20%, 50% and 50% in O26, O119 and O127 respectively. Our results agree with results recorded by Hussein *et al.* (2008) who found *mphA* resistance genes in a percentage 100% of the isolated *E. coli*. Meanwhile the results of the resistance *blaTEm* gene (at 516 bp), *ampC* gene (at 550 bp) and *Aada1* genes (at 484 bp) were higher than that recorded by Ashraf *et al.* (2018) who found that the *blaTEm*, *ampC* and *Aada1* genes were detected with a percentage of 4% and 26% and 12% of the isolated *E. coli* respectively. Also higher than that of Hinthong *et al.* (2017) who detected *blaTEm*, *Aada1* and *aacC* gene with a percentage of 61.3%, 3.3% and 4.9%, respectively and that of Wassef *et al.* (2014) who detected the *ampC* gene in the isolated *E. coli* with a percentage 66.7%. The variation between results may regards to geographical discrepancy in *ampC* β-lactamase types Pai *et al.* (2004). In general the variation between results was regarded to the dissemination of strains carrying resistance genes for antimicrobials whereas the antimicrobial drugs as aminoglycosides, beta-lactams, tetracycline chloramphenicol, sulfonamides, and trimethoprim has been acquired by *E. coli* strains from other microorganisms (Lietzau *et al.*, 2006).

In Fig. (15 and 16), DNA Sequence was initially performed to establish sequence identity to Gene Bank accessions. *S.* Typhimurium GH3 and *S.* Typhimurium GH9 were selected to study the similarity of virulence *stn* gene and *bcfC* gene with other types in Gene bank, while in Fig. (17 and 18), *E. coli* O26 GH12 and *E. coli* O26 GH13 were selected to study the similarity of virulence *fimH* gene and *TraT* gene with other types in Gene bank. Sequence alignments using the NCBI BLASTP program showed that of *S.* Typhimurium GH3 virulence *stn* had high genetic similarity (99.4%) of *S. Enteritidis* with accession-numbers: Cp018659.1 _*S*._ Enteritidis_93-0639 & Cp018640.1_S._ Enteritidis_70-1605 & Cp018661.1_*S*._ Enteritidis_ 95-0621 & Cp025554.1_S._ Enteritidis_ATCC_ BAA-708, while *S.* Typhimurium GH9 virulence *stn* had height genetic similarity with LS483489.1_*S*_

Poona_NCTC4840 and CP019201.1_*S*_ Muenster _420_CFSAN001201.

While *S.* Typhimurium GH3 virulence *bcfc* showed a high percentage of genetic similarity (99.6%) with accession-numbers: Cp031359.1_S._Heidelberg_5 & Cp012349.1_S._Slotedijk_ATCC_15791 & Cp019186.1_*S*._Pomona_ATCC_10722.

On the other hand the *E. coli* O26 GH12 and *E. coli* O26 GH13 virulence *TraT* were agree with other *E. coli* with accession-numbers: X06915.1_E._coli_F and Cp014273.1_E._coli_K_12_C3026 with a percentage of 99.7% and 100% respectively. While *E. coli* O26 GH12 virulence *fimH* was highly genetic similarity with accession-numbers: Cp007592.1_E._coli_O157:H16_Saintai & Cp001368.1_E._coli_O157:H7_TW14359 & Cp001164.1_E_coli_O157:H7_EC4115 with a percentage 98.4% for each one. Also *E. coli* O26 GH13 virulence *fimH* had a highly genetic similarity with *E. coli* with accession-numbers: Cp034843.1_E_coli_L103-2, Cp034734.1_E_coli_ L53, Cp033092.1_E_coli_ATCC_117755, FJ865813.1_E_coli_Top1371, FJ865736.1_E _coli_TB154A and FJ865622.1_E_coli_ECOR33.

Sequence alignments of antimicrobial resistance gene as *gyrA* (at 344 bp) for both *S.* Typhimurium and *E. coli* O 26 were performed by the NCBI BLASTP program after confirmation by PCR as showed in fig. (19). The main principle was the detection of the substitutions in terms of amino acid positions *gyrA* Ser 83, *gyrA* Asp 87 and *gyrA* Alar 179, which are located within the QRDR. The mutations induced a local conformation changes of the A subunits cause marked resistance to specific antibiotic as quinolones. On studied of the selected 2 strains of *Salmonella*, *S.* Typhimurium GH2 and *S.* Typhimurium GH4 in Table (11) and Fig.(20) showed that amino acid changes detected at amino acid 83 were Ser changed to phe results in one point mutant. Also the selected E._coli_ O26_ GH3 and E._coli_ O26_ GH13 in Table (12) and Fig.(21) had one point mutation at amino acid 83 whereas Ser changes into Leu. Our results agree with the results recorded by Yoshida *et al.* (1988) who found that amino acid changes detected in amino acid 83and the point mutations in codon TCG at Ser 83 and with the results recorded by Nakamura *et al.* (1989) who found that Mutations in the *gyrA* and *gyrB* subunits of DNA gyrase play a major role in conferring a high level of resistance to fluoroquinolone in Gram-negative bacteria, such as *E. coli* while the mutations in *gyrA* gene (at 344 bp) are more common in quinolone resistance of *E. coli*.

In this study some virulence genes of both *Salmonella* species and *E. coli* were detected but no records of symptoms within the examined dairy cattle herds, this referred to the pathogenicity is not dependent on one virulence factor but occurred due to the regulation and interaction between a numbers of virulence factors that affected by environmental conditions as host species, species stress, host health status, immune status of the individual, interaction with other bacteria, the infecting dose, the method of delivery of the organisms to the host. Therefore the examined dairy cattle herds considered carrier animals that shed and spread the *Salmonella* and *E. coli* microorganisms in the feces, milk and/or environment after ingestion feed or water contaminated with feces from other carrier or infected animals (cross contamination). Also the detected resistance genes regards to a problem in the role of corrected treatments. Thus we concluded that strictly purchasing cattle from good source, with its life history from birth, vaccination, treatment and diseases history, not from dealers of unknown sources. Strict control of the environment of farms by preventing contacts between dairy cattle, calves and the other carrier species such as dogs, birds, cats, people, pig, feral cats and wild birds. Also prevent contacts between the different carrier species and feed, water and all equipment used in the production of milk especially feral cats and wild birds. Only good sources of feed and good sources of water were used in the farms. Awareness should be created among the dairy farmers on the transmission of various diseases from dairy environment to dairy cattle and the preventive measures used. Governorates should cite the supervision of veterinarian in farms is strictly. Strict hygienic measures were applied during the waste management and effluent control. Antimicrobial drugs should be used when needed with an accurate dose, in specific times and for specific cases during a certain period under the supervisions of veterinarian. Applied the recommendations and the hygienic measurement of HACCP, biosafety and biosecurity for dairy farms especially for water, soil, udder, unhygienic milking utensil, and Milkers' hands to obtain a good health dairy cattle and calves and good hygienic safe milk for consumers.

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الكشف الجزيئي عن ميكروبات السالمونيلا والاشرشيا كولاي في مزارع الالبان مع الكشف عن جينات الضراوة وجينات المقاومة للمضادات الحيوية

جيهان محمد عمر محمد حسن ، حسن السيد محمد فرج

E-mail: dr.gehanomer@yahoo.com Assiut University web-site: www.aun.edu.eg

تم تجميع ٥٠٠ عينة بواقع ١٠٠ عينة لكل من البان ، العلف ، مساحات من ادوات الحلب (تانكات) ، مساحات من السقاية ومساحات من براز الابقار الحلاب من مزارع صغيرة في منطقة القابوطي وبحرالبقر بمدينة بورسعيد وذلك للكشف وعزل ميكروبات السالمونيلا والايشريشيا كولاي ودراسة جينات الضراوة وجينات المقاومة للمضادات الحيوية والتتبع الجيني لبعض الجينات. واظهرت النتائج ان نسبة عزل السالمونيلا والايشيريشيا كولاي كانت ١.٨%، ٢.٨% علي التوالي. وتم تصنيف عترات السالمونيلا المعزولة بيوكيميائيا وسيرولوجيا فكانت سالمونيلا تيفيموريم ، سالمونيلا انتريديتس وسالمونيلا سانتيبول بنسبة ٦٦.٦٧ % (٩/٦)، ٢٢.٢٢ % (٩/٢) ، ١١.١١ % (٩/١) علي التوالي بينما كانت ميكروب الايشيريشيا كولاي O127, O126 , O125, O119, O26, بنسبة ٣٥.٧١% (١٤/٥)، ١٤.٢٨ % (١٤/٢) ، ٢٨.٦٠% (١٤/٤) ، ٧.١٤% (١٤/١) ،١٤.٢٨% (١٤/٢) علي التوالي. وتم دراسة مدي حساسية عترات ميكروب السالمونيلا وميكروب الايشيريشيا كولاي المعزولة باستخدام ١٠ أنوع من المضادات الحيوية المختلفة وأظهرت النتائج ان درجة مقاومة عترات السلمونيلا وعترات الايشيريشيا كولاي للمضادات الحيوية المستخدمة تتراوح بين درجات مختلفة للمقاومة الي المقاومة بنسبة ١٠٠% . واظهرت نتائج التصنيف الجزيئ باستخدام اختبار تفاعل إنزيم البلمرة المتسلسل (PCR) عن وجود جينات الضراوة *invA stn* ، *bcfC* في ميكروب السالمونيلا، وعن تواجد جينات الضراوة *PhoA*, *TraT* و *fimH* ، لميكروب الايشيريشيا كولاي. وعن تواجد جينات المقاومة *ampC*، *aacC*، *mphA,* لميكروبات السالمونيلا وجينات المقاومة *ampC*، *blaTEm*، *mphA, Aada1, aacC,* لميكروبات الايشيريشيا كولاي وتم مناقشة نسبة تواجدهما. وتم دراسة التتبع الجيني لجينات الضراوة (*stn* and *bcfC*) لميكروب السالمونيلا و *(TraT* and *fimH)* لميكروب الايشيريشيا كولاي و مقارنتها بعترات متوافقة في بنك العترات واحتمالية حدوث طفرة في جين المقاومة (*gyrA*) لكل من ميكروب السالمونيلا وميكروب الايشيريشيا كولاي. وقد تم مناقشة النتائج وبيان أهمية الميكروبات المعزولة وخطورتها علي الثروة الحيوانية وعمل التوصيات للتقليل من مخاطرها في مزارع الألبان.