ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Molecular characterization and evaluation of different PCR primers for detection of *Salmonella* in clinical samples of Iraqi patients.

Khalidah Khalaf Jabbar*.

Directorate of Education Rasafa north of Baghdad, Ministry of Education, Iraq.

ABSTRACT

Salmonellosis is flare-ups including typhoid fever and human gastroenteritis are vital illnesses in tropical nations where clean conditions are regularly not kept up. In this current investigation, 12 *Salmonella* separates were identified by PCR from human fecal samples acquired during March 2016 to November 2017 from government medical centers and registered clinical research centers in Iraq. DNA was isolated from each organism and was amplified by PCR, followed by purification and DNA sequencing. The resulting sequences were then analysed to allow for identification of the bacterial species. The target genes (*invA*, *16s rDNA* and *flic*) produced amplicons at 276bp, 310bp and 382 bp which were shown to be 100% specific targeted *Salmonella* Spp bacteria. The detection limit of this PCR technique was assessed utilizing 14 spiked aliquots enrichment protocols. The information obtained in the results revealed that microbial concentrate from as few target cells for every gram of the example culture was required for this essay. We presume that the developed PCR primers have high sensitivity and specificity for recognition of *Salmonella* species in clinical samples. High occurrence of *Salmonella* species in the inspected blood/stool samples highlights the vital role played as a vehicle in ailment pervasiveness.

Keywords: *Salmonella* species, PCR primers, human samples, optimization

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Identification of bacteria is an important for basic biological research and applied clinical microbiology. Salmonellosis, caused by infection with bacteria from the genus *Salmonella*, is one of the most common food borne illnesses and is manifested by diarrhea, mild fever, nausea, and abdominal pains, with the symptoms developing in 12–48 hrs after consumption of contaminated food. Salmonella is a standout amongst the most widely recognized pathogens and a noteworthy reason for foodborne ailments in human around the world. Salmonellosis affects 1.3 billion people worldwide each year with an estimated 3 million annual deaths from non-typhoidal salmonelloses (NTS) [1]. Salmonella is a gram-negative facultative rod-shaped bacterium in the same proteobacterial family as Escherichia coli, the family Enterobacteriaceae, trivially known as "enteric" bacteria [2]. Salmonella infection places significant health and economic burden worldwide [3]. There are many different *Salmonella* serotypes found nearly 2541 as per the CDC report in 2010 [4] and their distribution can change over time [5] According to Ngan et al [6], *Salmonella Typhi* accounts for more than 25 million infections worldwide, resulting in approximately 200,000 deaths annually. Selected place for the current investigations in Iraq*, Salmonella enterica* and *Salmonella Typhimurium* were the most frequently isolated from humans [7, 8]. Among the many rapid methodologies being developed for the detection of Salmonella and other foodborne pathogens, the polymerase chain reaction (PCR) has been frequently studied over the past decade because, in addition to being rapid and facile, the method can be highly specific and sensitive [9] Finally, it is important to use tests that have been appropriately validated for use on clinical samples. Genetic identification systems may improve Salmonella identification. Several polymerase chain reaction (PCR) assays [10] have been developed by targeting various Salmonella genes, such as *inv*A, 16s rRNA, *flic*, *flib*, *hil*A, *sir*A , *ttr* , virulence-associated plasmids etc.,[11].Traditional methods of identifying bacteria, including culturing, can be time consuming and unreliable. Molecular based methods of sequencing provide quick, reproducible identification. One reason that the 16s rDNA gene sequencing is so widely used to identify salmonella bacteria is that this gene is highly conserved.

In the present study, the biochemical, serological and molecular techniques were employed to detect and characterize *Salmonella* bacteria in clinical isolates obtained from Ba'quba teaching hospital, Diyala Province is a governorate in eastern Iraq, Specialized Hospital Fevers & Chest Diseases, Al Qadisiyyah Governorate is A governorates of Iraq and Ibn Zuhr for Chest Disease Hospital (for Infectious Diseases), East Karradah, Rusafa DOH, Baghdad and PCR was performed in order to amplify the nucleotide sequence of identified genes among *Salmonella* isolates to understand the genetic links between those isolates which spread in this area.

MATERIALS AND METHOD

Sample Collection

Collected totally 50 samples [18 Blood (12 male/6 female), 22 urine (13 male/9 female) and 10 stool samples(6 male/4 female) of 20 male and 12 female of thirty two patients were attended during March 2016 to November 2017 tested for bacteriology in Ba'quba teaching hospital, Specialized Hospital Fevers & Chest Diseases, Al Qadisiyyah and Ibn Zuhr for Chest Disease Hospital finally sent for Baghdad CPHL for confirmation and further tests. Blood and urine samples collected with care in sterile disposable containers with screw caps in the required amount with the prior permission of patients and Hospital ethical committee.

Bacterial Identification

Unadulterated disengages of bacterial pathogen were initially portrayed by colony morphology, hemolytic response on blood agar medium, gram-stain and catalase test. 50 clinical examples of (blood, pee and feces tests) were immunized on Blood agar and MacConkey plates, hatched at 370C for 24 hours for most extreme recuperation of the detaches. Suspected bacterial provinces were recognized by standard bacteriological techniques given by Cheesbrough M. (1999) [12]. These were then subcultured into Deoxychocolate Citrate Agar. The separates were then subcultured in Kliger Iron Agar (KIA) and Simmon Iron Medium (SIM). These means were trailed by biochemical and serotyping distinguishing proof. The Salmonella positive specimens were then subcultured in supplement broth and put away in the fridge at 6oC for anti-microbial susceptibility testing.

ISSN: 0975-8585

Molecular Identification and Stereotyping

The Isolated bacterial culture were plated on Hekton Enteric Agar and cultured 48hours in a 37°C in incubator, these obtained bacterial cultures used to DNA extraction. The Prep man Isolation kit was used for isolation of bacterial DNA method developed by Anonymous (2000) [13].

The PCR mix consist of 10 µL of the bacterial DNA in a clean 1000 µL micro centrifuge tube 50 µL each (500 picomoles) *invA* primer 5'-CCT TTA GGC TTA GGG CCC CGA-3' and *16s r*DNA primer 5'-GGA TTC CCC GGC TTA GGC GGT-3' and *flic* primer 5'-AAC GGC AAG CCC CTA CCC GCA-3' 12.5 µL HotStar Taq master PCR mix (Shimadzu) and 9.5 µL of distilled water was added to the reaction mixture was place in a PCR machine, which was programmed according with manual. The PCR product was run in a 2.5% agarose electrophoresis get and the results were given in Figure (3). Samples showing an appropriate size band were saved for purification and DNA sequencing. PCR products were purified using the QIAquick PCR purification Kit (Qiagen Valencia, CA) and eluted with water. DNA sequences were analysed by submission to the ribosomal database project website [14] for identification.

Antibiotic susceptibility test

The control bacteria isolates were obtained from the National Medical Research Institute, Baghdad. The antimicrobial drugs used were obtained from Sigma Aldrich Company. Culture media was prepared by reconstituting commercial powder in distilled water and sterilized at 121°C for 15 minutes in an autoclave per the manufacturer's instructions [15]. The isolated microbial species were cultured on Hicrome agar Media. The isolates were tested by disc diffusion method for drug susceptibility according to National Committee for Clinical Laboratory Standards guidelines [16]. This was then impregnated with antimicrobial sensitivity discs using sterile forceps and then gently pressed down onto the agar. The antibiotic disc sensitivity was done using Ciprofloxacin (25µg), Nitrofurantoin (10µg), Ampicillin (30µg), Ceftriaxone (10µg) Norfloxacin (30µg), Azithromycin (30µg), Florfenicol (25µg), Tetracycline (20µg) and Nalidixic acid (20µg), obtained results were tabulated in Table (3). The criteria to select the antimicrobial agents were based on availability, CLSI guideline, the organisms' Gram reaction, and frequent prescription of drugs for the management of postnatal infections.

RESULTS AND DISCUSSION

Of the fifty clinical samples (blood, Urine and stool) from twenty male and twelve female patients in the specified hospitals analysed over a 20 months period to detect the presence of pathogenic bacteria *Salmonella* species and results were given in Table1 and corresponding frequencies of the samples were graphically represented in Figure (1) . Total 12 *salmonella* species were identified from the various clinical samples and their count and frequencies also tabulated in Table (1).

Table 1: Frequency of isolated Salmonella serovars in three types of clinical samples

Serovar Name	Blood Samples Frequency (n=18)		Urine Samples (n=22)		Stool Samples (n=10)	
	Male (n=12)	Female (n=6)	Male (n=13)	Female (n=9)	Male (n=6)	Female (n=4)
Salmonella Infantis	ND	2(33.3%)	ND	ND	2 (33.33%)	1 (25%)
Salmonella Newport	1 (8.3%)	ND	1 (7.69%)	1 (11.1%)	ND	ND
Salmonella Stanley	1(8.3%)	1(16.67%)	2(15.3%)	0	ND	2 (50%)
Salmonella Virchow	ND	ND	ND	1 (11.1%)	ND	1 (25%)
Salmonella Agona	ND	1(16.67%)	ND	0	ND	ND
Salmonella bongori	2 (16.7%)	1(16.67%)	1 (7.69%)	1 (11.1%)	ND	ND
Salmonella Bovismorbificans	ND	1(16.67%)	ND	1 (11.1%)	1	1 (25%)
Salmonella Derby	ND	ND	ND	ND	ND	1 (25%)
Salmonella enterica	4 (33.3%)	2 (33.3%)	4 (30.77%)	2 (22.2%)	4(87.8%)	1 (25%)
Salmonella Enteritidis	2(16.67%)	3 (50%)	5 (38.5%)	1 (11.1%)	3 (50%)	3 (75%)
Salmonella Typhi	6(50%)	1(16.67%)	4 (30.7%)	2 (22.2%)	2 (33.33%)	2 (50%)
Salmonella Typhimurium	2(16.7%)	1(16.67%)	2(15.3%)	1 (11.1%)	2 (33.33%)	1 (25%)

ISSN: 0975-8585

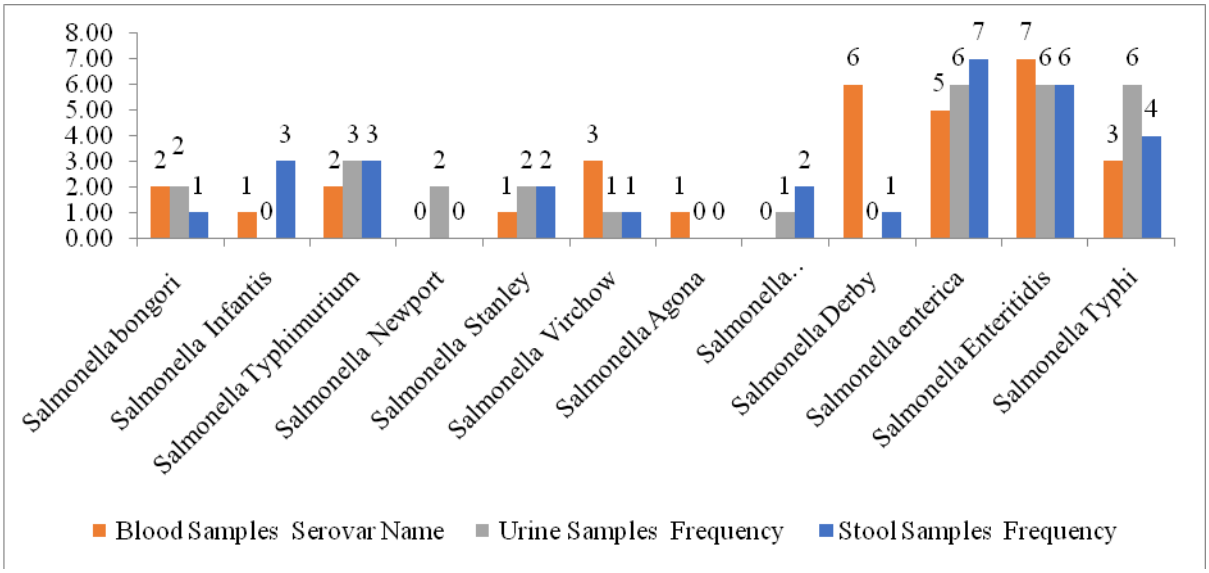


Figure 1: Schematic reprensetation of Salmonella Spp frequency in blood, urine & stool samples of Iraqi patients

- ***S. Infantis*** identified in five samples of blood and stool only. It was absent in urine samples. Out of 18 blood samples of male and female, it was only isolated in female samples with frequency 33.3%. in stool samples of male it was observed in 2 (33.3%) and in female only one out of four samples with frequency 25%
- ***S. enterica serovar Newport*** identified in only in three patients of male and female patients. It was absent in male blood and stool samples of both. This serover identified in one male sample of Blood (8.3%), male (7.69%) and female (11.1%) urine sample.
- ***Salmonella Stanley*** is an uncommon strain of Salmonella spp. It was observed in 4 patients out of fifty. Further, it was identified in one blood sample of male (8.3%), female sample (16.7%) and two male urine sample (15.3%), two of female stool (50%) having *S. Stanley* bacteria.
- ***S.enterica serotype Virchow*** is a food born pathogen, common serover observed in Israel[17], United states [18], Britain [19] etc., In the current study, this bacteria observed in two Iraqi female patients [one in Urine sample (11.1%) and one in stool sample (25%)].
- Another ***S. serotype Agona*** pathogen observed in only one female blood sample (16.6%), out of 50 collected samples. It is a nontyphoidal salmonella spp found in food items [20]. *S. Agona* disease observed in 32 cases observed in France in 2017,[21] and it is the 15th common pathogen observed in USA also [22].
- ***Salmonella bongori*** is the most common intestinal and danger pathogenic bacteria identified in cold blooded animals, but it may infect the human beings [23]. In the current investigation, this spp is identified in five samples. Two in male blood samples (16.7%), one in male urine samples (7.69%), where as in Urine samples of one male (7.69%) and one female sample (11.1%) respectively.
- Recently ***S.* serotype Bovismorbificans** identified in few human samples from Netherlands [24], Queensland [25], Finland [26] locations. *S.* Bovismorbificans is food born bacteria and causes salmonellosis [27]. Now it was reported in Iraq from the current study. Four isolates identified from one female blood (16.67%), one female Urine sample (11.1%), and one each in male and female stool samples.
- ***S.enterica subsp. enterica serovar Derby*** is the most prevalent species in pig and poultry mean, it was recently reported in Brazil clinical samples [28]. In the current study one isolate found in stool female sample (25%) only.
- ***Salmonella enterica* formerly known as *S. choleraesuis*** is an anaerobic, gram (-)ve, common gastrointestinal pathogenic bacteria. Surprisingly it as the commonly observed serover in all collected samples from Iraqi people in the current study. Totally 17 isolates found out of 85 isolates. This spp was observed in 6 blood and Urine samples (4 in male, 2 in female each), and five stool sample (four in Male and one in Female) with frequency of 33.3% in blood samples, 30.77, 22.2 % in Urine samples, 87.8% and 25% in stool samples respectively.

- ***S. Enteritidis*** is the most common stereo type in Salmonella spp. It is a much more common in both poultry and humans since 1980s. It was commonly reported in nontyphoidal salmonella infections in USA [29]. In the current study totally 17 isolates separated from fifty human samples. Out of 17, 2 (16.67%) from male blood, 3(50%) from female blood, 5 (38.5%) from male Urine, 1 (11.1%) from female Urine,3 (50%) from male stool and 3 (75%) from female stool samples respectively.
- ***Salmonella Typhi*** or *S*. enterica serotype Typhi is a bacteria mostly infect the intestinal tract and the blood. More than 2300 closely related salmonella serovars bacteria recognized so far. It causes Typhoid or enteric fever in Humans. Again 17 isolates contains this bacteria. Out of 17, 6 (50%) from male blood, 1(16.67%) from female blood, 4 (30.7%) from male Urine, 2 (22.2%) from female Urine, 3 (33.33%) from male stool and 2 (50%) from female stool samples respectively.
- Finally nine isolates of **S. enterica serotype Typhimurium** observed on three types of samples. It is the dominating pathogen in poultry and poultry products. S. Typhimurium causes enteric fever like illness. In the current study 9 isolates found in three types of samples from Man and Female adults. 2(16.7%) from male blood, 1(16.67%) from female blood, 2(15.3%), from male urine, 1 (11.1%) from female urine, 2 (33.33%) from male stool and 1 (25%) from female stool samples respectively.
- From the above points it was identified that most prevalent bacteria in the identifies samples are *Salmonella enterica* (17), *Salmonella Enteritidis* (17), *Salmonella Typhi* (17) and *Salmonella Typhimurium* (9) respectively.

Antibacterial Resistance

Later bacteria develop resistance to virtually any anti bacterial agent. Resistance has many consequences. Resistance also compels the use of more toxic or more expensive alternative drugs. The evaluation of antibacterial resistance typically reveals some organisms that are naturally resistance and that appear to be susceptible, thus defining the spectrum of activity for the specified agent. In the current investigation few common antibiotics were used to test the antibacterial resistance against the isolate of 12 salmonella serovers in three types of samples and average susceptibility values (%) are given ion Tables (2), the rate of bacterial isolates resistant (%) to selected antibiotics on Salmonella bongori 23.72 % (Blood samples), 25.86 % (Urine samples); Salmonella Infantis 42.08 % (Blood samples), 31.79 % (stool samples); Salmonella Typhimurium 52.32% (Blood samples), 51.32 % (Urine samples), 35.25% (stool samples); Salmonella Newport 42.90 % (Blood samples), 46.76 % (Urine samples); Salmonella Stanley 41.42% (Blood samples), 45.15 % (Urine samples), 18.96% (stool samples);Salmonella Virchow 28.95% (Urine samples), 21.07 % (stool samples); Salmonella Agona 41.95% (Blood samples); Salmonella Bovismorbificans 29.11% (Blood samples), 31.73% (Urine samples) 28.08% (stool samples); Salmonella Derby 27.96% (stool samples); Salmonella enterica 23.95% (Blood samples), 26.11% (Urine samples) 46.64% (stool samples); Salmonella Enteritidis 21.02% (Blood samples), 22.91% (Urine samples), 45.58% (stool samples); Salmonella Typhi 48.29% (Blood samples), 52.64% (Urine samples), 48.29% (stool samples) respectively. Salmonella Stanley, Salmonella Derby not identified in Blood samples, Salmonella Infantis, Salmonella Agona and Salmonella Derby not identified in urine samples, Salmonella bongori, Salmonella Newport and Salmonella Agona not identified in stool samples. Hence, Antibacterial susceptibility not reported against these samples.

It was identified that out of nine antibiotics tested in this study Ciprofloxacin (25µg), Nitrofurantoin (10µg) and Ampicillin (30µg) shows more than 70% susceptibility against all samlmonella isolates Salmonella Typhimurium and Salmonella Typhi, next antibiotic in order is Ceftriaxone (10µg) active against major isolates.

PCR results and gene sequencing

Gel analysis of PCR products showed 16 bands along in 14 bands in PCR amplicon for *invA* gene fragmentation at 272 bp Figure (2), out of 14 bands two bands are negative control and 12 are positive control represents 12 serovers, in the Figure (3), PCR amplification for *16s rDNA* gene fragments shows 16 bands including 4 negative and 12 positive control bands obtained at 310 bp, finally PCR amplification for *flic* gene fragments observed in Figure (4) shows 14 bands with two negative and 12 positive control at 382 bp along with reference sample M in all Figure (3-4). Approximate sized DNA bands were also obtained following PCR form of 12 bacterial isolates of blood, urine and stool samples. Among the three gene targets all 12 isolates were posively responded to *invA* and 16sr DNA genes. *flic* is moderately reflected in the current multi PCR technique.

ISSN: 0975-8585

Table 2: The Percentage of antibiotic susceptibility pattern of *Salmonella* serovars from Blood, Urine and Stood samples

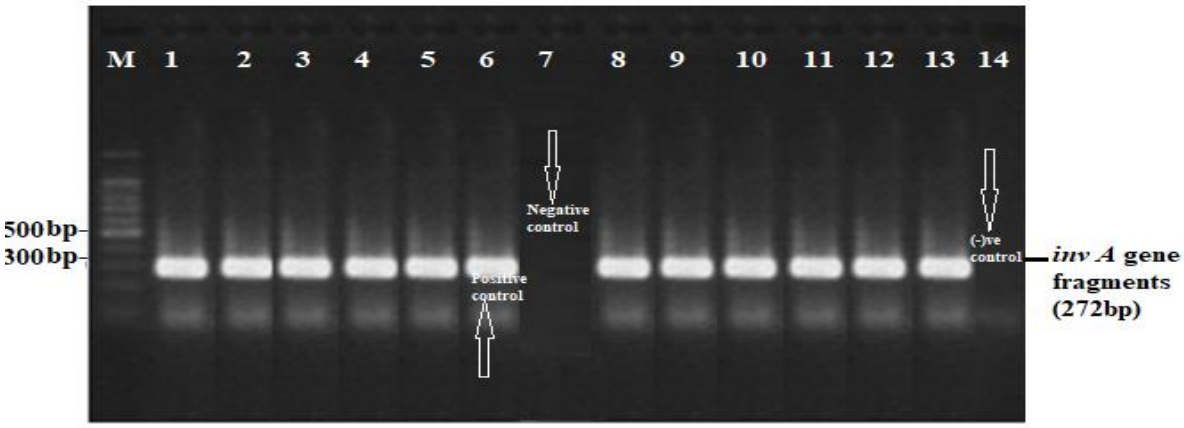
	% of Antibiotic susceptibility pattern in Blood Samples								
Serovar	Ciprofloxacin (25µg)	Nitrofurantoin (10µg)	Ampicillin (30µg)	Ceftriaxone (10µg)	Norfloxacin (30µg)	Azithromycin(30µg)	Florfenicol (25µg)	Tetracycline (20µg)	Nalidixic acid (20µg)
Salmonella bongori	34.50	35.19	35.89	24.05	16.11	16.43	16.76	17.10	17.44
Salmonella Infantis	61.20	62.42	63.67	42.66	28.58	29.15	29.74	30.33	30.94
Salmonella Typhimurium	76.10	77.62	79.17	53.05	35.54	36.25	36.98	37.72	38.47
Salmonella Newport	62.40	63.65	64.92	43.50	29.14	29.73	30.32	30.93	31.55
Salmonella Stanley	60.24	61.44	62.67	41.99	28.13	28.70	29.27	29.86	30.45
Salmonella Virchow	ND	ND	ND	ND	ND	ND	ND	ND	ND
Salmonella Agona	61.02	62.24	63.49	42.54	28.50	29.07	29.65	30.24	30.85
Salmonella Bovismorbificans	42.34	43.19	44.05	29.51	19.77	20.17	20.57	20.98	21.40
Salmonella Derby	ND	ND	ND	ND	ND	ND	ND	ND	ND
Salmonella enterica	34.84	35.54	36.25	24.29	16.27	16.60	16.93	17.27	17.61
Salmonella Enteritidis	30.57	31.18	31.81	21.31	14.28	14.56	14.85	15.15	15.45
Salmonella Typhi	70.24	71.64	73.08	48.96	32.80	33.46	34.13	34.81	35.51
	% of Antibiotic susceptibility pattern in Urine Samples								
Serovar	Ciprofloxacin (25µg)	Nitrofurantoin (10µg)	Ampicillin (30µg)	Ceftriaxone (10µg)	Norfloxacin (30µg)	Azithromycin(30µg)	Florfenicol (25µg)	Tetracycline (20µg)	Nalidixic acid (20µg)
Salmonella bongori	37.61	38.36	39.12	26.21	17.56	17.91	18.27	18.64	19.01
Salmonella Infantis	ND	ND	ND	ND	ND	ND	ND	ND	ND
Salmonella Typhimurium	74.64	76.13	77.66	52.03	34.86	35.56	36.27	36.99	37.73
Salmonella Newport	68.02	69.38	70.76	47.41	31.77	32.40	33.05	33.71	34.38
Salmonella Stanley	65.66	66.97	68.31	45.77	30.67	31.28	31.91	32.54	33.19
Salmonella Virchow	42.10	42.94	43.80	29.35	19.66	20.06	20.46	20.87	21.28
Salmonella Agona	ND	ND	ND	ND	ND	ND	ND	ND	ND
Salmonella Bovismorbificans	46.15	47.07	48.02	32.17	21.55	21.99	22.42	22.87	23.33
Salmonella Derby	ND	ND	ND	ND	ND	ND	ND	ND	ND
Salmonella enterica	37.98	38.74	39.51	26.47	17.74	18.09	18.45	18.82	19.20
Salmonella Enteritidis	33.32	33.99	34.67	23.23	15.56	15.87	16.19	16.51	16.85
Salmonella Typhi	76.56	78.09	79.65	53.37	35.76	36.47	37.20	37.95	38.70
	% of Antibioticsusceptibility pattern in Stool Samples								
Serovar	Ciprofloxacin (25µg)	Nitrofurantoin (10µg)	Ampicillin (30µg)	Ceftriaxone (10µg)	Norfloxacin (30µg)	Azithromycin(30µg)	Florfenicol (25µg)	Tetracycline (20µg)	Nalidixic acid (20µg)
Salmonella bongori	ND	ND	ND	ND	ND	ND	ND	ND	ND
Salmonella Infantis	46.24	47.16	48.11	32.23	21.60	22.03	22.47	22.92	23.38
Salmonella Typhimurium	51.27	52.30	53.34	35.74	23.94	24.42	24.91	25.41	25.92
Salmonella Newport	ND	ND	ND	ND	ND	ND	ND	ND	ND
Salmonella Stanley	27.57	28.12	28.68	19.22	12.88	13.13	13.40	13.66	13.94
Salmonella Virchow	30.64	31.25	31.88	21.36	14.31	14.60	14.89	15.19	15.49
Salmonella Agona	ND	ND	ND	ND	ND	ND	ND	ND	ND
Salmonella Bovismorbificans	40.84	41.66	42.49	28.47	19.07	19.46	19.84	20.24	20.65
Salmonella Derby	40.67	41.48	42.31	28.35	18.99	19.37	19.76	20.16	20.56
Salmonella enterica	67.84	69.20	70.58	47.29	31.68	32.32	32.96	33.62	34.30
Salmonella Enteritidis	66.3	67.63	68.98	46.22	30.96	31.58	32.22	32.86	33.52
Salmonella Typhi	70.24	71.64	73.08	48.96	32.80	33.46	34.13	34.81	35.51

ND=Not detected

ISSN: 0975-8585

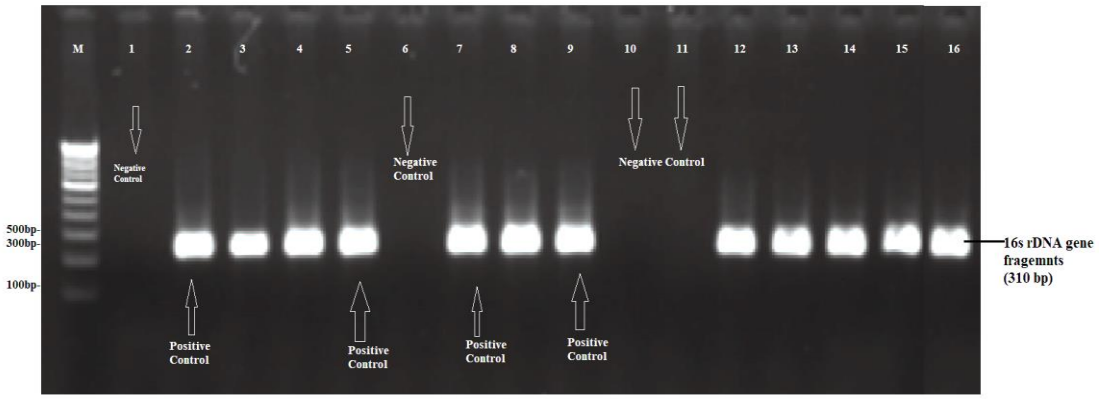
Table 3: Proportion of Salmonella isolates that were positive for the *invA*, 16s rDNA and *fliC* genes

Name of the *Salmonella* Serovar	% of salmonella Spp (+) ve for the targeted genes		
	invA	*16s rDNA*	***flic***
Salmonella bongori	76.10	67.47	23.67
Salmonella Infantis	67.60	78.64	61.27
Salmonella Newport	56.70	60.32	68.64
Salmonella Stanley	67.60	57.64	75.24
Salmonella Virchow	66.40	84.24	70.24
Salmonella Agona	57.40	61.72	61.08
Salmonella Bovismorbificans	48.67	57.68	56.84
Salmonella Derby	61.57	61.34	50.37
Salmonella enterica	60.37	50.37	49.67
Salmonella Enteritidis	76.84	42.37	46.34
Salmonella Typhi	61.27	22.67	60.24
Salmonella Typhimurium	78.40	86.67	67.34



(M-DNA ladder molecular weight marker (100 bp), lanes 1-6, and 8-13 are positive for *invA* gene (272bp); lanes 7& 14 are negative control for *Salmonella* Spp.

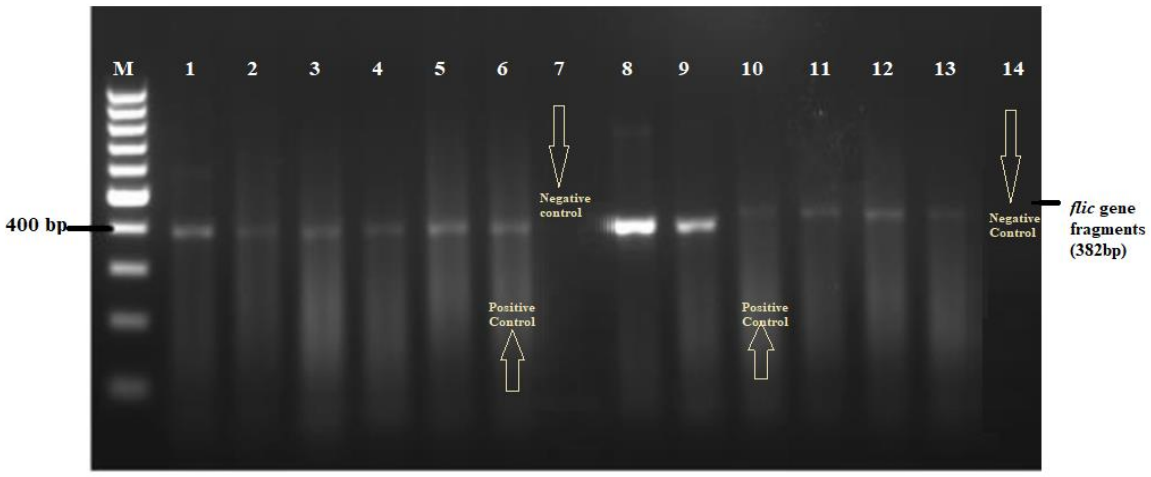
Figure 2: PCR amplification for *invA* gene fragments (272bp)



(M-DNA ladder molecular weight marker (100 bp), lanes 2-5, 7-9 & 12-16 are positive for*16s r*DNA gene (310bp); lanes 1, 6, 10&11 are negative control for *Salmonella* Spp.

Figure 3: PCR amplification for *16s rDNA* gene fragments (310bp)

ISSN: 0975-8585



[M-DNA ladder molecular weight marker (100 bp), lanes 1-6, and 8-13 are positive for *flic* gene (272bp); lanes 7& 14 are negative control for *Salmonella* Spp.]

Figure 4: PCR amplification for *flic* gene fragments (382bp)

CONCLUSION

The study suggested that, at present few male and female adults in Iraq suffering from an epidemic of Salmonella enterica stereo type serovers. However, to ensure the safety and health of their customers, food restaurants should inculcate food safety practices and habits in their staff and food processing. The critical control points to preventing food borne illness such as preventing cross contamination from the raw products food handlers should also be trained on hygienic food handling practices and safety. In the current research PCR experiment proved to be consistent way of identifying bacteria. From the anti bacterial resistance results Ciprofloxacin (25µg), Nitrofurantoin (10µg) and Ampicillin (30µg) were the most antibacterial agents for this type of diseases.

REFERENCES

[1] Bennett, A. R., Greenwood, D., Tennant, C., Banks, J. G and Betts, R. P. 1998. Rapid and definitive detection of *Salmonella* in foods by PCR. Lett. Appl. Microbiol. 26:437-441.

[2] Parry, C. M. .2006. Epidemiological and clinical aspects of human typhoid fever. In: Matroeni P and Maskell D (Eds.), *Salmonella* infections: Clinical, immunological and molecular aspects, New York: Cambridge University Press, :1-18.

[3] Chen, J., Zhang, L., Paoli, G.C., Tu, S. I and Shi, X. 2010. A real-time PCR method for the detection of Salmonella enterica from food using a target sequence identified by comparative genomic analysis. International Journal of Food Microbiology 137: 168–174

[4] CDC (Centers for Disease Control and Prevention) 2010. *Salmonella*. Available at: http://www.cdc.gov/*Salmonella*.

[5] Carter, J.D., Hird, D.W., Farver, T.B. and Hjerpe, C.A. 1986. Salmonellosis in hospitalized horses: seasonality and case fatality rates. J Am Vet Med Assoc 188: 163-167.

[6] Ngan G.J.Y., Ng L.M., Lin, R.T.P. 2010. Development of a novel multiplex PCR for the detection and differentiation of *Salmonella enterica* serovars Typhi and Paratyphi A Res Microbiol. 161: 243-248.

[7] Allos, G., Inventory of serotypes of Salmonella found in Iraq, Bull Soc Pathol Exot Filiales. 1978. Jul-Oct; 71(4-5):323-8.

[8] Singer, R.S., Mayer, A.E., Hanson, T.E. and Isaacson, R.E. 2009. Do microbial interactions and cultivation media decrease the accuracy of Salmonella surveillance systems and outbreak investigations. *J Food Prot***7.2** :707-713.

[9] Abubakar, I., Irvine, L., Aldus, C.F., Wyatt, G.M., Fordham, R., Schelenz, S., Shepstone, L., Howe, A., Peck, M., Hunter, P.R., 2007. A systematic review of the clinical, public health and cost-effectiveness of rapid

diagnostic tests for the detection and identification of bacterial intestinal pathogens in faeces and food. Health Technol. Assess. 11:1–216.

[10] Iida, K., Abe, A., Matsui, H., Danbara, H., Wakayama, S., Kawahara, K. 1993. Rapid and sensitive method for detection of *Salmonella* strains using a combination of polymerase chain reaction and reverse dot-blot hybridization. FEMS Microbiol. Lett., 114: 167-172.

[11] Al-Harthi, O. M. Halawani, E. M. and Hayam, S. A. 2012. Detection of Salmonella strains in clinical samples from Saudi Arabia by invA and hilA polymerase chain reaction (PCR)-based assays, African Journal of Microbiology Research. 6(25) :5410-5416.

[12] Cheesbrough, M. 1999. Microbiology: in Medical Laboratory Manual for Tropical countries. *ELBS edition. University Press*, Cambridge. 132: 26-58.

[13] Anonymous, Prepman Ultra Sample preparation reagent quick card, Applied Biosynthesis, Foster City, CA, 2000.

[14] http://rdp.cme.msu.edu.

[15] Onyango, 2008. In vitro Salmonella Drug Susceptibility, J Infect Developing Countries 2(2): 106-111.

[16] Cormican, M., Whyte, T., Hanahoe, B. 2001. Antimicrobial susceptibility testing in Ireland: An introduction to the methods of the National Committee for Clinical Laboratory Standards (NCCLS). Department of Medical Microbiology, University of College Hospital Galway and Department of Bacteriology. National University of Ireland Galway.

[17] Weinberger, M. et al, 2006. Salmonella enterica serotype Virchow: epidemiology, resistance patterns and molecular characterisation of an invasive Salmonella serotype in Israel, Clinical Microbiology and Infection, 12, Issue 10, :999-1005.

[18] Gambino-Shirley, K.J *et al*. 2018. Multistate Outbreak of Salmonella Virchow Infections Linked to a Powdered Meal Replacement Product-United States, 2015-2016, Clin Infect Dis. 31;67(6):890-896.

[19] Mani,V. Brennand,J. and Mandal,B. K. 1974. Invasive Illness with Salmonella Virchow Infection, Br Med J. 1974 Apr 20; 2(5911): 143–144.

[20] Adamma, Mba-Jonas *et al*., 2018. A Multistate Outbreak of Human Salmonella Agona Infections Associated With Consumption of Fresh, Whole Papayas Imported From Mexico—United States, 2011 , Clinical Infectious Diseases, 66, Issue 11, 17: 1756–1761.

[21] Jourdan-da, S. N. *et al*., 2018. Ongoing nationwide outbreak of Salmonella Agona associated with internationally distributed infant milk products, France, December 2017, Euro Surveill. 2018 Jan;23(2). doi: 10.2807/1560-7917.ES.2018.23.2.17-00852.

[22] Zhou, Z., McCann, A., Litrup, E., Murphy, R., Cormican, M., Fanning, S., *et al*. 2013. Neutral Genomic Microevolution of a Recently Emerged Pathogen, Salmonella enterica Serovar Agona. PLoS Genet 9(4): e1003471.

[23] Fookes, M., Schroeder, G.N., Langridge, G.C., Blondel, C.J., Mammina, C., and Connor, T.R. 32011. *Salmonella bongori* Provides Insights into the Evolution of the Salmonellae. PLoS Pathog 7(8): e1002191.

[24] Friesemalde, J.A., Hofhuis, A., Heck, M., Van, D., Kerkhof, H. and Jonge, R .2014. Large outbreak of Salmonella Thompson related to smoked salmon in the Netherlands, August to December 2012. Euro Surveill. 19(39):20918. .

[25] Stafford, R. J. McCall, B. J, Neill, A.S, Leon, D.S, Dorricott, G. J, Towner,C. D. A. 2002. Statewide outbreak of Salmonella bovismorbificans phage type 32 infection in Queensland. Commun Dis Intell Q Rep.;26(4):568-73.PMID: 12549525.

[26] Rimhanen, F.R., Niskanen, T., Lienemann, T., Johansson, T., Sjöman, M., Korhonen, T. A. 2011. Nationwide outbreak of Salmonella bovismorbificans associated with sprouted alfalfa seeds in Finland, 2009. Zoonoses Public Health. 58(8):589-96. DOI: 10.1111/j.1863-2378.2011.01408.x PMID: 21824360.

[27] Kontiainen, S., K. Sammalkorpi, and A. Siitonen. 1996. High frequency of septicemia in an outbreak of Salmonella Stanley infections. Scand. J. Infect. Dis. 28:207

[28] Peirano, G., Agersø, Y., Aarestrup, F.M. dos Reis, E.M. Prazeres, R. D. 2006. Occurrence of integrons and antimicrobial resistance genes among Salmonella enterica from Brazil. J Antimicrob Chemother 58: 305–359.

[29] Kendall, M.E., Crim, S. Fullerton, K, 2012. Travel-associated enteric infections diagnosed after return to the United States, Foodborne Diseases Active Surveillance Network (FoodNet), 2004–2009, Clin Infect Dis , 54 Suppl 5(pg. S480-7).