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ORIGINAL RESEARCH

EXAMINATION OF ISOLATED SALMONELLA SPECIES FROM FOOD SAMPLES FOR THE PRESENCE OF VIRULENCE GENES MOLECULARLY IN KARBALA, IRAQ

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ABSTRACT

Foodborne disease is a universal medical condition, and *One of the significant bacterial infections that may cause typhoid fever and salmonellosis worldwide is Salmonella*. Despite several innovations and sanitation efforts, these illnesses pose a significant risk to animal and human health. The high prevalence of these illnesses is caused by a variety of food products, including poultry and eggs that have been contaminated. This research aims to address the certainties mentioned above and the high prevalence of illnesses in Iraq that are highly drug resistant. This study focused on isolating and identifying species of Salmonella in food products gathered from several locations in Karbala, Iraq. The phenotypic isolation and characterization of Salmonella were conducted using the standard methodology of improvement, culture, and biochemical confirmation from BAM, the Biological Analytical Manual. Following a PCR; Polymerase chain reaction exposure, these isolates were electrophoretic ally resolved on 1 percent agarose. Using PCR to identify the invA gene in 250 specimens, fifteen genotypically verified Salmonella isolates were discovered; twenty percent of the positive isolates were from food samples, whereas different types of food products displayed eighty percent of the overall. Salmonellosis is a much more common illness in people and the major source of Salmonella spp. is food poisoning. Salmonella spp. Identification is highly cumbersome and takes a long time, especially in developing nations. It is necessary to use molecular detection techniques and understand the genotypic variation of certain genes responsible for Salmonella virulence. The study's objectives included detecting Salmonella spp. using 16S rRNA, determining if the invA gene for Salmonella identification, and genotyping several genes found in Salmonella spp. (invA, spvA, and spvC) that are associated with virulence in food specimens in Karbala, Iraq. For the 16S rRNA gene, the one hundred isolates returned positive, and 25 also tested positive for the salm3 (389 bp) gene. Twenty out of 284 bp of the invA genes, as determined by PCR, were found to be positive for the spvC gene 15. Although it is advised to utilize the invA gene to identify Salmonella in the specimens tested, most of our isolates lacked the virulence genes.

Keywords:*Salmonella,detection, genes, Food, virulence, molecularSamples*

INTRODUCTION

Salmonella spp. include bacteria that cause salmonellosis and are familiar sources of foodborne illnesses and incidents in modern and developing nations, with a vital public health issue (Smith *et al.*, 2015). Approximately three million individuals die from NTS, non-typhoidal salmonelloses yearly, afflicting 1.3 billion people globally (Bennett et al., 1998).

The diseases that resulted from subjection to Salmonella spp. Varied from mild symptoms to straightforward illness cases, they were occasionally fatal.

Various national species, including birds and certain solitary animals, approve of Salmonella spp. Raw foods, poultry, dairy products, and the environment have been shown to have Salmonella spp (Chiu et al., 2002). In addition, improper cooking, reheating of food, and eruptions have all been linked. Although the disease has been linked to a wide range of dietary sources, poultry has been identified as the sole leading cause of salmonellosis in individuals. In addition, ingesting tainted chicken meat and eggs can induce avian salmonellosis, which impacts the poultry business and people (Behravesh *et al.*, 2014).

Enteritis and typhoid from foodborne Salmonella poisoning can be more severe in patients with immune system disorders. Around the world, chicken has been linked to various Salmonella prevalences. Salmonella infection rates have increased globally due to a cumulative increase in the consumption of cattle and poultry foods (Henchion *et al*., 2017). The most frequent serovars related to human foodborne disease from far over 2500 serovars are "Salmonella enterica serovars Typhimurium (S. Typhimurium) and Enteritidis (S. Enteritidis)," according to several researchers (Rodpai et al., 2013). A groundbreaking technique for the diagnosis of harmful organisms with the use of DNA amplification using PCR. (Dorjiet al. 2014).

To identify and screen for genotypic virulence in Salmonella isolates from food and environmental specimens, many virulence genes that are targeted genes for PCR expansion of Salmonella, including spvA, invA, spvB, spvC, and sitC have been employed (Rodpai *et al* .2013). The research aims to analyze some of the local isolates for virulence genes and validate the occurrence of Salmonella spp. Recovered from food specimens utilizing "PCR virulence genes" (Salm3 spvA & spvC).

MATERIALS AND METHODS

A sum of 250 isolates believed to be Salmonella spp. have been cultured from meat specimens on Salmonella agar for twenty-fourth hours. In addition, pure colonies have been recognized using just a biochemical test described earlier by Chiu (2002). This work was based on previous research on identifying Salmonella from food specimens using the "REVEAL Kit (Neogen Corp. the US)."

Biochemical test:

Significant relevant biochemical assays, including the Methyl Red/ Voges-Proskauer test, Oxidase test, Urease test, Indol test, Kligler test, Citrate utilization, Catalase test, and Cytochrome oxidase tests, were carried out in accordance with Franchi (2009).

DNA Extraction

DNA was obtained using the boiling procedure, then 2 ml of the specimen in broth was quickly centrifuged for about 7 min at 13,000 rpm. These pellets were then rinsed twice with a sterile solution before the supernatant was removed. These pellets were then given 500 µl of the sterile solution, homogenized by vortexing, and heated for 5 minutes in a dry bath at 95°C. Next, for 10 minutes, the sample was centrifuged at 13,000 rpm while vortexed.

Finally, the DNA-containing supernatant was put in a separate tube and kept at -20°C. A Nanodrop spectrophotometer was used to evaluate the concentration also the clarity of the isolated DNA.

DNA Amplification

The PCR reaction was accomplishedin a 25µl reaction mixture which includes"1x PCR buffer (Promega, UK), 1.5mM Magnesium Chloride, 200µM of each dNTP, 20pMol" of every primer, "1.25U Taq DNA polymerase (Promega, UK)". The concentration of the DNA was reduced to be somewhere between 10-200ng/µl, 1 µl was applied in the PCR, as well as an "Eppendorf Mastercycler" Gradient was used for the amplification (Eppendorf, Hamburg). Tables 1 and 2.

Table 1:primes of *Salmonella spp* toxin genes used in this study

Primer Sequence	Target gene	Sequence	Amplified fragment size (bp)
16S rRNA	Genus specific	"5'-TGT TGT GGT TAA TAA CCG CA -3' 5'-CAC AAA TCC ATC TCT GGA -3'	571
Salm3	*Salm3*	5'-GCTGCGCGCGAACGGCGAAG-3' 5'-TCCCGCCAGAGTTCCCATT-3	389
spvC	*spvC*	5' GTC CTT GCT CGT TTA CGA CCT GAA T 3' 5' TCT CTT CTG CAT TTC GTC A 3'	244
spvA	*spvA*	5'-GTC AGA CCC GTA AAC AGT-3' 5'-GCA CGC AGA GTA CCC GCA-3'"	604

Table 2 PCR amplification of detection of *Salmonella spp*toxin genes

Target gene	**Amplification (35 cycles)**				
	Primary denaturation	**Secondary denaturation**	**Annealing**	**Extension**	**Final Extension**
16S rDNA	95 ◦C	94◦C	52◦C	72◦C	72◦C
Salm3	95 ◦C	94◦C	54◦C	72◦C	72◦C
spvC	95◦C	94◦C	50◦C	72◦C	72◦C
spvA	95◦C	94◦C	52◦C	72◦C	72◦C

RESULTS AND DISCUSSION

Currently, *Salmonella is detected by molecular techniques and biochemical and standards bacteriological.* Presumptive *Salmonella spp* colonies were substantiated using biochemical tests and molecular approaches by applying 16S rDNA partial sequencing. The "100 isolates tested positive for 16S rRNA gene while 25 were positive for *salm3* (389 bp) gene. PCR analysis of the *invA* gene (284bp)" showed that twenty are positive for *spvC* (244 bp) gene. 9 (Galan et al., 1992). Salmonella has been known to penetrate cultured epithelial cells through at least two other invasion genes, and there is no evidence that such invA gene has alterations. Less S. Typhimurium invA mutants may invade epithelial cells in vitro. Epithelial

cells' microvilli retain their standard structure in the presence of InvA mutants, yet their actin microfilament pattern is unaffected. No development of invasive gene bands could also be produced by mutant genes incapable of replicating on the surface of the intestinal mucosa, as well as by vulnerability to bactericidal host factors such as phagocytes. InvA mutants are severely hindered in their capacity to penetrate cells.

Wang et al. (2020) also calculated the prevalence of different Salmonella serovars in sick and healthy chickens. On serotyping and PCR-based tests, 84.7% were revealed for *S. Pullorum, S. Gallinarum,* and *S. Enteritidis. Salmonella ser. Pullorum was shown to be the most prevalent in both sick and healthy chickens.* Of these three isolates, the last one was found in healthy chickens. According to El-Sharkawy (2017), The predominance of S. enterica in broiler chicken is also a design element. Sixty-seven isolates of Salmonella enterica were obtained, which showed the appearance of *S. Typhimurium and S. Enteritidis*2. The prevalence of *Salmonella* was also found high in different spices.

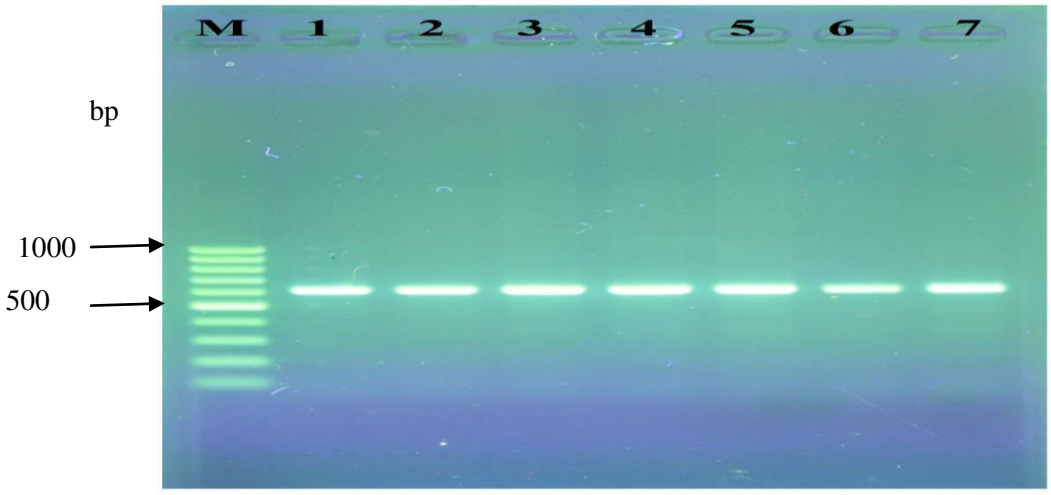

Figure 1 Amplicons of *16SrRNA* genedetected in *Salmonella spp*strains by polymerase chain reaction (PCR) technique. "M: 100 bp ladder; Lane 1": Positive; Lane 2–6: *Salmonella spp*isolates

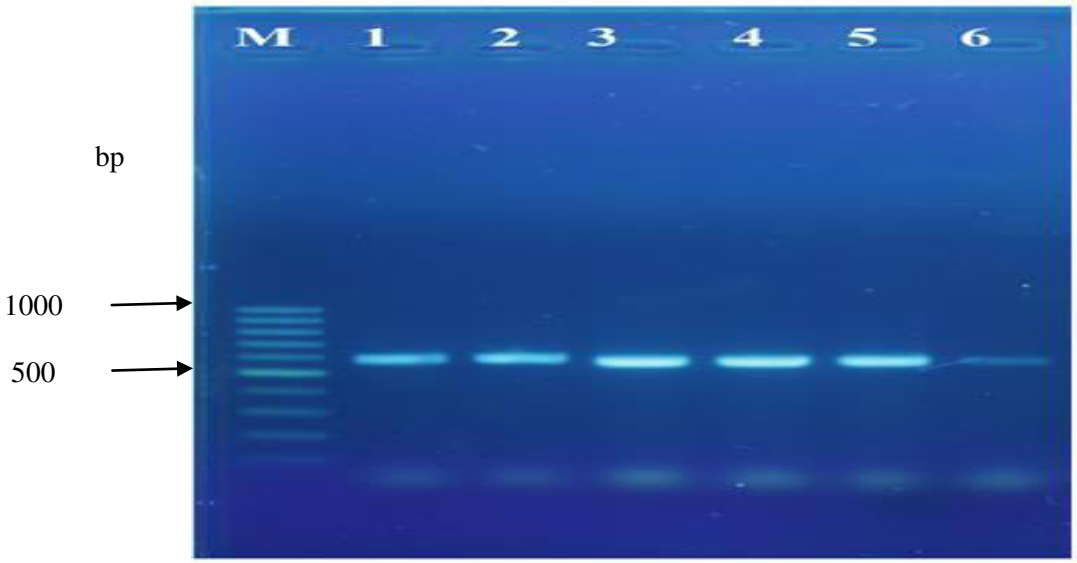

Figure 2 Amplicons of *spvA* genedetected in *Salmonella spp*strains by polymerase chain reaction (PCR) technique. M: 100 bp ladder; Lane 1: Positive; Lane 2–6: *Salmonella spp*isolates

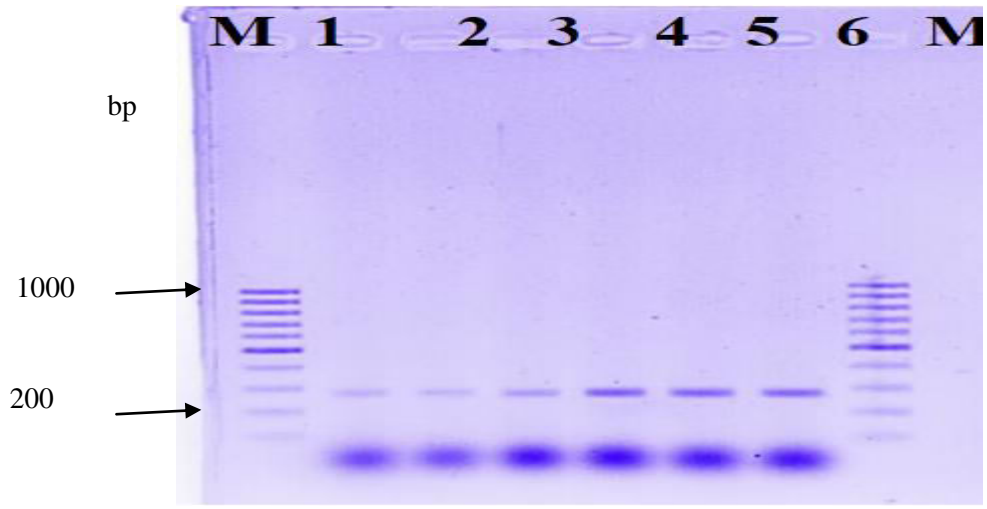

Figure 3 Amplicons of *spvCA* genedetected in *Salmonella spp*strains by polymerase chain reaction (PCR) technique. "M: 100 bp ladder; Lane 1": Positive; Lane 2–6: *Salmonella spp*isolates

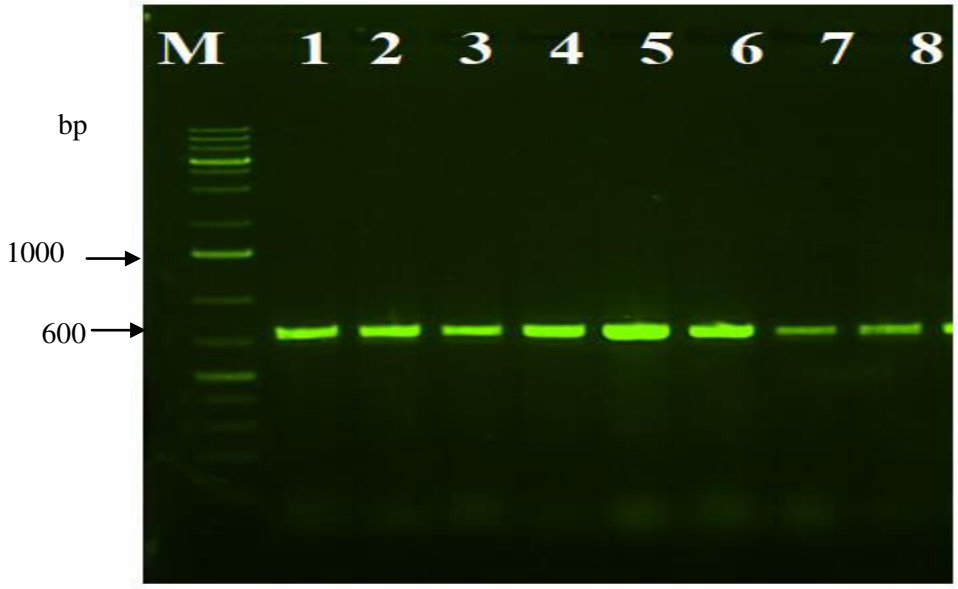

Figure 3 Amplicons of *spvCA* genedetected in *Salmonella spp*strains by polymerase chain reaction (PCR) technique. "M: 100 bp ladder; Lane 1": Positive; Lane 2–6: *Salmonella spp*isolates

CONCLUSION

Many of our food isolates included the salm3 virulence gene, which is significant for the nation's economy and reputation. As a result of the rising mortality and morbidity linked to salmonellosis and the potential for antibiotic resistance, food hygiene measures must be considered to reduce health risks and financial loss.To develop effective preventative strategies against Salmonella spp. in Iraq, more research on the precise distribution of virulence genes is crucial.

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