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Rapid Detection of Pathogenic *Salmonella* spp. in Green Leaves by Standard Microbiological Techniques and Molecular Methods in Erbil Province

Shean Ismail Salih [1], Blnd Ibrahim Mohammed [2,*], Ibrahim Ramadhan Ibrahim [3]

[1] Medical Microbiology Department, College of Health Sciences, Hawler Medical University, Erbil, Iraq
[2] Biology Department, College of Science, Salahaddin University-Erbil, Erbil, Iraq
[3] Fish Recourses and Aquatic Animals Department, College of Agriculture Salahaddin University, Erbil, Iraq

** Corresponding author: Blnd Ibrahim Mohammed, Biology Department, College of Science, Salahaddin University, Erbil, Iraq. E-mail: blnd.mohammed@su.edu.krd*

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Abstract

Salmonella is a group of Gram-negative rod shaped bacteria belongs to the Enterobacteriaceae family, 2,500 serotypes have been defined for *Salmonella*, are associated with 1.3 billion cases of gastroenteritis and 3 million deaths worldwide. For diagnosis of *Salmonella* spp. 100 vegetable samples was collected from four types of green leaves (Arugula, Leek, Celery and Cress) during February to April 2018, and then examined by cultural method using tow enrichment broth,. Afterward, *Salmonella* spp. were detected by PCR using Inv-A gene (796 bps). In this investigation, microbiological methods and molecular methods had been used for the diagnosis of *Salmonella* spp. Combination of both methods confirmed contamination of thirty samples which showed abnormal range for the contamination of vegetables in Erbil province. The present study concluded that both traditional and molecular based techniques like PCR are complementary. In this study, PCR study was carried out to determine the prevalence of *Salmonella* spp. at the level of Inv-A gene from vegetables, where microbiological findings were combined with the PCR results.

INTRODUCTION

The common healthy diet are vegetables which provide sufficient vitamins, minerals, and fibers for human and other animals [1]. Green leaves consider as source of foodborne illness by enteric pathogens including *Salmonella enterica, Listeria monocytogenes, and Escherichia coli* [2]. *Salmonella* spp. is common isolated foodborne bacteria which causes diseases through it is contamination in fresh fruits and vegetables. Data from other investigations concluded that *Salmonella* ssp. causes worldwide salmonellosis in human and animals [3].

Salmonella is a genus of Gram-negative rod shaped bacteria belongs to the Enterobacteriaceae family, *Salmonella* have about 2,500 serotypes associated with 1.3 billion cases of gastroenteritis and 3 million deaths worldwide [4]. The cultural methods are a good way, but not excellent for diagnosis of *Salmonella* as it is time-consuming which take up to 7 days, In addition, sensitivity of cultures may be affected by antibiotic treatment, inadequate sampling, variations of bacteremia and a small number of viable organisms in samples [5]. While PCR assay for the diagnosis of *Salmonella* is excellent way, but is moretimely manner. In addition, PCR diagnosis is more sensitive and can be done with inadequate sample size [6]. The aim of this study was the diagnosis of *Salmonella* ssp. at the level of Inv-A gene with combination between microbiological methods and PCR technique.

METHODS

Sample Collection

Vegetable samples were collected from north part of Iraq during February to April 2018, totally 100 samples were collected from four types of green leaves including (Arugula, Leek, Celery and Cress) only 25 samples were

collected from each type and then transported in sterile bags to the laboratory for analysis.

Culturing of Samples

Detection of *Salmonella* in leafy greens samples were performed according to the standard culture method ISO -6579: 2002. A 25 g sample was pre-enriched into 225 ml of buffered peptone water and then incubated at 37°C for 24 h. Subsequently, 0.1 ml of the pre-enrichment culture was added to 10 Ml of Rappaport-Vassiliadis broth and then followed by incubation for 24 h at 41.5°C.

DNA Extraction

Bacterial genomic DNA were extracted with the use of bacterial isolation kit (Bioscience, Germany), prior to the extraction, a loopful from each sample were cultured in 10 mL of phosphate buffer peptone water (Acumedia) for 18 h [7].

Primer Design and PCR Conditions

Primers were specified for the Inv-A gene from *Salmonella* spp. as previously described by Fratamico and Strobaugh (1998). Also, the set of primer has been confirmed by SnapGene Program.

Primers: [8].

F 1: CGG TGG TTT TAA GCG TAC TCTT

R 1: CGA ATA TGC TCC ACA AGG TTA

The Inv-A gene (796 bps) for the detection of the *Salmonella spp.* has been amplified by conventional PCR techniques, while the conditions for the PCR has been selected with the aid of gradient PCR techniques. Reaction mixture was prepared from MasterMix (Bioscience, Gemany) Table 1.

Table 1. PCR Condition Containing all Cycles Regarding to the Temperatures and Time [8]

Steps	Temperature	Time	Cycles
Initial Denaturation	95 °C	6 min	1
Denaturation	95 °C	30 sec	40
Primer Annealing	59-60 °C	35 sec	40
Elongation	74 °C	45 sec	40
Final Elongation	72 °C	4 min	1

Amplicons was separated by gel electrophoresis in order to confirm the size, location and quality of the PCR specific product for specific primers. The electrophoresis tank was prepared using a standard method. PCR Amplicons have been analyzed for the size of amplified target gene by using 1.5 % agarose gel electrophoresis. Furthermore, 6 µL from each amplified PCR mixture was mixed with 3 µL of loading dye and 3 µL of SYBR® Safe™ DNA Gel Stain (Quick-Load®)/ 100 mL of agarose and then runned using 1× TAE buffer at 93 V for 40 minutes, GeneRuler 1 kb DNA ladder (Quick-Load®) and images were captured through a Canon D100 (Canon Co., Japan).

RESULTS

In this investigation, microbiological methods and molecular methods were applied for the diagnosis of *Salmonella spp.* Combination of both methods confirmed contamination of thirty samples with abnormal range for vegetables in Erbil province. Result illustrated that PCR is more reliable and quicker than microbiological diagnosis as indicated in Table 2. Molecular technique (PCR) considered as the modern way for the bacterial diagnosis while microbiological methods less sensitive and both methods are more complementary than parallel (Fig 1).

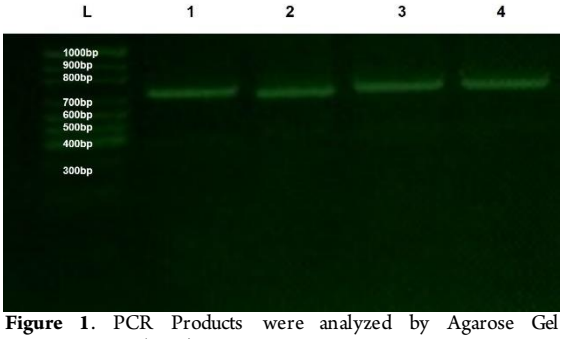

Figure 1. PCR Products were analyzed by Agarose Gel Electrophoresis (1.5%) under UV Light. Lane L: Ladder and Lane 1-4: Positive Result with Indicate the Presence of *Salmonella spp.* in Vegetables

Table 2. Difference Aspects of Traditional Microbiological Techniques in Comparison with PCR DNA based Techniques

Aspects	Microbiology	Molecular
Time	Slow 1-5 days	Fast 4-8 hours
Accurate rate	Low	High
Cost	Few	More

DISCUSSIONS

Molecular tools are increasingly relevant to diagnosis of pathogenic bacteria. The sequencing of the complete genomes of pathogens is allowing great advances in studying the biology, and improving diagnosis and control of pathogens [9]. Using nucleic acid as targets, and new methods polymorphism evaluation in this nucleic acid, can improve specificity, sensitivity, and speed of diagnosis and offer opportunity for examining the relationships of genotype with phenotype of various pathogens. Progress in techniques can be valuable in epidemiological studies as well as identification of disease outbreaks or the presence of pathogens. Therefore, molecular biology can be a routine tool for improved methods of diagnosis and control of *Salmonella spp.* pathogens [9, 10]. However the present study has the same conclude like other researches, suggesting high sensitivity of PCR over traditional techniques [11].

CONCLUSION

The present investigation concluded that combination of microbiological method (culture method) and molecular technique (PCR) could shorten time for the diagnosis of *Salmonella spp.* However, the use of either method alone would result in the failure, therefore, it would be more pertinent to use both methods for

maximizing the diagnosis rate of *Salmonella* spp. from contaminated vegetables.

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