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MOLECULAR IDENTIFICATION OF SALMONELLA ENTERICA SEROVAR FROM HUMAN BLOOD BY POLEMERASE CHAIN REACTION

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ABSTRACT

Salmonellosis is one of the major zoonotic diseases all over the world. The present study was carried out to report the prevalence of the genetic types of Salmonella obtained from patients blood who was suffering from food poisoning. Isolates were subjected to PCR using specific Salmonella primers by using PCR we identified the Salmonella strains named as Salmonellaenterica.

KEYWORDSDIARRHEA, PCR, *SALMONELLA*

INTRODUCTION

One of the most common infectious diseases found the world that is Salmonellosis. The genus *Salmonella* are gram negative and facultative anaerobic, rod-shaped bacteria [(1)]. The isolation and identification of *Salmonella* species from clinical samples is obtained by traditional cultural techniques which require 7 days to complete (stone el al, 1994). Now in recent years, diagnosis have been reducing the time required for identified *Salmonella* infections and more rapid method to identify *Salmonella* from food poisoning patient[(2)]. *Salmonella* usually pass through the food chair from various food sources and finally human, occasionally causing *Salmonellosis*[(3)].

The present study is based on investigating Salmonella using PCR to analysis and identifies the genetic relationship of *Salmonella* isolates from patient with food poisoning signs.

MATERIALS AND METHODS

Sampling Blood were collected from patients with food poisoning signs (Fever, vomiting, abdominal pain and diarrhea). The collected samples were labeled and directed to the laboratory for bacteriological examination.

Isolation and Identification of *Salmonella*

The collected samples is firstly inoculated in the nutrient broth, so that multiples cells should be obtained now take a loop full of broth and streaked on Salmonella – shigella agar (S.S. agar) and Xylose lysine Deoxycholate agar (XLD – agar) and incubated for 24 hrs. at 37^0C. Colonies of growth were picked up and confirmed morphologically (4) and biochemically(5).

ISOLATON OF DNA

Total genomic DNA from the bacteria was isolated by N-cetyl-N, N, N-tri-methyl ammonium bromide (CTAB) method

DNA Isolation Protocol

- 1 ml bacterial culture, centrifuge at 10000 rpm 2 min. at 4^0C.
- Wash the pellet with sterile distilled water. (Centrifuge at 1000rpm 20 min. at 4^0C)
- 675 µl of extraction buffer was added and incubated at 37°C for 30 min.
- 75µl of SDS (20%) was added and incubated at 65°C for 2 hours.
- Centrifuged at 10000 rpm for 10 min at 4°C
- Clear solution was collected in a sterile microcentrifuge tube.
- Equal volumes of Chloroform: Isoamyl alcohol (24:1) was added.
- Centrifuged at 10000 rpm for 10 min. at 4°C
- The aqueous phase was removed and taken in a sterile microcentrifuge tube.
- 0.6 volumes of isopropyl alcohol was added and incubated at room temperature for 1hour.
- Centrifuged at 10000 rpm for 10 min.
- Pellet was washed in 500µl of 70% ethanol.
- Centrifuged at 10000 rpm for 10 min at room temperature.
- Pellet was dried and dissolved in 20 µl sterile distilled water.

Quantification of Isolated DNA

The quantity of the isolated DNA was checked in UV-VIS spectrophotometer (VivaspecBiophotometer, Germany). From the stock 1µl DNA was mixed with 49-µl sterile distilled water to get 50 times dilution. The A260/A280 ratio was recorded to check the purity of DNA preparation.

PCR AMPLIFICATION

Reagents and the Optimal PCR Reaction Mixture

PCR amplification of 16s region was done in 20 µl of reaction mixture containing PCR buffer, 1X (Kappa, SA); $MgCl_2$, 3 mM; dNTP mix, 0.25 mM; *Taq*DNA polymerase, 0.05 U; primer, 1 picomol and template DNA, 50 ng. Sterile nuclease free water is used as negative control.

TABLE 1.1 REAGENTS AND THE OPTIMAL PCR REACTION MIXTURE

PCR components	Volume (µl)
Nuclease free water	10.75
10X reaction buffer with $MgCl_2$ (1.5mM)	2.00
dNTP mix (2.5mM)	2.00
Primer 16S F (10picomoles/ µl)	2.00
Primer 16S R (10picomoles/ µl)	2.00
Taq DNA polymerase (5U)	0.25
Template DNA (50ng/ µl)	1.00
Total volume	20.0

TABLE 1.2 OLIGONUGLEOTIDE PRIMERS

Oligonucleotide	Sequences (5'- 3')	GC %	Tm Value	Length	Product Size
16S FP	AGA GTT TGA TCC TGG CTC AG	50	51.0 ^{0}C	20	1500 bp
16S RP	AAG GAG GTG ATC CAG CCG CA	60	56.0 ^{0}C	20	

RESULTS

After performing all the test related to my present work, it is found that the Morphology and Bio-chemical test of *Salmonella* species shows in green table below –

TABLE 1.3 MORPHOLOGY, MOTILITY & BIOCHEMICAL TEST OF *SALMONELLA* SPECIES

Sr. No.	Tests	Results
1	Gm-Staining	-ve
2	Motility	+ve
3	Indole	-ve
4	Methyl Red	+ve
5	Vogues-Proskour	-ve
6	Simmon's Citrate	+ve
7	Hydrogen Sulphide	+ve
8	Catalase	+ve
9	Glucose (Gas production)	+ve
10	Lactose	-ve
11	Sucrose	+ve
12	Mannitol	-ve
13	Arabinose	+ve

-ve - negative

+ve – Positive

In this work molecular genetic study has been carried out to identify the genetic characters of *Salmonella* isolated from infected patients. By using PCR it has been identified that the name of *Salmonella* species is *Salmonellaenterica.*

TABLE 1.4 BACTERIAL IDENTIFICATION OF *SALMONELLA* SPECIES USING PCR

Sr. No	Sample ID	Identification (BLAST)	Percentage similarity
1	*Salmonella*entero	*Salmonellaenterica*	95%

16S rRNA gene Sequence

5’TACATTTGCCGCAGTCTACCATGCAGTCGACGGTAACAGGAAGCAGCTTGCTG
CTTCGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGA
GGGGGATAACTACTGGAAACGGTGGCTAATACCGCATAACGTCGCAAGACCAAA
GAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCCAGATGGGATTAGCTTGT
TGGTGAGGTAACGGCTCACCAAGGCGACGATCCCTAGCTGGTCTGAGAGGATGA
CCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGG
GGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGA
AGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGTGTTGTGGTTAATAA
CCGCAGCAATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCA
GCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCG
CACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAAC
TGCATTCGAAACTGGCAGGCTTGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGT
AGCGGTGAAATGCGTATAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCT
GGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGAT
ACCCTGGTAGTCCACGCCGTAAACGATGTCTACTTGGAGGTTGTGCCCTTGAGGC
GTGGCTTCCGGAGCTAACGCGTTAAGTACACCGCCTGGGGAGTACGGCCGCAAG
GTTAAAACTCAATTGAATTGACGGGGGCCCCGCACAAGCGGTGGAGCATGTGGT
TTATTTCGATGCAACGCGAATAACCTTACCTGGTCTTGACATCCACAGAAGAATC
CAGAGATTGATTTTGTGCCTTCGGGAACTGTGAGAACAGTGCTGCATGCCTAGCC
GTCAGCTCGGTTTGATAAATGTTGGTTAAGTCCCGCAACGAGCGCTACCTTTAAT
CCTTTTGTTGTCATGCGTAGCCGTAACTTCAAAGCAACTGCCATGATATCTGAAG
AGTTGGATGACTCAGTCACTTTGACCTTAGACCAGGCTTAACCGCAAGTTCAATG
GCAGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGA
GCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACT
CCATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGT
TCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAA
GTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGG
AAGTCGAACAAGTGGCGTTGCGCCA3’

These sequence had been send to NCBI Gen-Bank (National Center for Biotechnology Information) for registration, and we get the two a cession numbers, there are

1) LC068760 2) LC068761

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