

Original Research Article

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## Genetic Diversity Profiles of *Salmonella typhimurium* Isolated from Foods of Animal Origin

Yasarla Suresh\*, Chinnam Bindhu kiranmayi, Thummati Srinivasa Rao,  
M. Srivani and Metta muralidhar

Department of Veterinary Publichealth and epidemiology, N.T.R. C.V.Sc, Gannavaram, India

\*Corresponding author

### ABSTRACT

*Salmonella* is a foodborne pathogen having a worldwide public health concern. The present study was undertaken to characterize *Salmonella typhimurium* of animal origin based on cultural isolation and genetic diversity studies by employing ERIC-PCR and REP-PCR. A total of 516 samples comprising poultry cloacal swabs (249), raw foods of animal origin (118 chicken samples, 65 mutton and 30 pork), 17 poultry liver swabs and 37 poultry farm water samples were examined for presence of *Salmonella* serovars. A total of 21 *Salmonella* were isolated among them, 7 were *S. typhimurium*. For all 7 isolates REP and ERIC PCR were employed to study genetic diversity among *Salmonella* isolates. ERIC PCR and REP-PCR analysis revealed a greater degree of heterogeneity among *S. typhimurium* isolates from different sources. ERIC-PCR and REP-PCR genotyping distinguished 7 isolates. The discriminatory power of ERIC-PCR and REP-PCR for *Salmonella typhimurium* isolates was found to be highly significant (>0.9) i.e. 1.0, for both.

#### Keywords

Enterobacteriaceae,  
Gram-negative,  
Lactose non  
fermenting, Rod-  
shaped bacteria

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### Introduction

*Salmonella* species is responsible for a wide range of acute and chronic diseases in both poultry and humans. Contaminated poultry products are among the most important sources for foodborne outbreaks in humans. *Salmonella* is reported more frequently from poultry and poultry products than from any

other animal species. *Salmonellae* are Gram-negative, lactose non fermenting, rod-shaped bacteria belonging to the family Enterobacteriaceae. *Salmonella* is the most important agent implicated in outbreaks of food-borne diseases around the world (Lacey, 1993). Fuzihara *et al.*, 2000) Typing of bacteria can be used to determine whether isolates recovered from different patients or

from the environment are related and thus, provide evidence for a common source of transmission of the agent.

Traditional epidemiological methods include biotyping, serotyping and phage typing of isolates, as well as antimicrobial resistance testing, although these methods do not always give enough information for epidemiological purposes. Among the molecular methods for typing bacteria, the REP-PCR, ERIC-PCR and BOX-PCR genomic fingerprinting have been found to be extremely reliable, reproducible, rapid and highly discriminatory (Olivera *et al.*, 2007). ERIC sequences have been used for determining the genetic diversity among different pathogenic bacteria in the Enterobacteriaceae family including *Salmonella*.

This technique relies on the amplification of genomic DNA fragment using single primer pair, which is complimentary to the short repetitive sequences and generation of reproducible and complex fingerprints (Anjay *et al.*, 2015). The REP-PCR relies on primers that are complementary to the short repetitive sequence elements, dispersed throughout the bacterial genome, to generate DNA fingerprints that allow discrimination between strains (Versalovic *et al.*, 1991).

### **Materials and methods**

A total of 7 *Salmonella typhimurium* isolates which were isolated from the different foods of animal origin and one *Salmonella typhimurium* standard culture (ATCC 14028) was used as standard for ERIC and REP-PCR.

### **Assessment of genetic diversity**

Assessment of the genetic relatedness among the different *Salmonella* isolates was done by performing molecular techniques such as ERIC-PCR and REP-PCR.

### **Genotyping by ERIC-PCR**

*S. typhimurium* (7) isolates were selected and fingerprinted using ERIC-PCR assay as described by Ye *et al.*, (2011). Genotyping of *Salmonella* isolates was done using the oligonucleotide primers, ERIC-1 (5'-ATG TAA GCT CCT GGG GAT TCA C-3') and ERIC-2 (5'-AAG TAA GTG ACT GGG GTG AGC G-3') primer pair targeting the amplification of conserved ERIC sequences in the chromosomal DNA of *Salmonella* isolates, leading to amplicon patterns specific for an individual organism (Versalovic *et al.*, 1991). ERIC-PCR was carried out in 25 µl optimized reaction mixture under standardized thermal cycling conditions (Table 1). PCR products were subjected to 1.5% agarose gel electrophoresis and visualized using Gel Documentation unit (BIORAD, USA).

### **Genotyping by REP-PCR**

*S. typhimurium* (7) isolates obtained from different sources were subjected to REP-PCR fingerprinting using single oligonucleotide primer (GTG)<sub>5</sub> (GTGGTGGTGGTGGTG) as described by Prasertsee *et al.*, (2016) with slight modifications. PCR reactions were optimized in 25 µl volume reaction mixture under standardized thermal cycling conditions given in (Table 1). PCR products were subjected to 1.5% agarose gel electrophoresis and visualized using Gel Documentation unit (BIORAD, USA).

### **Results and Discussion**

ERIC-PCR typing revealed 2-10 fragments per isolate, ranging in size from ~120 bp to ~2000 bp, whereas REP-PCR typing revealed 4-10 fragments resolved per isolate, ranging in size from ~140 bp to ~1400 bp (Figure 1 & 2).

Of the 7 *S. typhimurium* analyzed, 6 ERIC-PCR patterns & 7 REP patterns were obtained.

The binary score demonstrating the variety of 6 ERIC (E1-E6) and REP PCR genotypes (R1-R7) were obtained. A pair of *S. typhimurium* isolates (3 and 4) that had identical ERIC-PCR pattern (E3) were distinguishable in REP-PCR pattern (R3 & R4) (Table 2). The two *S. typhimurium*

isolates sharing identical ERIC-PCR were recovered from chicken meat samples (C 4 and C 5) of different birds slaughtered in the same chicken retail shop. Dendrograms were constructed based on ERIC and REP-PCR profiles (Fig. 1–3) using dollop program of phylip 3.6 version.

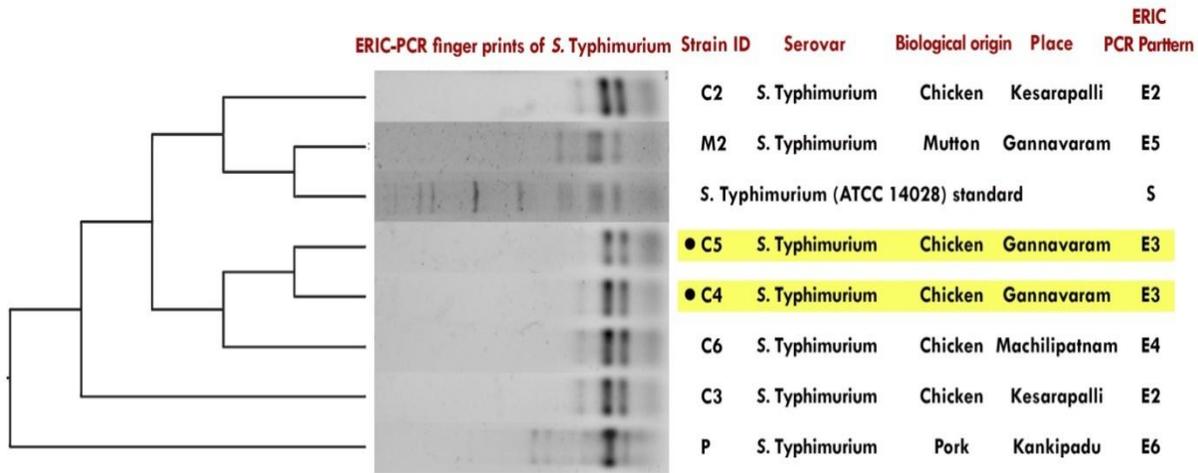
**Table.1** Standardized thermal cycling parameters for ERIC and REP-PCR of *Salmonella* isolates

Standardized cycling parameters			
Steps	ERIC-PCR	Rep-PCR	No. of cycles
<b>Initial denaturation</b>	95°C for 5 min	95°C for 5 min	1
<b>Denaturation</b>	94°C for 1 min	94°C for 45 sec	40
<b>Annealing</b>	50°C for 1 min	40°C for 1 min	
<b>Extension</b>	72°C for 2 min	65°C for 10 min	
<b>Final extension</b>	72°C for 10 min	65°C for 20 min	1
<b>Hold /stand by</b>	4°C for 10 min	4°C for 10 min	----

**Table.2** Genetic diversity of *S. typhimurium* from different sources by using ERIC-PCR and REP-PCR

Species	Biological origin	Number of Isolates	Number of ERIC-types	Number of REP-types
<i>S. typhimurium</i>				
<b>n=7</b>	Chicken	5	4	5
	Mutton	1	1	1
	Pork	1	1	1
<b>Total</b>		<b>7</b>	<b>6</b>	<b>7</b>

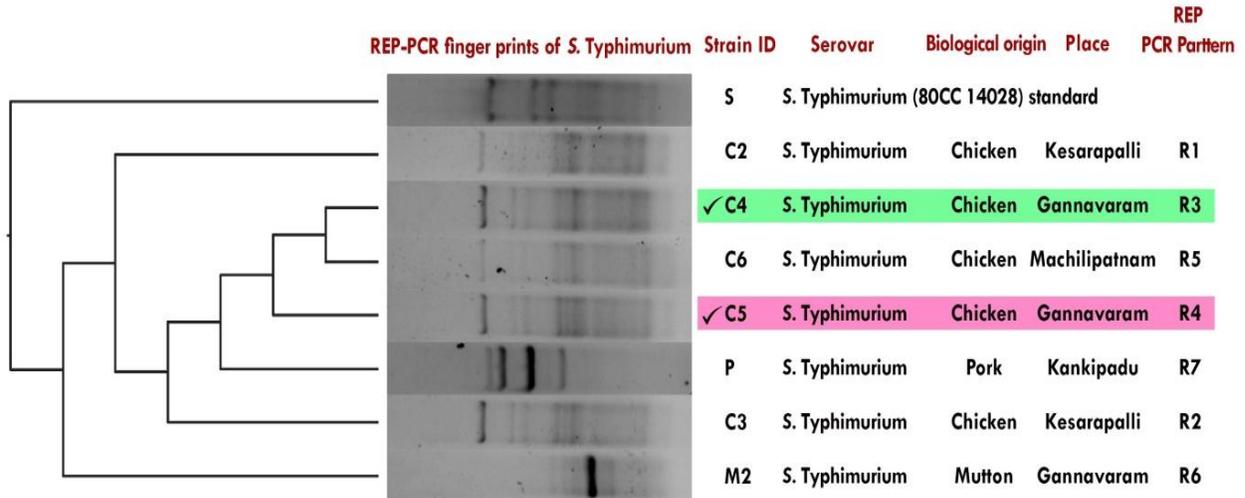
**Fig.1** Dendrogram analysis of ERIC-PCR finger printing of *S. typhimurium* isolated from different sources. The dendrogram was generated by branch and bound method using dollop program of PHYLIP 3.6 version.



The circle (●) in front of the strain ID indicates strains that had same ERIC-PCR pattern (E3) but had different REP-PCR patterns (R3 and R4)

Fig-26: Dendrogram analysis of ERIC-PCR finger printing of *S. Typhimurium* isolated from different sources. The dendrogram was generated by 'Branch and Bound method' using dollop program of PHYLIP 3.6 version

**Fig.2** Dendrogram analysis of REP-PCR finger printing of *S. typhimurium* isolated from different sources. The dendrogram was generated by branch and bound method using dollop program of PHYLIP 3.6 version



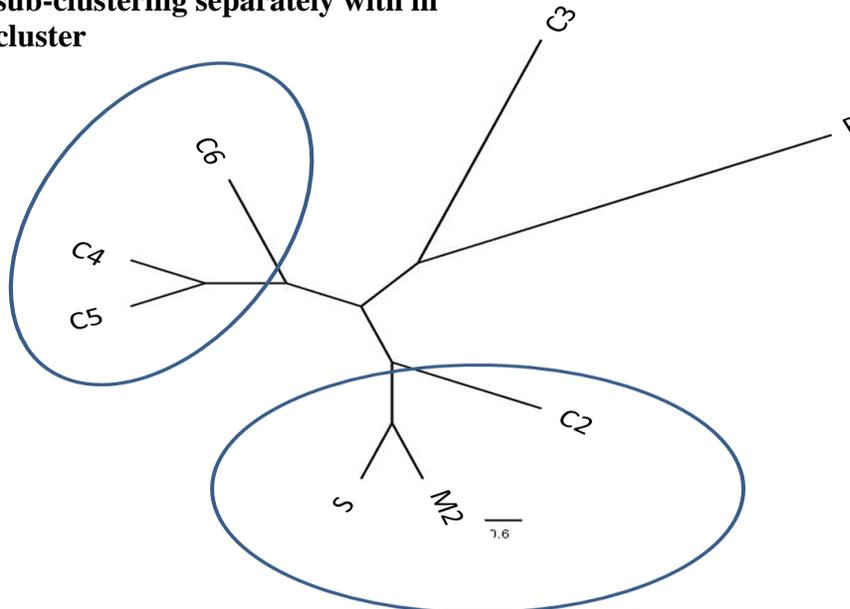
The circle (✓) in front of the strain ID indicates strains that had different REP-PCR patterns (R3 and R4) but had same ERIC-PCR pattern (E3)

Fig-28: Dendrogram analysis of REP-PCR finger printing of *S. Typhimurium* isolated from different sources. The dendrogram was generated by 'Branch and Bound method' using dollop program of PHYLIP 3.6 version

**Fig.3** Cluster analysis of ERIC-PCR fingerprints of *S. typhimurium* isolates from different sources. An unrooted phylogenetic tree constructed using dollop program of phylip 3.6 version (Branch-and-Bound algorithm)

**CLUSTER I**

Isolates recovered from chicken, sub-clustering separately with in cluster

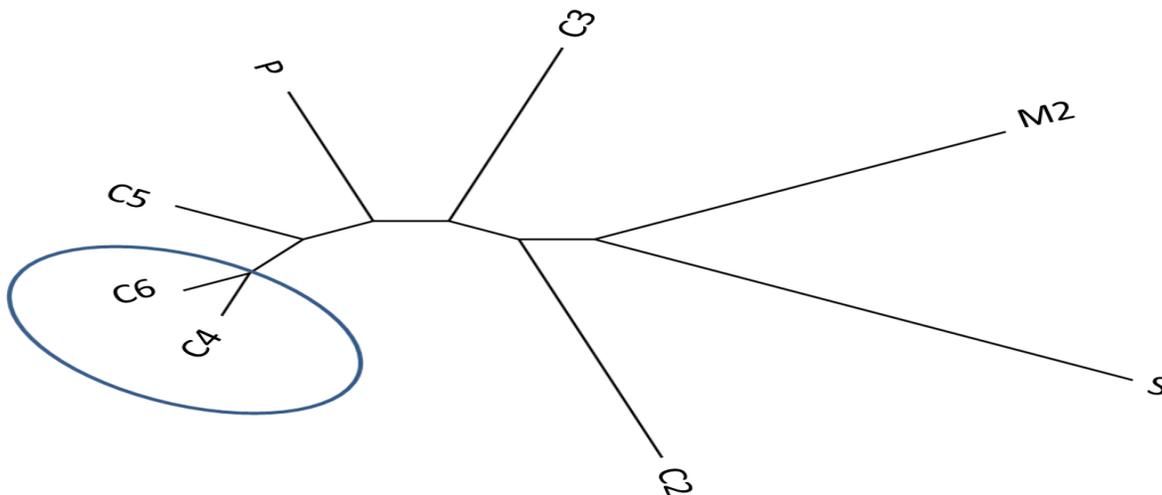


**CLUSTER II**

Isolate from Chicken clustering separately from isolate of mutton and reference standard.

C- Chicken, M- Mutton, S- *Salmonella typhimurium* ATCC 14028

**Fig.4** Cluster analysis of REP-PCR fingerprints of *S. typhimurium* isolates from different sources. An unrooted phylogenetic tree constructed using dollop program of phylip 3.6 version (Branch-and-Bound algorithm)



C- Chicken, M- Mutton, S- *Salmonella typhimurium* ATCC 14028

Clustering of chicken isolates, C4 and C6

Dendrogram analysis of ERIC-PCR profiles discriminated *S. typhimurium* isolates into two major clusters (each with three isolates) i.e. CL1 (C6, C5, C4) and CL 2 (C2, M2 and reference standard *S. typhimurium* ATCC 14028) for a 70% similarity cut-off. Two isolates (C3, P) were found to be unclustered (UC) with other isolates. Within the cluster CL1, isolate C6 clustered separately from that of other two isolates (C5, C4). Within the cluster CL2, isolate C2 clustered separately from that of other two isolates (M2 and reference standard) (Figure-3). Cluster analysis indicated wide genetic diversity among the isolates.

Dendrogram analysis of REP-PCR profiles discriminated *S. typhimurium* isolates into a major cluster with two isolates (C4, C6) for a 70% similarity cut-off, while the other five isolates along with the reference standard were found to be unclustered (UC) with other isolates (Figure 4). Cluster analysis indicated wide genetic diversity among the isolates.

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