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16-23S rRNA Spacer Region Polymorphism in Gangetic River Water Isolates of *Salmonella*

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Abstract

Salmonella is one of the major pathogenic bacteria present in contaminated water. 16-23S rRNA spacer region has been reported to be polymorphic at serovar level in *Salmonella*. *Salmonella* isolates obtained from Ganges river water were studied for 16-23S rRNA spacer region polymorphism. Thirty three isolates belonging to eight serovars (*S*. Typhimurium, *S*. Abuja, *S*. Pantypridd, *S*. Lagos, *S*. Chinkual, *S*. Zwickau, *S*. Goldenberg and *S*. Oritamerin) were studied for the polymorphism. Out of 33 isolates, 15 different profiles were observed no serovar specific profile. Our findings indicate that 16-23S rRNA spacer region is not specific at serovar level, but can be used for differentiation of different *Salmonella* isolates.

Keywords: Ganges River, *Salmonella*, Spacer Region Polymorphism, 16-23s rRNA

1. Introduction

The Ganges is a major river in Indian sub-continent and is considered to be Sacred and has been declared as National River by Indian Government in Year 2008. The fertile soil of the river basin measuring around one million square kilometers is a key to agricultural economy of the country and supports one of the highest human density populations. The large quantity of pollutant such as untreated sewage material in large volume (approximately one billion liters per day) [1] are disposed off in river Ganges that leads to accumulation of hazardous pathogens like *Shistosoma*, *Escherichia coli* O157:H7, *Shigella and Salmonella* [1-3] and expose the surrounding population to these diseases.

Salmonella is considered a dreaded pathogen causing several diseases such as endocarditis [4], Typhoid and Paratyphoid fever [5], Pneumonia [6] and meningitis [7]. Identification and serovar differentiation are prime requisite for controlling Salmonellosis. Conventional serovar typing is cumbersome and time consuming process [8], but with the development of molecular biology techniques like PCR genome has been targeted for this purpose. In the genome of the bacteria rRNA region has been extensively used for taxonomic purpose [9,10]. In *Salmonella*, 16-23S rRNA region has been studied and reported to be serovar specific [11], as he differentiated many serovars of *Salmonella* using 16-23S rRNA polymorphism. The basis of differentiation of serovars was based on variation in number and type of t-RNA sequences found within the spacer region of 16-23S rRNA [12]. The present study was undertaken to differentiate eight serovars of *Salmonella* isolated from Ganges river water by targeting 16-23S rRNA region.

2. Material and Method

Thirty three isolates of *Salmonella* were obtained from six different locations (stations) viz, Haridwar, Hastinapur, Kanpur, Garhmukteshwar, Varanasi and Narora. These isolates were serotyped at National *Salmonella* Centre I.V.R.I. (Izatnagar) India, and were characterized as *S*.Typhimurium, *S*. Abuja, *S*. Pantypridd, *S*. Lagos, *S*. Chinkual, *S*. Zwickau, *S*. Goldenberg and *S*. Oritamerin (**Table 1**). Genomic DNA was isolated from all isolates by C-TAB method [13].

3. 16-23 S rRNA Spacer Region PCR

Five µl (40ng) of genomic DNA was used for 50µl of PCR reaction mixture, containing 20 pmol of each primer (Primer1 (5' GAA GTC GTA ACA ACG 3') and Primer2 (5'CAA GGC ATC CAC CGT 3'), 200 µM of dNTPs, 3.0U of Taq polymerase and 1.5 mM final concentration of $MgCl_2$. All chemicals used in PCR were procured from MBI-fermentas. PCR program was as follows-initial denaturation (94℃ for 5 min.) followed

Table 1. Description of *Salmonella* isolates (serovars, place of isolation, band profile and Molecular type) isolated from various locations from river Ganges.

S.No.	No. of isolate	Serovar	Place of isolation	Band Size (base pairs)	Molecular Type
1	G-29	*Salmonella*Typhimurium	Narora	529, 740	M1
2	G-33	*Salmonella* Typhimurium	Narora	529, 740	M1
3	G-36	*Salmonella* Typhimurium	Narora	529, 740	M1
4	G-40	*Salmonella* Typhimurium	Narora	529, 740	M1
5	G-23	*Salmonella* Typhimurium	Narora	529, 740	M1
6	G-28	*Salmonella* Typhimurium	Narora	529, 740	M1
7	G-35	*Salmonella* Typhimurium	Narora	529, 740	M1
8	G-37	*Salmonella* Typhimurium	Narora	529, 740	M1
9	G-38	*Salmonella* Typhimurium	Narora	529, 740	M1
10	G-47	*Salmonella* Typhimurium	Narora	529, 740	M1
11	G-4	*Salmonella* Abuja	Hastinapur	645	M2
12	G-6	*Salmonella* Abuja	Hastinapur	645, 414	M3
13	G-7	*Salmonella* Pantopyroid	Kanpur	645, 414, 334	M4
14	G-12	*Salmonella* Lagos	Haridwar	414, 334	M5
15	G-13	*Salmonella* Lagos	Haridwar	414, 334, 744, 645	M6
16	G-15	*Salmonella* Lagos	Haridwar	645, 414, 334	M4
17	G-17	*Salmonella* chinkaul	Varanasi	645	M2
18	G-19	*Salmonella* Abuja	Hastinapur	645	M2
19	G-22	*Salmonella* Abuja	Hastinapur	645	M2
20	G-25	*Salmonella* Zwickau	Narora	744, 645	M7
21	G-39	*Salmonella* Oritamerin	Narora	645	M2
22	G-1	*Salmonella* Typhimurium	Haridwar	1820, 1487, 750, 603, 532	M8
23	G-10	*Salmonella* Typhimurium	Hastinapur	1820, 1487, 750, 603, 532	M8
24	G-14	*Salmonella* Typhimurium	Garhmukteshwar	1820, 1487, 750, 603, 532	M8
25	G-11	*Salmonella* Typhimurium	Hastinapur	738, 594	M9
26	G-16	*Salmonella* Typhimurium	Haridwar	1820, 1487, 750, 603, 532	M8
27	G-20	*Salmonella* Typhimurium	Garhmukteshwar	1141, 705, 566, 193, 144	M10
28	G-21	*Salmonella* Typhimurium	Garhmukteshwar	2408, 1441, 705, 560	M11
29	G-45	*Salmonella* Typhimurium	Narora	538, 733	M12
30	G-46	*Salmonella* Typhimurium	Narora	538, 733	M12
31	G-50	*Salmonella* Typhimurium	Narora	2435, 1758, 1431, 538, 733	M13
32	G-52	*Salmonella* Typhimurium	Narora	1758, 1431, 538, 733	M14
33	G-55	*Salmonella* Typhimurium	Narora	1291, 538, 733	M15

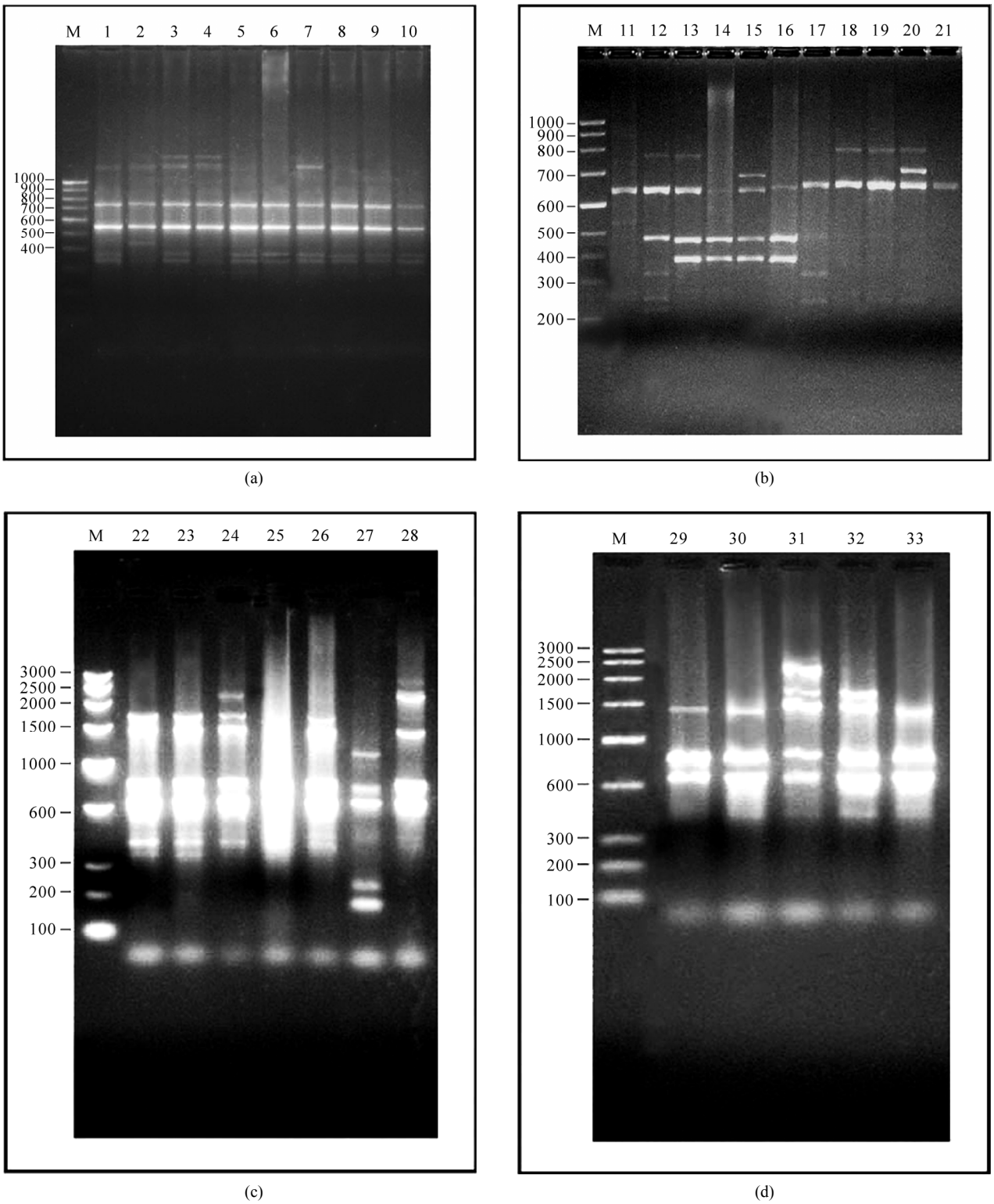

Figure 1. 12-23 SrRNA Spacer region amplicon of Gangatic isolates of *Salmonella*. (a) M: Marker 100 bp ladder (Baglore genei, India) Lane 1-10 *Salmonella* isolates (G29, G33, G36, G40, G23, G28, G35, G37, G38, G47); (b) M: Marker 100 bp (Baglore genei, India) Lane 11-21 *Salmonella* isolates (G4, G6, G7, G12, G13, G15, G17, G19, G22, G25, G39); (c) M: DNA Marker (100-3000) bp low range DNA rular (Baglore genei, India) Lane 22-28 *Salmonella* isolates (G1, G10, G14, G11, G16, G20, G21); (d) M: DNA Marker (100-3000) bp low range DNA rular (Baglore genei, India) Lane 29-33 *Salmonella* isolates (G45, G46, G50, G52, G55).

by 30 cycles composed of denaturation (94℃ for 1 min.), annealing (54℃ - 2min) and polymerization (72℃ - 2 min.). Final extension was carried out at 72℃ for 10 minutes. Five µl of amplified product was loaded on 1.5% agarose gel and electrophoresis was conducted in 1XTAE at 5V/cm. for two hours. Each sample was amplified thrice with negative control to avoid possibility of artifacts. Molecular weight of amplicon was measured by comparing 100bp ladder (Banglore Genei) using Bio1-D Software.

4. Results and Discussion

In all serovars, 16-23S r-RNA Spacer region was amplified. After separation of PCR product on 1.5% agarose gel only intense bands were scored. The relative molecular weights of amplicons were compared to the band with Standard molecular weight marker (100 bp ladder B.Genei India). Size of bands ranged from 144 to 2435 bp. All the isolates could be grouped into 15 profiles (M1-M15). M1 was most common and observed in 11 isolates. Eight profiles (M1, M8, M9, M10, M11, M12, M14 and M15Profile) were observed in 24 isolates of *Salmonella enteric* serovar Typhimurium. Two profiles M2 and M3 were observed in four isolates of *S*.Abuja. Three isolates of *S*.Lagos exhibited three different profiles M4, M5 and M6. Two isolates of *S*.Chinkaul and one isolate of *S*. Oritamerin shared common profile M2.One isolate of *S*. Zwickau obtained from Narora had altogether different profile M7 (**Figure 1**, **Table 1**). Comparison of similar and dissimilar bands of amplicon with Jaccord's coefficient value revealed genetic variability in different isolates. On cluster analysis using Unweighted Pair Group Methods with Arithmetic Mean (UPGMA) the genetic similarity between 33 different isolates of Salmonella ranged from 0 to 100% and five major clusters were produced five major clusters at the level of zero similarity index.

In this study eight serovars of *Salmonella* were isolated by molecular methods from Ganges river water at six different locations (stations). *Salmonella enterica* serovar Typhimurium is considered a life threatening pathogen with zoonotic importance [14,15] and is found to be commonly associated with multiple drug resistance in human clinical cases [16]. *Salmonella* Oritamerin which is common in other countries [17] but not so frequently reported in India were present in Ganges water possibly due to the congregation of vast human population from across the diverse geographical boundries during

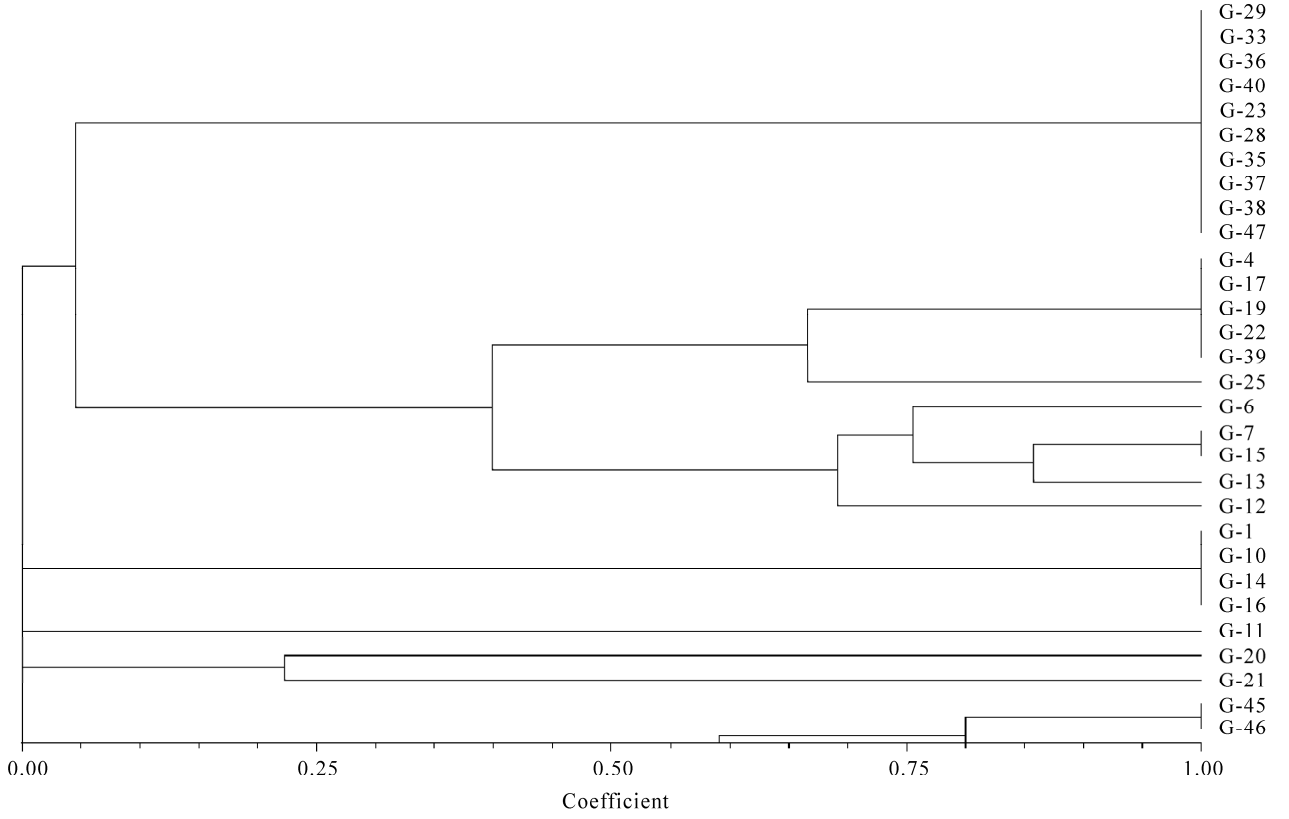

Figure 2. Dendrogram visualizing genetic variation in 16-23S rRNA spacer regions of isolates of *Salmonella* isolated from Ganges water.

festivals such as Kumbh.

On amplification of spacer region the product size varied from 529 to 2435 bp, which is very similar to the product size range reported by Jensen and Hubner [18]. The Variation of rRNA loci in *Salmonella* genome [19] and high genetic diversity of intergenic spacer region [20] could be attributed as possible reasons for high genetic variation shown by the isolates. We did not observed any serovar specific profile in the present study. *S*. Abuja and *S*. Chinkaul serovars shared the common profile M2 and *S*, Typhimurium the most common serovars observed in this study exhibited eight profiles. With these findings we suggest that 16-23S rRNA spacer region is not serovar specific but has a high genetic variation can be used for molecular typing of field isolates of *Salmonella*.

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