Association of Three Plasmid-Encoded *spv* Genes Among Different *Salmonella* Serotypes Isolated from Different Origins

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Received: 30 July 2012 / Accepted: 2 October 2012 / Published online: 20 October 2012
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Abstract The virulence plasmid associated *Salmonella* plasmid virulence (*spv*) locus is strongly concomitant with strains that cause non typhoid bacteremia. The *spv* region contains three genes required for the virulence, the positive transcriptional regulator *spvR* and two structural genes *spvB* and *spvC*. The purpose of this study was to investigate the presence of these three genes among *salmonella* serotypes isolated from different sources. A collection of 60 *salmonella* serotypes from different sources were used. Polymerase chain reaction was carried out for the presence of these genes using specific primers. The prevalence of *spvB*, *spvC*, and *spvR* genes were 26 (43.3 %), 44 (73.3 %), and 28 (46.6 %), respectively. The findings revealed that the distribution of these genes was dissimilar among these serotypes. Many of the human pathogenic *salmonella* strains which can be transmitted by animals may have these genes and can be very injurious for public health.

Keywords *Spv* genes · *Salmonella* serotype · PCR

Introduction

During the last decades, the isolation of *Salmonella* has been increased throughout the world [1]. Members of the genus *Salmonella* infect a wide variety of hosts and cause diseases in many animal species and in human beings according to their antigenic profiles [2, 3]. Salmonellosis is associated with medium to severe morbidity and even mortality in farm animals, representing major economic

losses in the food and animal industries [4]. Certain serovars are particularly associated with systemic infections. A common feature of these serovars is the presence of the plasmid-encoded *spv* genes [5]. One main function of the *spv* operon is to potentiate the systemic spread of the pathogen [6]. The *spv* locus is strongly associated with strains that cause non-typhoid bacteremia, but are not present in typhoid strains, indicating that the pathogenesis and immunology of typhoid have fundamental differences from the syndrome of non-typhoid bacteremia [7]. *Spv* genes are found in nearly all natural isolates of *Salmonella* that are host adapted to animals (*Salmonella dublin*, *S. choleraesuis*, *S. gallinarum-pullorum*, and *S. abortusovis*) but not in *S. typhi*. Plasmid-encoded *spv* genes are also found in the common broad-host-range serovars, *S. typhimurium* and *S. enteritidis*, but only a variable proportion of isolates carries these virulence plasmids [8]. The *spv* locus consists of five genes, designated *spvRABCD* [9], three of which are required for the virulence phenotype: the positive transcriptional regulator *spvR* and two structural genes *spvB* and *spvC*. The *spvD* gene is missing in the chromosomal locus as found in serovar Arizona [10] and it has been shown that the *spvA* gene is dispensable for virulence in orally inoculated mice [11]. *SpvB* prevents actin polymerization by ADP-ribosylation of actin monomers. The C-terminal domain of *SpvB* contains ADP-ribosyltransferase activity that covalently modifies G-actin monomers and prevents their polymerization into F-actin filaments [12]. Since F-actin is continuously formed and depolymerized in the cell, the activity of *SpvB* in the host cell cytoplasm leads to loss of the F-actin cytoskeleton [13], furthermore, *SpvB* is required for *Salmonella* proliferation in a subset of monocyte derived human macrophages, and is responsible for the late apoptosis seen in host cells during *Salmonella* infection [14]. *SpvC* has

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been shown to have phosphothreoninylase activity that irreversibly inactivates host cell MAPkinases by removal of phosphate and modification of the target threonine [15]. The role of this activity in the virulence phenotype of SpvC has not been determined. Both SpvB and SpvC are translocated by the SPI-2 TTSS [16]. *spvR* encodes a MetR/LysR-type transcriptional activator and is transcribed separately from the *spvABCD* structural genes. SpvR binds to both the *spvR* and *spvA* promoters and is required for expression of the *spvABCD* genes [17]. The *spv* genes from different serovars are highly homologous [8] and allow us to decide whether the pathogenesis of the isolates from positive clinical samples is attributable to chromosome or plasmid born virulence factor [18]. The present study aimed to investigate the prevalence of two main virulence plasmid-born genes in *spv* locus, *spvB*, *spvC* and regulator *spvR* gene and their association among different serovars of *Salmonella enterica* isolated from human and animals.

Materials and Methods

Bacterial Strains

A total of 60 *Salmonella* strains were recovered from specimens submitted to the diagnostic microbiology laboratory of the School of Veterinary Medicine between 2004 and 2005 in different outbreaks or individual cases in Shiraz, Iran. Human fecal isolates derived from patients with gastroenteritis were obtained from a diagnostic laboratory in Shiraz, Iran. Strains which were previously identified by conventional methods and serotyped by the *Salmonella* reference center are listed in Table 2.

DNA Preparation

A loopful of colonies of each isolate on agar plate was picked and suspended in 200 μ L of distilled water. After vortexing, the suspension was boiled for 5 min, and 50 μ L of the supernatant was collected after spinning at 14,000 rpm for 10 min. The DNA concentration of the boiled extracts was determined with spectrophotometer [19].

PCR Assay

PCR amplifications were performed in a final volume of 25 μ L in PCR tubes. The reaction mixtures consisted of 2 μ L of the DNA template, 2.5 μ L 10 $\times$ PCR buffer (75 mM Tris–HCl, pH 9.0, 2 mM MgCl₂, 50 mM KCl, 20 mM (NH₄)₂SO₄), (CinaGen, Iran), 1 μ L dNTPs (50 μ M), (CinaGen, Iran), 1 μ L (1U Ampli Taq DNA polymerase), (CinaGen, Iran), 1 μ L (25 pmol) from the

forward and reverse primers (CinaGen, Iran), of both primer pairs (Table 1) and the volume of the reaction mixture was completed to 25 μ L using distilled deionized water. The thermal cycler (MJ mini, BioRad, USA) was adjusted under the following conditions: Initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing as shown in Table 1 for 1 min and extension at 72 °C for 1 min. Final extension was carried out at 72 °C for 10 min and the PCR products were stored in the thermal cycler at 4° C until they were collected. Amplified products were separated by electrophoresis in 1.5 % agarose gel stained with ethidium bromide. Visualization was undertaken using a UV transilluminator (BTS-20, Japan) and the 50 bp plus DNA ladder was used as molecular size marker Fig. 1.

Results

Among 60 different *salmonella* isolates, the prevalence of *spvB*, *spvC*, and *spvR* genes were 26 (43.3 %), 44 (73.3 %), and 28 (46.6 %), respectively. *S. colindal* detected from poultry and *S. Virchow* obtained from animals did not show the presence of these virulence genes. Furthermore, 14 serotypes (four *S. enteritidis* and two *S. typhimurium* isolated from poultry and five *S. abortusovis*, two *S. typhimurium*, and one *S. enteritidis* detected from animals) were positive for occurrence of these three virulence genes concurrently. All strains with *spvR* gene had one or both of the other *spv* genes. Just one *S. infantis* isolated from poultry had both *spvB* and *spvC* genes, while four other analogous strains did not have these genes. The detailed results of PCR detection of three *spv* virulence genes in different *Salmonella* serotypes with various sources are presented in Table 2.

Discussion

Yet, more than 2,500 serovars of *S. enterica* have been identified using the Kauffman-White typing scheme, of which about 20 serovars [22] cause the vast majority of cases of disorders, as these strains are widely distributed amongst domestic food animals and so enter the food chain [23]. A number of central gene regulatory functions govern the life and growth of *salmonellae*, and these have been studied for decades [24]. Non-typhoid *Salmonella* strains associated with extra-intestinal infections in humans and animals carry an additional locus termed *spv* including five genes [5, 25]. Genetic analysis demonstrates that the *spvR* and *spvBC* genes are required for the virulence phenotype of the *spv* locus [11]. Based on previous studies, of the most commonly isolated serovars from stool cultures only Typhimurium and Enteritidis can carry virulence plasmids

Table 1 Nucleotide sequences used as primers in the PCR reaction for three *spv* genes in *Salmonella* serotypes

Name of primer	Sequence (5'–3')	Target gene	Annealing temperature	Product size (bp)	References
spvC1	CGGAAATACCATCTACAAATA	<i>spvC</i>	42 °C	669	[20]
spvC2	CCCAAACCCATACTTACTCTG				
spvBF	ATGTTGATACTAAATGGTTTTTCA	<i>spvB</i>	55 °C	1,776	[21]
spvBR	CTATGAGTTGAGTACCCTCATGTT				
spvRF	ATGGATTTCATTAATAAAAAATTA	<i>spvR</i>	55 °C	894	[21]
spvRR	TCAGAAGGTGGACTGTTTCAGTTT				

Table 2 Prevalence of *spvB*, *spvC* and *spvR* genes in *Salmonella* serotypes

Source/serotype	No. of isolates	No. (%) positive for virulence genes		
		<i>spvB</i>	<i>spvC</i>	<i>spvR</i>
Poultry				
<i>S. Enteritidis</i>	15	7 (46.6)	14 (93.3)	9 (60)
<i>S. Typhimurium</i>	9	2 (22.2)	7 (77.7)	4 (44.4)
<i>S. Infantis</i>	5	1 (20)	1 (20)	0 (0)
<i>S. Colindal</i>	1	0 (0)	0 (0)	0 (0)
Animals				
<i>S. Abortusovis</i>	12	8 (66.6)	10 (83.3)	7 (58.3)
<i>S. Enteritidis</i>	7	2 (28.5)	6 (85.7)	3 (42.8)
<i>S. Typhimurium</i>	5	5 (100)	3 (60)	3 (60)
<i>S. Virchow</i>	1	0 (0)	0 (0)	0 (0)
Human				
<i>S. Enteritidis</i>	3	0 (0)	3 (100)	2 (66.6)
<i>S. Bardo</i>	2	1 (50)	0 (0)	0 (0)
Total	60	26 (43.3)	44 (73.3)	28 (46.6)

Fig. 1 Agarose gel electrophoresis of PCR product of three *spv* genes. Lanes 1 and 2 PCR product of *spvB* gene, Lanes 3 and 4 PCR product of *spvR* gene, Lanes 5 and 6 PCR product of *spvC* gene, Lane 7 50 bp DNA marker

that encode the *spv* operon, indicating that *spv* genes are not required to cause gastroenteritis in people [7]. Few studies have been conducted on the prevalence of these genes in different serovars of *Salmonella* in all parts of the world, especially in Iran [26, 27]. This study is the first report of distribution of *spvB*, *spvC* genes in *S. abortusovis* and *S. infantis* isolated from animals and poultry in Iran. In epidemiologically unrelated isolates from different states in the United States, 76 % of the blood isolates carried *spv* genes, about twice the percentage of fecal isolates [7]. In the present study, the prevalence of these three genes, *spvB*, *spvC* and *spvR* in all of the *salmonella* serotypes were 43.3 % (26/60), 73.3 % (44/60) and 46.6 % (28/60) respectively. Zahraei et al. [27] revealed that *spvB* and *spvC* genes were present in 90 % of the isolates (18/20). They reported that the prevalence of these genes in poultry and bovine *S. enteritidis* isolates were 88.6 and 100 % respectively, while in the present study the presence of *spvB* and *spvC* genes in poultry isolated *S. enteritidis* was 46.6 % (7/15) and 93.3 % (14/15) respectively. Another

study has reported the lack of *spvC* gene in 7.2 % (8/110) of the *S. enteritidis* samples from human, pig, and poultry sources [28]. Zahraei et al. [27] showed that the *spv* genes were not detected in 5.9 % of isolates of the *S. enteritidis* as compared with 7.4 % of the isolates reported by Pan and Liu [29]. A study reported in 2000 has shown that among a total of 17 isolates of *S. enteritidis* and *S. typhimurium*, all samples possessed *spvC* [30]. Ling [31] analyzed 152 isolates of *S. enteritidis* from human feces in which only 4 isolates (8 %) lacked *spvC*. Although, Zahraei et al. [27], have proposed the increase in *spv* genes in *S. enteritidis* in recent years these prevalence diversities of one gene may be due to genetic differences between strains from different geographic areas. Findings showed that, among animal *S. enteritidis* isolates, 28.57 % (2/7) and 85.7 % (6/7) had *spvB* and *spvC* virulence genes, respectively. Prevalence of *spvR* gene in poultry and animal *S. enteritidis* isolates was 60 % (9/15) and 42.8 % (3/7) respectively and 46.6 % (28/60) in total of strains, which is against the results of Bacci et al. [32] that showed 100 % presence of *spvR* gene in different serovars. They reported that some plasmid-negative strains had *spv* genes, and which be due to the creation of episome mode [32]. Unlike them, we suggested that the lack of some *spv* genes in *salmonella* serotypes could be due to the existence of incomplete *spv* locus, rather than the transfer of genes into the chromosomal DNA. Among twelve *S. abortusovis* serovars studied in this study, distribution of *spvB*, *spvC* and *spvR* genes was 66.6, 83.3 and 53.3 % respectively and 41.6 % (5/12) of them had three *spv* genes simultaneously, which may increase the propensity of these strains to be of major clinical relevance in coordination with the results of Gebreyes et al. [33]. Furthermore, all 28 strains with *spvR* gene simultaneously had one or both of the other *spv* genes. Although *spvR* is a positive transcriptional regulator of *spvABCD*, it seems that these serovars could express their related *spv* genes and may be more virulent in pathogenicity [8]. *S. colindal* and *S. virchow* serotype in the present study did not show the presence of *spv* genes. To clarify the subject, more investigations are proposed for the presence or absence of *spv* locus genes in these serovars. One of two *S. bardo* isolated from human showed only the presence of *spvB* gene. In addition, three human *S. enteritis* serovars did not have *spvB* gene, but two of them indicated concurrent presence of *spvC* and *spvR*. However, one out of five *S. infantis* strains obtained from poultry had both *spvB* and *spvC* genes and neither had *spvR* gene. The evidence taken from this study confirms the results of other studies which show a higher distribution for the virulence plasmid from animal-origin isolates than that of human-origin [26, 27]. The findings of the present study suggest a dissimilar distribution of *spvB*, *spvC* and *spvR* genes among *Salmonella* serotypes from different sources. Genetic variations in

Salmonella could be derived from the transfer of virulence plasmid from animal-origin strains to human-origin strains or vice versa, which remains to be investigated. It is becoming clear that epidemiological investigations about bacterial virulence factor genes are essential to understand the biology of bacterial abnormalities, rapid diagnosis of infections, for treatment of their disorders and producing ideal vaccines against them. Results demonstrated that the prevalence of *spv* genes is different among various *salmonella* serovars. Strains isolated from animals showed a higher prevalence of these genes.

Acknowledgments This work was supported by a Grant from Shiraz University. The authors are grateful to Mr. Shahed for his excellent technical assistance.

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