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Full Length Research Paper

Phylogenetic analysis of virulence factor gene of *Salmonella* isolated from clinically symptomatic Chickens

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Salmonella enterica **is a major cause of human food-borne disease, which is mainly associated with the consumption of contaminated poultry meat and eggs. Understanding of the pathogenic mechanisms of** ***Salmonella*** **is an important strategy to lower the prevalence of** ***Salmonella*** **in poultry flocks. The invasion proteins encoded by** ***invA*** **and** ***invE*** **on the** ***Salmonella*** **pathogenicity island-1 (SPI-1), are important virulence factors that play a role in invasion and systemic spread in chickens. To explore the relationship between** ***Salmonella*** **pathogenicity and variance of the virulence factor genes, sequences of the** ***invA*** **genes and** ***invE*** **genes of 33 strains of** ***Salmonella*** **isolated and amplified from chickens from the north of China between 2000 and 2010, which identified by microbiology method were determined and analyzed in comparison with the sequences of reference** ***Salmonella*** **strains from GenBank. As our results show,** ***invA*** **genes were successfully amplified in all 33 strains, and 32 strains were found to harbor the** ***invE*** **genes. The** ***InvA*** **nucleotide sequences from 12 of 33 strains isolated from chickens are 72.9 to 97.6% identical, and they shared 78.9 to 97.2% identity to the sequences from reference** ***Salmonella invA*** **genes from GenBank; The nucleotide sequence similarities were >95.3% for** ***InvE*** **genes from 23 of 33 strains isolated from chickens and shared 89.6-98.6% similarity to that of reference strains from GenBank. The phylogenetic trees constructed from the** ***InvA*** **and** ***InvE*** **genes showed that most of the 33 strains were distinct from reference strains and clustered into different groups.**

Key words: *Serovar enteritidis*, virulence factor gene, phylogenetic analysis

INTRODUCTION

Salmonella is a well-known zoonosis pathogen that can cause food poisoning, acute gastroenteritis, or diarrhea, and even death (Xia et al., 2009; Bangtrakulnonth et al., 2004; Herikstad et al., 2002). The infected poultry are among the most common reservoir of salmonellae that can be transmitted through the food chain to humans.

Understanding of the pathogenic mechanisms of *Salmonella* plays an important role to lower the prevalence of *Salmonella* in poultry flocks. It was reported that those virulence factors such as virulence plasmid, endotoxin or intestinotoxin play a major role in systemic virulence of *Salmonella* (Zhao et al., 2003). Other result demonstrated that the invasion proteins, who encoded by a series genes *invA, invB, invC, invD* or *invE*, are involved in host cell invasion of Salmonella and correlated the pathogenicity of bacteria (Zaporojets et

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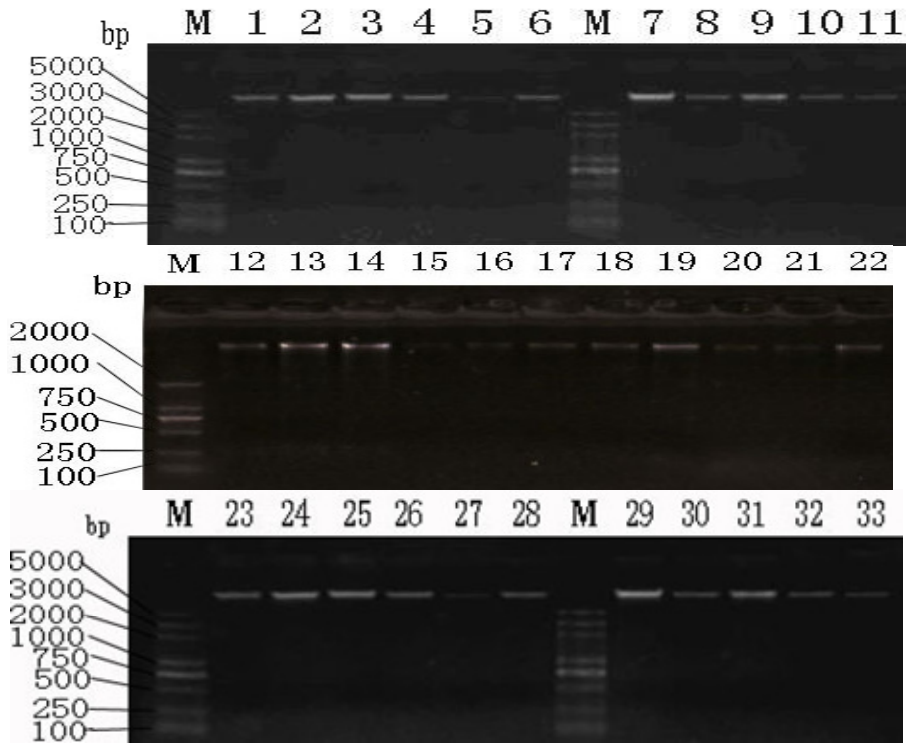

Figure 1. Salmonella genomic DNA 1~33Salmonella genomic DNA; M: Maker.

al., 2003). The *invA* and *invE* are major virulence factors of salmonella for its important role in the pathogenicity of bacteria (Brumell et al., 2003; Hoorfar et al., 1999; Schrank et al., 2001). Little is reported about both *InvA* and *invE*. In our study, *Salmonella paratyphoid, Salmonella gallinarum* or *Salmonella typhimurium,* which have close relation to animal medicine, public health and food security, were investigated to cloning the genes of *InvA* and *invE* and analyze the sequence of them. This study provided a scientific experimental data for quick test, epidemiology or pathogenic mechanism of Salmonella in molecular biology.

MATERIALS AND METHODS

Bacterial strains

33 strains salmonella were isolated from clinically symptomatic chickens from QinHuangdao, Beijing and Liaoning between 2000 and 2010.The strains from Beijing numbered 1 to 11, the number of strains from Liaoning is from 12 to 15, the serial number of QinHuangdao strains is from 16 to 33.Those strains were identified by biochemistry methods and stocked in the Hebei key laboratory of preventive veterinary, and the strain 13 is *S. paratyphoid*, strain 18 and 19 are *S. gallinarum*, the others are *S. typhimurium*.

Salmonella genomic DNA was extracted from bacteria cells using genome extraction Kit (Takara Blotechnology dalian co.,ltd)according to the manufacturer's instructions. Two pairs of primer sets for PCR were designed from the reported conservative nucleotide sequences for Salmonella in Genbank. A 234 bp DNA section of *invA* was amplified from the genomic DNA with two primers PAF (5′-AACTTTATTGGCGGTATTTC-3′) and PAR (5′-CGTAACAACCAATACAAATG-3′), another 511bp DNA section of *invE* was amplified with two further primers PEF (5′-GGCGGAAGTACAGAAATTTG-3′) and PER (5′-ACGTTGGTAGCCATAACTGG-3′) by PCR. The E. coli DH5α was a negative control. Nucleotide sequences of these DNA sections were determined by Sangon Biological Engineering Technology and Service Co., Shanghai, China.

RESULTS

Extraction of genome

DNA of Salmonella

Salmonella genomic DNA was extracted from bacteria cells using genome extraction Kit according to manufacturer's instructions (Figure 1). The genomic DNA of 33 strains was extracted successful.

PCR amplifications of *invA* and *invE* genes

Using specific primers, a 234bp DNA section of ***i****nvA* genes were successfully amplified in all 33 strains (Figure 2), and 32 strains were found to harbor a 511bp DNA section of the *invE* genes (Figure 3).

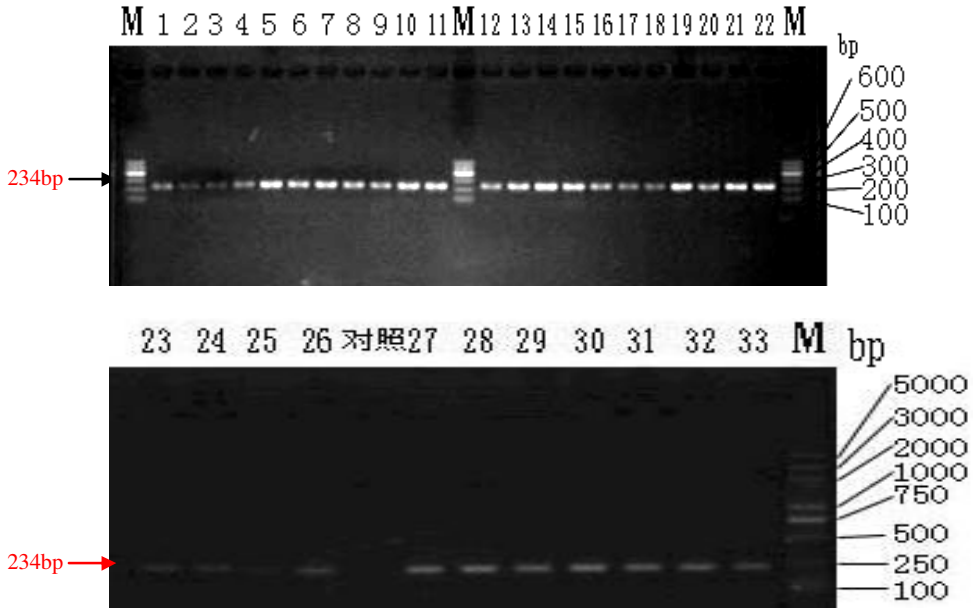

Figure 2. PCR amplification of *invA* gene from salmonella spp. 1~33 PCR amplification result of salmonella spp; M: Maker; control: *E. coli* DH5α.

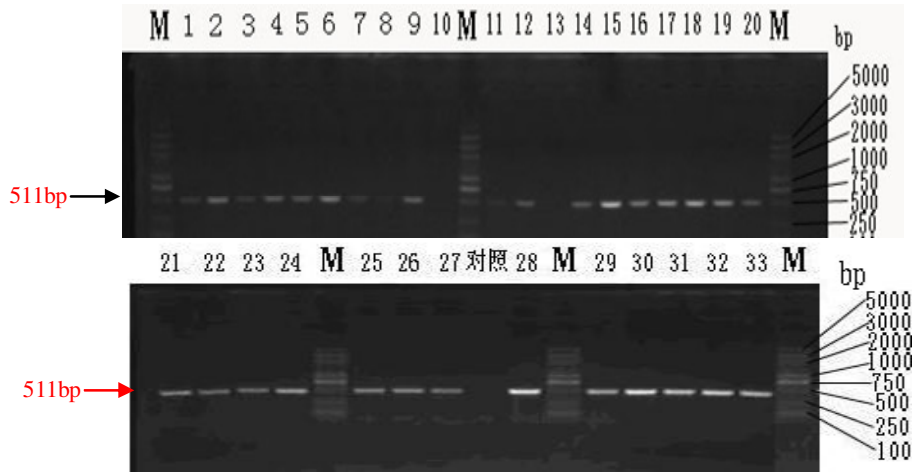

Figure 3. PCR amplification result of *invE* gene from salmonella spp. 1~33 strains of *Salmonella* spp; M: Maker; control: *E .coli* DH5α.

Sequencing of PCR amplification products of virulence genes invA and invE

The *InvA* nucleotide sequences from 12 of 33 and *InvE* genes from 23 of 33 strains were determined.

Homology Analysis of nucleotide sequences of the *invA* and *invE* genes from salmonella with different serum types

Sequence comparisons showed nucleotide identities of72.9~97.6% among the invA genes of Salmonella isolated 12 of 33strain, and they shared 78.9~97.2% identity to the sequences from reference *Salmonella invA* genes from GenBank (*S. gallinarum*, No.U43273; *Salmonella enterica*, No.U43272; *Salmonella bongori* strain, No. DQ644632; *S. typhimurium*, No. M90846; *Salmonella choleraesuis* strain, No. EU348367). The nucleotide sequence similarities were >95.3% for the *InvE* genes from 23 of 33 strains isolated from chickens and shared 89.6~98.6% similarity to that of reference strains from GenBank (Figure4).

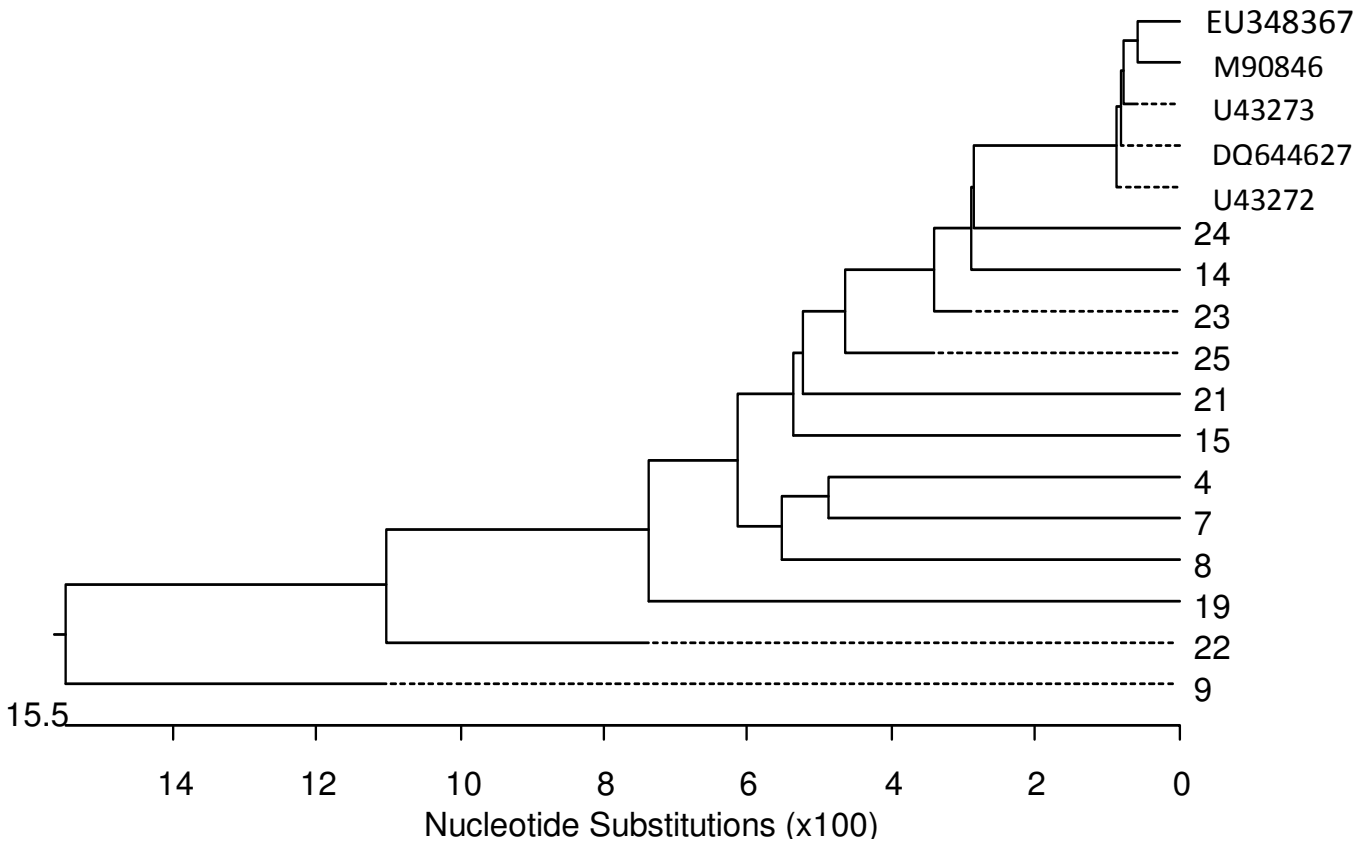

Figure 5. Phylogenetic tree of *Salmonella* spp.based on nucleotide sequence of *invA* genes.

The phylogenetic trees constructed from the *InvA* genes showed that, those strains isolated from Beijing (1~11) and Qinhuangdao (16~33) have closer relations, and share 86.0% identity; The nucleotide sequence similarities of the *invA* genes from Qinhuangdao strains were 97.6%. The 12 of 33 strains were distinct from reference strains and clustered into different groups. The phylogenetic trees constructed from the *InvE* genes demonstrated that the 23 of 33 strains were clustered into different groups. *S. typhimurium* (15) and *S. pullorum* (18) from Qinhuangdao was clustered in same group, and shared homology of 100%; all *S. typhimurium* were not distinct to other strains (Figure 5).

DISCUSSION

S. enterica encodes a type III secretion system (TTSS) within a pathogenicity island located at centisome 63 (SPI-1), which is essential for its pathogenicity. The T3SS is an injection device that can transfer bacterial virulence proteins directly into host cells. The apparatus is made up of a basal body that spans both bacterial membranes and an extracellular needle that possesses a channel that is thought to act as a conduit for protein secretion (Brumell et al., 2003). The Salmonella protein *InvA* is one of the most highly conserved proteins of this core of the T3SS components. *InvA* is critical to the functioning of the T3SS. The invasion protein *InvE*, which is also encoded within SPI-1, is essential for the translocation of bacterial proteins into host cells. The InvE is essential for triggering cellular responses that lead to bacterial entry. So both *invA* and *invE* have closer relation to the pathogenicity of *Salmonella* (Kingsley et al., 2003).

As our results show, the DNA section of the invA gene were amplified from the 33strains Salmonella that contain 30 strains *S. typhimurium*, 1 *S. paratyphoid* and 2 strains *S. gallinarum*; It demonstrated the *invA* gene is highly conserved gene in *Salmonella*. The DNA section of the *invE* gene was not amplified from Salmonella *paratyphoid,* and got from the others. This showed that the *invE* genes have less conservative than the *invA* in *Salmonella*. The *InvA* nucleotide sequences from 12 of 33 strains isolated from chickens are 72.9 to 97.6% identical, and they shared 78.9~97.2% identity to the sequences from reference *Salmonella invA* genes from GenBank; The nucleotide sequence similarities were>95.3% for *InvE* genes from 23 of 33 strains isolated from chickens and shared 89.6 to 98.6% similarity to that of reference strains from GenBank. There is no

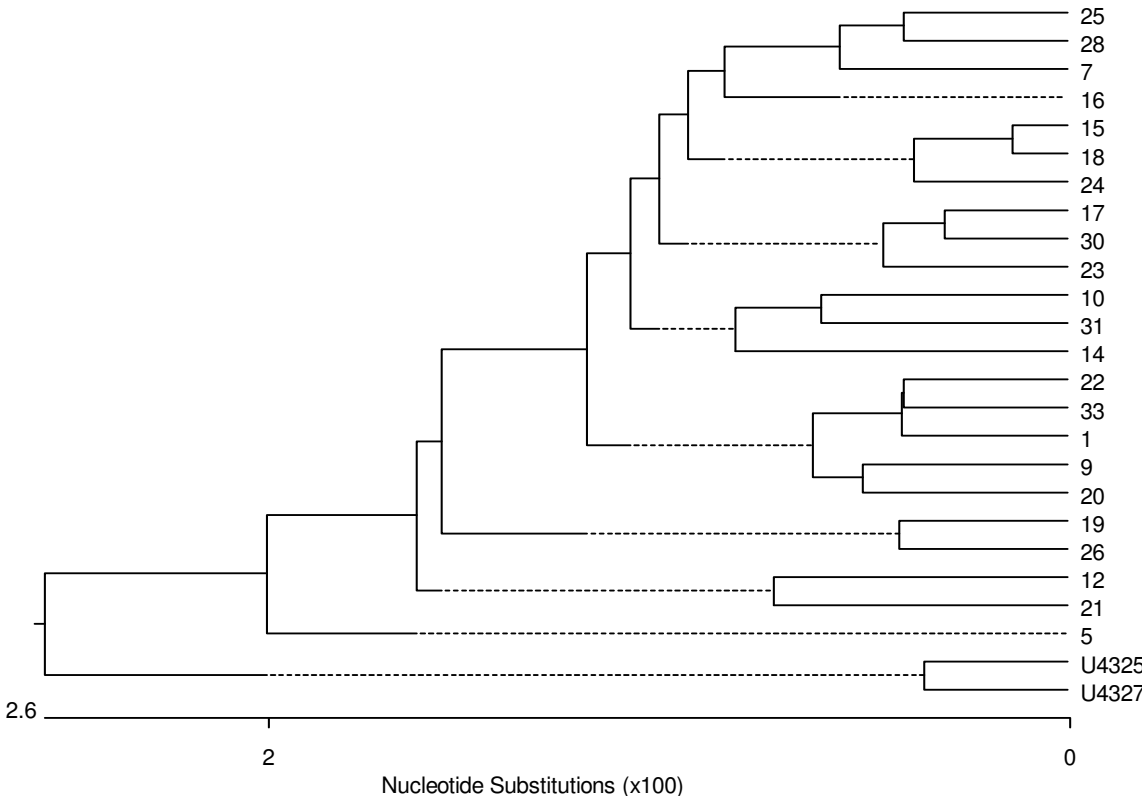

Figure 6. Phylogenetic tree of Salmonella spp.based on nucleotide sequence of *invE* genes.

homologous sequence in other germ searched by BLAST from Genbank. The phylogenetic trees constructed from the *InvA* and *InvE* genes showed that most of the 33 strains were distinct from reference strains and clustered into different groups. All *S. typhimurium* were not distinct to other strains, and clustered into different group. Those confirmed the variation of the virulence genes both *invA* and *invE*, and the variation located in important domain of *Salmonella* genome (Figure 6).

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