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Serotype Distribution and Detection of Enterotoxin Gene of Chicken Source of Salmonella in the Eastern Part of Hebei Province

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Abstract: To explore the pathogenic mechanism of Salmonella, serotype was identified and enterotoxin gene *stn* was detected for 47 strains suspected Salmonella in the Eastern part of Hebei Province. According to the bacterial culture characteristic, physicochemical properties and analysis results of K-3401 semi automatic bacteria identification instrument and identification for another time by Chengdu Institute of Biological Products, enterotoxin gene *stn* was detected by PCR. The 17 strains of *Salmonella gallinarum*, 14 strains of *Salmonella typhimurium*, 4 strains of *Salmonella pullorum* 2 strains of *Salmonella paratyphi* A, 2 strains of Salmonella group BO, 5 strains of *Salmonella bovismorbificans*, 3 strains of *Salmonella enteritidis* were detected from 47 strains of chicken source of Salmonella. The 45 strains of *stn* gene were amplified successfully in all 47 strains. The carrying rate was 95.7%. Homology of *stn* gene of test strains of Salmonella was between 94 and 100%. Evolutionary tree display that different serotypes of Salmonella enterotoxin *stn* gene divided into 5 groups. There was no homology with other bacterium. Chicken source of Salmonella contained 7 kinds of serotypes in the eastern part of Hebei Province. Among them, *Salmonella gallinarum* was 36.2% (17/47), *Salmonella typhimurium* which was advantages serotypes was 29.8% (14/47). The carrying rate of enterotoxin gene *stn* which had relation with bacterial pathogenicity was 95.7% (45/47).

Key words: Salmonella in the Eastern part of Hebei Province, serotype distribution, enterotoxin stn, polymerase chain reaction, physicochemical

INTRODUCTION

Salmonellae was a large class of important pathogenic bacteria in Enterobacteriaceae which could cause a series of disease to infected animal and is a important pollutional sources of livestock and poultry products and cause food borne disease for human by alimentary infection.

There were as many as 2500 kinds of Salmonella serotypes and new serotype emergence every year most of that serum had strong pathogenicity to human and animal (Fang *et al*., 2010). In recent years except the report about a large number of livestock poultry infected with different serotypes of Salmonella, many wild animal such as mink, Nipponia nippon were infected (Daochao *et al*., 2009) that may be associated with the Salmonella self having strong pathogenicity much serotypes and the variant strains increasing year by year. In recent years, there were so many reports about humans and various animal suffering from Salmonella invasion which usually could cause human and animal food poisoning, acute gastroenteritis and dysentery and other diseases even to death. Therefore, there was important significance to explore the pathogenic mechanism of Salmonella. At present the study on causative agent of Salmonella such as virulence island (Wang and Tang, 2008), Viantigen, resistance plasmid *pRST98spv* virulence gene, *PhoQ* gene (Liu *et al*., 2008). Virulence Plasmid (VP), endotoxin and enterotoxin were virulence factor of Salmonella which had been reported. Salmonella enterotoxin was a virulence factor which could cause saimonella-enteritis to animal and enhance bacterial invasive power. According to the domestic and foreign research, exotoxin, especially enterotoxin which existenced in culture filtrate of Salmonella had closely relation with diarrhoea (Li and Takayuke, 1995). Serotype was identified and enterotoxin gene *stn* was detected for 47 strains suspected Salmonella in the Eastern part of Hebei Province in order to provide theoretical basis for the research of the pathogenic mechanism of Salmonella.

MATERIALS AND METHODS

Material bacterial strains: Test strains were isolated from organ of chicken which died to Salmonella from chicken

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Table 1: Background material of strains

No.	Strain	Source of strain	Tissue	Days	Collection date
1, 2	HQ010915(1-2)	Some chicken farm in Changli of Hebei	Liver mucilage of oviduct	120	2001/09/15
3, 4	HQ020508(1-2)	Some chicken farm in Funing of Hebei	Liver	20	2002/05/08
5-9	HQ030710(1-5)	Some chicken farm in Changli of Hebei	Liver	30	2003/07/10
10-12	HQ030906(1-3)	Some chicken farm in Changli of Hebei	Liver	14	2003/09/06
13-16	LT080910(1-4)	Some chicken farm in Laoting of Hebei	Liver	190	2008/09/10
17, 18	HQ090511(1-2)	Some chicken farm in Changli of Hebei	Liver	35	2009/05/11
19-21	BJ090524-G(1-3)	Some chicken farm in Beijing	Liver	35	2009/05/24
22, 23	BJ090524-X(2-3)	Some chicken farm in Beijing	Heart	35	2009/05/24
24-29	BJ090504-(1-6)	Some chicken farm in Beijing	Liver	7	2009/05/04
30-33	LN091007-(1-4)	Some chicken farm in Liaoning	Liver	7	2009/10/07
34	HQ100516-1	Some breeding chicken farm in Qinghuangdao of Hebei	Liver	130	2010/05/16
35	HQ100516-2	Some breeding chicken farm in Qinghuangdao of Hebei	Vitellus	130	2010/05/16
36	HQ100622-1	Some breeding chicken farm in Qinghuangdaoof Hebei	Liver	125	2010/06/22
37	HQ100622-2	Some breeding chicken farm in Qinghuangdao of Hebei	Vitellus	125	2010/06/22
38	HQ100622-3	Some breeding chicken farm in Qinghuangdao of Hebei	Spleen	125	2010/06/22
39	HQ100712-1	Some breeding chicken farm in Qinghuangdao of Hebei	Spleen	120	2010/07/12
40	HQ100712-2	Some breeding chicken farm in Qinghuangdao of Hebei	Liver	120	2010/07/12
41	HQ100817-1	Some breeding chicken farm in Qinghuangdao of Hebei	Liver	140	2010/08/17
42	HQ100817-2	Some breeding chicken farm in Qinghuangdao of Hebei	Vitellus	140	2010/08/17
43, 44	HQ100824(1-2)	Some breeding chicken farm in Qinghuangdao of Hebei	Liver	135	2010/08/24
45	HQ100824-3	Some breeding chicken farm in Qinghuangdao of Hebei	Vitellus	135	2010/08/24
46	HQ100824-4	Some breeding chicken farm in Qinghuangdao of Hebei	Liver	135	2010/08/24
47	HQ100824-5	Some breeding chicken farm in Qinghuangdao of Hebei	Liver	135	

farm in the Eastern part of Hebei Province. The strains were numbered 1-11 (Table 1). Reference strains for sequence analysis *Salmonella typhimurium* (indexed No. AF170176), *Salmonella enterica* serovar (indexed No. CP000880), *Salmonella enterica* serovar (indexed No. CP000886), *Salmonella enterica* serovar (indexed No. CP001363). Quality control of bacterial strains by K-B Method *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853 (these strains were purchased from China Institute of Veterinary Drug control).

Main agent: Salmonellae serum (Department of Health Lanzhou Institute of Biological Products, valid until February 2013 year) and Salmonella CHROM agar medium (Beijing Land Bridge Limited Liability Company).

Main instrument: K-3401 semi-automatic bacteria identification instrument (Jinan Daxin Pharmaceutical Equipment Technology Limited Company) *EDC-810* gene amplification instrument (Dongsheng Innovative Biotechnology Limited Company) BIO-BEST200E gel imaging analysis system (American SIM International Group).

Bacterial identification: Using the conventional sterile method, colony was collected with inoculating loop from slant in tube which was preserved and then was inoculated in SS agar, Agar Mac-Conkey, plain agar, blood agar. Questionable colonies were colleted and pured from SS agar. Pured cultures of bacteria was inoculated into triple sugar iron agar for 24 h, conserved. Culture from triple sugar iron agar in which emerged response which had relation with salmonella was dipped and inoculated micro-biochemical tube for identification. It was initially identified as salmonella by analysis results with K-3401 semi-automatic bacteria identification instrument then was identified again by Chinese Medicine Center Salmonella Laboratory Chengdu Institute of Biological Products.

Extraction of genome DNA: The culture of salmonellae was inoculated into sterile LB broth and cultured for 12 h in constant temperature oscillator by 120 r min^{-1}. The 1.5 mL bacterial liquid (about containing 5×10^6 cultured bacteria) was centrifugated by 13000 rpm for 30 sec. Collecting thalline then mixing thalline which was suspended in 0.5 mL TE buffer with 1 mL purified resin Incubating for 3 min at ordinary temperature, mixing once, centrifugating for 3 sec by 5000 rpm, last collecting precipitation. Suspending purified resin with 1 mL GN binding liquid, mixing, centrifugating for 3 sec by 50000 rpm, collecting precipitation. Rinsing purified resin twice with 0.5 mL rinsing liquid, mixing, centrifugating for 3 sec by 5000 rpm, collecting precipitation. Suspending purified resin with 0.8 mL dehydrated alcohol, putting them into centrifugal purification column, centrifugating for 1 min by 13000 rpm, collecting alcohol, dropping waste liquid. Centrifugating again for 1 min by 13000 rpm to dorp alcohol exhaustively. Putting centrifugal purification column into a 1.5 mL or 2 mL clean centrifuge tube, opening cover placed 2-3 min to make alcohol evaporate. Adding 100 uL TE buffer in purified resin (do not stuck in the wall of the tube), putting for 3 min at ordinary

temperature centrifugating for 2 min by 13000 rpm. Repeat the same step for 1 or 2 times. The liquid in centrifuge tube was genome DNA. Taking 5 μL to electrophoresis (1% agarose, 120 V) and conserving the other at -20°C.

Designion of primer: Designion of primer refer to the GenBank *stn* gene, upstream primer 5-TGA GCG CTT TAA TCT CCT TC, downstream primer 3'-TTA CTG GCG TTT TTT TGG CA. The size of the fragment which was amplified was 975 bp. The synthesis of primers was did by Shanghai Biological Engineering Technology Services Limited Company.

PCR amplification and product sequence determination: PCR amplification system were: 2×Taq Master Mix 12.5 μL the upstream and downstream primer (10 mol l μL) 0.5 μL, DNA templates, 2 μL, double distilled water was added to 25 μL, amplification conditions: 94°C 5 min; 5 min; 94°C 30 sec, 55°C 30 sec, 72°C 60 sec, 30 circulation at last extening for 7 min at 72°C. After amplification, taking 5 μL PCR products carried out detection by garose gel electrophoresis. At the same time, takeing *Escherichia coli* ATCC25922 and *Bacillus aeruginosus* ATCC 27853 as control. PCR products was send Shanghai Shenggong Biological Engineering Limited Company to sequence.

RESULTS

Bacterial identification: Most of isolated strains were grown in ordinary culture medium formed smaller, round, protuberant, smooth, protruding, neat edge, colorless colonies. And in blood agar plate colonies were formed gray or milky white, translucent, rounded protuberances, smooth which sizes were like needle tip. The individual colony appeared β hemolytic. In SS culture medium, colonies were smaller, colorless, transparent or black in center and colorless and transparent at edge, regular edge and the color was similar with culture medium. In triple sugar iron agar slant was red in which colony was inoculated, bottom surface was yellow at beginning at last was black, agar underlayer was broken or emerged bubbles at the last bottom. Through biochemical tests showed that the biochemical characteristics of most test strains are basically the same: they can decompose and utilize glucose, mushroom polysaccharide, rhamnose, mannose and produce acid gas they can not decompose lactose, raffinose, sucrose, maltose, sorbitol, inositol glucose, sorbitol, xylose, melampyrin, Arabia alcohol, inositol, esculin, salicin, adonitol, β-galactosyl, melizitose, melibiose and amygdaloside. They were positive in H_2S test, M.R. test, nitrate reduction test, semisolid, mucic acid salts using catalase. They are negative in indole test, urea agar test, citrate salt hydrogenize, enzyme, V-P test, amylase, acetate using, malonate using, tartrate using, esterase, lecithinase, dextrinase, arginine dihydrolase and other. According to the bacterial culture characteristic, physicochemical properties and analysis results of K-3401 semi automatic bacteria identification instrument, the bacteria was saimonella and identification for another time by Chengdu Institute of Biological Products, 47 strains Salmonella involved 7 kinds of serotypes, the results in Table 2.

Extraction of genome DNA and amplification of gene: Salmonella genomic DNA was extracted from bacteria cells using resin type TM genome DNA extraction kit according to the manufacturer's instructions and collected genome DNA bands that show that DNA are successfully extracted from 47 strains of chicken source

Table 2: Serotypes of Salmonella in the Eastern part of Hebei Province

No.	Strain	Date of isolation	Spot of isolation	Serotype
1, 2	HQ010915(1-2)	2001/09/15	Some breeding chicken farm in Changli of Hebei	S. group B O
3, 4	HQ020508(1-2)	2002/05/08	Some breeding chicken farm in Funing of Hebei	*S. gallinarum*
5-9	HQ030710(1-5)	2003/07/10	Some breeding chicken farm in Changli of Hebei	*S. bovismorbificans*
10-12	HQ030906(1-3)	2003/09/06	Some breeding chicken farm in Changli of Hebei	*S. enteritidis*
13-16	LT080910(1-4)	2008/09/10	Some breeding chicken farm in Laoting of Hebei	*S. gallinarum*
17, 18	HQ090511(1-2)	2009/05/11	Some breeding chicken farm in Changli of Hebei	*S. typhimurium*
19-21	BJ090524-G(1-3)	2009/05/24	Some breeding chicken farm in Beijing	*S. gallinarum*
22, 23	BJ090524-X(2-3)	2009/05/24	Some breeding chicken farm in Beijing	*S. gallinarum*
24-29	BJ090504-(1-6)	2009/05/04	Some breeding chicken farm in Beijing	*S. gallinarum*
30-33	LN091007-(1-4)	2009/10/07	Some breeding chicken farm in Liaoning	*S. pullorum*
34, 35	HQ100516(1-2)	2010/05/16	Some breeding chicken farm in Changli of Hebei	*S. typhimurium*
36-38	HQ100622(1-3)	2010/06/22	Some breeding chicken farm in Changli of Hebei	*S. typhimurium*
39, 40	HQ100712(1-2)	2010/07/12	Some breeding chicken farm in Changli of Hebei	*S. typhimurium*
41, 42	HQ100817(1-2)	2010/08/17	Some breeding chicken farm in Changli of Hebei	*S. typhimurium*
43, 44	HQ100824(1-2)	2010/08/24	Some breeding chicken farm in Changli of Hebei	*S. typhimurium*
46, 47	HQ100824(4-5)	2010/08/24	Some breeding chicken farm in Changli of Hebei	*S. paratyphi*

From the results in 47 strains of chicken source Salmonella in the Eastern part of Hebei Province, 17 strains were *Salmonella gallinarum*, 14 strains were *Salmonella typhimurium*, 4 strains were *Salmonella pullorum*, 2 strains were *Salmonella paratyphi* A, 2 strains were Salmonella group BO, 5 strains were *Salmonella bovismorbificans*, 3 strains were *Salmonella enteritidis*

Table 3: Homology of enterotoxin gene of different serotype of Salmonella

Percent identity	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	-	100.0	98.1	98.1	97.1	95.6	97.5	97.4	97.9	97.4	98.0	95.9	97.2	97.3	97.1	98.7	99.0	99.3	95.4	96.3	99.3
2	0.0	-	98.1	98.1	97.1	95.6	97.5	97.4	97.9	97.4	98.0	95.9	97.2	97.3	97.1	98.7	99.0	99.3	95.4	96.3	99.3
3	1.2	1.2	-	98.3	96.3	95.4	96.9	97.7	98.0	97.9	97.8	95.7	97.7	98.1	97.6	98.2	98.4	98.5	95.2	96.5	98.5
4	0.3	0.3	0.9	-	95.8	97.1	96.1	97.3	97.5	97.7	97.3	97.4	96.6	97.1	97.1	98.3	98.5	98.6	96.9	98.2	98.6
5	2.1	2.1	2.2	1.8	-	94.3	97.9	97.2	97.1	97.4	97.3	94.7	97.3	96.9	96.6	97.1	96.9	96.8	94.1	94.0	96.8
6	1.8	1.8	2.8	1.9	2.2	-	95.1	96.3	96.4	96.7	96.3	98.5	95.3	96.2	96.2	96.5	96.2	96.1	97.8	97.1	96.1
7	1.9	1.9	1.8	1.7	1.7	1.7	-	97.9	98.3	97.7	98.2	95.4	97.6	98.0	96.9	97.5	97.3	97.2	94.4	94.3	97.2
8	2.1	2.1	1.8	1.9	1.4	1.7	0.9	-	98.7	98.7	98.5	96.6	98.2	98.5	97.9	98.0	97.8	97.7	95.6	95.5	97.7
9	1.3	1.3	1.7	1.4	1.3	1.3	0.4	0.6	-	98.8	99.2	96.7	98.3	99.3	98.5	98.6	98.4	98.3	95.8	95.7	98.3
10	1.7	1.7	2.0	1.7	0.9	1.5	0.8	0.5	0.6	-	98.5	97.1	98.0	98.6	98.2	98.3	98.1	98.0	96.0	95.9	98.0
11	1.6	1.6	2.0	1.6	1.5	1.4	0.9	1.3	0.5	1.0	-	96.6	98.4	98.9	98.0	98.6	98.4	98.3	95.6	95.5	98.3
12	1.6	1.6	2.7	1.7	2.1	0.9	1.5	1.5	1.2	1.4	1.3	-	95.6	96.5	96.3	96.8	96.6	96.5	98.0	97.7	96.5
13	2.3	2.3	1.8	2.1	1.3	2.3	1.2	1.4	1.3	1.3	1.4	2.2	-	98.2	97.8	98.1	97.9	97.8	95.0	94.9	97.8
14	1.8	1.8	1.7	2.0	1.4	1.9	0.4	0.8	0.4	1.0	0.5	1.8	1.3	-	98.3	98.0	97.8	97.7	95.6	95.5	97.7
15	2.0	2.0	2.2	1.9	1.5	2.0	1.5	1.3	1.1	1.3	1.4	1.9	1.6	1.6	-	97.0	97.6	97.5	96.3	95.6	97.5
16	0.7	0.7	1.6	0.6	1.6	1.4	1.4	1.7	1.1	1.3	1.3	1.3	1.6	1.6	1.7	-	99.6	99.5	96.2	96.6	99.5
17	0.5	0.5	1.4	0.4	1.8	1.6	1.6	1.9	1.3	1.5	1.5	1.5	1.8	1.8	2.0	0.4	-	99.7	96.0	96.8	99.7
18	0.2	0.2	1.3	0.3	1.9	1.8	1.7	2.1	1.4	1.6	1.6	1.6	1.9	1.9	2.1	0.5	0.3	-	95.9	96.9	100.0
19	2.4	2.4	3.4	2.5	2.9	1.6	2.8	2.9	2.4	2.8	2.7	1.9	3.1	3.0	2.1	2.1	2.3	2.4	-	97.2	95.9
20	1.3	1.3	1.9	1.0	2.9	2.4	2.8	2.9	2.4	2.8	2.7	2.2	3.1	3.0	2.8	1.6	1.4	1.3	2.7	-	96.9
21	0.2	0.2	1.3	0.3	1.9	1.8	1.7	2.1	1.4	1.6	1.6	1.6	1.9	1.9	2.1	0.5	0.3	0.0	2.4	1.3	-

S. group BO (1) seq; *S. gallinarum* (4) seq; *S. bovismorbificans* (5) seq; *S.enteritidis* (10) seq; *S. gallinarum* (3) seq; *S. gallinarum* 15) seq; *S. gallinarum* (20) seq; *S. gallinarum* (22) seq; *S. gallinarum* (25) seq; *S. pullorum* (30) seq; *S. typhimurium* (34) seq; *S. typhimurium* (35) seq; *S. typhimurium* (39) seq; *S.typhimurium* (41) seq; CP000880.1*Salmonella enterica* ssp. *ari*; CP000886.1*Salmonella enterica* ssp. *Par*; CP001363.1*Salmonella enterica* ssp. *en*; *S.typhimurium* (43) seq; *S. paratyphi* (46) seq

Salmonella. Then, taking genome DNA as template, amplified specific bands whose size was about 975 bp (stn) and was basically the same with expection.

The 45 of 47 strains of Salmonella carried *stn* gene, HQ020508-1, Q020508-2 (both of them were *Salmonella gallinarum*, numbered 3 and 4) was not amplified from enterotoxin gene, carrying rate of *stn* gene was 95.7%. The 17 strain were *Salmonella gallinarum* and carrying rate was 88.24%. The 14 strains were *Salmonella typhimurium* 4 strains were *Salmonella pullorum*, 2 strains was *Salmonella paratyphi* A, 2 strain were Salmonella group BO, 5 strains were *Salmonella bovismorbificans* 3 strains were *Salmonella enteritidis* carrying rate of *stn* gene were all 100% (Fig. 1).

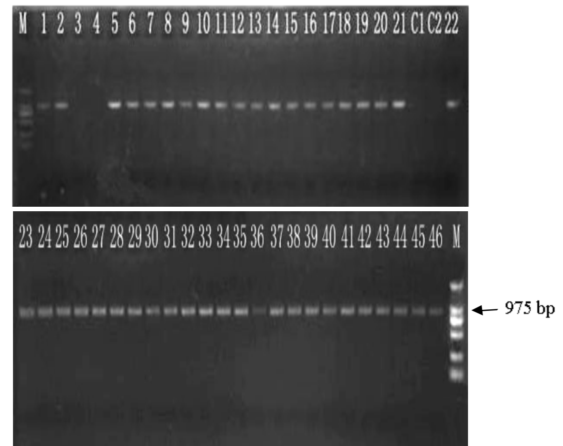

Fig. 1: Amplification of *stn* gene, 1-45: amplification of *stn* gene, M: Maker, C1, C2: *Bacillus aeruginosus* ATCC 27853 control, *Escherichia coli* ATCC25922 control

Homology comparison of different serotypes of Salmonella virulence gene: The test strains were 7 serotypes by detection with DNAStar Software. Taking each of them to compare the homology with reference strains (2 kinds of serotype): homology of *stn* gene of test strains was between 94 and 100% such as the homology of the test strain of *Salmonella enteritidis* (No. 10) and *Salmonella paratyphi* A (No. 46) was 94% and the homology Salmonella group BO (No. 1) and *Salmonella gallinarum* (No. 4) was 100%. The homology of *stn* gene of test strains and reference strains were between 95.9 and 99.3%. The homology of the test strain *Salmonella typhimurium* (No. 43) and the reference strains of *Salmonella typhimurium* (indexed No. AF170176) was 95.9%. The homology of test strain Salmonella group BO (No. 1) and the reference strains of *Salmonella typhimurium* (indexed No. AF17017) was 99.3%. This indicated the homology of enterotoxin gene of different serotypes of Salmonella was higher (Table 3).

From evolutionary trees researcher could find that different serotypes of Salmonella enterotoxin *stn* gene were divide into 5 groups. The test strains of *Salmonella typhimurium* (No. 41) was group, the test strains of *Salmonella typhimurium* (No. 43) was group, the test strains of *Salmonella typhimurium* (No. 34) was group.

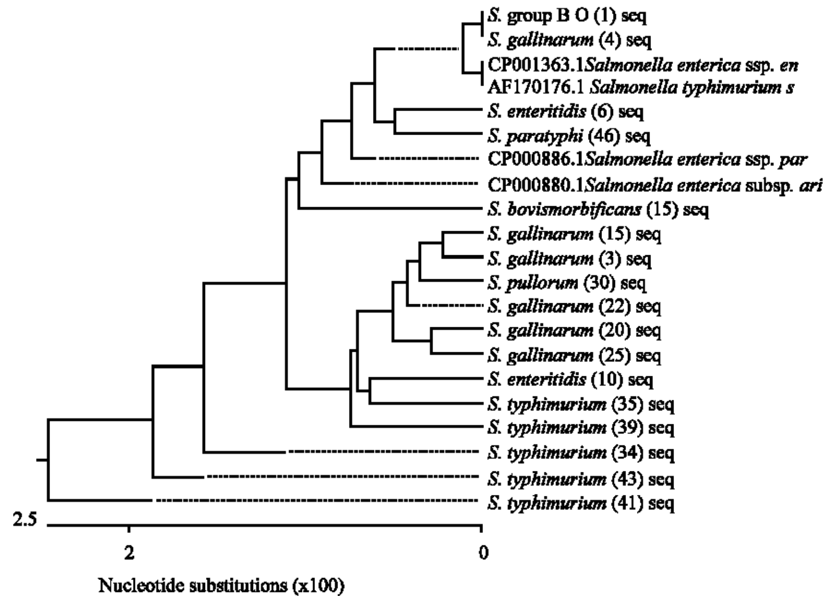

Fig. 2: The phylogenetic tree of nucleotide sequence of *stn* genes

Salmonella gallinarum (No. 15, 3, 22, 20, 25), *Salmonella pullorum* (No. 30), *Salmonella enteritidis* (No. 10), *Salmonella typhimurium* (No. 35, 39) were group IV, Salmonella group B O (No. 1), *Salmonella gallinarum* (No. 4), *Salmonella bovismorbificans* (No. 5, 6) and *Salmonella paratyphi* A (No. 46) were group V. The 5 test strains of group V and 4 reference strains had closely phylogenetic relationship and were the same group but the phylogenetic relationship with the other 12 test strains were not closely (Fig. 2).

DISCUSSION

Distribution of serotype of chicken source salmonella: Salmonellosis was a type of acute, septic, infectious disease which was caused by a certain serotypes of Salmonella. There were as many as 2500 kinds of Salmonella serotypes and every year there are new serotypes.

China had reported 37 group, 255 serotypes and variant serotypes and the majority of serotypes have strong pathogenic to human and animal since the end of 1991 year. This study collected 47 strains of different age of chicken source Salmonella from the eastern part of Hebei Province and researched serotype distribution. This study confirmed that the isolation of 47 strains of Salmonella belong to 7 serotypes. Among them, 17 strain were *Salmonella gallinarum* which was advantages serotypes and percentage was 36.2%. In addition, 14 strain were *Salmonella typhimurium* and percentage was 29.8%. The 4 strains were *Salmonella pullorum* and percentage was 8.5%, 2 strains were Salmonella group BO and percentage was 4.3%. The 5 strains were *Salmonella bovis-morbificans* and percentage was 10.5%, 3 strains were percentage was *Salmonella enteritidis* and percentage was 6.4%. The 2 strains were *Salmonella paratyphi* A and percentage was 4.3%. The most strains from Beijing, Qinhuangdao cities and counties were *Salmonella gallinarum* and *Salmonella typhimurium*. The strains were all *Salmonella pullorum* from some chicken farm in Liaoning. Basing on study, researchers found that the Salmonella infected chickens is *Salmonella gallinarum*, accounted and percentage is 36.2% (17/47) and infected grown up chickens mainly is *Salmonella typhimurium* and percentage is 29.8% (14/47). In addition, the serotype of Salmonella group BO *Salmonella bovismorbificans*, *Salmonella paratyphi* A were reported seldomly.

About *stn* gene of *Salmonella*: Bacterial infecting host was usually by adhesion to tissues of host at first and then invaded cell of host by virulence factor and caused the host to illness and death. These virulence factors encoded by bacterial genes and was products of bacterial gene (Zhao *et al*., 2003). The react process of enterotoxin included stimulation of adenylate cyclase and cAMP levels of intracellular rise. The increasing of cAMP levels leaded to small intestinal juice liquid secrete excessly which could cause diarrhea when the amount of secretion was more than reabsorption capacity of intestinal. In early 1975, koupal isolated enterotoxin from Salmonella firstly (Jones and Falkow, 1996). With the development of

research of molecular Bacteriology, people's knowledge on bacterial toxins, invasins and virulence island and other virulence factors are constantly in-depth (Kingsley *et al*., 2003; Zaporojets *et al*., 2003) (Althouse *et al*., 2003). The study of Huang and Xu (2001a) confirmed enterotoxin stnof salmonella is an important virulence factor. Studies show that (Huang and Xu, 2001b) many different types of Salmonella can produce enterotoxin, especially the study on *Salmonella typhimurium* producing enterotoxin is the most widely. Chopra *et al*. (1994) cloned stn sequence successfully from *Salmonella typhimurium* for the first time. Thereafter, the reporte about Salmonella enterotoxin gene *stn* was less.

This experiment detected enterotoxin *stn* gene by the method of PCR amplification and obtained the gene sequence by sequencing. Looking from the result, enterotoxin *stn* gene was amplified successfully from 7 kinds of different serotypes of Salmonella. It indicated that enterotoxin *stn* virulence genes of Salmonella have stable conservative. The gene sequence analysis of enterotoxin stn of Salmonella indicated that enterotoxin *stn* gene sequence from 7 kinds of serotypes and *stn* gene of Salmonella sequence from GenBank have high homology >95.9%. The homology of the test strain of Salmonella group BO (No. 1) and the reference strains of *Salmonella typhimurium* (indexed No. AF170176) was 99.3% which was relatively high. The homology of the test strain which had no difference with differente serotype were between 94 and 100%. From the evolutionary tree researcher could see the different serotypes of enterotoxin *stn* gene of Salmonella divided into 5 groups which had no homologous relation with other bacteria by BLAST retrieval. Therefore, enterotoxin *stn* gene could exist widespread in different serotypes. The carrying rate of enterotoxin *stn* gene of test strains was 95.7%, that demonstrate *stn* gene is likely to be the main feature of salmonella.

The serotype was identified for 47 strains chicken source Salmonella in the Eastern part of Hebei Province. Referring respectively *Salmonella enteritidis*, *Salmonella typhimurium* and *Salmonella pullorum*, *Salmonella gallinarum* and Salmonella group BO which was reported less, *Salmonella bovismorbificans*, *Salmonella paratyphi* A that was common in the country and was closely related to public health. The 45 of 47 strains of Salmonella were amplified successfully enterotoxin *stn* gene and carrying rate was 95.7%.

CONCLUSION

The results showed that by sequencing and analysis for enterotoxin stn of the mentioned bacterial, the homology of stn nucleotide sequence of 7 different serotypes of Salmonella strains is higher between 94 and 100%. From the evolutionary tree researcher could see the different serotypes of enterotoxin *stn* gene of Salmonella divided into 5 groups which had no homologous relation with other bacteria by BLAST retrieval. It described that enterotoxin *stn* gene of Salmonella in different serotypes of Salmonella is highly conserved. If study further, stn is expected to be as a new target genes for the detection of salmonellae after *invA*, *16S rRNA*, *sefA* gene, Hin/H2 region, *arfA*, *fimA* gene (Pan *et al*., 2009).

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