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# Correlation Analysis of Drug Resistance and Integron Gene Types of Food-borne Salmonella from Jilin Province

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**Abstract:** Objective: To detec integron genes and the drug resistance of two foodborne salmonella from different sources in Jilin Province, explore the correlation between drug resistance and integron. Method: The minimum inhibitory concentration (MIC) method was used to detect drug resistance of 278 salmonella strains in Jilin Province. Real-time PCR was used to detect type I, II and III integron genes, and the correlation between drug resistance and integron gene carrier rate was compared. Result: The total drug resistance rate of 278 strains of food-borne Salmonella was 89.57% (249/278). The positive rates of type I integron, type II integron and type III integron were 51.44% (143/278), 4.32% (12/278) and 14.39% (40/278) respectively. The drug resistance rate of foodborne disease samples was 95.8% (207/216), type I integron was 58.8% (127/216), type II integron was 5.56% (12/216), and type III integron gene was 17.59% (38/216). The drug resistance rate of food (meat, egg) samples was 67.7% (42/62), type I integron was 25.81% (16/62), type III integron gene was 3.23% (2/62), and type II integron gene was detected. Conclusion: Through the surveillance of Salmonella drug resistance and Integron gene carrying rate in food safety risk surveillance and food-borne disease surveillance in Jilin Province, The gene carrying rate of type I and III Integron of Salmonella from food-borne diseases was significantly higher than that of food (meat and eggs). Integron gene system plays an important role in drug resistance transmission of foodborne pathogens. It is suggested that attention should be paid to the monitoring of salmonella drug resistance to ensure food safety and human health.

**Keywords:** Food Safety, Salmonella, Drug Resistance Monitoring, Integron Genes

## 1. Introduction

Salmonella is one of the important intestinal bacteria in public health, and is one of the important target bacteria in national food safety surveillance and food poisoning [1]. Antibiotics are an effective way to treat salmonella infection. However, the abuse of antibiotics leads to more and more serious problems of Salmonella drug resistance, especially multi-drug resistance, the rate of multiple drug resistance has increased from 20% to 70%, which poses a serious threat to human life and health [2].

In addition to their own gene mutations, bacteria can acquire resistance from the outside through horizontal transfer of Antibiotic Resistance Genes, which is also an important pathway for the production of clinically resistant strains. Integrons are bacterial genetic elements that can capture, rearrange, and express mobile gene cassettes. Integrons are DNA elements that helped drive the global antibiotic-resistance crisis. They are best known for their role in disseminating antibiotic-resistance genes among pathogens [3]. Their ability to rapidly spread resistance phenotypes makes it important to consider what other integron-mediated traits might impact human health in the future, such as increased pathogenicity, virulence or resistance to novel antimicrobial strategies. Integrons play an important role in the transmission of drug resistance of Salmonella [4]. Integrons can carry one or more drug-resistance genes, which can be transferred horizontally within or between Salmonella species, leading to the broadening of drug-resistance spectrum and the enhancement of drug-resistance [5, 6]. The purpose of this study was to investigate the relationship between drug resistance and integrons of two food-borne Salmonella strains isolated from Jilin province.

# 2. Materials and Methods

## *2.1. Materials*

### *2.1.1. Strains*

From 2014 to 2017, 278 strains of Salmonella were isolated from food safety risk surveillance program and food-borne disease surveillance program in Jilin Province. 216 strains of Salmonella were isolated from faecal samples of patients from the food-borne disease surveillance program in Jilin Province, sixty-two strains of Salmonella were isolated from food (meat and eggs) samples of the food safety risk surveillance program in Jilin Province.

### *2.1.2. Culture Medium and Reagent Sources*

Buffer peptone water (BPW) was purchased from BD Company; disodium Selenite Cystine (SC) and tyrosinase soybean peptone Agar (TSA) were purchased from Beijing Land Bridge; Salmonella chromogenic medium purchased from Comarca, France; API20E and Vitek 2 Gram Negative Identification Test Card were purchased from Meyrié, France; bacterial genomic DNA Extraction Kit was purchased from Kaijie company. The freeze-dried bacterial quantitative drug susceptibility minimum inhibitory concentration (MIC) test kits were produced by Shanghai Xingbai Biotechnology Co., Ltd. All media are within the validity period. Quality Control Strain ESCHERICHIA coli (ATCC 25922) was purchased from Guangzhou Huankai Biotechnology Co., Ltd. 2 × Fast Probe Mixture (Fast Taq DNA Polymerase, PCR Buffer, dNTPs, $Mg^{2+}$; Low ROX), $ddH_2O$ were purchased from CWBIO.

### *2.1.3. Instrumentation*

VITEK 2 (Bio-Merieux), Thermo 96-well plate (14 kinds of antibiotics), fluorescence PCR (AB-Vi7), incubator, turbidimeter (Bio-Merieux). Applied Biosystems ABI Viia7.

## *2.2. Method*

### *2.2.1. Salmonella Strain Recheck*

Culture methods for strains isolated from food refer to Food microbiology test, Salmonella Test GB 4789.4-2016; methods for isolation of samples from patients with diarrhea: a certain amount of samples were inoculated with SC growth medium and inoculated on Salmonella chromogenic medium for 24 hours incubation at 36°C, biochemical and serological identification of the suspected colonies.

### *2.2.2. Drug Sensitivity Test*

The strains were inoculated on TSA Plate and cultured at 37°C for 24 hours. 3-5 new bacterial colonies were selected and suspended in 10 mL normal saline. Adjusted the bacterial solution to 0.5 MC, it means the bacterial solution was $1 \times 10^8$ CFU/mL. First, a Micropipette is used in the Biosafety cabinet to absorb the turbid bacterial solution 10μL, added to the 12 mL broth tube, mixed and slowly poured into a v-shaped aseptic tank, then add the dilution solutionadded to the 96-hole drug sensitive plate (100μL per hole) with eight micropipettes and covered with a plastic cover. The final inoculation concentration was about $1 \times 10^5 \sim 2 \times 10^5$ CFU/mL. The positive control hole joins 100μ l bacterium suspension then, the entire process notices the asepsis operation.

After inoculating, put the susceptibility kit into the incubator at 36 ± 1°C for 16 ~ 18 hours, and read the MIC (minimum inhibitory concentration) value. The quality control strain was ESCHERICHIA coli ATCC25922.

### *2.2.3. Integron Gene*

According to the references [7, 8], the type I, II and III Integron primers were designed. The detailed sequences are shown in Table 1.

***Table 1.*** *The detailed sequences of the type I, II and III Integron primers.*

| Target Gene | Primer sequence (5,-3,) | Segment Length /BP | Gene Coding |
|---|---|---|---|
| Intl 1 | F1: GCCGTGGTTCTGGGTTTT<br>R1: GAGTGGCGGAGGGTGTG<br>pb1: FAM-TCCGTGGATCGGTCGAATGCGT-BHQ1 | 1013 | Gene ID: 58463195 |
| Intl 2 | F2: CCTTGTAAGTTGTCGTCTTGCTG<br>R2: AAGATTTTGATTTTGATAATGGCTG<br>Pb2: FAM-ACAACTCATTGAGCAAGCGCGGC-BHQ1 | 977 | Gene ID: 57334186 |
| Intl 3 | F3: GGCTTCGTGATGCCTGCTT<br>R3: CCGTGGTTCTGGGTTTTTG<br>pb3: FAM-TCAGCGCGCCTTCAAACGTGC-BHQ1 | 1010 | Gene ID: 58391950 |

20μL REAL-TIME PCR reaction system: Fast TaqMan Mixture Premix 10 μl, upstream and downstream Primers 1 μl, probe 0.5 μl, DEPC water (4.2) 5.5 μl, DNA template 2 μl. Run A REAL-TIME PCR.

REAL-TIME PCR reaction conditions: pre-denaturation at 95°C for 3 min, denaturation at 95°C for 5s, annealing extension at 55°C for 1 min. Fluorescent light is collected here, 40 cycles in all.

### *2.2.4. Statistical Method*

Use SPSS 21.0 software. The count data were expressed as a percentage (%), and the comparison between the two groups was made by $\chi^2$ test. Test Level $\alpha$=0.01, if P<0.01, it shows that there is significant difference between the two groups.

# 3. Result

## *3.1. Drug Resistance of Two Food-borne Pathogens*

### *3.1.1. Detection of Drug Resistance of Salmonella in Food-borne Disease Surveillance in Jilin Province*

The total drug resistance rate of 278 strains of food-borne Salmonella in Jilin Province was 89.57% (249/278). The

total drug resistance rate of Salmonella was 95.8% (207/216) in 216 stool samples from patients with foodborne diseases. The highest resistance rate was nalidixic acid 83.3% (180/216), followed by ampicillin 82.4% (178/216) and tetracycline 70.0% (149/216)(see table 2).

***Table 2.*** *Resistance of Salmonella to antibiotics in two kinds of samples.*

| Antibacterials | Salmonella in patient faeces n=216 | | Salmonella in raw meat and eggs n=62 | | $\chi^2$ value | P value |
|---|---|---|---|---|---|---|
| | Number of drug resistance (strain) | Drug resistance rate (%) | Number of drug resistance (strain) | Drug resistance rate (%) | | |
| Ampicillin, | 178 | 82.4 | 15 | 24.2 | 76.91 | <0.001 |
| Ceftazidime | 1 | 0.5 | 0 | 0 | | 1.00 |
| Ampicillin Sulbactam | 49 | 22.7 | 2 | 3.2 | 12.18 | <0.001 |
| Imipenem | 1 | 0.5 | 0 | 0 | | 1.00 |
| Tetracycline | 149 | 70.0 | 21 | 33.9 | 25.0 | <0.001 |
| Nalidixic acid | 180 | 83.3 | 20 | 32.2 | 62.26 | <0.001 |
| Cefoxitin | 4 | 1.9 | 0 | 0 | | 0.58 |
| Chloramphenicol | 68 | 31.5 | 7 | 11.3 | 9.97 | 0.002 |
| Cefotaxime | 47 | 21.8 | 0 | 0 | 16.24 | <0.001 |
| Cefazolin | 60 | 27.8 | 16 | 25.8 | 0.09 | 0.76 |
| Gentamicin, | 26 | 12.0 | 5 | 8.6 | 0.77 | 0.38 |
| Compound SULFA | 86 | 39.8 | 8 | 12.9 | 15.59 | <0.001 |
| Azithromycin | 31 | 14.4 | 0 | 0 | 10.02 | 0.002 |
| Ciprofloxacin | 40 | 18.5 | 8 | 12.9 | 1.06 | 0.30 |

#### *3.1.2. Detection of Drug Resistance of Salmonella in Food Safety Risk Surveillance in Jilin Province*

The highest resistance rate was tetracycline (Tet) 33.90% (21/62), followed by naphtholic acid (NAL) 32.3% (20/62) and CEFAZOLIN (CFZ) 25.8% (16/62), which were sensitive to Ceftazidime, Imipenem, cefoxime, cefotaxime and Azithromycin (see table 2).

### *3.2. Detection of Integron Genes in Two Kinds of Food-borne Salmonella*

#### *3.2.1. Analysis of Integron I Gene of Two Kinds of Food-borne Salmonella*

In this study, type I integron gene of food-borne Salmonella was detected. The results showed that the percentage of type I integron gene of food-borne Salmonella was 51.44% (143/278). The positive rate of Salmonella type I integron was 58.8% (127/216) in stool samples from patients with foodborne diseases and 25.81% (16/62) in food (meat and eggs), Chi-square test, $\chi^2$ value 20.99, Salmonella type I integron gene carrying rate in stool samples and food (meat and eggs) from patients with foodborne diseases was statistically significant (P<0.01). Food-borne Salmonella has multiple drug resistance to clinical antibiotics, and the multiple drug resistance of bacteria is generally closely related to class I integron.

#### *3.2.2. Analysis of Integron II and III Genes in Two Kinds of Food-borne Salmonella*

In this study, type II integron gene detection was carried out for two kinds of food-borne Salmonella. The results showed that the percentage of Type II integron gene carrying was 4.32% (12/278) in Jilin Province, the percentage of Salmonella Type II integron in stool samples from patients with foodborne diseases was 5.56% (12/216). Salmonella type II integron in food (meat and eggs) was not detected. The results showed that 14.39% (40/278) of food-borne Salmonella had type III Integron gene in Jilin province, and 17.59% (38/216) of food-borne disease patients had type III Integron gene, the gene carrying rate of type III Integron in food (meat and eggs) was 3.23% (2/62), $\chi^2$ value was 8.06, the results indicated that there was a significant difference in carrying rate of type III Integron gene of Salmonella from food-borne diseases and Food Safety (P<0.01). See Table 3.

***Table 3.*** *Analysis of Class I, II and III integron genes in two kinds of food-borne Salmonella.*

| Integral subtype | Salmonella in patient faeces | Salmonella in raw meat and eggs | $\chi^2$ Value | P Value |
|---|---|---|---|---|
| Class I integron | 58.8% (127/216) | 25.81% (16/62) | 20.99 | P<0.01 |
| Class II integron | 5.56% (12/216) | 0 | 3.6 | P>0.05 |
| Class III Integron | 17.59% (38/216) | 3.23% (2/62) | 8.06 | P<0.01 |

## 4. Discuss

### *4.1. Drug Resistance of Two Kinds of Food-borne Salmonella and Its Significance*

The total drug resistance rate of 278 strains of food-borne Salmonella was 89.57% (249/278). The drug resistance rate of Salmonella in the samples of patients with foodborne diseases was 95.8% (207/216), the highest was NAL 83.3% (180/216), followed by ampicillin 82.4% (178/216) and tetracycline 70.0% (149/216);, as well as some resistance to other antibiotics. The drug resistance rate of Salmonella in food (meat and eggs) was 67.7% (42/62). The drug resistance rate of Tet was 33.90% (21/62), NAL was 32.3% (20/62) and CFZ was 25.8% (16/62), but it is sensitive to

CEFTAZIDIME, Imipenem, Cefoxitin, CEFOTAXIME and Azithromycin.

The resistance rate of Salmonella to most antibiotics in the samples of patients with foodborne diseases was much higher than that of Salmonella in food (meat and eggs). There were significant differences in the resistance rates of Salmonella to Ampicillin, ampicillin Sulbactam, tetracycline, nalidixic acid, chloramphenicol, cefotaxime, sulfamethoxazole, Azithromycin. There was no significant difference in the resistance rates of Salmonella to Cefoxitin, Cefazolin, ceftazidime, Imipenem, gentamicin and Ciprofloxacin among the food-borne disease patients. There were significant differences in the resistance rates of Salmonella in the food-borne disease patients. to Ampicillin, ampicillin Sulbactam, tetracycline, nalidixic acid, chloramphenicol, cefotaxime, sulfamethoxazole, Azithromycin. but, no significant difference in the resistance rates of Salmonella to Cefoxitin, Cefazolin, ceftazidime, Imipenem, gentamicin and Ciprofloxacin among the food-borne disease patients.

### *4.2. Analysis of I, II and III Integron Genes in Two Kinds of Food-borne Salmonella*

The results of detection of type I, II and III Integron genes of 278 food-borne Salmonella strains by fluorescence PCR showed that, The percentage of type I integron was 51.44% (143/278), type II integron was 4.32% (12/278), and type III Integron was 14.39% (40/278). In the detection results of Salmonella from patients with foodborne diseases, the positive rate of type I integron gene was 58.8% (127/216), type II integron gene was 5.56% (12/216), type III integron gene was 17.59% (38/216). In the detection results of Salmonella in food (meat and eggs), the positive rate of type I integron gene was 25.81% (16/62), type III integron gene was 3.23% (2/62), type II integron gene was not detected. Salmonella from food-borne disease patients compared with Salmonella from food (meat and eggs), type I and type III integron genes were significantly different. The positive rate of type I and III Integron of Salmonella from food-borne disease patients was much higher than that of food (meat and eggs). The integron gene system plays an important role in drug resistance of food-borne pathogens. Salmonella from food-borne diseases has multiple drug resistance to clinical antibiotics, the multiple drug resistance of bacteria is closely related to type I and type III Integron.

## 5. Conclusion

Salmonellosis remains one of the most frequent food-borne pathogens, constituting a worldwide major public health concern [9]. People infected with Salmonella can experience typhoid fever, nausea, vomiting, abdominal cramps and diarrhea. In recent years, Salmonella has a high proportion of antibiotic resistance and genetic characteristics [10]. Salmonella strains have been found to be highly resistant to monohydroxylactam, aminoglycoside, sulfa, tetracycline, chloramphenicol and quinolones [11, 12]. The mechanism of bacterial resistance induced by integron gene system has attracted more and more attention. Integration subsystem can carry a variety of drug resistance genes and has a wide range of hosts [15], which has become one of the important mechanisms for the generation and transmission of drug resistance.

In this study, the drug resistance profile of Salmonella in foodborne diseases was significantly different from that in food safety monitoring. The antimicrobial resistance of patients with foodborne diseases is much higher than that of salmonella in food samples (meat, eggs). This is consistent with the research results of some scholars [13, 14]. The results of drug sensitivity test and type I, II and III integron of two foodborne salmonella in Jilin province were analyzed. The integron gene system may mediate the spread of gene-level drug resistance in foodborne Salmonella.

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# Biography

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