Kafkas Univ Vet Fak Derg
23 (5): 803-807, 2017
DOI: 10.9775/kvfd.2017.17821

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
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Research Article

Class 1 Integrons and the Antibiotic Resistance Profile of *Salmonella* Infantis Strains from Broiler Chickens

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Article Code: KVFD-2017-17821 Received: 30.03.2017 Accepted: 06.06.2017 Published Online: 07.06.2017

Citation of This Article

Kaya İB, Şahan Yapıcıer Ö, Akan M, Diker KS: Class 1 integrons and the antibiotic resistance profile of *Salmonella* infantis strains from broiler chickens. *Kafkas Univ Vet Fak Derg,* 23 (5): 803-807, 2017. DOI: 10.9775/kvfd.2017.17821

Abstract

Salmonella infections are one of the most important diseases and cause economic problems in poultry. The zoonotic feature of the agent and leading to food-borne infections are also important in public health issues. Increasing antibiotic resistance causes difficulties of controlling *Salmonella* infections, in recent years. Among non-typhoidal *Salmonella* serotypes the rate of *Salmonella* Infantis are increasing in Turkey. With this increase, it is important to know the antimicrobial resistance patterns of *Salmonella* Infantis strains as seen in other *Salmonella* serotypes. In this study, we aimed to investigate *Salmonella* Infantis strains which were isolated from feces of healthy broiler chickens for the presence of antibiotic resistance and frequency of Class 1 integrons that is responsible for the transfer of antibiotic resistance as plasmids, transposons. For this purpose a total of 150 S. Infantis strains which were isolated and identified according to the ISO 6579-2002 and Kauffmann-White serotyping scheme were used. Antimicrobial resistance of the strains was determined by the disc diffusion method following to the recommendations of CLSI 2011 standard protocol and also Class 1 integrons were investigated by PCR. According to the results, high rate of multi-drug antibiotic resistance (89.3%), high rate of sensitivity (100%) to cefotaxime, ciprofloxacin, gentamicin, ceftazidime were observed and also Class 1 integrons were determined in all isolates. In conclusion, the presence of Class 1 integron is all strains of *Salmonella* Infantis showed the potential importance of these strains as recipient for antibiotic resistance.

Keywords: *Antimicrobial resistance, Class 1 integron, Salmonella Infantis*

Broyler Tavuklardan Elde Edilen *Salmonella* Infantis Suşlarının Antibiyotik Direnç Profilleri ve Sınıf 1 İntegron Varlığı

Özet

Salmonella infeksiyonları kanatlı hayvanlarda görülen ve ekonomik kayıplara neden olan en önemli hastalıklardan biridir. Etkenin zoonotik karakteri ve gıda kaynaklı infeksiyonlara neden olması da halk sağlığı açısından önem göstermektedir. Son yıllarda artan antibiyotik dirençliliği *Salmonella* infeksiyonlarının kontrolünü güçleştirmektedir. Türkiye'de non-tifoidal *Salmonella* serotiplerinden *Salmonella* Infantis'in oranı artış göstermektedir. Bu artış ile birlikte, diğer *Salmonella* serotiplerinde yaygın olarak görülen antimikrobiyal dirençliliğin *Salmonella* Infantis suşlarında da bilinmesi önem taşımaktadır. Bu çalışmada, sağlıklı broyler tavukların dışkılarından izole edilen *Salmonella* Infantis suşlarında antibiyotik dirençliliği ve plasmidler, transpozonlar gibi antibiyotik dirençliliğinin taşınmasında rol oynayan sınıf 1 integronların sıklığının araştırılması amaçlandı. Bu amaçla ISO 6579-2002 ve Kauffmann-White serotiplendirme şemasına göre identifiye edilen 150 adet *Salmonella* Infantis suşu kullanıldı. Suşların antibiyotik dirençleri CLSI 2011 standart protokolüne göre disk difüzyon yöntemi ile belirlendi ve PZR ile sınıf 1 integronlar araştırıldı. Elde edilen sonuçlara göre yüksek oranda çoklu antibiyotik dirençliliği (%89.3), yüksek oranda (%100) sefotaksim, siprofloksasin, gentamisin, seftazidime karşı duyarlılık ve incelenen suşların tamamında sınıf 1 integron varlığı tespit edildi. Sonuç olarak, *Salmonella* Infantis suşlarının hepsinde sınıf 1 integronların varlığı, bu suşların potansiyel direnç alıcı haline gelmesi bakımından önem taşıdığını göstermektedir.

Anahtar sözcükler: *Antimikrobiyal direnç, Salmonella Infantis, Sınıf I integron*

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INTRODUCTION

Non-typhiodal *Salmonella* infection is one of the most significant health problem in poultry all over the world particularly in developing countries [1]. *Salmonella* is also the most important zoonotic pathogen in poultry [2] *Salmonella* may be transmitted to the raw chicken meat by improper evisceration of the intestine at the slaughter-house which leads to food-borne infections in humans. On the other hand, *Salmonella* isolates carrying the antibiotic resistant genes are also important for both human and animal health [3]. According to the European Food Safety Authority (EFSA), among *Salmonella* serovars *Salmonella* Infantis is the most commonly reported serovar in poultry flocks [4]. In Turkey, the prevalence of *S.* Infantis serovar from healthy broiler chicken flocks is 77.2% by percentage [5].

In Argentina, Australia, Brazil, Netherlands, Finland, Canada, Hungary, Japan, New Zealand and Russia this serovar has increasingly been reported and multidrug-resistant (MDR) *Salmonella* has been observed for more than 20 years [6,7]. Most of the antimicrobial resistance genes are integrated in mobile genetic elements such as plasmids, transposons and integrons [1,8,9]. Typically, Class 1 integrons have *intI1* gene which encodes integrase, a recombination specific site, a promoter and gene cassettes. They can be inserted into transposons/plasmids and carry antibiotic resistance genes from bacteria to bacteria. Class 1 integrons harbor more than 100 gene casettes some of which are encoding resistance to β-lactamases, rifampicin, trimethoprim, aminoglycosides, quinolones and chloramphenicol [10,11]. Class 1 integrons are the most frequent integron type classified in MDR *Salmonella* serotypes [12,13].

In recent years, several studies have been conducted to investigate integrons in *Salmonella* [8,9,12,13]. For example a study in Germany demonstrated that all *S*. Derby isolates obtained from pigs had Class 1 integrons. Another study in Thailand showed that 84 of 183 *Salmonella* strains (*S*. Anatum, *S*. Kedougou, *S*. Stanley, *S*. Weltevreden, *S*. Rissen, *S*. Baiboukoum) which were isolated from humans and pork had Class 1 integrons and 18 of the strains carried resistance gene cassettes [12,13]. Nevertheless, information of antimicrobial resistance related integrons of *S.* Infantis is limited in Turkey. Because of the relative prevalence of *S*. Infantis to other *Salmonella* serotypes in poultry production has increased in recent years, in this study multiple antibiotic resistance and the presence of Class 1 integrons related with antibiotic resistance was investigated in *S.* Infantis strains.

MATERIAL and METHODS

Salmonella Strains and Antimicrobial Susceptibility Tests

A total of 150 *S.* Infantis strains selected among 206 *S*. Infantis strains which were isolated from feces of healthy broiler chickens between 2012-2013 were used in this study. 150 strains were selected from four different regions (57 from Black Sea region, 39 from Central Anatolia, 30 from Aegean and 24 from Mediterranean region) of Turkey according to the capacity of flocks. Briefly, a 10% systematic sampling method was used for the flocks which were below 10.000 broiler chickens *(Table 1)*. The isolation method and serotyping was performed according to the ISO 6579-2002 and Kauffmann-White scheme, respectively. Antimicrobial resistance of the strains was detected by the disc diffusion method following to the recommendations of CLSI 2011 standard protocol. Discs were used as follows: ampicillin (AMP: 10 µg); cefotaxime (CTX: 30 µg); chloramphenicol (C: 30 µg); ciprofloxacin (CIP: 5 µg); gentamicin (GM: 10 µg); kanamycin (K: 30 µg); nalidixic acid (NA: 30 µg); streptomycin (S: 10 µg); tetracycline (TE: 30 µg); trimethoprim- sulfamethoxazole (SXT: 5 µg); sulfonamide (S3: 250 µg) and ceftazidime (CAZ: 30 µg). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as positive control in all tests.

DNA Extraction

DNA was extracted from the isolates with a commercial kit (Genomic DNA Purification, Thermo Fisher Scientific, USA) following the manufacturer's recommended protocol. Then, all samples were kept in -20°C until the PCR assay was performed.

PCR Amplification of the intI1 Gene

Integrons were investigated by the detection of *intI1* gene [14]. PCR amplification was performed containing 0.2 µM of each primer (int1-F 5'-GCC TTG CTG TTC TTC TAC-3', int1-R 5'-GAT GCC TGC TTG TTC TAC-3'), 0.2 mM dNTPs (10 mM dNTP mix; Thermo Fisher Scientific, USA), 3 mM of $MgCl_2$ (Thermo Fisher Scientific, USA), 2.5 µL PCR reaction buffer, 2U of Taq DNA polymerase (Thermo Fisher Scientific; EP0402), and nuclease-free water to a final volume of 25 µl. In the reaction, 2 µL of DNA was used as template. The amplification was performed as follows: strand separation at 94°C for 3 min, followed by 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min. Finally, there was a 7 min at 72°C for further strand extension. Ten microliters of the amplified PCR products were analyzed by electrophoresis on 1.5% agarose gel (Promega Corporation, USA) with 4 µL of SafeView Classic (Applied Biological Materials, Canada) in Gel Electrophoresis Apparatus with 180V for 60 min. Positive (AVMA-SI14/2 *Salmonella* Infantis strain) and negative *(Leptospira* spp., *Listeria* spp., *Bordatella* spp., *Ornithobacterium rhinotracheale*, *Streptococcus* spp. and *Pseudomonas* spp.) strains were obtained from strain collection of Ankara University Faculty of Veterinary Medicine Department of Microbiology.

RESULTS

The isolates showing resistance to at least four and more antibiotic drug resistance were assessed as multiple-

Table 1. *Distribution of Salmonella Infantis strains according to the regions*

Regions/City	Number of Total Flocks	Number of Flocks ≤ 10.000	Number of Flocks with 10% Sampling	Number of Isolates
Black Sea/Bolu	378	70	7	57
Central Anatolia/Çorum	60	3	0.3*	7
Central Anatolia/Ankara	20	9	0.9*	19
Central Anatolia/Afyonkarahisar	70	2	0.2*	13
Aegean/İzmir	55	4	0.4*	30
Mediterranean/İçel	92	41	4	24

** The fractional results of 10% sampling calculations were accepted as one flock*

Table 2. *The antimicrobial resistance prevalence of Salmonella Infantis isolates*

Antimicrobials	Number of Isolates	Resistance Rates (%)
AMP	10	6.6
C	11	7.3
K	66	44
S	117	78
SXT	122	81.3
S3	139	92.6
TE	140	93.3
NA	142	94.6
CTX	0	0
CIP	0	0
GM	0	0
CAZ	0	0

Table 3. *Antimicrobial resistance patterns of 134 MDR Salmonella Infantis isolates*

Multiple Resistance Patterns	Number of Isolates (%)
K, NA, S, TE, SXT, S3	46 (30.6)
K, NA, TE, SXT, S3	6 (4)
K, NA, S, TET, S3	5 (3.3)
NA, S, TE, S3	8 (5.3)
NA, TE, SXT, S3	13 (8.6)
NA, S, TE, SXT, S3	43 (28.6)
AMP, C, NA, S, TE, SXT, S3	2 (1.3)
AMP, K, NA, S, TE, SXT, S3	1 (0.6)
AMP, C, NA, S, TE, S3	1 (0.6)
AMP, NA, S, TE, SXT, S3	1 (0.6)
AMP, C, K, NA, S, TE, SXT, S3	4 (2.6)
C, K, NA, S, TE, SXT, S3	3 (2)
C, NA, S, TE, SXT, S3	1 (0.6)

resistant strains. One hundred forty two (94.6%) isolates were resistant to nalidixic acid, 140 (93.3%) isolates were resistant to tetracycline, 139 (92.6%) isolates were resistant to sulfonamide and 122 (81.3%) isolates were resistant to trimethoprim- sulfamethoxazole *(Table 2)*. Also, all isolates were sensitive to cefotaxime, ciprofloxacin, gentamicin and ceftazidime (100%). Furthermore, 134 (89.3%) multi-drug resistance isolates were demonstrated *(Table 3)*.

All samples were investigated for the presence of *intI1* gene and thus the Class 1 integron *(intI1* gene) was detected in all isolates. Beside this, all multi-drug resistant isolates have been found to have Class 1 integron.

DISCUSSION

Increasing antibiotic resistance is an important public health problem and resistance continues to spread due to many factors. Antibiotics used for therapeutic and prophylactic purposes for broilers as stimulating growth in production. Some antibacterial drugs, such as ampicillin, tetracycline, chloramphenicol, enrofloxacin, neomycin, which are used in antibacterial treatment of *Salmonella* infections to become resistant by inhibiting the microflora of the digestive system and these bacteria play an important role in the transfer of resistance genes and cause serious infections that affect the food chain when spreading in nature [15-18]. For all these reasons, after January 2006, antibiotic feed additives are not allowed to be imported to Turkey by law [19].

In the present study, antibiotic susceptibility and presence of Class 1 integron of 150 *S.* Infantis strains that were isolated from healthy broiler chickens feces were detected. Nograd y et al.[20] also conclude that *S.* Infantis strains, which had multiple resistance spread through the poultry meat among humans and this supported that MDR has become a public health concern. According to the European Food Safety Authority (EFSA) report, it was emphasized that *S.* Infantis strains detected in poultry resistant to tetracycline, ampicillin and sulphonamides and 31% of MDR of these isolates [4]. Similar studies related to antibiotics that was used by poultry industry, performed by other researchers. Kudaka et al.[21] 99.2% of broiler isolates were identified as *S*. Infantis and antimicrobial resistance strains were detected as tetracycline, streptomycin and trimetoprim and especially sulphanamide. In our study, the resistance of nalidixic acid, tetracycline and sulfonamide against *S.* Infantis is 94.6%, 93.3%, 92.6% respectively. The high resistance rate of *S.* Infantis to quinolones was known in

Germany and Hungary, but in our study we had an opposite result of 100% sensitivity of ciprofloxacin [19]. Resistance to ciprofloxacin was reported to be 66.7% to 100%, especially in Germany, Slovakia, Bulgaria and Austria [4], while the study strains showed sensitivity to ciprofloxacin. Also high frequency of resistance to ciprofloxacin and nalidixic acid for *S.* Infantis was reported in several reports in Iran too [22,23]. Quinolones were used frequently in treatment regimens and also as a feed additives in poultry industry [24]. In recent years several studies have been reported about third-generation cephalosporin resistance patterns of *Salmonella* was isolated from domestic animals and human [25-27]. In parallel with this situation, Kameyama et al. emphasized that 3rd-generation cephalosporin-resistant *S.* Infantis associated with β-lactamases is increasing in worldwide [28]. Although cefotaxime and ceftazidime resistance were found in their research [28], our isolates were 100% sensitive.

Furthermore, in this study, it was observed that multiple antibiotic resistance was directly proportional to Class 1 integron presence. In our study *S.* Infantis strains were investigated for the presence of *intI1* gene and Class 1 integron was detected in all isolates. Previously, several laboratories mentioned that antibiotic resistance genes are chromosomally encoded and contain integrons. Integrons play an important role in the dissemination of antimicrobial resistance through horizontal transmission and it was known that prevalence of integron increases in *Salmonella* isolates [29,30]. Firoozeh et al.[8] showed that poultry MDR *Salmonella* isolates were carried Class 1 integron 91.4%, also Asgharpour et al.[1] reported that *S.* Infantis strains contain 36% Class 1 integron in Iran. Naghoni et al.[31] were detected Class 1 integron from *S. enterica* serovars and Kudaka et al.[21] was revealed that presence of Class 1 integron in *S.* Infantis isolated from broiler chickens as our study. Several other researches have been reported in many different countries about Class 1 integron but *S.* Infantis studies were limited [32-36]. This situation is similar in Turkey. To our knowledge there is only one study conducted to determine the association of antibiotic resistance with integrons of *S.* Infantis strains [37].

In our study, it was revealed that the antibiotic resistance rates were high in *S.* Infantis strains and a great number of these isolates have been found as MDR. Therefore, the more prudent usage of antibiotics and epidemiological studies are needed which can reveal the mechanism of spreading resistance and resistant strains must be included in prevention strategies.

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