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Research Article

Antibiotic Resistance of *Salmonella* Enteritidis of Human and Chicken Origin

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Abstract: The aim of this study was to examine the relationship between antibiotic resistance patterns among *Salmonella enterica* subsp. *enterica* Serovar Enteritidis isolates (*Salmonella* Enteritidis) of human and poultry origin. Antibiotic resistance of 97 *Salmonella* Enteritidis isolates from 25 chicken meat, 25 chicken intestine and 47 human fecal samples was examined using the National Committee for Clinical Laboratory Standards (NCCLS, 1997) disk diffusion method. Resistance patterns of the isolates were as follows: Of the 25 chicken meat isolates 4 were resistant to some antibiotics. Two isolates (CM15 and CM22) were resistant to ampicillin (AMP), cefuroxime sodium (CXM), ceftriaxone (CRO), trimethoprim-sulphamethazole (SXT) and ampicillin-sulbactam (SAM). Of the other 2 isolates, CM20 showed resistance to ceftriaxone (CRO) and CM23 was resistant to chloromphenicol (C) and cephalexime (CL). Eight human isolates showed 4 different resistance patterns. Five (HF3, HF19, HF20, HF22, and HF25) were resistant to ampicillin (AMP), ampicillin-sulbactam (SAM), tetracycline (TE) and chloromphenicol (C), while resistance to chloromphenicol (C) and cephalexime (CL) was observed in HF32 and HF46. HF13 was resistant to both ampicillin (AMP) and ampicillin-sulbactam (SAM). One of the chicken intestinal isolates was found to be resistant only to cefuroxime sodium (CXM). There was no relationship between antibiotic resistance patterns for *Salmonella* isolates of different origins. Multiple antibiotic resistance patterns were observed in some isolates. It is important that some isolates of chicken intestine and meat origin showed resistance to antibiotics being used in humans.

Key Words: Antibiotic resistance, *Salmonella* Enteritidis, human feces, chicken intestine, chicken meat

İnsan ve Tavuk Orijinli *Salmonella* Enteritidis İzolatlarının Antibiyotik Direnci

Özet: Bu çalışmanın amacı, insan ve kanatlı orijinli *Salmonella* Enteritidis izolatları arasında antibiyotik direnç paternlerinin ilişkisini incelemektir. Yirmibeş tavuk eti, 25 tavuk bağırsağı ve 47 insan dışkı örneğinden 97 *Salmonella* Enteritidis izolatı National Committee for Clinical Laboratory Standards (NCCLS, 1997) disk diffüzyon metodu kullanılarak incelenmiştir. İzolatların dirençlilik paternleri şu şekildedir: 25 tavuk eti izolatının 4'ü bazı antibiyotiklere dirençli bulundu. İki izolat (CM15 ve CM22) ampicillin (AMP), cefuroxime sodium (CXM), ceftriaxone (CRO), trimethoprim-sulphamethazole (SXT) ve ampicillin-sulbactam (SAM)'a dirençliydi. Diğer iki izolattan, CM20 ceftriaxone (CRO)'na dirençlilik gösterdi. CM23 chloromphenicol (C) ve cephalexime (CL)'e karşı dirençliydi. Sekiz insan izolatı dört farklı direnç paterni gösterdi. Chloromphenicol (C) ve cephalexime (CL)'e direnç HF32 ve HF46'da gözlenirken, bunlardan 5 tanesi (HF3, HF19, HF20, HF22, HF25) ampicillin (AMP), ampicillin-sulbactam (SAM), tetracycline (TE) ve chloromphenicol (C)'e dirençliydi. HF13 ampicillin (AMP) ve ampicillin-sulbactam (SAM)'a dirençliydi. Kanatlı bağırsağı izolatları arasında, sadece biri cefuroxime sodium (CXM)'a dirençli bulundu. Farklı orijinli *Salmonella* izolatlarına ait antibiyotik direnç paternleri arasında identikallik (birbiriyle aynılık) yoktu. Bazı izolatlarda çoklu antibiyotik dirençlilik paternleri gözlendi. Tavuk bağırsağı ve tavuk eti orijinli bazı izolatların insanlarda kullanılan antibiyotiklere karşı direnç göstermiş olması önemlidir.

Anahtar Sözcükler: Antibiyotik direnci, *Salmonella* Enteritidis, insan dışkısı, tavuk bağırsağı, tavuk eti

Introduction

Salmonellae have been responsible for the majority of human bacterial food-poisoning cases worldwide in recent years (1-6). The aim of this study was to determine the antibiotic resistance profile similarities/differences of poultry meat, poultry intestine, and human fecal isolates (7-9). Therefore, we selected antibiotics that have been widely and solely used in the treatment of *Salmonella enterica* subsp. *enterica* Serovar Enteritidis (*Salmonella* Enteritidis) in humans (10-12). As is also widely known, poultry meat and poultry products have been shown to be major sources of this serovar (1,9,13-15).

Determination of the antibiotic resistance profiles of *Salmonella* Enteritidis isolates from different sources is important in order to predict possible clonal relations among the isolates (3,8). In this study we initially determined the antibiotic resistance profiles of human

isolates to antibiotics that have been widely and solely used in the treatment of *Salmonella* Enteritidis in humans (12). Then we examined whether the source of the determined resistances to these antibotics was poultry meat or poultry meat products (16). This study was performed to determine and compare the resistance patterns of *Salmonella* Enteritidis isolates of human and chicken origin against the most commonly used antibiotics in humans.

Materials and Methods

A total of 97 *Salmonella* Enteritidis strains, consisting of 25 chicken meat, 47 human fecal (kindly provided by Dr. Suna Gedikoğlu, Uludağ University, Faculty of Medicine, Bursa, Turkey) and 25 chicken intestine isolates were used. All the isolates had been serotyped before in different *Salmonella* reference centers (WHO Collaborating Center for Reference and Research on *Salmonella*, Institut Pasteur, France, and Ankara University, Faculty of Medicine, Department of Microbiology, Ankara).

The following antibiotic disks were used: ampicillin (10 µg) (AMP), ampicillin-sulbactam (10/10 µg) (SAM), cephalexime (30 µg) (CL), ceftriaxone (30 µg) (CRO), cefuroxime sodium (30 µg) (CXM), ciprofloxacin (5 µg) (CIP), tetracycline (30 µg) (TE), trimethoprim-sulphamethazole (1.25/23.75 µg) (SXT), and chloromphenicol (30 µg) (C).

The antimicrobial sensitivity test was performed using the disk diffusion method described by the National Committee for Clinical Laboratory Standards (NCCLS, 1997) (17). Briefly, *Salmonella* Enteritidis isolates were plated on Xylose Lysine Tergitol$_4$ (XLT$_4$ Agar) (Difco; 0234-17-9) agar and were incubated for 24 h at 37 ºC. One colony was selected and inoculated into Brain-Heart Infusion (BHI) broth to prepare pure cultures and to adjust the inoculum turbidity to 0.5 McFarland. Each *Salmonella* culture (100 µl) was distributed evenly, using a glass streaker, onto Mueller Hinton agar (Difco; 0252-17-6) and antibiotic disks were placed onto the agar. Plates were incubated for 18 h at 37 ºC and the inhibition zones were measured to interpret the results. Tests were carried out in duplicate for accuracy. *Escherichia coli* ATCC 25922 was used as a reference strain.

Results

Different resistance patterns were observed in 13 out of 97 *Salmonella enterica* subsp. *enterica* Serovar Enteritidis (*Salmonella* Enteritidis) isolates. Antibiotic resistance patterns are shown in detail in Table.

Table. Resistance patterns of *Salmonella* Enteritidis isolates from chicken meat, human feces and chicken intestines.

Strain code	Antibiotics								
	AMP[††]	CXM[I]	CRO[ŝ]	CIP[O]	SXT[ŝ]	TE[Δ]	C[δ]	CL[θ]	SAM[ô]
CM* 15	R[§]	R	R	S[†]	R	S	S	S	R
CM20	S	S	R	S	S	S	S	S	S
CM22	R	R	R	S	R	S	S	S	R
CM23	S	S	S	S	S	S	R	R	S
HF[¶] 3	R	S	S	S	S	R	R	S	R
HF13	R	S	S	S	S	S	S	S	R
HF19	R	S	S	S	S	R	R	S	R
HF20	R	S	S	S	S	R	R	S	R
HF22	R	S	S	S	S	R	R	S	R
HF25	R	S	S	S	S	R	R	S	R
HF32	S	S	S	S	S	S	R	S	R
HF46	S	S	S	S	S	S	S	R	S
CI** 20	S	R	S	S	S	S	S	S	S

*Chicken meat
**Chicken intestine
[ŝ]Trimethoprim-sulphamethazole
[Δ]Tetracycline
[†]Sensitive
[δ]Clorompheniçol
[¶]Human feces
[ŝ]Ceftriaxone
[I]Cefuroxime sodium
[δ]Cephalexime
[ô]Ampicillin-sulbactam
[O]Ciprofloxacin
[ô]Resistant
[††]Ampicillin

Out of 25 chicken meat isolates, 4 were resistant to some antibiotics. Two isolates (CM15 and CM22) were resistant to ampicillin (AMP), cefuroxime sodium (CXM), ceftriaxone (CRO), trimethoprim-sulphamethazole (SXT) and ampicillin-sulbactam (SAM). Of the other 2 isolates, CM20 showed resistance to ceftriaxone (CRO) and CM23 was resistant to chloromphenicol (C) and cephalexime (CL). Eight human isolates showed 4 different resistance patterns. Five (HF3, HF19, HF20, HF22, and HF25) were resistant to ampicillin (AMP), ampicillin-sulbactam (SAM), tetracycline (TE) and chloromphenicol (C), while resistance to chloromphenicol (C) and cephalexime (CL) was observed in HF32 and HF46. HF13 was resistant to both ampicillin (AMP) and ampicillin-sulbactam (SAM). One of the chicken intestinal isolates was found to be resistant only to cefuroxime sodium (CXM).

Discussion

Out of 25 chicken meat isolates, 4 were resistant to some antibiotics. A similiar resistance pattern in *Salmonella* Enteritidis from chicken carcases has been reported by Arvanitidou et al. (14). These resistance patterns in *Salmonella* of chicken meat origin should be considered a great risk for the treatment of human salmonellosis.

Of the 47 human fecal isolates, 8 were found to have 4 different resistance patterns as previously reported in other studies (8,16). Five (HF3, HF19, HF20, HF22, and HF25) were resistant to AMP, SAM, TE and C, while C and CL resistances were observed in HF32 and HF46 (18,19). The other isolate (HF13) was found to be resistant to both AMP and SAM (18,19).

One chicken intestinal isolate was resistant only to CXM. This antibiotic is not used for therapeutic purposes or as a feed additive in poultry. CXM has been widely used in the treatment of certain human infections, bovine mastitis, and feline and canine upper respiratory tract infections. Perhaps a *Salmonella* Enteritidis clone previously acquired resistance to cefuroxime while it was in a mammalian host, and then this resistant strain somehow found a way into a poultry environment and was transferred to the poultry gut.

It is interesting that there was no identical antibiotic resistance pattern among chicken meat, human and chicken intestinal isolates. The reason for this is unclear. In order to reveal if there are any isolates of identical antibiotic resistance patterns from chicken meat, human faeces, and chicken intestines, we are continuing to produce isolates from chicken meat and chicken intestines to increase the number of isolates and to carry out wide-scale investigations.

However, it is important for public health that we have determined some resistance similarities between CM and HF isolates. These resistances observed in HF isolates could have been transferred by various genetic means from CM isolates (18,19).

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