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Extended spectrum beta-lactamase activity and multidrug resistance of *Salmonella* serovars isolated from chicken carcasses from different regions of Turkey

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Summary: This research was conducted to investigate the extended spectrum beta-lactamase activity and multidrug resistance of *Salmonella* serovars isolated from chicken carcasses. For this purpose, 99 *Salmonella* isolates from 930 chicken carcasses were tested against 12 different antimicrobials. The resistance rates of *Salmonella* isolates to antimicrobials were as follows: 35.3% (35/99) to ampicillin, 33.3% (33/99) to tetracycline, 29.2% to amoxicillin-clavulanic acid, 18.1% (18/99) to nalidixic acid, 17.1% (17/99) to chloramphenicol, 16.1% (16/99) to aztreonam, 12.1% (12/99) to trimethoprim-sulfamethoxazole, 4% (4/99) to gentamicin, 1.0% (1/99) to ceftazidime. Of the isolates 46.4% (46/99) were found to be resistant to two or more antimicrobials as a multidrug resistance. Extended spectrum beta-lactamase activity was detected in 1.0% (1/99) of the isolates. Furthermore, *S.* Typhimurium 26.2% (28/99), *S.* Infantis 16.1% (16/99), *S.* Hadar 12.1% (10/99) and *S.* Branderburg 9.0% (9/99) were found to be the predominant serovars. In conclusion, antimicrobial resistance and also multidrug resistance rates of *Salmonella* isolates in this study, indicated that monitoring of antimicrobial resistance profiles is important for Salmonella infections to plan treatment strategies.

Keywords**:** Chicken carcass, ESβLs, Multidrug resistance, *Salmonella* serovar.

Türkiye'nin çeşitli bölgelerindeki tavuk karkaslarından izole edilen *Salmonella* seovarlarının çoklu ilaç dirençliliği ve geniş spektrumlu beta laktamaz aktivitesi

Özet: Bu çalışma, tavuk karkaslarından izole edilen *Salmonella* serovarlarının çoklu ilaç dirençliliği ve geniş spektrumlu beta laktamaz aktivitesinin araştırılması amacıyla yürütülmüştür. Çalışmada, 930 tavuk karkasından izole edilen 99 *Salmonella* izolatı 12 farklı antimikrobiyale karşı test edilmiştir. *Salmonella* izolatlarının antimikrobiyallere karşı direnç oranları sırasıyla; ampisiline %35.3 (35/99), tetrasikline %33.3 (33/99), amoksisilin-clavulanic asite %29.2 (29/99), nalidiksik asite %18.1 (18/99), kloramfenikole %17.1 (17/99), aztreonama %16.1 (16/99), trimetoprim-sulfametoksazole %12.1 (12/99), gentamisine %4 (4/99), seftazidime %1.0 (1/99) olarak belirlenmiştir. İzolatların %46.4 (46/99)'ünün çoklu ilaç dirençli olarak iki veya daha fazla antibiyotiğe direnç gösterdiği tespit edilmiştir. Geniş spektrumlu beta laktamaz aktivitesi %1.0 (1/99) oranında bulunmuştur. Ayrıca, *S.* Typhimurium %26.2 (28/99), *S.* Infantis %16.1 (16/99), *S.* Hadar %12.1 (10/99) ve *S.* Branderburg %9.0 (9/99) çalışmadaki predominat serovarlardır. Sonuç olarak, antimikrobiyal direnç ve çoklu ilaç direnç oranları, direnç profillerinin monitorize edilmesinin *Salmonella* enfeksiyonlarının tedavi stratejilerinin planlanması için önemli olduğunu göstermiştir.

Anahtar sözcükler**:** Çoklu ilaç direnci, GSBL, *Salmonella* serovar, Tavuk karkası

Introduction

Salmonella species (particularly *Salmonella* Typhimurium *and Salmonella* Enteritidis) are recognized worldwide as a significant cause of human and animal diseases. This bacterium is one of the leading foodborne pathogens with infections leading to gastroenteritis including diarrhea, abdominal cramps, fever, nausea, and vomiting in human beings (3). The gastrointestinal tract of animals, mainly chickens, are considered as the major source of this pathogen and human illnesses are usually linked to exposure to contaminated animal-derived products such as poultry meat and poultry meat products (23).

Resistance in *Salmonella* isolates to commonly used antimicrobials is increasing both in the veterinary and public health area due to their extensive use both in humans and in veterinary medicine such as gentamicin, streptomycin, ampicillin, nalidixic acid, chloramphenicol, tetracycline, ceftazidime, amoxicillin, trimethoprim-sulfamethoxazole, cefotaxime, aztreonam, ceftriaxone, cefotaxime/clavulanic acid, ceftazidime/clavulanic acid, erythromycin, enrofloxacin. Several *Salmonella* serotypes may acquire resistance to antimicrobials in food animals before transmission to humans through the food chain. Increasing resistance rates and growing up of multidrug resistant strains (MDR) makes more difficult to treatment of *Salmonella* infections. (21, 34). Therefore, surveillance for antimicrobial resistance in humans and food animals is important for detecting changes in susceptibility to implement control measures on the use of antimicrobial drugs and to prevent the further spread of multidrug-resistant strains (34). Furthermore, extended spectrum beta-lactamases (ESβLs) that have the ability to hydrolyze β-lactam antibiotics, have emerged as a major contributor of drug resistance (8, 33). The ESβLs provide resistance to the penicillins, cephalosporins, aztreonam but do not cephamycins or carbapenems. ESβL producing organisms are frequently multiple drug resistant and have been isolated from foods of animal origin (19, 20, 37). ESβL-production (e.g., TEM, CTX-M, PER, SHV) in *Salmonella* strains that was first reported in 1988 have increased and emerged worldwide in recent years (9, 15, 22). More than 340 β-lactamases have been detected and many of these were found in *Salmonella*. Most ESβL-carrying *Salmonella* strains have been reported in Latin America, the Western Pacific, Europe and North America. Also an increase in ESβL-producing strains has been reported in poultry in recent days (31, 37). The aim of this study was to determine the ESβL-production, antimicrobial resistance for commonly used in veterinary and human medicine and also to detect multidrug resistance situation of *Salmonella* serovars isolated from chicken carcasses in Turkey.

Material and Method

Isolation and serotyping: From January 2008 to January 2010, a total of ninety nine *Salmonella* isolates were isolated from 930 chicken carcasses (300 carcasses were collected from Eskisehir, 240 Balıkesir, 160 Bandırma, 150 Istanbul and 80 Ankara) that were collected from five slaughterhouses from different regions, which are important for poultry breeding and have a large capacity of slaughtering, of Turkey. Carcasses were obtained from the packing plants of the slaughterhouses. *Salmonella* spp. were isolated by conventional culture methods as described in ISO 6579 as follows: 25 g of chicken carcass was aseptically placed into a sterile stomacher bag with a filter that contained 225 ml of buffered peptone water (Biomeriéux, Marcy l'Etoile, France) and was incubated at 37°C for 18 h. Subsequently, 1 ml of this pre-enrichment culture was used to inoculate 10 ml of Rappaport-Vassiliadis soy peptone broth (Oxoid, CM0866) and was incubated for 24 h for primary enrichment at 42°C. After enrichment, the samples were inoculated on to Xylose-Lysine-Terigitol-4 (Beckton Dickinson, 223420) and Xylose Lysine Deoxycholate Agar (Beckton Dickinson, 278850) and incubated at 35°C for 24 h. The suspicious colonies were identified with biochemical test: Triple Sugar Iron (Oxoid, CM0277), Lysine Iron Agar (Oxoid, CM0381), urea hydrolysis (Oxoid, CM0053B), H_2S, indole production, ONPG (β-galactosidase), lysine decarboxylase and Voges-Proscauer tests. Then all presumptive *Salmonella*-positive isolates were confirmed serologically with polyvalent and monovalent specific somatic and flagellar antisera (Beckton-Dickinson) and serotyped using slide agglutination according to the Kauffman-White scheme, in Ankara University, Faculty of Veterinary Medicine, Department of Microbiology (2, 14).

Antimicrobial susceptibility test: Antimicrobial profile of each *Salmonella* serovar was determined by the disk diffusion method (Kirby-Bauer) according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (4). The following antimicrobial drugs: Ampicillin (AMP, 10 µg, Oxoid CT0003B), Amoxicillin clavulanic acid (AMC, 20/10 µg, BD BBL231628), Aztreonam (ATM, 30 µg, Oxoid CT0264B), Cefotaxime (CTX, 30 µg, Oxoid CT0166B), Cefotaxime/Clavulanic acid (CTC,30+10 µg, Bioanalyse CTC-40), Ceftazidime (CAZ, 30 µg, Oxoid CT0412B), Ceftazidime/clavulanic acid (CZC, 30+10 µg, Bioanalyse CZC-40), Ceftriaxone (CRO, 30 µg, Oxoid CT0417B), Chloramphenicol (C, 30 µg, Oxoid CT0013B), Gentamicin (CN, 10 µg, Oxoid CT0072B), Nalidixic acid (NA, 30 µg, Oxoid CT0031B), Streptomycin (S, 10 µg, Oxoid CT0047B), Tetracycline (TE, 30 µg, Oxoid CT0054B), Trimethoprim/ Sulfamethoxazole (SXT, 25 µg, Oxoid CT0052B) were used for antimicrobial susceptibility testing. *Escherichia coli* ATCC 25922 was used as quality control strain in all tests.

ESβL detection: Extended spectrum β-lactamase production was detected using a double-disc synergy test. The presence of ESβL was assayed using the following antibiotic discs (Oxoid, UK): cefotaxime 30 µg, cefotaxime/clavulanic acid 30/10 µg, ceftazidime 30 µg and ceftazidime/clavulanic acid 30/10 µg. According to the CLSI criteria for ESβL detection, each isolate with an inhibition zone diameter of ≤22 mm for ceftazidime or ≤27 mm for cefotaxime was considered to be a potential ESβL producer or screen positive. A zone diameter increase of ≥5 mm for either antimicrobial agent when tested in combination with clavulanic acid versus when tested alone was considered as an ESβL-producing organism. *Klebsiella pneumoniae* ATCC700603 and *E. coli* ATCC25922 were used as positive and negative control respectively for quality control in the ESβL tests (4). Multiple antibiotic resistances (MARs) index for each resistance pattern was calculated as "MAR index=Number of resistance antibiotics/total number of antibiotics tested" (29).

Table 1. Antimicrobial resistance profiles of *Salmonella* serovars.
Tablo 1. *Salmonella* serovarlarının antimikrobiyal direnç profilleri.

Salmonella serovar (N)	Antimicrobial resistance profile	MAR Index
Agona (2)	C	0.083
Branderburg (4)	-	0.000
Branderburg (4)	AMC, AMP	0.166
Branderburg	AMC, AMP, NA, ATM	0.333
Chincol (2)	-	0.000
Corvallis (2)	-	0.000
Corvallis*	CAZ	0.083
Corvallis	AMC, AMP	0.166
Corvallis	AMC, AMP, TE	0.250
Corvallis	AMC, AMP, TE, NA	0.333
Dabou (2)	-	0.000
Emek (2)	-	0.000
Enteritidis (2)	-	0.000
Enteritidis	SXT, AMC, AMP	0.250
Enteritidis (3)	C, AMC, AMP, ATM	0.333
Essen (2)	NA, AMC, AMP, TE	0.333
Hadar (5)	-	0.000
Hadar (2)	TE	0.083
Hadar (2)	AMP	0.083
Hadar (2)	CN, TE	0.166
Hadar	SXT, AMC, AMP, NA, TE	0.416
Infantis (3)		0.000
Infantis (4)	TE	0.083
Infantis (9)	SXT, NA, TE	0.250
Kentucky (5)	-	0.000
Kentucky	C, AMC, AMP, ATM	0.333
Kentucky	NA, AMC, AMP, TE	0.333
Kentucky	NA, AMC, AMP, TE, SXT	0.416
Kingston (2)	CN, TE	0.166
Seftenberg (2)	-	0.000
Typhimurium (4)	-	0.000
Typhimurium (10)	C, AMC, AMP, ATM	0.333
Typhimurium (7)	TE	0.083
Typhimurium (4)	AMC, AMP	0.166
Typhimurium (1)	C, AMP, ATM	0.250
Virchow (2)	NA	0.083

N: number of serovars exhibited same resistance profile; *ESβL active *Salmonella* serovar.
N: aynı direnç profilini gösteren serovarların sayısı; *GSBL gösteren *Salmonella* serovarı.

Results

In this study, the presence of *Salmonella* spp. was 10.6% (99/930) and the number of strains from Eskisehir, Balıkesir, Istanbul, Bandırma and Ankara were 25, 22, 19, 18 and 15, respectively. Also fifteen *Salmonella* serovars were identified (Table 1). A total of 99 *Salmonella* spp. isolated from 930 chicken carcasses were tested against 12 antimicrobials. According to the results, 64.6% (64/99) of all isolates were determined to be resistant to at least one agent and 46.4% (46/99) of the isolates were found multi-resistant from 2 to 5 out of 12 antimicrobials tested. Among the tested antimicrobials ampicillin 35.3% (35/99) has the highest resistance rate. Detailed results for all other tested antimicrobials and resistance patterns of MDR strains are presented in Table 2 and *Salmonella* serovars with antimicrobial resistance patterns and MAR indexes are shown in Table 1. Also, *Salmonella* serovars with antimicrobial resistance rates are shown in Table 3. Extended spectrum β-lactamase production was detected in only one isolate 1.0% (1/99).

Table 2. Antimicrobial resistance patterns of 46 MDR *Salmonella* isolates
Tablo 2. 46 MDR *Salmonella* izolatının antimikrobiyal direnç örnekleri

Multiple resistance patterns	Number of isolates (%)
AMC, AMP	9 (9.09)
CN, TE	4 (4.04)
SXT, NA, TE	9 (9.09)
SXT, AMC, AMP	1 (1.01)
C, AMP, ATM	1 (1.01)
AMC, AMP, TE	1 (1.01)
C, AMC, AMP, ATM	14 (14.14)
NA, AMC, AMP, TE	4 (4.04)
NA, AMC, AMP, ATM	1 (1.01)
SXT, NA, AMC, AMP, TE	2 (2.02)

Table 3. Antimicrobial resistance rates of 99 *Salmonella* isolates.
Tablo 3. 99 *Salmonella* izolatının antimikrobiyal direnç oranları.

Antimicrobials		Number of isolates	Resistance rates (%)
Penicillins	AMP	35	35.3
	AMC	29	29.2
Tetracycline	TE	33	33.3
Quinolone	NA	18	18.1
Amphenicole	C	17	17.1
Monobactame	ATM	16	16.1
Sulfonamide	SXT	12	12.1
Aminoglycosides	CN	4	4.0
	S	0	0
Cephalosporins	CAZ	1	1.0
	CRO	0	0
	CTX	0	0

Discussion and Conclusion

In recent years, numerous studies have been conducted on the prevalence of *Salmonella* spp. in chicken carcasses. The prevalence of *Salmonella* serovars identified in this study was also similar to the findings from other studies in Turkey (10,16, 12). Isolation rates of *Salmonella* spp. from chicken samples (10.6%) were found to be almost similar to other studies in Turkey (8-18%) (5, 12, 36) and other countries (2.7-22%) (1, 17, 28). On the other hand, there are studies observed higher prevalence of *Salmonella* (38-72%) from chicken samples (6, 7, 30).

The prevalence of antimicrobial resistance among *Salmonella* isolates has increased worldwide during the last two decades, predominantly as a result of emerging MDR strains and increasing antimicrobial resistance in *Salmonella* spp. is a major health problem. The occurrence of MDR in *Salmonella* isolates is important for safety and microbiological quality of chicken meat and also considerable for transmission of genetic elements to other intestinal bacteria (11, 32) In the present study, 64.6% (64/99) of all isolates were determined to be resistant to at least one antimicrobial and 46.4% (46/99) of the isolates were detected as MDR. There are number of studies reported different rates of MDR in *Salmonella* spp. (1, 5, 7, 10, 17, 32). Dogru et al. (10) found MDR rate as 68.7%; Arslan and Eyi (5) reported that rate as 62%; Temelli et al. (32) reported it as 82.83% in Turkey. Firoozeh et al. (11) showed that 74.1% of *Salmonella* spp. were MDR in Iran. Padungtod and Kaneene (23) found that rate as 32% in Thailand. Lestari et al. (17) reported that MDR was 52.4% of the *Salmonella* isolates. The differences in the rates of resistance to antibiotics made us to think that these strains may be show regional differences and these findings verify that poultry is an important reservoir for MDR *Salmonella* isolates and suggest that successful treatment of *Salmonella* infection becomes more difficult caused by these MDR strains.

According to the resistance rates determined in our study, ampicillin was found to have the highest rate (35.3%) and that was followed by tetracycline (33.3%) and amoxicillin-clavulanic acid (29.2%) (Table 3). This antibiotic resistance ranking was similar to some studies (11, 21, 23, 34) but rates were found to be lower. The reasons behind these relatively lower resistance rates obtained in this study for these antimicrobials used in the treatment of various poultry diseases need to be investigated in further studies. There was a low level of resistance to ceftazidime and also no resistance to ceftriaxone and cefotaxime. Beside this, the highest susceptibility rate was detected against to cephalosporin, streptomycin and gentamicin. These results were also in-parallel to other studies (5, 16, 23, 36).

Beta-lactams and fluoroquinolones are generally used to treat invasive *Salmonella* infections but ESβLs continue to be a major problem worldwide, conferring resistance to the extended spectrum cephalosporins (33). Also ESβL producing *Salmonella* isolates resistant to several antibiotic agents, especially 3rd generation cephalosporins were reported. Although reports of ESβLs associated with *Salmonella* compared to those for other species in the *Enterobacteriacea* was relatively rare, there were some studies that investigate the ESβLs of *Salmonella* spp. in human and animal isolates (13, 23, 24, 25, 26, 27, 35). In this study, only one *S.* Corvallis strain (1.0%) was detected as an ESβL active while Arslan and Eyi (5) found that result as negative. In Turkey, more studies should be done about ESβL producing *Salmonella* isolates.

In conclusion, detection of the high resistance rates and MDR to commonly used antimicrobial agents for the chicken *Salmonella* isolates analyzed in this study suggested that resistance rates need to be monitored regularly for planning successful and proper treatment strategies.

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