

Antibiotic Resistance of *Salmonella* spp. Isolated from Raw Chicken Wings

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Abstract

The use of antibiotics in food animals creates an important source of antimicrobial resistant bacteria that can spread to humans through the food chain. Strains of *Salmonella* spp. with resistance to antimicrobial drugs are now widespread in all countries. The present study analysed the prevalence and antimicrobial resistance of *Salmonella* spp. isolates in raw chicken wings. Out of 200 fresh raw chicken wing packages 102 (51%) samples were positive. Antibiotic resistance test was performed on 200 isolates out of 336 after being confirmed. All the isolates showed multiple resistance against the antibiotics investigated with the average 0.371 multiple antibiotic resistance (MAR) index. None of the isolates were resistant to amoxicillin/clavulanate and cefoxitin. Only 2 isolates showed intermediate resistance to imipenem. The major resistance was observed against nalidixic acid (95%), trimethoprim/sulfamethoxazole (92%), tetracycline (92%), streptomycin (90%) and trimethoprim (81%). Even though only 4 isolates were resistant against ciprofloxacin, high percentage of intermediate resistance (92%) was detected. Some of the isolates were also resistant to gentamicin (7%), cefoperazone (2%), ampicillin (24%), chloramphenicol (24%), cephalosporin (7%) and cefotaxime (39%). According to our results high prevalence and the increase in antibiotic resistant *Salmonella* spp. is of concern and constitutes a threat to public health.

Keywords: *Salmonella*, Chicken, Antibiotic resistance

Piliç Kanatlarından İzole Edilen *Salmonella* spp.'nin Antibiyotik Direnci

Öz

Gıda amaçlı hayvan yetiştiriciliğinde antibiyotik kullanımı, gıda zinciri yoluyla insana geçiş yapan, antibiyotiklere dirençli bakterilerin en önemli kaynağı konumundadır. Günümüzde antimikrobiyal ilaçlara dirençli *Salmonella* spp. tüm ülkelere yayılmış durumdadır. Bu çalışma ile kanatlı etlerindeki *Salmonella* prevalansı ve izolatların antimikrobiyal direnç durumları araştırılmıştır. İncelenen 200 piliç kanat paketinden 102 tanesi (%51) pozitif olarak değerlendirilmiş ve konfirme edilen 336 izolatın 200'ünün antibiyotik duyarlılık durumları belirlenmiştir. Buna göre tüm izolatların ortalama 0.371 çoklu antibiyotik direnci (MAR) indeksi ile çoklu antibiyotik direncine sahip olduğu görülmüştür. İzolatların hiçbirisi amoxicillin/clavulanate ve cefoxitin'e direnç göstermemiştir. Yalnızca 2 izolatın imipenem'e dirençli olduğu saptanmıştır. Asıl direncin nalidixic acid (%95), trimethoprim/sulfamethoxazole (%92), tetracycline (%92), streptomycin (%90) ve trimethoprim (%81) antibiyotiklerine karşı şekillendiği gözlenmiştir. Her ne kadar sadece 4 izolat ciprofloxacin'e karşı tam dirençli olsa da orta düzeydeki direnç oranının yüksek olduğu (%92) ortaya konmuştur. İzolatlar ayrıca gentamisin (%7), cefoperazone (%2), ampicillin (%24), chloramphenicol (%24), cephalosporin (%7) ve cefotaxime (%39)'e karşı direnç geliştirmiştir. Çalışmamızın bulguları *Salmonella* spp.'nin yüksek prevalansı ve antibiyotik direncindeki artış nedeniyle halk sağlığına yönelik önemli bir tehdit oluşturduğuna dikkati çekmiştir.

Anahtar sözcükler: *Salmonella*, Piliç, Antibiyotik direnci

INTRODUCTION

Salmonellosis is known as a chief foodborne disease in humans, resulting in 16 million cases of typhoid fever, 1.3 billion cases of gastroenteritis and 3 million deaths around the world annually [1]. While numerous potential vehicles of transmission exist, poultry meat is considered

one of major sources of this organism [2]. Intestinal salmonellosis typically resolves in five to seven days and does not require treatment with antibiotics. However, in some cases, especially among patients of old ages and those who are immunocompromised, bacteraemia occurs. When infection spreads beyond the intestinal track appropriate antimicrobial therapy can be very



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important [3]. In the past, recommended regimens for the treatment of *Salmonella* included ampicillin, trimethoprim-sulfomethoxazole or chloramphenicol [4]. Unfortunately, in the early 1990s, multidrug resistant strain of phage type 104 (DT 104) *S. typhimurium* was isolated and found to be resistant to five antimicrobial agents; ampicillin, streptomycin, chloramphenicol, sulfonamide and tetracycline. Because of increased resistance to conventional antibiotics, extended spectrum cephalosporins and fluoroquinolones have become the drugs of choice for the treatment of infections caused by multidrug resistant *Salmonella* serotypes [5]. Antibiotic usage in food animals leads to the development and spread of organisms that are resistant to fluoroquinolones, third- and fourth generation cephalosporins, and vancomycin, among others [6]. Humans are exposed to antimicrobial-resistant bacteria and resistant genes that are present in food chain [7]. As mentioned above, when antimicrobials are indicated for treatment of *Salmonella* infection, clinicians often treat with fluoroquinolones and third generation cephalosporins. However, these same classes of antimicrobial agents are also administered to food animals, which leads to the inevitable development of resistant bacteria [6]. If antimicrobial resistant bacteria or antimicrobial resistant genes become incorporated into human bacterial population through antibacterial resistant bacteria from food animals, the efficacy of human drugs will be threatened [8].

Surveillance of antimicrobial resistance in food animals is essential for providing information on the magnitude and trends in resistance and for monitoring the effect of interventions, especially because the prevalence of resistance varies widely between and within countries and over time [9]. This study aimed to determine the prevalence of *Salmonella* in raw chicken wings and to describe the characteristics of the isolates, which would contribute to the understanding of antimicrobial resistance in poultry meat chain.

MATERIAL and METHODS

Sample Collection

Total of 200 packaged fresh raw chicken wings were aseptically collected from the retail markets in Eastern Anatolia between March-August 2017. Packaged raw chicken wings were transported to the laboratory under cold chain and analysed within 2 h.

Isolation and Identification of *Salmonella* spp.

Standard cultivation method was performed for isolation of *Salmonella* spp. as recommended by ISO 6579 [10]. Around 50 g chicken wings from each package were added into 450 mL of Buffered Pepton Water (Oxoid CM059). After the overnight incubation at 37°C, 0.1 mL aliquots were inoculated into tubes containing 10 mL

Rappaport Vassiliadis Enrichment Broth (RV) (Oxoid CM0669) and incubated at 42°C for 48 h. One loopful of enriched RV broth was streaked onto Modified Brilliant Green agar (Oxoid CM0329) and Xylose Lysine Deoxycholate agar (Oxoid CM0469) and incubated at 37°C for 18-24 h. Suspected colonies with typical *Salmonella* morphology were initially determined as Gram negative then confirmed biochemically by Lysine Iron agar (Oxoid CM0381), Triple Sugar Iron agar (Oxoid CM0277), Indole (Tryptone Water, Merck 110859), Methyl Red (MR-VP Medium, Oxoid CM0043), Voges-Proskauer (MR-VP Medium, Oxoid CM0043), Citrate utilization (Simmons Citrate Agar, Oxoid CM0155), Oxidase (Microbact Oxidase Strips, Oxoid MB0266) and Urease (Oxoid, Urea Agar Base CM0053, 40% Urea Solution SR0020) tests. After being identified serologically by *Salmonella* Antisera Omnipotent serum (Denka Seiken Co. Ltd., 292537) *Salmonella* spp. isolates were stored at +4°C on Tryptone Soya agar (TSA, Oxoid CM0131) slope for antibiotic resistance tests.

Antimicrobial Resistance Testing

Antimicrobial resistance test of the *Salmonella* spp. isolates was performed by agar disc diffusion method on Mueller Hinton agar according to the Clinical and Laboratory Standards Institute [11] and European Committee on Antimicrobial Susceptibility Testing [12]. Isolates were inoculated onto Mueller Hinton agar after setting their density according to McFarland 0.5. Antibiotic discs of 5.5 mm diameter impregnated with ampicillin (10 µg), cefazolin (30 µg), gentamicin (10 µg), imipenem (10 µg), trimethoprim/sulfomethoxazole (25 µg), chloramphenicol (30 µg), tetracycline (30 µg), trimethoprim (5 µg), amoxicillin-clavulanate (30 µg), cefoperazone (75 µg), cefotaxime (30 µg), ceftiofuran (30 µg), nalidixic acid (30 µg), streptomycin (10 µg), ciprofloxacin (5 µg) were placed to be four antibiotic discs for each plate. After the 24 h incubation at 35°C, the diameter of the inhibition zones was measured and graded as susceptible (S), intermediate resistant (IR) and resistant (R) according to the reference zone of inhibition of particular antibiotic.

Moreover, the multiple antibiotic resistance (MAR) index was calculated for all *Salmonella* isolates according to the protocol designated by Krumperman [13], using the formula a/b where "a" is the number of antimicrobials to which an isolate was resistant and "b" is the total number of antimicrobials to which the isolate was exposed.

RESULTS

A total of 200 fresh raw chicken wing packages were analysed and *Salmonella* spp. were isolated from 102 (51%) samples by conventional culture technique. Antibiotic resistance test was performed on 200 isolates out of 336 after being confirmed.

Among the 15 antibiotics investigated, all the isolates

Table 1. Antimicrobial resistance percentages *Salmonella* spp. isolated from raw chicken wings

Antibiotics	Concentration (µg/disc)	Susceptible (%)	Intermediate Resistant (%)	Resistant (%)
Amoxicillin/Clavulanate (AMC)	30 µg	100	-	-
Cefoxitin (FOX)	30 µg	100	-	-
Imipenem (IPM)	10 µg	99	1	-
Gentamicin (CN)	10 µg	92	1	7
Cefoperazone (CFP)	75 µg	81	17	2
Ampicillin (AMP)	10 µg	75	1	24
Chloramphenicol (C)	30 µg	76	-	24
Cefazolin (KZ)	30 µg	70	23	7
Cefotaxime (CTX)	30 µg	46	15	39
Ciprofloxacin (CIP)	5 µg	6	92	2
Streptomycin (S)	10 µg	5	5	90
Trimethoprim/Sulfomethoxazole (SXT)	25 µg	7	1	92
Tetracycline (TE)	30 µg	6	2	92
Nalidixic acid (NA)	30 µg	2	3	95
Trimethoprim (W)	5 µg	17	2	81

were susceptible to cefoxitin and amoxicillin/clavulanate. Only 2 isolates showed intermediate resistance to imipenem while rest of them were susceptible. The major resistance was observed against nalidixic acid (95%), trimethoprim/sulfomethoxazole (92%), tetracycline (92%), streptomycin (90%) and trimethoprim (81%). Even though only 4 isolates were resistant against ciprofloxacin, high percentage of intermediate resistance (92%) was detected. Data have been presented in *Table 1*. All of the *Salmonella* isolates exhibited multiple antibiotic resistance with average 0.371 MAR index (*Table 2*).

DISCUSSION

The prevalence of *Salmonella* spp. and their antimicrobial resistance profiles varies widely between and within countries and over time. In this study, 51% of the tested samples were positive for in question microorganisms which is considered very high. This finding could indicate a potential breakdown of hygiene at various stages of the food processing and distribution chain of chicken meat. Most importantly, all of the isolates exhibited multi antibiotic resistance ranging from 3 to 9 with average 0.371 MAR index. Other researchers have also reported high percentages of resistance or multi resistance [1,14-20].

The World Health Organization has developed and applied criteria to rank antimicrobials according to their relative importance in human medicine. This ranking allows stakeholders to focus risk management efforts on drugs used in food animals those are the most important to human medicine and need to be addressed most urgently, such as fluoroquinolones, macrolides and third- and fourth generation cephalosporins [21]. Considering the ranking, our study revealed crucial findings; most

of the isolates showed a significant resistance against streptomycin (90%), nalidixic acid (95%) which are “Critically Important” and tetracycline (92%), trimethoprim sulphamethoxazole (92%), trimethoprim (81%) which are “Highly Important”. Under “Critically Important” group of antibiotics, the resistance to cefotaxime (39% Resistance, 15% Intermediate Resistance) and ampicillin (24%) was also worrisome. Improved resistance against nalidixic acid and ciprofloxacin (2% Resistance, 92% Intermediate Resistance, “Critically Important”) which are belong to quinolones and cefotaxime which is belong to 3rd generation cephalo-sporins is particularly important taking into consideration those antibiotics are being used for the treatment of Salmonellosis in humans. It is also known that nalidixic acid resistance plays a role in the initial steps of the development of ciprofloxacin resistance. Our isolates also exhibited decreased susceptibility against ampicillin (24%, “Critically Important”) and chloram-phenicol (24%, “Important”) which have been the drugs of choice for the cure of human Salmonellosis for decades.

High incidence of resistance against streptomycin, tetracycline, trimethoprim, trimethoprim sulfomethoxazole, nalidixic acid have also been declared from Spain [14], Vietnam [22], Brazil [15], Pakistan [16], Turkey [17], China [18], Albania [19], Ireland [23], Jordan [24].

Antimicrobial resistance poses a threat to human health and the food animal reservoir is an important source. Ensuring the future effectiveness of antimicrobials in therapy for human disease and protecting public health requires immediate development of implementation of risk-management strategies by government authorities for the use of fluoroquinolones, 3rd and 4th generation cephalosporins and macrolides in food producing animals.

Table 2. Antibiotic resistance profiles of *Salmonella* spp. isolates

No	Antibiotic Resistance Profile	MAR Index	No	Antibiotic Resistance Profile	MAR Index	No	Antibiotic Resistance Profile	MAR Index	No	Antibiotic Resistance Profile	MAR Index
1	SXT, TE, NA	0.200	51	SXT, TE, W, NA, S	0.333	101	SXT, TE, CTX, NA, S	0.333	151	SXT, TE, W, NA, S	0.333
2	SXT, TE, NA, S	0.266	52	SXT, TE, W, CTX, NA, S	0.400	102	SXT, TE, NA, S	0.266	152	SXT, TE, W, CTX, NA, S	0.400
3	SXT, TE, CTX, NA, S	0.333	53	SXT, TE, W, NA, S	0.333	103	SXT, TE, NA	0.200	153	SXT, TE, W, NA, S	0.333
4	AMP, SXT, C, TE, W, NA, S, CIP	0.533	54	SXT, TE, NA, S	0.266	104	AMP, SXT, C, TE, W, NA, S, CIP	0.533	154	SXT, TE, NA, S	0.266
5	AMP, KZ, C, CTX, NA, S	0.400	55	SXT, TE, W, CTX, NA	0.333	105	AMP, KZ, SXT, C, TE, W, CTX, NA, S	0.600	155	SXT, TE, W, CTX, NA	0.333
6	AMP, KZ, C, CTX, NA, S	0.400	56	TE, CTX, NA, S	0.266	106	AMP, KZ, C, CTX, NA, S	0.400	156	SXT, TE, W, CTX, NA, S	0.400
7	AMP, KZ, SXT, C, TE, W, CTX, NA, S	0.600	57	SXT, TE, W, NA, S	0.333	107	AMP, KZ, C, CTX, NA, S	0.400	157	SXT, TE, W, NA, S	0.333
8	SXT, C, TE, W, NA, S	0.400	58	SXT, C, TE, W, NA, S	0.400	108	SXT, C, TE, W, NA, S	0.400	158	SXT, C, TE, W, NA, S	0.400
9	SXT, C, TE, W, CTX, NA, S	0.466	59	SXT, C, TE, W, CTX, NA, S	0.466	109	SXT, C, TE, W, CTX, NA, S	0.466	159	SXT, C, TE, W, CTX, NA, S	0.466
10	SXT, C, TE, W, NA, S	0.400	60	SXT, C, TE, W, NA, S	0.400	110	SXT, TE, W, NA, S	0.333	160	TE, CTX, NA, S	0.266
11	SXT, TE, W, NA, S	0.333	61	SXT, TE, NA, S	0.266	111	SXT, C, TE, W, NA, S	0.400	161	SXT, TE, NA, S	0.266
12	SXT, TE, W, CTX, NA, S	0.400	62	SXT, TE, CTX, NA, S	0.333	112	SXT, TE, W, CTX, NA, S	0.400	162	SXT, TE, CTX, NA, S	0.333
13	SXT, TE, W, NA, S	0.333	63	TE, CTX, NA, S	0.266	113	SXT, TE, W, CTX, NA, S	0.400	163	SXT, C, TE, W, NA, S	0.400
14	SXT, TE, W, NA	0.266	64	SXT, TE, NA, S	0.266	114	SXT, TE, W, NA	0.266	164	SXT, TE, NA, S	0.266
15	SXT, TE, W, CTX, NA, S	0.400	65	TE, W, CTX, NA, S	0.333	115	SXT, TE, W, NA, S	0.333	165	TE, W, CTX, NA, S	0.333
16	SXT, TE, W, NA, S	0.333	66	TE, NA, S	0.200	116	SXT, TE, W, NA, S	0.333	166	TE, NA, S	0.200
17	AMP, SXT, TE, W, NA, S	0.400	67	TE, NA, S	0.200	117	AMP, SXT, TE, W, NA, S	0.400	167	TE, NA, S	0.200
18	AMP, KZ, SXT, TE, W, CTX, NA, S	0.533	68	SXT, TE, W, NA, S	0.333	118	AMP, KZ, SXT, TE, W, CTX, NA, S	0.533	168	SXT, TE, W, NA, S	0.333
19	AMP, SXT, TE, W, NA, S	0.400	69	SXT, TE, W, NA, S	0.333	119	AMP, SXT, TE, W, NA, S	0.400	169	SXT, TE, W, CTX, NA, S	0.400
20	AMP, KZ, SXT, TE, W, NA, S	0.466	70	SXT, TE, W, NA, S	0.333	120	AMP, KZ, SXT, TE, W, NA, S	0.466	170	SXT, TE, W, NA, S	0.333
21	SXT, TE, W, NA, S	0.333	71	SXT, TE, NA	0.200	121	SXT, TE, W, NA, S	0.333	171	SXT, TE, NA	0.200
22	SXT, TE, W, CTX, NA, S	0.400	72	SXT, TE, W, CTX, NA, S	0.400	122	SXT, TE, W, NA, S	0.333	172	SXT, TE, W, NA, S	0.333
23	SXT, TE, W, NA, S	0.333	73	SXT, TE, W, NA, S	0.333	123	SXT, TE, W, CTX, NA, S	0.400	173	AMP, SXT, C, TE, W, CTX, NA, S	0.533
24	AMP, CN, SXT, W, CFP, CTX, S	0.466	74	SXT, TE, W, NA, S	0.333	124	AMP, CN, SXT, W, CFP, CTX, S	0.466	174	SXT, TE, W, NA, S	0.333
25	SXT, TE, W, CTX, NA, S	0.400	75	AMP, SXT, C, TE, W, CTX, NA, S	0.533	125	AMP, CN, SXT, W	0.266	175	SXT, TE, W, NA, S	0.333
26	AMP, CN, SXT, W	0.266	76	SXT, TE, W, CTX, NA, S	0.400	126	AMP, CN, SXT, W	0.266	176	SXT, TE, W, CTX, NA, S	0.400
27	AMP, CN, SXT, W	0.266	77	AMP, SXT, TE, W, NA, S	0.400	127	SXT, TE, W, CTX, NA, S	0.400	177	AMP, SXT, TE, W, NA, S	0.400
28	AMP, CN, SXT, W, CTX	0.333	78	AMP, SXT, TE, W, CTX, NA, S	0.466	128	AMP, CN, SXT, W, CTX	0.333	178	AMP, SXT, TE, W, CTX, NA, S	0.466
29	AMP, CN, SXT, W	0.266	79	AMP, KZ, SXT, TE, W, CTX, NA, S	0.533	129	AMP, CN, SXT, W	0.266	179	AMP, KZ, SXT, TE, W, CTX, NA, S	0.533
30	SXT, TE, W, NA, S	0.333	80	SXT, TE, NA, S	0.266	130	SXT, TE, W, NA	0.266	180	SXT, TE, NA, S	0.266
31	SXT, C, TE, W, CTX, NA, S	0.466	81	SXT, TE, CTX, NA, S	0.333	131	SXT, C, TE, W, CTX, NA, S	0.466	181	SXT, TE, CTX, NA, S	0.333
32	SXT, TE, W, NA, S	0.333	82	CN, SXT, C, TE, W, NA, S	0.466	132	SXT, TE, W, NA, S	0.333	182	SXT, TE, W, CTX, NA, S	0.400
33	SXT, TE, W, NA	0.266	83	CN, SXT, C, TE, W, NA, S	0.466	133	AMP, KZ, SXT, TE, W, CTX, NA, S	0.533	183	CN, SXT, C, TE, W, NA, S	0.466
34	SXT, TE, W, NA, S	0.333	84	SXT, TE, W, NA, S	0.333	134	SXT, C, TE, W, CTX, NA, S	0.466	184	SXT, TE, W, NA, S	0.333
35	SXT, TE, W, NA, S	0.333	85	SXT, TE, W, CTX, NA, S	0.400	135	SXT, TE, W, NA, S	0.333	185	CN, SXT, C, TE, W, NA, S	0.466
36	SXT, C, TE, W, NA, S	0.400	86	SXT, TE, W, CTX, NA, S	0.400	136	SXT, C, TE, W, NA, S	0.400	186	SXT, TE, W, CTX, NA, S	0.400
37	SXT, C, TE, W, CTX, NA, S	0.466	87	SXT, TE, W, NA, S	0.333	137	SXT, TE, W, NA, S	0.333	187	SXT, TE, W, NA, S	0.333
38	SXT, C, TE, W, NA, S	0.400	88	SXT, C, TE, W, CTX, NA, S	0.466	138	SXT, C, TE, W, NA, S	0.400	188	SXT, TE, W, NA	0.266
39	SXT, TE, W, NA, S	0.333	89	SXT, TE, W, CTX, NA, S	0.400	139	SXT, TE, W, NA, S	0.333	189	SXT, TE, W, CTX, NA, S	0.400
40	SXT, TE, W, CTX, NA, S	0.400	90	STX, TE, NA, S	0.266	140	AMP, SXT, TE, W, NA, S	0.400	190	SXT, TE, W, NA, S	0.333
41	SXT, TE, W, NA, S	0.333	91	SXT, TE, W, CTX, NA, S	0.400	141	SXT, TE, W, NA, S	0.333	191	SXT, TE, W, CTX, NA, S	0.400
42	AMP, SXT, TE, W, NA, S	0.400	92	SXT, TE, W, NA	0.266	142	AMP, SXT, TE, W, NA, S	0.400	192	SXT, C, TE, W, CTX, NA, S	0.466
43	AMP, KZ, SXT, TE, W, CTX, NA, S	0.533	93	SXT, TE, NA, S	0.266	143	SXT, TE, W, NA, S	0.333	193	SXT, TE, NA, S	0.266
44	AMP, SXT, TE, W, NA, S	0.400	94	SXT, TE, CTX, NA, S	0.333	144	SXT, TE, W, CTX, NA, S	0.400	194	AMP, SXT, C, TE, W, NA, S, CIP	0.533
45	SXT, C, TE, W, NA, S	0.400	95	SXT, TE, CTX, NA, S	0.333	145	SXT, C, TE, W, NA, S	0.400	195	SXT, TE, CTX, NA, S	0.333
46	SXT, C, TE, W, CTX, NA, S	0.466	96	AMP, SXT, C, TE, W, NA, S, CIP	0.533	146	SXT, C, TE, W, CTX, NA, S	0.466	196	SXT, TE, CTX, NA, S	0.333
47	SXT, C, TE, W, NA, S	0.400	97	AMP, SXT, C, TE, W, NA, S	0.466	147	SXT, C, TE, W, NA, S	0.400	197	AMP, SXT, C, TE, W, NA, S	0.466
48	SXT, TE, W, NA, S	0.333	98	AMP, SXT, C, TE, W, CFP, CTX, NA, S	0.600	148	SXT, TE, W, NA, S	0.333	198	AMP, SXT, C, TE, W, CFP, CTX, NA, S	0.600
49	SXT, TE, W, CTX, NA, S	0.400	99	SXT, TE, W, NA, S	0.333	149	TE, CTX, NA, S	0.266	199	STX, TE, NA, S	0.266
50	SXT, TE, W, NA, S	0.333	100	AMP, SXT, C, TE, W, NA, S	0.466	150	SXT, TE, W, NA, S	0.333	200	AMP, SXT, C, TE, W, NA, S	0.466

Average: 0.371

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