Acta Veterinaria-Beograd 2025, 75 (3), 309-325
UDK: 636.5.09:[616.98:579.842.11
615.33.015.8
DOI:10.2478/acve-2025-0024

Research article

HIGH LEVEL FLUOROQUINOLONE RESISTANCE AND MULTIDRUG RESISTANCE IN *SALMONELLA* SPP. ISOLATED FROM POULTRY, TURKEY FLOKS AND SLAUGHTERHOUSES IN ALGERIA

Amina DJEMAIOUNE[1*], Dalibor TODOROVIĆ[2], Katarina NOVOVIĆ[3], Svetlana MIKOVIĆ DRAMLIĆ[4], Zineb CHERAK[5], Esma BENDJAMA[1], Oussama KHALOUTA[6], Bakir MAMACHE[1], Branko JOVČIĆ[7], Maja VELHNER[2], Ammar AYACHI[5]

[1]LESPA, Department of Veterinary Sciences, Institute of Veterinary and Agronomic Sciences, University of Batna 01, Algeria; [2]Scientific Veterinary Institute "Novi Sad", Novi Sad, Serbia; [3]University of Belgrade, Institute for Molecular Genetics and Genetic Engineering, Belgrade, Serbia; [4]Institute of Public Health of Serbia, Belgrade, Serbia; [5]Batna University, Laboratory of Biology of Bioactive Molecules and Cellular Physiopathology, Batna, Algeria; [6]University of Souk Ahras, STV Laboratory, Souk Ahras, Algeria; [7]University of Belgrade, Faculty of Biology, Belgrade, Serbia.

(Received 07 May, Accepted 01 September 2025)

In this work, *Salmonella* spp. was detected in poultry and turkey farms, slaughterhouses and hatcheries in the Sétif Province in Algeria. Eighty single isolates per farm were analyzed by establishing the resistotype and detected resistance genes underlining the mechanism of resistance. In one case, serotypes *S*. Virchow and *S*. Ivory were found in the same sample and both isolates were resistant to nalidixic acid. *S*. Enteritidis was detected in four broiler breeder flocks, three hatcheries, 12 flocks of layers, 12 broiler flocks while five slaughterhouses yielded 10 isolates. The wide distribution of *S*. Enteritidis in the primary production and food chain in Algeria requires special measures in the management practice on poultry farms. All isolates except five were resistant to nalidixic acid and pefloxacin which means that these salmonellae phenotypically express reduced sensitivity to ciprofloxacin. Five isolates were multidrug resistant. Two *Salmonella* Galinarum biotype gallinarum isolates from flocks of laying hens were resistant to quinolones, aminoglycosides and sulfonamides. One of these isolates was also resistant to trimethoprim alone and in combination with sulafmethoxazole. One *S*. Enteritidis isolate was resistant to ampicillin, nalidixic acid, pefloxacin and colistin. Especially worrying is the high level of resistance to ciprofloxacin in nine isolates (six, *Salmonella* Galinarum biotype gallinarum, two, *S*. Kentucky and one *Salmonella enterica* subsp. *enterica* isolate) due to mutations in the enzymes DNA gyrase and topoisomerase IV. Resistance genes were identified in 21 isolates. All resistance genes detected are commonly conferring resistance to ampicillin, streptomycin, gentamicin, tetracycline, sulfonamides and trimethoprim antibiotics.

Keywords: poultry, *Salmonella*, resistotype, resistance genes, mutations.

*Corresponding author: e-mail: amina.djemaioune@univ-batna.dz

INTRODUCTION

Poultry meat is a significant source of protein-rich food in Africa [1]. Accordingly, the implementation of food safety management systems is required not only in food factories and other manufacturing units, but also at the farm level [2,3]. The rise of antimicrobial resistance in bacteria, especially in low-income countries, is a major concern. Therefore, it is necessary to implement harmonized surveillance in order to track antimicrobial resistance as efficiently as possible and to improve laboratory capacity in developing countries including serological typing and resistotyping of *Salmonella* [4]. One of the most significant Campaigns is the Global Salm-Surv 2000-2007, organized by the World Health Organization (WHO). The enhanced, laboratory-based survey involved the following African countries: Botswana, Cameroon, the Central African Republic, Democratic Republic of the Congo, Côte d'Ivoire, Mauritania, Madagascar, Mauritius, Morocco, Senegal, South Africa, Sudan, Tunisia, and Uganda [4]. Of crucial importance is the research as well, since it is helping to better understand the global epidemiology of *Salmonella* infections worldwide [5].

Poultry farming is highly developed in northern Algeria and other African countries, but it faces numerous challenges, such as infection of poultry with the host-specific *Salmonella Galinarum* biotype gallinarum and *Salmonella Galinarum* biotype pullorum [6]. In addition, other non-typhoid *Salmonella* serovars are widespread in poultry flocks and pose significant public health concerns [7-11]. Many problems in poultry farming occur due to difficulties in establishing and maintaining good management, including the use of antibiotics. To address these problems and improve the poultry industry in Algeria, it is important to continuously conduct research and carefully analyze and evaluate the obtained results to improve poultry production and avoid unnecessary antibiotic therapy.

One of the major obstacles in the livestock and poultry industries in developing countries is the use of antibiotics originally intended for human medicine. In response, the WHO has drawn up a list of antibiotics according to their importance and provided recommendations for their cautious use or avoidance, in both human and veterinary medicine. It is important to carefully consider these recommendations in order to mitigate the challenges faced by the industry. The following classes of antibiotics have the highest priority: 3rd, 4th and 5th cephalosporin generation, glycopeptides, macrolides and ketolides, polymixins and quinolones [12]. Since bacteria have been around for millions of years, they have adapted over the course of evolution to neutralize numerous substances with potentially harmful effects. Additionally, they can acquire resistance to literally all antibiotics developed by pharmaceutical companies, including new generations of antimicrobials, within a very short time [13]. Some of these bacteria cause nosocomial infections, and many of them are well – established in the farm environments. They are difficult to deal with not only because of their resistance to antibiotics, but also because of their virulence and ability to adapt to various hosts and environments. This refers to *Salmonella* species as well, since almost

all of them have a broad host range and can adapt to antibiotic treatment in the animal and human intestines. This biological phenomenon occurs due to certain mutations, horizontal gene transfer, and other genetic mechanisms that allow the development of new *Salmonella* pathovars, or cause persistent infections [14,15]. Under these circumstances, the treatment of human patients and animals is at risk.

For all these reasons, research was conducted to identify *Salmonella* serotypes in Algerian poultry farms, to establish antimicrobial resistance phenotypes, and to detect their resistance genes. In the long run, such studies will help to introduce a more prudent use of antibiotics, and to increase awareness of safe farming practices and the One Health approach in Africa.

MATERIAL AND MATHODS

Sampling strategy and *Salmonella* detection

This study includes a total of 145 broilers farms, 107 laying hen farms, 32 broiler breeder farms, 48 turkey farms, five slaughterhouses, and three hatcheries. The choice of these establishments was motivated by the size of the poultry industry and the frequent occurrence of infectious gastrointestinal pathologies as reported by local veterinary practitioners. In total, 332 samples were collected from chicken farms including feces, liver, heart, and oviduct for the isolation and identification of *Salmonella* spp. From slaughterhouses, 60 samples of neck skin and 40 samples of fluff feathers from hatcheries were collected. Sampling was done in Sétif Province, Algeria. This region covers over 6500 km^2. Sampling took place from September 2020 to June 2022.

Salmonella was isolated from 79 establishments. Due to the constraints set by farm owners, only one isolate per farm was collected. Therefore all *Salmonella* numbers are also indicating the number of the farm or hatchery. In one farm of broiler chickens (isolates number 14a and 14b) *S*. Virchow and *S*. Ivory were identified yielding a total of 80 *Salmonella* for the research. Out of 80 *Salmonella* isolates, 61 were from poultry farms, and four isolates were from turkey flocks. Twelve *Salmonella* spp. were detected from slaughterhouses, and three *Salmonella* spp. were isolated from hatcheries.

Isolation and identification of *Salmonella* spp.

The samples were analyzed for the presence of *Salmonella* using ISO 6579-1:2017 conventional culture based method (Microbiology of the food chain — Horizontal method for the detection, enumeration, and serotyping of *Salmonella* — Part 1: Detection of *Salmonella spp.*, 2017).

The presumptive *Salmonella* colonies were subjected to biochemical tests such as triple sugar iron and urea-indole test. All of these *Salmonella* spp. were identified with MALDI-TOF. To differentiate between *Salmonella Galinarum* biotype pullorum and

Salmonella Galinarum biotype gallinarum serotypes, miniaturized biochemical tests based on Ornithine Decarboxylase (ODC) and Rhamnose (RHA), Api 20 E strips (Biomerieux, France) were used for these tests [16].

Isolates were stored in deep agar. Serological typing of *S*. Enteritidis, *Salmonella Galinarum* biotype gallinarum, *S*. Infantis and *S*. Typhimurium was done using sera from Staten serum Institute Denmark in the Scientific Veterinary Institute "Novi Sad" in Novi Sad, Serbia. Other serotypes were determined in the Institute of Public Health of Serbia "Dr Milan Jovanović – Batut", Belgrade, Serbia, National Reference Laboratory for *Salmonella*, *Shigella*, *Vibrio cholerae*, and *Yersinia enterocolitica*.

Antimicrobial susceptibility testing

Kirby-Bauer disk diffusion test was performed using Mueller Hinton agar (Biokar diagnostics, France) with the following antibiotic disks: ampicillin 10 μg (AMP), amoxicillin/clavulanic acid 20 μg + 10 μg (AMC), chloramphenicol 30 μg (CHL), ciprofloxacin 5 μg (CIP), gentamicin 10 μg (GEN), nalidixic acid 30 μg (NAL), streptomycin 10 μg (STR), sulfonamides 300 μg (SA), tetracycline 30 μg (TET), trimethoprim 5 μg (TMP), trimethoprim/sulfamethoxazole 1.25 / 23.75 μg (SXT), cefpodoxime 10 μg (CPD), cefotaxime 30 μg (CTX), ceftazidime 30 μg (CAZ), cefoxitin 30 μg (FOX), and pefloxacin 5 μg (PEF). The disks were from BioRad (Marnes-la-Coquette, France). For quality control, *Escherichia coli* ATCC 25922 were used. Results were interpreted according to CLSI M100, 2022 and EUCAST 2022 recommendations. Isolates were assigned as multidrug resistant if resistance was found to more than three antibiotics of different classes [17]. All *Salmonella* spp. isolates with the zone diameter breakpoint of $\leq$ 20 mm for ciprofloxacin [18], were culture on Mueller Hinton agar supplemented with 1 mg/L ciprofloxacin (Sigma Aldrich, St Louis, MO, USA) in order to confirm resistance phenotype. Growing colonies were used for further experiments. Also, MacConkey plates for agar diffusion test supplemented with 2mg/L of colistin sulfate salt (Sigma Aldrich, St Louis, MO, USA) were prepared to culture isolates to detect resistance to colistin.

Polymerase chain reaction (PCR) and sequencing

The master mix kit One Taq Hot Start 2x Master Mix M0484, (New England BioLabs, Frankfurt am Main, Germany) was used for resistance gene detection by PCR. The primers used in the study are listed in Table 1. DreamTaq DNA Polymerase (Thermo Fisher Scientific, the Netherlands) was used for amplification of the *gyrA* and *parC* genes for sequencing. The obtained amplicons were purified using the commercial kit GeneJET PCR purification kit (Thermo Fisher Scientific, the Netherlands).

Table 1. Primers used in the study (annealing temperature, size of the PCR product and references included)

Target genes	Primer sequences	Annealing °C	Fragment sizes (bp)	References
Quinolone resistance genes				
gyrA	fw:tgt cc gaga tgg cct gaa gc rw:cgt taa tca ctt ccg tca g	55	432	[19]
gyrB	fw:gaa atg acc cgt cgt aaa gg rw:tac agt ctg ctc atc aga aag	58	671	[19]
parC	fw:atg agc gat atg gca gag cg rw:tga ccg agt tcg ctt aac ag	52	374	[19]
parE	fw:gac cga gct gtt cct tgt gg rw:gcg taa ctg cat cgg gtt ca	52	454	[19]
Aminoglycoside resistance genes				
strA	fw: tga ctg gtt gcc tgt cag agg c rv: cca gtt gtc ttc ggc gtt agc a	64	646	[20]
strB	fw: atc gtc aag gga ttg aaa cc rv: gga tcg tag aac ata ttg gc	56	509	[21]
aadA1	fw: cga ctc aac tat cag agg ta rv: ctt ttg tca gca aga tag cc	55	384	[22]
aadA2	fw: cgg tga cca tcg aaa ttt cg rv: cta tag cgc gga gcg tct cgc	55	249	[23]
aac(3)-I	fw:ggg cat cat tcg cac atg tag gc rv:cat cac ttc ttc ccg tat gcc c	64	429	[24]
aac(3)-II	fw:tga aac gct gac gga gcc tc rw: gtc gaa cag gta gca ctg ag	58	369	[24]
aac(3)-III	fw:gtg cat cgc agc gca aac ccc rw: caa gcc act gca ccg caa acc g	64	436	[24]
aac(3)-IV	fw:gtg tgc tgc tgg tcc aca gc rw:agt tga ccc agg gct gtc gc	58	628	[24]
Sulfonamide resistance genes				
sul1	fw: cta ggc atg atc taa ccc tcg gtc t rv: atg gtg acg gtg ttc ggc att ctg	55	840	[25]
sul2	fw: aca gtt tct ccg atg gag gcc g rv: ctc gtg tgt gcg gat gaa gtc a	55	704	[20]
sul3	fw: gag caa gat ttt tgg aat cg rv: cat ctg cag cta acc tag ggc ttt gga	51	789	[26]
Trimethoprim resistance genes				
dfrA1	fw: gat att cca tgg agt gcc a rv: acc ctt ttg cca gat ttg	50	414	[27]
dfrA5/dfrA14	fw: gat tgg ttg cgg tcc a rv: ctc aaa aac aac ttc gaa gg	50	383	[27]
dfrA7/dfrA17	fw: cag aaa atg gcg taa tcg rv: tca cct tca acc tca ac	50	345	[27]
dfrA12	fw: ttt atc tcg ttg ctg cga tg rv: taa acg gag tgg gtg tac gg	60	457	[28]
dfrB1/B2	fw: caa agt agc gat gaa gcc a rv: cag gat aaa ttt gca ctg agc	50	205	[27]
Tetracycline resistance genes				
tet(A)	fw: gct aca tcc tgc ttg cct tc rv: cat aga tcg ccg tga aga gg	55	210	[29]
tet(B)	fw: ttg gtt agg ggc aag ttt tg rv: gta atg ggc caa taa cac cg	55	659	[29]
Beta lactam resistance genes				
bla_{TEM}	fw: gtg cgg tat tat ccc gtg tt rv: aac ttt atc cgc ctc cat cc	58	416	[30]
Colistin resistance				
mcr1	fw: agt ccg ttt gtt ctt gtg gc rv: aga tcc ttg gtc tcg gct tg	58	320	[31]
mcr2	fw: caa gtg tgt tgg tcg cag tt rw: tct agc ccg aca agc ata cc	58	715	[31]

Obtained, purified DNA was then sent to Macrogen in Amsterdam, the Netherlands, for sequencing of the *gyrA* and *parC* genes. The sequences were analyzed to detect point mutations applying the Basic Local Alignment Search Tool-nucleotide program-BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn).

RESULTS

Prevalence of *Salmonella* spp. in Sétif Province (Algeria)

In this study, 80 *Salmonella* isolates were obtained from 340 samples collected on poultry and turkey farms, slaughterhouses and hatcheries in Sétif Province. In general, a single isolate per farm/establishment was included in the study. The majority of isolates originated from broiler farms (n= 29; 36.25%) and laying hen farms (n=25; 31.25%). Fewer isolates were recovered from breeder broiler farms (n=7; 8.75%), turkey farms (n=4; 5%), slaughterhouses (n=12; 15%), and hatcheries (n=3; 3.75%) (Table 2). Only in one broiler flock two *Salmonella* serotypes were found: *S*. Virchow, and *S*. Ivory. Serological typing revealed that of the 80 *Salmonella* isolates, 41(51.25%) belonged to serotype Enteritidis, making it the most prevalent serotype in this study. Twenty isolates (25%) were Gallinarum, and four (5%) each *S*. Infantis, and *S*. Virchow serotypes. Two (2.5%) isolates from broilers and layer hens were *S*. Kentucky and two (2.5%) isolates from broiler breeders and broilers were *S*. Ohio. One turkey flock and a flock of broiler chickens yielded *S*. Typhimurium (2.5%). It was not possible to determine the serotype of four (5%) isolates, while seven isolates did not survive and were not available for the research (Table 2).

Salmonella Enteritidis has been isolated in many locations in Sétif Province: it was found in four broiler breeder flocks (S3, S55 and S75, S87), three hatcheries (S24, S50 and S62), 12 flocks of layers, 12 broiler flocks as well as five slaughterhouses from which a total of ten *S*. Enteritidis were isolated (Table 2).

Table 2. Summary of *Salmonella* isolates, serology types and resistotype, from Setif-Algeria, period 2021-2022

No	Origin	Antigenic formula	Serotype	Resistotype
S1	Broilers	6,7:r:1,5	*S*. Infantis	NAL, COL, PEF
S2	Laying hens	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S3	Broiler breeders	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S4c[4]	Slaughterhouse	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S5	Laying hens	9,12:-:-	*S*. Gall. biotype gallinarum	NAL, PEF
S6	Broilers	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S7d	Slaughterhouse	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S8e	Slaughterhouse	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S9e	Slaughterhouse	9,12:-:-	*S*. Gall. biotype gallinarum	NAL, PEF
S10	Broilers	-:g,m:-	*Salmonella enterica* subsp. *enterica*[1]	NAL, PEF

No	Origin	Antigenic formula	Serotype	Resistotype
S11	Broilers	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S12	Laying hens	9,12:g,m:-	*S*. Enteritidis	AMP, NAL, PEF
S13	Broilers	9,12:-:-	*S*. Gall. biotype gallinarum	NAL, PEF
S14a, S14b	Broilers	6,7:r:1,2+16:r:1,6	*S*. Virchow, S. Ivory	NAL
S16	Laying hens	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S17	Broilers	6,7:b:l,w	*S*. Ohio	-[3]
S18	Broilers	-:g,m:-	*Salmonella enterica* subsp. *enterica*[1]	NAL, STR, PEF
S19f	Slaughterhouse	6,7:r:1,5	*S*. Infantis	NAL, PEF
S20c	Slaughterhouse	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S21	Laying hens	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S22	Broilers	9,12:g,m:-	*S*. Enteritidis	-
S23	Broilers	6,7:b:l,w	*S*. Virchow	NAL, PEF
S24	Hatchery	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S26	Broilers	9,12:-:-	*S*. Gall. biotype gallinarum	NAL, PEF
S27	Laying hens	9,12:-:-	*S*. Gall. biotype gallinarum	AMP, CIP[5], GEN, NAL, STR, SA, PEF
S28	Laying hens	9,12:g,m:-	*S*. Enteritidis	AMP, NAL, PEF
S29	Turkey	-:g,m:-	*Salmonella enterica* subsp. *enterica*[2]	CIP, NAL, TET, PEF
S30	Broilers	6,7:b:l,w	*S*. Virchow	NAL, PEF
S31	Broiler breeders	6,7:b:l,w	*S*. Ohio	NAL, PEF
S32	Broilers	9,12:g,m:-	*S*. Enteritidis	-
S33	Turkey	9,12:-:-	*S*. Gall biotype gallinarum	CIP,NAL, PEF,COL
S34	Broilers	8,20:i:z6	*S*. Kentucky	CIP, NAL, TET, PEF
S36f	Slaughterhouse	9,12:g,m:-	*S*. Enteritidis	AMP, NAL, COL, PEF
S38	Laying hens	8,20:i:z6	*S*. Kentucky	AMP, CIP, NAL, TET, COL, PEF
S39	Broilers	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S40	Broilers	9,12:-:-	*S*. Gall. biotype gallinarum	NAL, PEF
S42	Laying hens	9,12:g,m:-	*S*. Enteritidis	AMP, NAL, PEF
S43	Broilers	-:g,m:-	*Salmonella enterica* subsp. *enterica*[1]	NAL, PEF
S44	Turkey	1,4,[5],12:i:1,2	*S*. Typhimurium	NAL, PEF
S45e	Slaughterhouse	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S46	Laying hens	9,12:-:-	*S*. Gall. biotype gallinarum	NAL, PEF
S47	Laying hens	9,12:-:-	*S*. Gall. biotype gallinarum	NAL, PEF
S48	Laying hens	9,12:g,m:-	*S*. Enteritidis	AMP, NAL, PEF
S49	Laying hens	9,12:g,m:-	*S*. Enteritidis	AMP, NAL, PEF
S50	Hatchery	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S51	Laying hens	9,12:-:-	*S*. Gall. biotype gallinarum	AMP, NAL, SA, TET, TMP, SXT, PEF
S52c	Slaughterhouse	9,12:g,m:-	*S*. Enteritidis	AMP, NAL, PEF
S53	Laying hens	9,12:-:-	*S*. Gall. biotype gallinarum	NAL, PEF
S54g	Slaughterhouse	9,12:g,m:-	*S*. Enteritidis	NAL, PEF

No	Origin	Antigenic formula	Serotype	Resistotype
S55	Broiler breeders	9,12:g,m:-	*S*. Enteritidis	PEF
S56	Turkey	9,12:-:-	*S*. Gall. biotype gallinarum	NAL, , PEF
S57	Laying hens	9,12:-:-	*S*. Gall biotype gallinarum	CIP,NAL, PEF
S58	Laying hens	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S59	Broilers	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S60	Laying hens	9,12:-:-	*S*. Gall. biotype gallinarum	NAL, PEF
S61	Broilers	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S62	Hatchery	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S63	Broilers	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S64	Laying hens	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S65d	Slaughterhouse	9,12:g,m:-	*S*. Enteritidis	AMP, NAL, PEF
S68	Broiler breeders	6,7:r:1,2	*S*. Virchow	NAL
S69	Broiler breeders	9,12:-:-	*S*. Gall. biotype gallinarum	CIP, NAL, PEF, COL
S70	Broilers	9,12:-:-	*S*. Gall. biotype gallinarum	CIP, NAL, PEF
S71	Laying hens	9,12:-:-	*S*. Gall. biotype gallinarum	AMP, CIP, GEN, NAL, STR, SA, PEF
S72	Broilers	6,7:r:1,5	*S*. Infantis	-
S74	Broilers	6,7:r:1,5	*S*. Infantis	-
S75	Broiler breeders	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S76	Laying hens	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S77	Laying hens	9,12:-:-	*S*. Gall. biotype gallinarum	NAL, PEF
S78	Laying hens	9,12:-:-	*S*. Gall. biotype gallinarum	NAL, PEF
S80	Broilers	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S81	Broilers	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S82	Broilers	1,4,[5],12:i:1,2	*S*. Typhimurium	NAL, PEF
S83c	Slaughterhouse	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S84	Broilers	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S85	Broilers	9,12:g,m:-	*S*. Enteritidis	AMP, NAL, PEF
S86	Laying hens	9,12:g,m:-	*S*. Enteritidis	NAL, PEF
S87	Broiler breeders	9,12:g,m:-	*S*. Enteritidis	NAL
S88	Laying hens	9,12:-:-	*S*. Gall. biotype gallinarum	NAL

[1]Slide agglutination with the O antigen was not possible
[2]Slide agglutination was not possible; expression of the second flagellar antigen is missing
[3]Isolate is susceptible to antibiotics
[4]Slaughterhouses are marked with the isolate number and letters c,d,e,f, g, so that each slaughterhouse has its own letter.
[5]High level resistance to CIP, highlighted
Isolates number 15, 25, 37, 41, 66, 67, 79 did not survive in laboratory and could not be included in the research.
Antibiotic abbreviations: **AMP** – ampicillin, **CIP** – ciprofloxacin, **COL** – colistin, **GEN** – gentamicin, **NAL** – nalidixic acid, **PEF** – pefloxacin, **SA** – sulfonamides, **STR** – streptomycin, **TET** – tetracyclines, **TMP** – trimethoprim, **SXT** – trimethoprim/sulfamethoxazole.

Antimicrobial resistance module

All isolates except six were resistant to nalidixic acid and pefloxacin (Table 2). These results imply that *Salmonella* isolates from poultry industry in Sétif Province have decreased susceptibility to CIP according to both CLSI and EUCAST criteria [18-32]. In addition, nine isolates express high-level CIP resistance, which is particularly worrying. Those isolates were *S*. Gall. biotype gallinarum originating from four layer farms, two broiler farms and one broiler breeder flock. One isolate from a broiler flock and one from layer chickens were serologically identified as *S*. Kentucky in this study. Both isolates were highly resistant to ciprofloxacin as well as to tetracycline (Table 2). Therefore, single or double mutations in the target genes, *gyrA* and *parC*, were identified in these ciprofloxacin resistant isolates (Table 3).

Table3. Mutations on gyrase and topoisomerase IV

Isol. No	Serotype	Poultry/turkey	gyrA gene	parC gene
S27	*S*. Gall. biotype gallinarum[2]	Laying hens	D87A	S80R
S29	*Salmonella enterica* subsp. *enterica*[1]	Turkey	S83F, D87N	T57S, S80I
S33	*S*. Gall biotype gallinarum[2]	Turkey	D87A	-
S34	*S*. Kentucky	Broilers	S83F, D87N	T57S, S80I
S38	*S*. Kentucky	Laying hens	S83F, D87N	T57S, S80I
S57	*S*. Gall biotype gallinarum	Laying hens	D87A	-
S69	*S*. Gall biotype gallinarum[2]	Broiler breeders	D87A	-
S70	*S*. Gall biotype gallinarum[2]	Broilers	D87A	-
S71	*S*. Gall biotype gallinarum[2]	Laying hens	D87A	S80R

[1]Slide agglutination was not possible; expression of the second flagellar antigen is missing.
[2] Isolates with the single mutation on *gyrA* gene had zone diameter to CIP of 20-22mm. Amino acids are as follows: **D** (aspartic acid), **A** (alanine), S (serin), **F** (phenylalanine), **N** (asparagine), **R** (arginine), **T** (threonine).

Four isolates (S27, S51, S71 from laying hens, and S36 from a slaughterhouse) were multidrug resistant, and two of them (S27 and S71) were resistant to gentamicin. In both isolates, aminoglycoside N-acetyltransferase gene *aac(3)-II* confers resistance to GEN (Table 4) through enzymatic modification of the drug. Resistance to colistin was detected in *Salmonella* Gall. biotype Gallinarum isolates from broiler breeders and a turkey flock respectively (S69, S33), from *S*. Kentacky of laying hens (S38), and *S*. Enteritidis from a slaughterhouse (S36) (Table 2). None of these isolates carried the plasmid-mediated colistin resistance genes *mcr-1* or *mcr-2*. In the studied strain collection, only isolates (S27, S71, and S51 from the flocks of laying hens) carried *sul3* or *sul1* genes (Table 3). Only the isolate S51 (*Salmonella Galinarum* biotype gallinarum) from a flock of laying hens carried the *dfrA12* gene, and subsequently this isolate was resistant to TMP and SXT. The bla_{TEM} gene conferring resistance to ampicillin was

identified in thirteen isolates (in nine isolates of *Salmonella* Enteritidis, three *Salmonella Galinarum* biotype gallinarum isolates, and one *S*. Kentucky isolate), while the *tet*(A) gene was found in isolate S29 with no serotype determined (derived from turkeys); *S*. Kentucky, S34 (derived from broilers), and *Salmonella Galinarum* biotype gallinarum, S51 (derived from laying hens), (Table 4).

Table 4. Resistance gene detection of *Salmonella* spp. isolates from poultry in the Setif district Algeria

Isolate no*	Poultry establishment	*Salmonella* serotype	Resistance phenotype	Resistance gene detection
S12	Laying hens	*S*. Enteritidis	AMP, NAL, PEF	*bla*$_{TEM}$
S18	Broilers	*Salmonella enterica* subsp. *enterica*[1]	NAL, STR, PEF	*strA*, *strB*
S27	Laying hens	*S*. Gall. biotype gallinarum	AMP, CIP, GEN, NAL, STR, SA, PEF	*bla*$_{TEM}$, *aac(3)-II*, *strA*, *strB*, *sul3*
S28	Laying hens	*S*. Enteritidis	AMP, NAL, PEF	*bla*$_{TEM}$
S29	Turkey	*Salmonella enterica* subsp. *enterica*[2]	CIP, NAL, TET, PEF	*tetA*
S33	Turkey	*S*. Gall. biotype gallinarum	CIP, NAL, PEF, COL	-
S34	Broilers	*S*. Kentucky	CIP, NAL, TET, PEF	*tetA*
S36	Slaughterhouse	*S*. Enteritidis	AMP, NAL, COL, PEF	*bla*$_{TEM}$
S38	Laying hens	*S*. Kentucky	AMP, CIP, NAL, TET, COL, PEF	*bla*$_{TEM}$
S42	Laying hens	*S*. Enteritidis	AMP, NAL, PEF	*bla*$_{TEM}$
S48	Laying hens	*S*. Enteritidis	AMP, NAL, PEF	*bla*$_{TEM}$
S49	Laying hens	*S*. Enteritidis	AMP, NAL, PEF	*bla*$_{TEM}$
S51	Laying hens	*S*. Gall. biotype gallinarum	AMP, NAL, SA, TET, TMP, SXT, PEF	*bla*$_{TEM}$, *sul1*, *tetA*, *dfrA12*
S52	Slaughterhouse	*S*. Enteritidis	AMP, NAL, PEF	*bla*$_{TEM}$
S57	Laying hens	S. Gall. biotype gallinarum	CIP, NAL, PEF	-
S58	Laying hens	*S*. Enteritidis	NAL, PEF	-
S65	Slaughterhouse	*S*. Enteritidis	AMP, NAL, PEF	*bla*$_{TEM}$
S69	Broiler breeders	*S*. Gall. biotype gallinarum	CIP, NAL, PEF, COL	-
S70	Broilers	*S*. Gall. biotype gallinarum	CIP, NAL, PEF	-
S71	Laying hens	*S*. Gall. biotype gallinarum	AMP, CIP, GEN, NAL, STR, SA, PEF	*bla*$_{TEM}$, *aac(3)-II*, *strA*, *strB*, *sul3*
S85	Broilers	*S*. Enteritidis	AMP, NAL, PEF	*bla*$_{TEM}$

***** Since single isolates per farm were included in the study, isolate number is also farm number. In this collection of isolates, five were susceptible to antibiotics while 75 isolates were resistant to nalidixic acid and to pefloxacin (only one isolate was resistant only to PEF) as summarized in Table 2. High resistance to CIP was detected in nineout of 80 isolates. [1]Slide agglutination with the O antigen was not possible; [2]Slide agglutination was not possible, and expression of the second flagellar antigen is missing.

DISCUSSION

In this research the presence of *Salmonella* Enteritidis in various poultry establishments in Sétif Province was confirmed. Due to its virulence and its ability to invade internal organs and spread rapidly in the environment, this serotype is a major public health concern. Moreover, it is of importance to the poultry industry due to its vertical transmission and endless contamination of poultry farms, which are difficult to clean and control the infection [3]. In addition, it has recently been proven that *Salmonella* can be imported via day-old chicks or poultry and distributed intercontinentally [33]. This discovery resulted from the analysis of 30,015 *Salmonella* genomes originating from 98 countries, deposited to the EnteroBase since 2020, which were analyzed by multilocus sequence typing (MLST). Subsequently, phylogenetic analysis based on single nucleotide polymorphism (SNP), and phylodynamic analysis was done to determine the maximum likelihood of *S*. Enteritidis distribution in the given time span [33].

The presence of *Salmonella Galinarum* biotype gallinarum in Algerian poultry flocks is also of great importance since these strains are adapted to the host and cause fowl typhoid outbreaks in poultry flocks. The adaptation to the host is attributed to the *Salmonella* Pathogenicity Island SPI-19, which encodes the type VI secretion system (T6SS), an important virulence factor involved in the colonization of the poultry gut [34,35]. As *Salmonella Galinarum* biotype gallinarumis is a direct descendant of *S*. Enteritidis and both are pathogens that survive in the environment for long periods of time, they are significant for the poultry industry worldwide.

This resistance to fluoroquinolones occurs due to the point mutations in the quinolone resistance region-QRDR of the genes *gyrA* and *gyrB*, which encode gyrase, and the *parC* and *parE* genes encoding topoisomerase IV [36,37]. These enzymes are essential for bacterial replication, and are considered the primary mechanism of quinolone resistance, except in *S*. Typhimurium DT104, where it is the efflux pump [38]. After first-step mutations, gyrase mutants are less susceptible to quinolones and this biological process can lead to the emergence of secondary mutants. Multiple mutations in the *gyrA* gene and/or in the *gyrB*, *parC* and *parE* genes lead to clinically relevant resistance to fluoroquinolones. Therefore, in nine *Salmonella* isolates highly resistant to ciprofloxacin from farms in Sétif Province, single or double mutations were found simultaneously in the *gyrA* and *parC* genes as expected. Nevertheless, these are not the only resistance mechanisms that *Salmonella* develop against quinolones. Important mechanisms also include mutations that reduce the accumulation of the drug in the cell (e.g. efflux mechanism), and some *Salmonella* may also contain plasmids with genes encoding proteins that protect their DNA gyrase from these drugs (named plasmid-mediated quinolone resistance-PMQR), [38-40]. It is emphasized that PMQR genes were not evaluated in this study as the phenotypic tests (high resistance to NAL) did not indicate their presence so far [41]. However, their potential role should not be

excluded, and future studies should include targeted screening for PMQR genes to better understand quinolone resistance.

S. Kentucky ST198 is commonly isolated from patients, poultry and food in the Mediterranean basin including Algeria. The isolates from human patients are resistant to CIP, but also to extended-spectrum cephalosporins, carrying $bla_{\text{CTX-M-1}}$ or $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-48}}$ or $bla_{\text{CMY-2}}$ genes [42,43]. Therefore, *S*. Kentucky resistant to CIP is an international clone and the epidemiological relationship between strains must be closely monitored all around the globe.

Important is also resistance to aminoglycoside antibiotic-gentamicin as it was conferred by the aminoglycoside N-acetyltransferase-*aac(3)-II*gene in two *Salmonella* isolates. This gene is located on transmissible plasmids in *Escherichia coli* [44] or it can be found on a chromosomal genomic island (SGI) in multidrug resistant *Salmonella* Typhimurium DT104 [45]. Since *aac(3)-II*gene are located on mobile genetic elements, all *Salmonella* isolates carrying these and similar antibiotic resistance genes are significant from an epidemiological point of view. Therefore, it would be important to examine the whole genome sequences of multidrug-resistant and fluoroquinolone-resistant isolates from Algeria, in order to compare the genomes and detect possible clonal spread in the future. The search for resistance to colistin and possible plasmid-mediated mechanism needs to be continued in a more comprehensive manner by utilizing MIC analysis of a large number of isolates to more accurately determine the presence of colistin-resistant isolates, and the underlying mechanism of resistance.

Resistance to sulfonamides occurs when bacteria produce variants of the dihydropteroate synthase (DHPS) enzymes, which are the targets for sulfonamides. DHPS is encoded by the plasmid-borne resistance genes *sul1*, *sul2* and/or *sul3* [46]. The *sul3* gene was first discovered in 2003 by Perreten and Boerlin in *E. coli* isolates from pigs in Switzerland [26]. Resistance to trimethoprim is plasmid-mediated as well. The responsible dihydrofolate resistance genes (*dfr* genes) are organized as a gene cassette, and are usually located within class 1 and class 2 integrons. Bacteria possessing these genes encode DHFR enzymes that overcome the antibiotic attack because thymine synthesis continues unhindered [46]. Therefore, in the future mobile genetic elements carrying resistance genes have to be determined and epidemiological relationship of *Salmonella* isolates comprehensively investigated.

In summary, most isolates from poultry and turkey flocks in Sétif Province are resistant to nalidixic acid and pefloxacin, which indicates lower susceptibility to CIP. Of absolute concern is the increasing trend of high resistance to fluoroquinolones due to the overuse of enrofloxacin in the poultry industry in Algeria. The animal treatment with antibiotics against *Salmonella* is not recommended not only due to its transient therapeutic effect, the development of antimicrobial resistance, and the destruction of intestinal microbiome, but also because it is difficult to correctly determine the therapeutic dose of antibiotics in farm conditions [47]. If *Salmonella* already has a reduced susceptibility to quinolones, the concentration of mutation prevention is

likely to increase, leading to a higher level of mutations [39]. Once a mutation occurs in the *gyrA* gene, a subsequent mutation in this gene leads to high level of resistance to fluoroquinolones, rendering this antibiotic completely ineffective. This is the reason why prolonged use of fluoroquinolone antibiotics in livestock and poultry must be avoided or even better discontinued [48-50].

CONCLUSION

This study confirms the widespread presence of multidrug-resistant *Salmonella* spp. in poultry systems in Sétif, Algeria, with high rates of quinolone resistance. The detection of mutations in *gyrA*/*parC* genes highlights the urgent need for antimicrobial stewardship and enhanced surveillance across animal production. Only by continuously monitoring the emergence, transmission, and persistence of resistance in primary human food production can the risk of the spread of multidrug-resistant bacteria in humans and animals be realistically assessed.

Acknowledgment

This stady was funded by Ministry of Science, Technological Development and Innovation of Republic of Serbia by the Contract of implementation and funding of research work of NIV-NS in 2025, Contract No: 451-03-136/2025-03/200031.

Authors' contributions

AD conducted experiments, analyzed the results, and participated in writing the manuscript. DT, KN, and SM conducted experiments and analyzed the results. ZC, EB, OK, and BM conducted experiments. BJ analyzed the results and performed the editing of the manuscript. MV analyzed the results, wrote the manuscript, and performed the final editing. AA analyzed the results and approved the final draft of the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ORCID iDs

Amina Djemaioune https://orcid.org/0009-0005-5048-1126
Dalibor Todorović https://orcid.org/0000-0001-6707-9292
Katarina Novović https://orcid.org/0000-0003-3727-4163
Svetlana Miković Dramlić https://orcid.org/0009-0001-5808-6076
Zineb Cherak https://orcid.org/0000-0002-5921-7955
Esma Bendjama https://orcid.org/0009-0000-8284-9937
Oussama Khalouta https://orcid.org/0009-0006-5025-9194
Bakir Mamache https://orcid.org/0000-0003-0410-3149

Branko Jovčić https://orcid.org/0000-0002-9500-3786
Maja Velhner https://orcid.org/0000-0001-7360-7805
Ammar Ayachi https://orcid.org/0000-0002-4539-6964

REFERENCES

1. Daghir N, Diab-El-Harake M, Kharroubi S: Poultry production and its effects on food security in the Middle Eastern and North African region. J Appl Poult Res 2021, 30:100110.
2. Kussaga JB, Jacxsens L, Tiisekwa BPM, Luning PA: Food safety management systems performance in African food processing companies: a review of deficiencies and possible improvement strategies. J Sci Food Agric 2014, 94:2154–2169.
3. Velhner M, Milanov D, Kozoderović G: *Salmonella* spp. in poultry: a constant challenge and new insights. J Hellenic Vet Med Soc 2018, 69:899–910.
4. Hendriksen RS, Mikoleit M, Carlson VP, Karlsmose S, Vieira AR, Jensen AB, Seyfarth AM, DeLong SM, Weill FX, Lo Fo Wong ADM, Angulo FJ, Wegener HC, Aarestrup FM: WHO Global Salm-Surv External Quality Assurance System for Serotyping of *Salmonella* Isolates from 2000 to 2007. J Clin Microbiol 2009, 47:2729–2736.
5. Wain J, Hendriksen RS, Mikoleit ML, Keddy KH, Ochiai RL: Typhoid fever. Lancet 2015, 385:1136–1145.
6. Sato Y, Sato G, Tuchili L, Pandey GS, Nakajima A, Chimana H, Sinsungwe H: Status of *Salmonella* gallinarum-pullorum infections in poultry in Zambia. Avian Dis 1997, 41:490–495.
7. Elgroud R, Zerdoumi F, Benazzouz M, Bouzitouna-Bentchouala C, Granier SA, Frémy S, Brisabois A, Dufour B, Millemann Y: Characteristics of *Salmonella* Contamination of Broilers and Slaughterhouses in the Region of Constantine (Algeria). Zoonoses Public Health 2009, 56:84–93.
8. Smith SI, Seriki A, Ajayi A: Typhoidal and non-typhoidal *Salmonella* infections in Africa. Eur J Clin Microbiol Infect Dis 2016, 35:1913–1922.
9. Djeffal S, Bakour S, Mamache B, Elgroud R, Agabou A, Chabou S, Hireche S, Bouaziz O, Rahal K, Rolain JM: Prevalence and clonal relationship of ESBL-producing *Salmonella* strains from humans and poultry in northeastern Algeria. BMC Vet Res 2017, 13:132.
10. Djeffal S, Mamache B, Elgroud R, Hireche S, Bouaziz O: Prevalence and risk factors for *Salmonella* spp. contamination in broiler chicken farms and slaughterhouses in the northeast of Algeria. Vet World 2018, 11:1102–1108.
11. Djeffal SD, Bakir M, Rachid E, Chaffia B, Omar B, Rolain JM, Diene SM: Prevalence and genotypic characterization of *Salmonella* spp. from chicken meats marketed in the province of Skikda, Algeria. J Infect Dev Ctries 2021, 15:523–529.
12. World Health Organization: CIA list 6th rev. WHO.
13. Ventola CL: The antibiotic resistance crisis. Pharm Ther 2015, 40:277–283.
14. Feasey NA, Dougan G, Kingsely RA, Heyderman RS, Gorodn MA: Invasive non-typhoidal *salmonella* disease: an emerging and neglected tropical disease in Africa. Lancet 2012, 379:2489–2499.
15. Tanner JR, Kingsley RA: Evolution of *Salmonella* within hosts. Trends Microbiol 2018, 26:986–988.

16. Quinn PJ, Carter ME, Markey B, Carter GR: Clinical Veterinary Microbiologie. Wolf/ Mosby, London, 1994.
17. Schwarz S, Silley P, Simjee S, Woodford N, van Duijkeren E, Johnson AP, Gaastra W: Editorial: assessing the antimicrobial susceptibility of bacteria obtained from animals. J Antimicrob Chemother 2010, 65:601–604.
18. Clinical and Laboratory Standards Institute: Performance standards for antimicrobial susceptibility testing. 32nd ed. CLSI Document M100, Wayne, Pennsylvania, USA, 2022.
19. Kehrenberg C, Friederichs S, de Jong A, Michael GB, Schwarz S: Identification of the plasmid-borne quinolone resistance qnrS in *Salmonella enterica* serovar Infantis. J Antimicrob Chemother 2006, 58:18–22.
20. Kehrenberg C, Schwarz S: Occurrence and linkage of genes coding for resistance to sulfonamides, streptomycin and chloramphenicol in bacteria of the genera Pasteurella and Mannheimia. FEMS Microbiol Lett 2001, 205:283–290.
21. Kikuvi GM, Schwarz S, Ombui JN, Mitema ES, Kehrenberg C: Streptomycin and chloramphenicol resistance genes in Escherichia coli isolates from cattle, pigs, and chicken in Kenya. Microb Drug Resist 2007, 13:62–68.
22. Dolejská M, Bierošová B, Kohoutová L, Literák I, Čížek A: Antibiotic-resistant *Salmonella* and *Escherichia coli* isolates with integrons and extended-spectrum beta-lactamase in surface water and sympatric black-headed gulls. J Appl Microbiol 2009, 106:1941–1950.
23. Faldynova M, Pravcova M, Sisak F, Havlickova H, Kolackova I, Cizek A, Karpiskova R, Rychlik I: Evolution of antibiotic resistance in *Salmonella enterica* serovar Typhimurium strains isolated in the Czech Republic between 1984–2002. Antimicrob Agents Chemother 2003, 47:2002–2005.
24. Jakobsen L, Sandvang D, Hansen LH, Bager-Skjot L, West H, Jorgensen C, Hansen DS, Pedersen MD, Monnet LD, Frimodt-Moller N, Sorensen JS, Hammerum MA: Characterization, dissemination and persistence of gentamicin resistant Escherichia coli from Danish university hospital to the waste water environment. Environ Int 2008, 34:108–115.
25. Prüller S, Rensch U, Meemken D, Kaspar H, Kopp PA, Klein G, Kehrenberg C: Antimicrobial susceptibility of Bordetella bronchiseptica isolates from swine and companion animals and detection of resistance genes. PLoS ONE 2015, 10(8):e0135703.
26. Perreten V, Boerlin P: A new sulfonamide resistance gene (sul3) in Escherichia coli is widespread in the pig population of Switzerland. Antimicrob. Agents Chemother 2003, 47:1169–1172.
27. Frech G, Kehrenberg C, Schwarz S: Resistance phenotypes and genotypes of multiresistant *Salmonella enterica* subsp. *enterica* serovar Typhimurium var. Copenhagen isolates from animal sources. J Antimicrob Chemother 2003, 51:180–182.
28. Todorović D, Velhner M, Grego E, Vidanović D, Milanov D, Krnjaić D, Kehrenberg C: Molecular characterization of multidrug-resistant Escherichia coli isolates from bovine clinical mastitis and pigs in the Vojvodina Province, Serbia. Microb Drug Resist 2018, 24:95–103.
29. Ng LK, Martin I, Alfa M, Mulvey M: Multiplex PCR for the detection of tetracycline resistant genes. Mol Cell Probes 2001, 15:209–215.
30. Rodriguez-Villalobos H, Malaviolle V, Frankard J, de Mendonça R, Nonhoff C, Stuelens MJ : In vitro activity of temocillin against extended spectrum β-lactamase-producing Escherichia coli. J Antimicrob Chemother 2006, 57:771–774.31.
31. Technical University of Denmark protocols. EURL-AR.

32. Li S, He Y, Ames Mann D, Deng X: Global spread of *Salmonella* Enteritidis via centralized sourcing and international trade of poultry breeding stocks. Nat Commun 2021, 12:5109.
33. Blondel CJ, Yang HJ, Castro B, Chiang S, Toro CS, Zaldívar M, Contreras I, Andrews-Polymenis HL, Santiviago CA: Contribution of the type VI secretion system encoded in SPI-19 to chicken colonization by *Salmonella enterica* serotypes Gallinarum and Enteritidis. PLoS ONE 2010, 5(7):e11724.
34. Xian H, Yuan Y, Yin C, Wang Z, Ji R, Chu C, Jiao X, Li Q: The SPI-19 encoded T6SS is required for *Salmonella* Pullorum survival within avian macrophages and initial colonization in chicken dependent on inhibition of host immune response. Vet Microbiol 2020, 250:108867.
35. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. EUCAST, Version 12.0, 2022.
36. Hopkins KL, Arnold C, Threlfall EJ: Rapid detection of gyrA and parC mutations in quinolone-resistant *Salmonella enterica* using Pyrosequencing® technology. J Microbiol Methods 2007, 68:163–171.
37. Velhner M, Kozoderović G, Grego E, Galić N, Stojanov I, Jelesić Z, Kehrenberg C: Clonal spread of *Salmonella enterica* serovar Infantis in Serbia: Acquisition of mutations in the topoisomerase genes gyrA and parC leads to increased resistance to fluoroquinolones. Zoonoses Public Health 2014, 61:364–370.
38. Giraud E, Cloeckaert A, Kerboeuf D, Chaslus-Dancla E: Evidence for active efflux as the primary mechanism of resistance to ciprofloxacin in *Salmonella enterica* serovar Typhimurium. Antimicrob. Agents Chemother 2000, 44:1223–1228.
39. Jacoby GA: Mechanisms of resistance to quinolones. Clin Infect Dis 2005, 41(Suppl. 2):S120–S126.
40. Strahilevitz J, Jacoby GA, Hooper DC, Robicsek A: Plasmid-mediated quinolone resistance: a multifaceted threat. Clin Microbiol Rev 2009, 22:664–689.
41. Skov R, Matuschek E, Sjölund-Karlsson M, Åhman J, Petersen A, Stegger M, Torpdahl M, Kahlmeter G: Development of a pefloxacin disk diffusion method for detection of fluoroquinolone-resistant *Salmonella enterica*. J Clin Microbiol 2015, 53:3411–3417.
42. Le Hello S, Harrois D, Bouchrif B, Sontag L, Elhani D, Guibert V, Zerouali K, Weill FX: Highly drug – resistant *Salmonella enterica* serotype Kentucky ST198-X1: a microbiological study. Lancet Infect Dis 2013, 13:672–679.
43. Le Hello S, Bekhit A, Granier SA, Barua H, Beutlich J, Zajac M, Münch S, Sintchenko V, Bouchrif B, Fashae K, Pinsard JL, Sontag L, Fabre L, Garnier M, Guibert V, Howard P, Hendriksen RS, Christensen JP, Biswas PK, Cloeckaert A, Rabsch W, Wasyl D, Doublet B, Weill FX: The global establishment of a highly-fluoroquinolone resistant *Salmonella enterica* serotype Kentucky ST198 strain. Front Microbiol 2013, 4:395.
44. Jakobsen L, Sandvang D, Hansen LH, Bagger-Skjøt L, Westh H, Jørgensen C, Hansen DS, Pedersen BM, Monnet DL, Frimodt-Møller N, Sørensen SJ, Hammerum AM: Characterisation, dissemination and persistence of gentamicin resistant Escherichia coli from a Danish university hospital to the waste water environment. Environ Int 2013, 34:108–115.
45. Doublet B, Weill FX, Fabre L, Chaslus-Dancla E, Cloeckaert A: Variant *Salmonella* genomic island 1 antibiotic resistance gene cluster containing a novel 3-N-aminoglycoside acetyltransferase gene cassette, aac(3)-Id, in *Salmonella enterica* serovar Newport. Antimicrob Agents Chemother 2004, 48:3806–3812.

46. Sköld O: Sulfonamide resistance: mechanisms and trends. Drug Resist Updat 2000, 3:155–160.
47. Manyi Loh C, Mamphweli S, Meyer E, Okoh A: Antibiotic use in agriculture and its consequential resistance in environmental sources: Potential public health implications. Molecules 2018, 23:795.
48. Piddock LJV: Fluoroquinolone resistance in *Salmonella* serovars isolated from humans and food animals. FEMS Microbiol Rev 2002, 26:3–16.
49. Nelson JM, Chiller TM, Powers JH, Angulo FJ: Fluoroquinolone-resistant Campylobacter species and the withdrawal of fluoroquinolones from use in poultry: A public health success story. Clin Infect Dis 2007, 44:977–980.
50. United States Food and Drug Administration: Final Decision of the Commissioner Docket No. 2000N – 1571. Withdrawal of Enrofloxacin for Poultry. FDA, 2017. Available: http://www.fda.gov/AnimalVeterinary/SafetyHealth/RecallsWithdrawals/ucm042004.htm.

VISOK NIVO REZISTENCIJE NA FLUOROKINOLONE I VIŠESTRUKA REZISTENCIJA KOD *SALMONELLA* SPP. IZOLOVANIH IZ ŽIVINE, JATA ĆURAKA I KLANICA U ALŽIRU

Amina DJEMAIOUNE, Dalibor TODOROVIĆ, Katarina NOVOVIĆ, Svetlana MIKOVIĆ DRAMLIĆ, Zineb CHERAK, Esma BENDJAMA, Oussama KHALOUTA, Bakir MAMACHE, Branko JOVČIĆ, Maja VELHNER, Ammar AYACHI

U ovom radu, *Salmonella* spp. je otkrivena na farmama živine i ćuraka, klanicama i inkubatorima u provinciji Setif u Alžiru. Osamdeset pojedinačnih izolata po farmi analizirani su geni otpornosti koji podvlače mehanizam otpornosti. U jednom slučaju, serotipovi *S*. Virchow i *S*. Ivory pronađeni su u istom uzorku i oba izolata su bila otporna na nalidiksinsku kiselinu. *S*. Enteritidis je otkrivena u četiri jata brojlera za uzgoj, tri inkubatora, 12 jata koka nosilja, 12 jata brojlera, dok je pet klanica dalo 10 izolata. Široka rasprostranjenost *S*. Enteritidis u primarnoj proizvodnji i lancu ishrane u Alžiru zahteva posebne mere u upravljačkoj praksi na farmama živine. Svi izolati osim pet bili su otporni na nalidiksinsku kiselinu i pefloksacin, što znači da ove salmonele fenotipski pokazuju smanjenu osetljivost na ciprofloksacin. Pet izolata je bilo otporno na više lekova. Dva izolata *Salmonella* Galinarum biotipa gallinarum iz jata kokošaka nosilja bila su otporna na hinolone, aminoglikozide i sulfonamide. Jedan od ovih izolata bio je takođe otporan na trimetoprim sam i u kombinaciji sa sulafmetoksazolom. Jedan izolat *S*. Enteritidis bio je otporan na ampicilin, nalidiksinsku kiselinu, pefloksacin i kolistin. Posebno je zabrinjavajući visok nivo rezistencije na ciprofloksacin kod devet izolata (šest, *Salmonella* Galinarum biotip gallinarum, dva, Južni Kentaki i jedan izolat *Salmonella enterica* subsp. *enterica*) zbog mutacija u enzimima DNK giraza i topoizomeraza IV. Geni rezistencije su identifikovani kod 21 izolata. Svi otkriveni geni rezistencije obično dovode do rezistencije na ampicilin, streptomicin, gentamicin, tetraciklin, sulfonamide i trimetoprim antibiotike.