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**Antibiotic resistance and distribution of *SodCI*, *sopE*, *sefA* genes among *Salmonella enteric* serotype Enteritidis isolates from poultry**

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**Αντιβιοανθεκτικότητα και κατανομή των γονιδίων *SodCI*, *sopE*, *sefA* σε στελέχη *Salmonella enterica* serotype Enteritidis ορνίθειας προέλευσης**

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**ABSTRACT.** Present work aimed to examine the antibiotic resistance of the *Salmonella enterica* serotype Enteritidis (SE) isolates from poultry, to study the plasmid-mediated ampicillin resistance and to detect and determine the distribution of *sodCI*, *sopE* and *sefA* genes. Thirty-five SE isolates from one-day chicks, layers and broilers were studied for susceptibility/resistance to sixteen antimicrobial agents; 23 (65.7%) of them showed resistance to ampicillin, 5 (14.3%) to ampicillin and tetracycline, 4 (11.45%) to tetracycline and 1 (2.9%) isolate showed multi-drug resistance. Ampicillin (Amp<sup>R</sup>) and ampicillin/tetracycline (Amp<sup>R</sup>Te<sup>R</sup>) resistance was easily transferred by conjugation, and all isolates except two possessed a common band. The molecular mass of the plasmid carrying ampicillin resistance was approximately determined at 41kb after DNA digestion with *Bam*HI, *Hind*III, *Eco*RI, *Eco*RV and *Pst*I restriction enzymes and ligation of *Eco*RI fragments to pET29c. For the detection of TEM-1 or/and TEM-2 β-lactamases, two pairs of primers were used in a polymerase chain reaction (PCR). The PCR products showed the presence of *bla*<sub>TEM-1</sub> gene in all isolates. The presence of *SodCI*, *sopE*, *sefA* genes was also examined by PCR. Twenty-two (62,8%) isolates carried the *sodCI* gene, thirty-four (97,2%) isolates carried the *sopE* gene and all isolates carried the *sefA* fimbrial locus.

**Keywords:** *S. enteritidis*; plasmid analysis; *sodCI*, *sopE* and *sefA* genes; *bla*<sub>TEM-1</sub>, *bla*<sub>TEM-2</sub> genes

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**Περίληψη.** Η παρούσα εργασία είχε ως στόχο να προσδιορίσει την αντιβιοανθεκτικότητα στελεχών *Salmonella enteric* serotype Enteritidis (SE) ορνίθειας προέλευσης, να μελετήσει την πλασμιδιο-εξαρτώμενη ανθεκτικότητα στην αμπικιλίνη και να προσδιορίσει την κατανομή μεταξύ των στελεχών των γονιδίων *sodCI*, *sopE* και *sefA*. Τριάντα πέντε SE στελέχη που απομονώθηκαν από νεοσσούς ηλικίας μίας ημέρας, από όρνιθες ωοτοκίας και κρεοπαραγωγής μελετήθηκαν ως προς την ανθεκτικότητα/ευαισθησία σε δεκαέξι αντιβιοτικά. Είκοσι τρία στελέχη (65,7%) εμφάνισαν ανθεκτικότητα στην αμπικιλίνη, πέντε στελέχη (14,3%) στην αμπικιλίνη και την τετρακυκλίνη, τέσσερα στελέχη (11,45%) στην τετρακυκλίνη και ένα (2,9%) εμφάνισε πολυανθεκτικότητα. Η ανθεκτικότητα στην αμπικιλίνη ( $Amp^R$ ) και το συνδυασμό αμπικιλίνης/τετρακυκλίνης ( $Amp^R$ Te $^R$ ) μεταφέρθηκε εύκολα με τη σύζευξη σε όλα τα στελέχη τα οποία – με εξαίρεση δύο- εμφάνισαν μία κοινή πλασμιδιακή ζώνη. Η μοριακή μάζα του πλασμιδίου που έφερε το γονίδιο ανθεκτικότητας στην αμπικιλίνη προσδιορίστηκε στα 41kβ μετά από πέψη του DNA με τα ένζυμα περιορισμού *Bam*HI, *Hind*III, *Eco*RI, *Eco*RV και *Pst*I. Η επαλήθευση του μεγέθους της μοριακής μάζας έγινε με την επίδεση (ligation) στο πλασμίδιο pET29c, των τμημάτων DNA τα οποία προέκυψαν μετά από πέψη με το ένζυμο περιορισμού *Eco*RI. Για την ανίχνευση των TEM-1 ή/και TEM-2 β-λακταμασών, χρησιμοποιήθηκαν δύο εξειδικευμένα ζεύγη εκκινητών με τη μέθοδο της αλυσιδωτής αντίδρασης της πολυμεράσης (PCR). Τα προϊόντα της PCR έδειξαν την παρουσία του γονιδίου *bla*<sub>TEM-1</sub> σε όλα τα στελέχη. Ελέγχθηκε επίσης η παρουσία των γονιδίων *of SodCI*, *sopE*, *sefA* με εξειδικευμένους εκκινητές της PCR. Είκοσι δύο (62,8%) στελέχη έφεραν το γονίδιο *sodCI*, τριάντα τρία (97,2%) το γονίδιο *sopE* και όλα τα στελέχη ήταν φορείς του γονιδίου *sefA*.

**Λέξεις ευρετηρίασης:** *S. enteritidis*, ανάλυση πλασμιδίου, γονίδια *sodCI*, *sopE*, *sefA* genes, *bla*<sub>TEM-1</sub>, και *bla*<sub>TEM-2</sub>

## INTRODUCTION

*Salmonella enterica* serotype Enteritidis (SE) is one of the two most frequent etiological agents of human foodborne salmonellosis. Contaminated poultry facilities is the main vehicle of SE, ranging from 29% to 34% of all *Salmonella* infections (Sarna et al. 2002, CDSC 2004; Altekruze et al., 2006; Braden, 2006; Jain and Chen, 2006; Linam and Gerber, 2007; CDC 2013; Henriques et al., 2013).

Most SE isolates harbour serovar-associated factors as the virulence, the fibrial and the antibiotic resistance genes (Lu et al., 1999; Anđu-Küçüker et al., 2000; Bakshi et al., 2003). Virulence genes encode products that assist organisms to express the virulence in the host cells. Some genes as *Sod* and *Sop* are associated with the survival in the host system or in the actual manifestation of pathogenic processes and others as *Sef* genes, are involved in adhesion and invasion (Murugkar et al., 2003). As for *Sef* genes, the *sef14* fimbriae operon contains four structure genes (*sefABCD*) required for the translocation and biogenesis and the *sefA* gene, encodes the main subunit of the SEF14 fimbrial protein in SE (Turcotte and Woodward, 1993; Lopes et al., 2006; Zhu et al., 2010). Virulent serovars of *S. enterica* possess two different periplasmic [Cu, Zn] superoxide dismutases, the SodCI and the

SodCII. The SodCI dismutase, encoded by *SodCI* gene, seems to be of higher importance as it is involved in the defence against oxidative stress, to which SE is exposed when residing intracellularly in macrophages or neutrophils. SopE is a SPI-1-dependent translocated protein that modulates host cell RhoGTPase function. It is located on a cryptic  $\lambda$ -like phage in serotypes Enteritidis, Dublin, Hadar and Gallinarum (Hopkins and Threlfall, 2004). Although not studied in such a detail, *sodCI* gene has been reported to be highly associated to the virulence activity of SE strains, while *sopE* gene is relatively randomly distributed among *S. enterica* serovars (Karasova et al., 2009).

Antibiotics are used in livestock, including chickens, to treat or prevent disease and to promote growth. The correlation between the use of antibiotics and the emergence of resistance has been documented. Moreover, the increasing rate of antibiotic resistance has been reported from 1993 (Threlfall et al., 1993) until recently (Smith et al., 2002; Maripandi and Al-Salamah, 2010; Melendez et al., 2010). A variety of antibiotics such as ampicillins, tetracyclines, sulfonamides and streptomycin, have been used for the treatment of salmonellosis leading to the emergence of resistant or multi-resistant strains (Glynn et al., 1998; Metzger et al., 1998; Buyd

et al., 2002; Snow et al., 2007; Yang et al. 2002). The resistance genes involved in *Salmonella* strains are usually plasmid encoded and they can be transmitted to other bacteria (Foley and Lynne, 2008). These conjugative plasmids have a high molecular weight and as they can disseminate genetic information within cells and species, are of great significance (Rychlik et al., 2006).

In Greece, the most detailed data about antibiotic resistance in humans have been published from '80s to '90s. The frequency of resistance SE isolates, started from a low level (7.9%) in 1987, increased to 30.4% in 1991 and reached a plateau or even decreased in 1997 (Vatopoulos et al., 1994; Tassios et al., 1997; Tassios et al., 1999; Markogiannakis et al., 2000). In 1998, Arvatitidou et al., found that chicken carcasses from the hospitals in Thessaloniki were contaminated by SE strains at a frequency up to 69% while the same period the ampicillin resistant isolates from poultry, were found up to 91.6%. The majority of the SE resistant isolates of poultry origin, were resistant to ampicillin or/and tetracycline (Zdragas, 2001). Similar high prevalence of ampicillin resistant SE isolates from poultry have been reported from many countries in Europe such as France (Llanes et al., 1999), the U.K. (Yates and Amyes, 2005; Papadopoulou et al., 2009), or worldwide as in Korea (Suh and Song, 2006) Pakistan (Mirmomeni et al., 2007) and Tynisia (Abbassi-Ghossi et al., 2011).

The aim of the present work was to examine the antibiotal resistance of the SE isolates from poultry, to study the plasmid-mediated ampicillin resistance and to detect and determine the distribution of *sodCI*, *sopE* and *sefA* genes.

## MATERIALS AND METHODS

### Bacterial strains

Thirty-five SE isolates obtained from one-day old chicks with high or low mortality ratio, from layers with sporadic egg-peritonitis and from clinically healthy broilers by random sampling in slaughter-houses (G1 Group) or from healthy meat producer broilers, randomly selected from thirty flocks (Group 2). For the selective enrichment of all strains, the

modified Rappaport-Vassiliadis broth was used (Vassiliadis, 1983). Isolation of the SE strains was performed according to ISO 6579-2002. Isolated strains were identified biochemically and checked serologically by Poly A-S (Prolab) antiserum.

### Antimicrobial resistance testing

Routine screening for antibiotic susceptibility was performed using Mueller–Hinton agar (Biorad-Marnes-la-Coquette. France) and the disk diffusion method according to the current recommendations of the CLSI (2009), against: Enrofloxacin (ENR), Tetracycline (TE) 30µg, Trimethoprim Sulphamethoxazole (SXT) 1,25/23,75µg, Erythromycin (E) 30µg, Cephalothin(CEF) 30µg, Nalidixic Acid (NA) 30µg, Kanamycin (K) 30µg, Ampicillin (AM) 10µg, Streptomycin (S) 10µg, Floumequin (UB) 30µg, Apramycin (APR) 30µg, Amoxycillin (AMX) 30µg, Ceftriaxone (CRO) 30µg, Neomycin (N) 30ui, Gentamicin (GM) 10µg, Penicillin (P) 10ui. Minimal Inhibitory Concentration (MIC) was performed to resistant strains according to the current recommendations of the Clinical Laboratory and Standards Institute (CLSI, 2009) in a series of twofold antibiotic concentrations in Brain Heart Infusion Broth (Scharlau).

### Conjugation

SE isolates, *E. coli* N99 and TOP10F' recipient cells were grown in LB broth to logarithmic phase. Conjugation experiments were carried out in Brain Heart Infusion (BHI) broth by the filter method (Ekat-eriniadou et al., 1994) at a 1:10 donor/recipient ratio. In *E. coli* TOP10F' cells culture, 50µg/ml S was added. Transconjugants were selected on MacConkey agar containing 50µg/ml AM or 50µg/ml AM + 30µg/ml TE, after 24-48h incubation at 37°C. The transfer frequency was expressed as the number of the transconjugant colonies per donor colony.

### Plasmid isolation

Single colonies of the donors and the transconjugants were transferred in 70 ml of BHI and LB broth respectively with 50µg/ml AM or 50µg/ml AM + 30µg/ml TE and were incubated overnight. Plasmid DNA was extracted by the Concert™ High Purity – Plasmid Midiprep System kit (Life Technologies,

GibcoBRL). The isolated DNA was electrophoresed in 0.7% agarose gels at 70 V for 6 h or in 0.7% agarose gels at 90 V for 4 h, stained with ethidium bromide, and analyzed under UV illumination by the TEX-20M (Life Technologies, GibcoBRL) system (Balis et al., 1996).

#### Plasmid analysis by Restriction Fragment Length Polymorphism - RFLP

The approximate molecular mass of the plasmid was determined by RFLP analysis and confirmed by ligation. Plasmid DNA from ten SE AM<sup>R</sup> isolates was digested with *Bam*HI, *Eco*RI, *Hind*III, *Eco*RV and *Pst*I (Brown et al., 1993; Llanes et al., 1999). DNA fragments were subjected to electrophoresis in 1% w/v agarose gel with ( $\lambda$ ) *Hind*III and 1Kb DNA ladder as markers. Restriction endonuclease analysis was repeated three times for *Eco*RV and *Eco*RI to better determine the plasmid molecular mass. To confirm the estimated plasmid molecular mass, the *Eco*RI fragments of the AM<sup>R</sup> plasmid DNA, at a concentration of 120 fmol each, were ligated to 30 fmol of plasmid pET29c (Invitrogen) previously digested with *Eco*RI. In all reactions, 1 Unit of T4 DNA ligase (TAKARA BIO Inc) was used. After 16h incubation at 15°C, the product was transformed to *E. coli* competent cells (Sambrook and Russell, 2001).


#### Detection of *bla*<sub>TEM-1</sub> and *bla*<sub>TEM-2</sub> genes

The presence of *bla*<sub>TEM-1</sub> and *bla*<sub>TEM-2</sub> genes was examined by PCR. Oligonucleotide primers were used to amplify a 291-bp fragment for the *bla*<sub>TEM-1</sub> gene and a 489-bp fragment for the *bla*<sub>TEM-2</sub> gene (Table 1). Amplification reaction mixtures were prepared at a final volume of 25  $\mu$ l containing 1x PCR Taq Polymerase Buffer (Invitrogen), 2.5 mmol MgCl<sub>2</sub> (Invitrogen), 0.2 mmol dNTPs, 1  $\mu$ mol of each primer, 100 ng of genomic DNA and 1.5 U of Taq polymerase (Invitrogen). The temperature cycling for amplification was performed in a PTC-200 Peltier Thermal Cycler (MJ Research) as follows: initial denaturation 95°C for 5 min followed by 30 cycles at 95°C for 1 min denaturation, 48°C for 30sec annealing, 72°C for 30sec extension and 5 min final extension at 72°C. The resulting amplification products were separated by the electrophoresis in 1.5% w/v agarose gel, stained with ethidium bromide and visualized under UV light.

#### Identification of *sodCI*, *sopE* and *sefA* genes

The presence of plasmid-mediated virulence genes was examined by PCR. Oligonucleotide primers were used to amplify a 912-bp fragment for the *sodCI* gene, a 722-bp fragment for the *sopE* gene and a 498-bp fragment for the *sefA* fimbrial locus (Table

**Table 1.** Primers used in PCR for the detection of *sodCI*, *sopE*, *sefA*, *bla*<sub>TEM1</sub>, *bla*<sub>TEM1/2</sub>  $\beta$ -lactamases genes in *S. enteritidis*

Primer	Target gene	Sequence 5' 	3'	Amplified Fragment Size (bp)
VL1F	<i>sodCI</i> <sup>[31]</sup>	CTTGCAAACATATACCTGC		912
VL1R		GACTATCTGAATGCTTA		
VL2F	<i>sopE</i> <sup>[31]</sup>	TCAGGGAGTGTTTTGTATATATTTA		722
VL2R		GTGACAAAATAACTTTATCTCCCC		
VL3F	<i>sefA</i> <sup>[31]</sup>	ATGCGTAAATCAGCATCTGCAGTAG		498
VL3R		TTA GTT TTG ATA CTG CTG AAC GTA		
TEM1F	<i>bla</i> <sub>TEM1</sub> <sup>[60]</sup>	GCA CGA GTG GGT TAC ATC GA		291
TEM1R		GGT CCT CCG ATC GTT GTC AG		
TEM1/2F	<i>bla</i> <sub>TEM1/2</sub> <sup>[29]</sup>	GAG TACT CA CCA GTC ACA GAA AAC		489
TEM1/2R		TTAGTTTTGATACTGCTGAACGTAG		



**Table 2.** Antibiotic resistance of *S. enteritidis* strains

a/a	Isolates	Antibiogramme	M.I.C.
<b>Group 1*</b>			
1.	3, 4, 6, 8, 16E, 17/E, 358, 162	AM	50µg/ml AM
2.	9, 6x, 334, 32, 34,	AM	100µg/ml AM
3.	10, 11E, 162, x <sub>1</sub> S <sub>1</sub>	AM/TE	50µg/ml AM, 30µg/ml TE
4.	x/15	AM/TE	100µg/ml AM, 30µg/ml TE
5.	197, 4x, 29, 294	TE	30µg/ml TE
6.	35	AM/GM/SXT/TE	AM/AMX/GM/SXT/TE
7.	146, 371	Sensitive (blank)	-
<b>Group 2**</b>			
1.	1G <sub>2</sub> - 10G <sub>2</sub>	AM	50µg/ml AM

\* Thirty-five isolates from one-day old chicks with a high mortality ratio, one-day old chicks with a low mortality ratio, layers with sporadic egg-peritonitis and clinically healthy broilers by random sampling in slaughter-houses.

\*\*Ten isolates from thirty flocks with healthy meat producer broilers, randomly selected.

1). Amplification reaction mixtures were prepared at a final volume of 25 µl containing 1x PCR Taq Polymerase Buffer (Invitrogen), 2.5 mmol MgCl<sub>2</sub> (Invitrogen), 0.2 mmol dNTPs, 1 µmol of each primer, 100 ng of genomic DNA and 2.5 U of Taq polymerase (Invitrogen). The temperature cycling for amplification was performed in a PTC-200 Peltier Thermal Cycler (MJ Research) as follows: initial denaturation 95°C for 5 min followed by 30 cycles at 95°C for 1 min denaturation, 58°C for 40sec annealing, 72°C for 40sec extension and 5 min final extension at 72°C. The resulting amplification products were separated by the electrophoresis in 1.5% w/v agarose gel, stained with ethidium bromide and visualized under UV light.

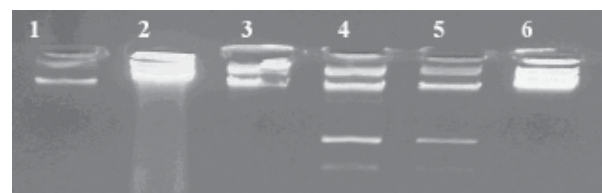
## RESULTS

All the thirty-five isolates of current study were identified serologically and biochemically as *Salmonella enteric* serotype Enteritidis (SE). Thirteen to twenty-five isolates of G1 group (52%) were resistant to ampicillin, five isolates (20%) to

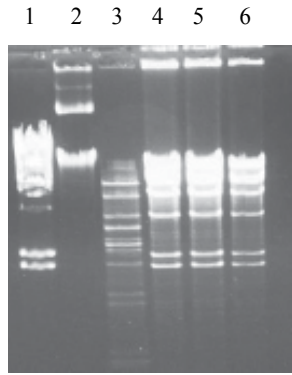
ampicillin and tetracycline, four isolates (16%) to tetracycline, one isolate (4%) appeared multi-drug resistance and two isolates (8%) were susceptible. All ten isolates (100%) from G2 group were resistant to ampicillin (results are shown in Table 2). It is also significant that six of the twenty-five (17.1%) SE isolates from the G1 group carried resistance to more than one antimicrobial.

From the MIC test it was pointed out that resistance to ampicillin was five or ten times higher than the disk concentration (50µg/ml or 100µg/ml instead of 10µg/ml). Ampicillin or ampicillin/tetracycline resistance was transferred to *E. coli* N99 and *E. coli*

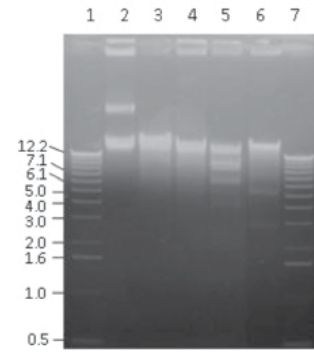
SE 4SE 10 SE X<sub>1</sub>S<sub>1</sub> Se162 SE 17/E SE1A  
AM<sup>R</sup> AM<sup>R</sup>TE<sup>R</sup> AM<sup>R</sup>TE<sup>R</sup> AM<sup>R</sup> AM<sup>R</sup>TE<sup>R</sup> AM<sup>R</sup>



**Figure 1.** Analysis of antimicrobial resistance plasmid DNA



**Figure 2a.** Plasmid DNA from SE AMR after treatment with EcoRV, EcoRI restriction enzymes. Lane 1:  $\lambda$ /HindIII DNA, lane 2: uncut pDNA, lanes 3-6: digestion with EcoRV, EcoRI, EcoRI, EcoRI, respectively



**Figure 2b.** Analysis of plasmid DNA after treatment with BamHI, PstI, EcoRI and HindIII restriction enzymes. Lanes 1 and 7: 1Kb DNA Ladder, lane 2: uncut pDNA, lanes 3-6: digestion with BamHI, PstI, EcoRI and HindIII, respectively.

TOP10F' recipients at a frequency from  $1 \times 10^{-4}$  up to  $2.5 \times 10^{-7}$ . DNA isolation and agarose gel electrophoresis of both SE donors and *E. coli* recipients showed that all isolates possessed a common band of the same molecular mass. Two isolates have more than one band (162 and 17/E Amp<sup>R</sup>Te<sup>R</sup>). These extra bands could be different conformations of the same plasmid. The results are shown in Figure 1.

Plasmid analysis of the ten SE AM<sup>R</sup> isolates after endonuclease digestion with *Bam*HI showed three bands, with *Hind*III two bands, with *Eco*RI seven bands, with *Eco*RV thirteen bands and with *Pst*I one band. Plasmid DNA from the same isolates was also digested with *Bam*HI, *Eco*RI, *Hind*III, *Eco*RV and *Pst*I after ligation to compare the plasmid molecular mass. The results of the digestion are shown in Figures 2a and 2b. The restriction endonuclease analysis revealed that ampicillin resistance was encoded by a 41 kb plasmid in all isolates.

Identification of the *bla*<sub>TEM-1</sub> and *bla*<sub>TEM-2</sub> genes associated with resistance to ampicillines was accomplished using PCR. Only *bla*<sub>TEM-1</sub> gene was detected in all isolates.

As for the *sod*CI, *sop*E and *sef*A genes, twenty-two (62.8%) isolates harboured the *sod*CI gene and among them fourteen (40%) belonged to the G1 group and eight (22.8%) to the G2 group. Thirty-four (97.2%) isolates carried the *sop*E gene and among them twenty-four (68.6%) belonged to G1 group and ten (28.6%) to the G2 group. All isolates carried the *sef*A gene.

## DISCUSSION

Animals are the consumers of almost half of Europe's production of antimicrobials. The prevalence of *S. Enteritidis* (SE) strains in poultry farming has as a result the appearance of this serotype in human food chain (Altekruse et al., 2006). The most recent data in European Union ([www.efsa.europa.eu/en/efsajournal/2597.pdf](http://www.efsa.europa.eu/en/efsajournal/2597.pdf)) indicate that *S. Enteritidis* (SE) is the most frequent serovar observed in confirmed human salmonellosis. In Greece, Arvanitidou et al, (1998) reported SE as the predominant serotype. Past decade, the most common *Salmonella* serotypes, from broiler flocks in EU were Enteritidis, Infantis, Mbandaka, Typhimurium and Hadar (van de Giessen et al., 2006; [www.efsa.europa.eu/en/efsajournal/2597.pdf](http://www.efsa.europa.eu/en/efsajournal/2597.pdf)). In the United States, during the Surveillance for Foodborne Disease Outbreaks 2009–2010, among the 225 confirmed *Salmonella* outbreaks with a serotype reported, Enteritidis was the most common serotype with 76 outbreaks (34%). In parallel with the increased gastroenteritis cases in humans of high-risk groups, the problem of the resistance to antibiotics has raised.

This study describes phenotypic and genotypic characteristics of thirty five isolates. All isolates were identified as *Salmonella enterica* serotype Enteritidis (SE). The results from the antibiotic resistance tests were similar to earlier studies in Greece (Balis et al., 1996,; Iordanidis and Georgopoulou, 1998; Vatopoulos et al., 1994) and in other countries (Dogru

et al., 2010; Hur et al. 2011). It is remarkable that ampicillin resistant SE strains were also isolated from healthy broilers. *S. Enteritidis* was one of the predominant serotypes carrying tetracycline and ampicillin resistance in Zdragas et al., (2012). Although healthy carriers of ampicillin resistant SE isolates transfer the resistance to other animals and they could contribute to the resistance transfer from animals to humans being a threat still their role is not absolutely clarified.

The restriction endonuclease analysis revealed that ampicillin resistance was encoded by a ca. 41 kb plasmid in all isolates while earlier studies showed that the ampicillin resistance has been correlated to plasmids with a molecular mass varying from 54.44 kb to 160 kb (Balis et al., 1996; Vatopoulos et al., 1994). The last ten years some studies – especially in Europe, resulted that while human and poultry strains possess many common structural features, they represent two distinct populations (Suh et al. 2006). This hypothesis seems to be enhanced from the fact that antibiotic resistance is transferred from plasmids with different molecular mass depending on the origin (human or animal). In Southern Italy, where SE isolates resistant to ampicillin and ampicillin/tetracycline have been found at a high frequency, the molecular mass of the isolated plasmid DNAs were estimated to be from 40kb to 128kb (Nastazi et al., 2000; Villa et al., 2002). In Taiwan, the size of the isolated plasmids ranged from 3.6 to 100 kb including a plasmid of 36kb (Chu et al., 2009). Present findings from India revealed that SE isolates possessed more than one plasmid. The plasmids size ranged between 0.43 and 115 MDa (Maripandi and Al-Salamah, 2010).

In Iran, six different plasmid patterns were detected among 49 isolates and a 68-kb plasmid was found in 98% of isolates (Morshed and Peighambari, 2010). The fact that in Greece also, there are plasmids of different molecular mass is probably a first indication that human and poultry strains possess plasmids of two distinct populations.

The distribution of virulence factors used by *Salmonella* to induce enteropathogenesis has not been fully explored. Although all the serovars of *S. enterica* are considered as potentially pathogenic, the fact that there are significant differences between virulence genes, even among the isolates of the same

serovar, makes more crucial to obtain as much data as we can. The *sodCI* gene appears to be functionally important as it is carried by selected strains belonging to some of the most highly pathogenic serotypes (Fang et al., 1999). Many investigations have demonstrated the contribution of the gene to the ability of *Salmonella* to cause disease in the host. It has been showed that the differences in their virulence are associated to the presence or absence of *sodCI* gene; a number of mutations in the gene confer different reductions in virulence for mice. The presence of *sodCI* gene in the genome of SE may influence its persistence in poultry flocks (Karasova et al., 2009).

The *sopE* gene, it has been detected in different phage types of SE and that may contribute for the expression of *Salmonella* invasion by stimulating membrane ruffling; it has been found to be encoded by a temperate phage. The presence of *sopE* gene has been associated with many *Salmonella* species such as *S. typhimurium*, *S. typhi*, *S. enteritidis*, *S. dublin*, *S. hadar*, *S. gallinarum* and it is associated with epidemic disease in both humans and animals (Hopkins and Threlfall, 2004; Rahman et al., 2004; Smith et al., 2010; Dione et al., 2011). In present study the great majority of the isolates (34/36 or 97.2%) harbored the *sopE* gene.

As for the SEF14 fimbriae, although they have not a demonstrable role in the pathogenesis or virulence of SE, may be important in the attachment to the host epithelium in the early stages of infection (Ogunniyim et al., 1997). In our study, all isolates carried the *sefA* fimbrial locus giving probably higher abilities to the attachment to the host epithelium.

## CONCLUDING REMARKS

In our study the prevalence and combinations of SE antimicrobial resistance were determined in isolates from chickens, which contribute the majority of food-borne salmonellosis found in humans. From the thirty-five isolates of the current study, thirty three (94.3%) appeared resistance to antimicrobials and from them twenty nine (82.9%) to ampicillin while ten (28.6%) to streptomycin.

Plasmid DNA of poultry origin carrying ampicillin resistance was identified and analyzed for the first time in Greece. DNA analysis showed one band, common



in all isolates, possessing the antimicrobial resistance genes. The plasmid isolation, ligation and RFLP analysis, revealed that the ampicillin resistance is encoded by a ca. 41 kb plasmid in all isolates. The fact that from poultry and human strains identified plasmids of different molecular mass possibly indicates that in Greece, the above strains possess plasmids of two distinct populations. Moreover, according to recent unpublished data the prevalence of the AM<sup>R</sup> isolates

is gradually reducing due to the limited use of this antibiotic in poultry industry, resulting to the improvement of current situation.

The possession of virulence-associated genes in these SE isolates and especially in the isolates from the healthy broilers, suggests that they could cause serious disease and give rise to public health problems if they were dispersed in the human population.

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