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## ***Salmonella* spp. in poultry: a constant challenge and new insights**

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**ABSTRACT.** The knowledge about virulence mechanisms, resistance to antimicrobial agents and the biofilm formation ability of *Salmonella* spp. in poultry industry has been expanded over the years. However, in spite of the research efforts and significant investments to improve management systems in poultry industry, it has become evident that none of the methods applied in all stages of food production chain are 100% effective in eliminating *Salmonella* spp. Different serovars are manifesting different mechanisms of invasiveness which depend on their ability to invade lower zones of the lamina propria, their ability to gain accesses to parenchymatous organs and survive in macrophages. The ubiquitous nature of *Salmonella* spp. due to their adaptation to animal and plant hosts, as well as their survival in hostile environments and their enhanced capacity to produce biofilms, contribute to a long lasting contamination of the environment, feed and animals. The emergency and spread of antimicrobial resistances in *Salmonella* spp. raise additional concerns.

**Keywords:** *poultry, Salmonella, pathogenesis, biofilm, resistance*

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## INTRODUCTION

Poultry farming presents one of the most important food manufacturing industries around the globe. Therefore, food safety standards are highly demanding and are generally better maintained in large scale production facilities than in small ones. In developing countries, rearing of backyard chicken flocks contributes to the continuous occurrence of some viral and bacterial diseases that are less likely present in well maintained farms. Except for a very few countries in the world, *Salmonella* spp. are detected in environmental specimens in practically all stages of the food production chain. Out of more than the 2600 serovars known today, only 10% are found in the commercial poultry and egg industry. Two of them, *S. Enteritidis* and *S. Typhimurium*, are of paramount importance to human health and can colonize the intestines of chickens (Velge et al., 2005). In most cases, the infected chickens either do not have clinical symptoms or the symptoms remain unnoticed.

With all this taken into account, it is evident that control programs for *Salmonella* spp. have to be implemented in all stages of the food production chain, starting from animal farms. According to European Directive 1003/2005, the occurrence of *S. Enteritidis* and *S. Typhimurium* in adult breeder flocks has to be  $\leq 1\%$ , in EU member states. However, this directive also targets serovars Hadar, Virchow and Infantis which are of public health significance in the EU (Carrique-Mas and Davies, 2008). It is very difficult to accomplish such a goal in developing countries, since implementing good management practice is expensive and requires the participation of educated staff. Even if biosecurity measures are well established on a farm, salmonellae can still be found in poultry and premises.

Other available measures to cope with *Salmonella* spp. in farms include the use of prebiotics and probiotics, antimicrobial therapy and vaccination of the birds. For serovars *S. Enteritidis* and *S. Typhimurium* commercial inactivated and attenuated vaccines have been developed and used widely. These vaccines target serogroups D and B respectively, but do not protect livestock against serovars from other serogroups. Therefore, vaccination against *S. Enteritidis* and *S. Typhimurium* could lead to the elimination of these two serovars on farms, opening

a vacant ecological niche, enhancing, thus, the emergence of new serovars, such as *S. Kentucky* or *S. Heidelberg* (Foley et al., 2011).

The framework of National control programs in European Union member states includes the vaccination of layer flocks during rearing which has to be mandatory in cases of 10% prevalence of *S. Enteritidis* (EC No 1168/2006 and EC No 1177/2006). Live vaccines could be used only in cases when the discrimination of vaccine versus wild type *Salmonella* is possible and the ban of antibiotic use in layers has been initiated (Carrique-Mas and Davies, 2008). Such high demands have motivated a number of research works aiming to find the best sampling strategy and the best monitoring systems for *Salmonella* spp. control all around the world.

The most convenient methods of taking samples for bacteriological analysis from poultry houses are using boot swabs or the "step on a drag swab" method (Buhr et al., 2007). Official sampling is carried out while birds are in the unit while own checks are carried out not only while livestock is in the unit but also after depopulation. Own check programs must be approved by the competent authorities. The sampling strategy aiming to detect and control *Salmonella* spp in adult breeding flocks of *Gallus gallus* is defined in Commission Regulation EU No 200/2010 and for laying hens in Commission Regulation (EU) No 517/2011. Reduction of the prevalence of the serovars *Enteritidis* and *Typhimurium* in flocks of turkeys is required and the sampling strategy is defined in Commission regulation (EC) No 584/2008. After cleaning and disinfection, swabs are collected from walls, floor, vents, drinking and feeding systems, changing rooms and other areas that may be exposed to external contamination. It is important to collect as many swabs as possible to determine the success of cleaning and disinfection. The same strategy applies for hatcheries which may become contaminated with the pathogen. In fact, *Salmonella* spp. can be effectively disseminated in the hatchery cabinet and chickens may become infected before removing from the hatchery (Bailey et al., 1998).

According to a longitudinal study of environmental *Salmonella* contamination in caged and free-range layer flocks carried out by Wales et al. (2007), the timing of taking samples has been shown to have a

significant influence on *Salmonella* spp. isolation. Flocks that remained longer on the premises yielded more isolates comparing to the new flocks. The temperature and the season also had an influence on *Salmonella* spp. populations, proving increased isolation rate during summer. The role of other animal reservoirs harboring *Salmonella* in and outside the farms is also significant (Guard-Petter, 2001). *Salmonella* spp. in wildlife vectors correlated well with the status of the flock and the same serovar and phage type could be found in wild predators caught around the farm and poultry. Cleaning and disinfection in cases when organic matter had been substantially removed and disinfectants were adequately applied and in proper concentration, had a positive influence on *Salmonella* control. However, the wildlife reservoirs, multiage farming and lack of “all in all out” strategy highlight the need for vaccination and the use of probiotics in flocks with high and low incidence of the pathogen’s load or even in cases that it is absent (Wales et al., 2007). Another study by Dewaele et al. (2012) which aimed to examine the *Salmonella enterica* serovar Enteritidis environmental contamination on persistently positive layer farms in Belgium during successive laying cycles showed that in contaminated poultry houses, neither vaccination nor cleaning and disinfection are considered as the only prerequisite for successful elimination of *Salmonella* spp. from the environment and that the chances for *Salmonella* spp. elimination were better in less contaminated poultry farms, comparing to those in highly contaminated environments. This is even more pronounced if rodents, flies and mites come into contact with poultry or equipment. In addition the authors concluded that there is a possibility that even if poultry houses are separately cleaned and disinfected, egg collection areas may still become a reservoir of *Salmonella* spp. In fact, the egg collection areas may become contaminated with a few serovars which are present on the entire farm.

### THE PATHOGENESIS, TISSUE INVASION AND IMMUNE RESPONSES

*Salmonella* spp. possesses an arsenal of genetic determinants responsible for colonization, adhesion, invasion and proliferation in host cells, including

fimbriae, flagella, toxins, surface lipopolysaccharides (LPS), etc. Virulence genes are organized in clusters and spread throughout the chromosome, such as *Salmonella* Pathogenicity Islands 1 and 2 (SPI-1, SPI-2), or located on virulence plasmids, such as *spv* genes (associated with invasive strains). *Salmonella* pathogenicity genomic islands carry genes that are required for successful infection in poultry (Wisner et al., 2012). Noninvasive strains cause gastroenteritis, while invasive strains may cause systemic bacteremia in humans and animals. The outcome of infection depends on virulence factors, the pathogenesis of *Salmonella* spp. and their interaction with the host organism (Foley et al., 2013). Unlike noninvasive strains, invasive *Salmonella* strains penetrate through the epithelial lining to the lower parts of the lamina propria. Also, invasive strains are commonly isolated from parenchymatous organs (spleen, liver, ovaries) and a small number of bacteria become internalized by macrophages (Berndt et al., 2007). The survival in the acidic environment of the stomach is enabled by the activation of more than 50 acid tolerance response proteins (Bearson et al., 2006). The first phase of the infection has to provide a chance for the bacteria to invade intestinal epithelial cells. This process is accomplished by proteins encoded by *Salmonella* Pathogenicity Island (SPI-1) type III secretion system (T3SS). These organelles produce a special structure in the bacterial envelope called “the needle complex” which delivers toxins and other effector proteins and injects them into the host cells (Kubori et al., 2000). Bacterial effector proteins modulate the host actin cytoskeleton and initiate the signal transduction pathways required for the internalization of the bacteria. In addition, invasive strains recruit their own systems responsible for survival in macrophages. *Salmonella* spp. become internalized in a specific membrane bound compartment called “*Salmonella* containing vacuole” (SCV). The maturation of the SCVs and their migration to the basal membrane disable the destruction of the bacteria by phagolysosomes. Such intracellular trafficking and intracellular pathogenesis is also accomplished by the activation of the second T3SS encoded by the SPI-2. Hence, the type III secretory system encoded by SPI-1 and SPI-2 enables the attachment, invasion and survival of the pathogen within the

host cell, as well as the avoidance of antimicrobial compounds (Hensel, 2000; Foley et al., 2013). Most of *Salmonella* serovars contain SPI-1 to -5, while other pathogenicity islands are not so common. The colonization of the gastrointestinal tract and of the internal organs of poultry is enabled by the type VI secretion system encoded by the SPI-19 locus present in serovar Gallinarum (Blondel et al., 2010). In mice infected with serovar Typhimurium, the SPI-6 was necessary for the intracellular replication of the pathogen in macrophages and its systemic dissemination. The experimental work indicates that T6SS encoded by both SPI-6 and SPI-19 gene clusters are genetically involved in bacterial pathogenesis and that T6SS-SPI-6 play a role in gastrointestinal colonization and systemic spread of serovar Typhimurium in chickens (Pezoa et al., 2013).

Besides *Salmonella* pathogenicity islands-1 and 2, *Salmonella* strains involved in extraintestinal non-typhoid disease with bacteremia carry additional virulence genes in a *spv* locus, contained on virulence plasmids (Guiney and Fierer, 2011). Genes *spv* were found in serovars Typhimurium, Enteritidis, Choleraesuis, Abortusovis, Dublin, Gallinarum/Pullorum and in subspecies *arizonae*. The plasmid genes in the *spv* locus include *spvABCD* operon which is positively regulated by the upstream *spvR* gene. Only *spvR*, *spvB* and *spvC* are responsible for *spv* related virulence phenotype. In spite of having different biochemical pathways of action, SpvB and SpvC proteins are eventually involved in late apoptosis of macrophages, enabling the intracellular proliferation of *Salmonella* spp. Subsequent uptake of apoptotic macrophages by surrounding macrophages, facilitates cell to cell spread of *Salmonella* spp. (Guiney and Lesnick, 2005; Derakhshandeh et al., 2013). Consequently, it potentiates the systemic spread of the pathogen instead of causing a self limited gastroenteritis.

Salmonellae have different invading capacities in the poultry intestine and parenchymatous organs. They trigger systemic and local immune response which is in good correlation with their virulence. Experimental work was conducted by Berndt et al., (2007) to measure the immune response in cecum after the infection of White Leghorn day old chickens with serovars Enteritidis, Typhimurium,

Hadar and Infantis. At 2, 4 and 7 days post infection (pi) serovars Hadar and Infantis showed diminished invading capabilities for liver, compared to serovars Enteritidis and Typhimurium. *S. Enteritidis* was the best invader of the lower zones of the lamina propria, while *S. Infantis* was found in epithelial lining and subepithelial region. The increase of granulocytes, TCR1 gd and CD8 $\alpha$ + in chicken cecum was most prominent for serovar Enteritidis, followed by serovars Typhimurium and Hadar, while Infantis provoked less significant immune cell influx. In the same study the reorganization of the extracellular matrix proteins, notably the increase of total fibronectin and tanascin-C, has been more pronounced after the infection of day old chickens with serovar Enteritidis comparing to the infection with the non invasive *Salmonella* Infantis. Furthermore, enhanced *Salmonella* spp. entry and the ability to disseminate in the gut epithelium support the concept that the most virulent strains utilize distinctive genetic mechanisms to invade the intestine and disseminate through the body, showing an important ability to provoke better immune responses in infected birds, as well (Berndt et al., 2009). It was experimentally shown that *S. Infantis* was found in higher numbers in avian macrophages in vitro comparing to *S. Typhimurium*, but the number of viable cells inside macrophages was higher for *S. Typhimurium* than for *S. Infantis* (Braukmann et al., 2015). Both serovars trigger active immune responses by activating genes involved in regulating immunological processes. The infection of avian macrophages with both serovars induced the increased expression of the immune mediators up to four hours post infection. The longer survival of serovar Typhimurium in macrophages was probably related to a higher and rapid SPI-2 genes activation, which explains the better invasiveness and the ability of causing systemic infection, something observed in serovar Typhimurium, but not in Infantis. The unfimbriated state of *Salmonella* spp. and *Escherichia coli* in chicken intestine are manifesting good colonizing ability in the intestine and oviducts of laying hens at 19 weeks of age as described by De Buck et al. (2004). However, the egg content, particularly the yolk and the egg shell, was contaminated by the wild type strain more efficiently.

Although the type 1 fimbriae deficient mutant caused prolonged bacteraemia in laying hens, the reduced egg shell contamination in mutant comparing to wild type strain, has shown that fimbriae are important for causing egg contamination in serovar Enteritidis (De Buck et al., 2004).

### THE PREVALENCE OF *SALMONELLA* SEROVARS IN POULTRY FLOCKS

The rise of *S. Enteritidis* during the 1980s and 1990s coincided with the extensive measures undertaken to eradicate *S. Gallinarum*. It is suggested that *S. Enteritidis* has taken the ecologic niche previously occupied by *S. Gallinarum* in poultry flocks, via the mechanism of competitive exclusion, due to their antigenic similarity (Rabsch et al, 2000). Clearing the commercial flocks from *S. Gallinarum* enabled *S. Enteritidis* to colonize chickens without signs of disease (Andino and Hanning, 2015). In addition, serovar Enteritidis has a wider spectrum of natural reservoirs which makes it easier to persist on the farms. It has been isolated from insects, rodents, nematodes, wild birds and other animal hosts living in and around hen houses. Thus, after adequate disinfection of houses and stocking with culture-negative chicken, *S. Enteritidis* can be reintroduced from hen house pests, especially mice (Guard-Petter, 2001).

In the United States of America, *S. Enteritidis* which was dominant in the 1990s, was supplanted by serovar Heidelberg in the period 1997-2006, but since 2007 *S. Kentucky* has been the most prevalent serovar isolated from poultry (Foley et al., 2011). However, these serovars are less common in humans, with serovars Enteritidis and Typhimurium being the leading causes of alimentary toxoinfections in the USA. There are several possible reasons for the prevalence of serovars Kentucky and Heidelberg: flock immunity against *S. Enteritidis* gained due to vaccination or exposure might have opened the space for these two antigenically different serovars to which the flocks were susceptible (Foley et al., 2011). The ability of *S. Heidelberg* to colonize the reproductive tract in chickens and enter eggs, poses a threat to public health as another important egg transmitted pathogen, besides *S. Enteritidis* and *S. Typhimurium* (Gast et al., 2004). Although *S. Kentucky* is not so commonly involved in human infections, it is

very successful in colonizing chicken. One of the reasons might be the acquisition of the virulence plasmids ColBM and ColV from the avian pathogenic *Escherichia coli* (APEC) (Johnson et al., 2010).

In the past few years, the emergence of *S. Kentucky* strains resistant to multiple antimicrobial drugs has become a new threat to human and animal health. The international trade has facilitated the spread of those strains to the domestic poultry in the region of Mediterranean basin (Le Hello et al., 2013).

The experimental infection of two day old broiler chickens has revealed that serovar Kentucky persisted longer in the cecum comparing to Typhimurium and the peak was noted at 25 days pi (Cheng et al., 2015). Compared to *S. Typhimurium*, the expression of genes regulated by RNA polymerase sigma S factor (rpoS) was more pronounced in serovar Kentucky in the ceca content. The expression of genes from the metabolic pathway and the role of curli production seem to be in correlation with the ability of serovar Kentucky to colonize and persist in poultry.

Unlike other serovars, *S. Gallinarum* biovars Gallinarum and Pullorum are restricted to avian species and do not pose a risk to human health. However, among poultry, they cause septicemic fowl typhoid and pullorum disease (respectively) with high mortality and morbidity. Strict control programs using serological tests and elimination of positive birds has lead to the eradication of diseases from commercial poultry in the United States of America, Canada and most of Western Europe, although outbreaks occasionally occur (Barrow and Freitas Neto, 2011).

In the European Union, harmonized *Salmonella* control programs have lead to the overall decrease in the prevalence of five serovars (*S. Enteritidis*, *S. Typhimurium*, *S. Infantis*, *S. Hadar*, *S. Virchow*) of the public health relevance. However, in 2015 there was a slight increase in *S. Enteritidis* incidence comparing to 2014, but *S. Infantis* was the most prevalent serovar among domestic fowl (EFSA 2016a).

### BIOFILM FORMING CAPACITY OF *SALMONELLA* SPP. IN POULTRY AND FEED INDUSTRY

Because of the profound ability to irreversibly bind to different types of biotic and abiotic surfaces,

the Most Prevalent Poultry-associated *Salmonella* serotypes (MPPSTs) usually have a capacity of biofilm (BF) formation on plant surfaces, in the host organism, as well as in a variety of materials commonly used in the poultry production and feed industry (Steenackers et al., 2012; White and Surette, 2006). Hence, BF formation is a common feature of bacteria and it is characterized as a complex surface associated community of microorganisms. Biofilm is defined as matrix-enclosed bacterial populations adherent to each other and/or on surfaces or interfaces (Donlan 2002; Donlan and Costerton 2002). Bacteria with the ability to form biofilms express different genes comparing to their planktonic counterparts, becoming increasingly resistant to antibiotics and disinfectants. Indeed, the resistance of bacteria in the BF may be 10 to 1000 times higher comparing to the bacteria in suspension, which is most often used for the examination of the effectiveness of disinfectants or other antimicrobial compounds, such as antibiotics (Mah and O'Toole, 2001). Hence, biofilm is the perfect microenvironment for the horizontal transfer of genetic material and the emergence of pathogens with new virulence factors and mechanisms of antibiotic resistance.

In a number of experimental studies, the ability of *Salmonella* to form BF on a variety of materials such as concrete, glass (Prouty and Gunn, 2003), cement (Joseph et al., 2001), stainless steel (Oliveira et al., 2007), plastic (Stepanović et al., 2004; Solomon et al., 2005), granite and rubber (Arnold and Yates, 2009) was confirmed (Solano et al., 2002; Steenackers et al., 2012). *Salmonella* spp. can rapidly colonize hydrophobic substrate, such as plastic, and they commonly produce a BF on them. Plastic materials are widely used on farms, in slaughterhouses and in food industry for the preparation of tanks, pipe-work, accessories and cutting surfaces (Diez-García et al., 2012). The microorganism easily forms a BF on galvanized steel, which is used for making transport containers for poultry (Ramesh et al., 2002). Various serovars of *Salmonella* spp. are characterized by a good ability to produce BF, which enables their persistence in poultry facilities, hatcheries, the water supply systems on farms, slaughterhouses, as well as in

processing and storage facilities of poultry products (Gradel et al., 2003; Gradel et al., 2004; McKee et al., 2008; Díez-García et al., 2012).

Biofilm is an important risk factor in feed contamination, and one of the critical points of controlling *Salmonella* spp. on poultry farms, having an increasing importance in the last decades (Cox and Pavic, 2010). The contamination of feed with *Salmonella* spp. may occur as a consequence of the use of contaminated raw materials or it may occur during the production process, by getting in contact with contaminated surfaces in production facilities. Biofilm formation is involved in both processes. The main components of the *Salmonella* BF-matrix, the protein surface aggregative fimbriae (curli) and the extracellular polysaccharide cellulose, are required for the colonization of plant surfaces and for the attachment to the surface of the feed factory environment. These biofilms allow the persistence of *Salmonella* spp. in feed and food factory environments for months, and even years (Vestby et al., 2009; Schonewille et al., 2012; Prunić et al., 2016).

In slaughterhouses and facilities for processing poultry carcasses, *Salmonella* spp. are found continuously, despite the regular use of strict measures for the control and reduction of pathogens (Rose et al., 2000; Joseph et al., 2001; Gradel et al., 2004; Marin et al., 2009). Research shows that conventional methods of disinfection are ineffective in eliminating *Salmonella* spp. from surfaces on which fresh meat processing is carried out (McKee et al., 2008). It is also experimentally evaluated that only two out of 13 commercially available disinfectants based on sodium hypochlorite, sodium chlorite and alkaline peroxide were effective against *Salmonella* biofilms formed on galvanized steel in the presence of organic matter (Ramesh et al., 2002). In field conditions, methods of cleansing and disinfection are often insufficient for *Salmonella* spp. elimination from poultry housing facilities (Marin et al., 2011; Davies and Breslin, 2003). The BF-matrix, particularly the extracellular polysaccharide cellulose, is considered to be an important factor for the protection against chemical agents.

The purpose of maintaining a dry environment in feed and food factories and low water activity in the finished product is to reduce pathogens, but these

measures are not effective in controlling *Salmonella* spp. In some *Salmonella* strains, including those of serovar Enteritidis, isolated from food products with low water activity, an increase in virulence and the reduction of the infective dose was found (Aviles et al., 2013; Andino et al., 2014). It is believed that the increasing virulence of *Salmonella* spp. in products with low water activity is the result of *rpoS* activation (the main stress response regulator), which directly affects the activation of virulence genes such as the *invA*, *hilA* and *sipC* (Aviles et al., 2013). However, experimental studies show that genes *invA* and *hilA* in *S. Enteritidis* are down regulated in low water activity, but the exact reason for the increased virulence of this serovar remains unknown (Andino et al., 2014).

Differences in the ability to produce the BF are established among different serovars, or strains of the same *Salmonella* serovar (Schonewille et al., 2012). However, *in vitro* conditions used in research on BF formation capacities, may not always reflect the conditions required for BF formation in the environment. Bacteria express important features that enable them to adapt under various challenges and the formation of the BF communities presents an important defense mechanism.

Biofilm is a risk that has been recognized recently as it causes long term contamination and persistency of some *Salmonella* serovars in all cycles of the poultry industry. It also presents actual research challenge in raising food producing animals and in safe food production. There are no effective measures to prevent or remove BF. Starting with the fact that multiple sources of contamination with *Salmonella* spp. are recognized, the only way to cope with *Salmonella* spp. in poultry production facilities is good management practice and high biological safety. Innovations in the field of BF control refer to the compounds that actually inhibit biosynthesis of signal molecules in BF, but they are not applicable in poultry and food industry at present.

## RESISTANCE TO ANTIMICROBIAL AGENTS IN *SALMONELLA* SPP. FROM POULTRY SPECIMENS

Multiple drug resistance (MDR) of *Salmonella* spp. in poultry is developing because of the established

practice of using antibiotics in animal husbandry. There is evidence that some resistant *Salmonella* strains have increased virulence, which could be a result of the integration of virulence and resistance plasmids and their co-selection or up regulation of the virulence or the improved fitness of the bacteria (Mølbak, 2005).

It is widely considered that antibiotics used in human medicine should be avoided for the therapy of animals. Such practice is well established in developed countries except for rare cases, as for the treatment of infections caused by susceptible bacteria (Garcia-Migura et al., 2014). However, travelling and trade have a high impact on establishing MDR microorganisms in their communities. Besides the restrictive use of antibiotics in developed countries, growth promoter use was also banned in the year 2006 and the overall resistance rate in commensal and pathogenic bacteria from food producing animals has been decreasing. In developing countries resistance to fluoroquinolones and extended spectrum beta lactams is still worrisome. It has been recorded that multiple drug resistant *S. Kentucky*, *S. Typhimurium* and *S. Infantis* have a worldwide distribution and that poultry present permanent (*S. Infantis*, *S. Typhimurium*) or transient reservoirs (*S. Kentucky*). Emerging strains of *S. Kentucky* resistant to carbapenems and fluoroquinolones may cause life threatening disease in humans and they are among the most dangerous *Salmonella* serovars that have been diagnosed recently (LeHello et al., 2013). The first report of the occurrence of extended spectrum  $\beta$ -lactamase (ESBL) resistant *S. Kentucky* from poultry specimens (whole chicken, farm dust and chicken neck skin) in Ireland was attributed to *bla*<sub>SHV-12</sub> and *bla*<sub>CMY-2</sub> genes. Even though cephalosporins are not applicable for the therapy of chickens in Ireland, there is a possibility that the use of amoxicillin has favored the selection of  $\beta$ -lactamase producers over the time (Boyle et al., 2010). *Salmonella Kentucky* designated CVM29188 isolated from a chicken breast sample in the year 2003 has shown resistance to streptomycin, tetracycline, ampicillin and ceftiofur. All the genes determining resistances (*strAB* and *tetRA*, *bla*<sub>CMY-2'</sub>, *sugE*) were found on two large transmissible plasmids. In addition, the pCVM29188\_146 plasmid

is genetically similar to the virulence plasmids found in avian pathogenic *Escherichia coli* (APEC). These APEC-like plasmids were probably exchanged among the two bacteria species in the intestinal environment and they also possess virulence elements that have contributed to their establishment in predominant *Salmonella* Kentucky strains in chicken intestines and meat (Fricke et al., 2009).

Serovar Infantis is a typical poultry *Salmonella* serovar. It is well established on poultry farms with a tendency of clonal spread of the multidrug resistance phenotype. Clonal spread of *Salmonella* Infantis in poultry and poultry meat was reported in Japan (Shahada et al., 2006), Hungary (Nógrády et al., 2007), Israel (Gal-Mor et al., 2010), Italy (Dionisi et al., 2011), Germany (Hauser et al., 2012), Serbia (Rašeta et al., 2014; Velhner et al., 2014) but also in humans in Argentina (Merino et al., 2003) and Brazil (Fonseca et al., 2006). All these clonal strains were resistant to three or more antimicrobials except for Serbia, where the predominant resistance phenotype was nalidixic acid (NAL) / tetracycline (TET), while an approximate 30% of the isolates was showing resistance to ciprofloxacin (CIP), with the minimal inhibitory concentration (MIC) of  $\geq 1\text{mg/L}$  (Velhner et al., 2014). The resistance to CIP was also found in some isolates of *Salmonella* Infantis from Hungary which belonged to the different pulsotype (Nógrády et al., 2007). The occurrence of novel multidrug resistant clones from human, food and poultry sources in Israel was established in 2007. These clones were resistant to NAL, TET, nitrofurantoin and trimethoprim/sulfamethoxazole (SXT). It was evident that the resistance to TET and SXT was encoded by a 280kb self-transmissible plasmid (pESI) and that new clones represented 33% of all *Salmonella* strains isolated in Israel (Aviv et al., 2014).

The most frequently detected serovars in poultry meat in the EU were *S. Infantis*, *S. Indiana* and *S. Enteritidis*. According to the epidemiological cut off breakpoints (ECOFFs), multi-drug resistance in *Salmonella* spp from broiler meat in the year 2014 fluctuated from high (Hungary) to low (France and Lithuania) or complete absence of resistance (Ireland). Resistance to colistin was 31.6% in *S. Enteritidis* while resistance to ciprofloxacin and nalidixic acid was 22.4%. No resistance toward

colistin was recorded in *S. Infantis*. High rate of multi-drug resistance was detected in some EU countries in *Salmonella* spp. isolates from turkey meat. In flocks of boilers, the most prevalent was *S. Infantis* with extremely high resistance rate to ciprofloxacin (except in Denmark and Spain). Second most frequently detected serovar in broilers was *S. Enteritidis* with overall resistance to ciprofloxacin and nalidixic acid of 23.3 and 24.6% respectively. Levels of resistance to ciprofloxacin were high in *Salmonella* spp. isolates from layer flocks in Cyprus, Hungary, Italy and Romania. However, trends in multi drug resistance were much lower in *Salmonella* spp. from layers comparing to broilers in the EU member states (EFSA 2016b). In the report of the National Antimicrobial Resistance Monitoring System (NARMS) USDA of 2011, it was documented that the most prevalent serovars from poultry in the USA were: Kentucky, Enteritidis, Heidelberg, Typhimurium var-5 and Infantis (NARMS-USDA, 2014). Resistance to beta lactam/inhibitor combination and cepheims was found in 17.9% of serovar Heidelberg isolates and in 0.7% of serovar Enteritidis isolates, regarding poultry. Resistance to (fluoro)quinolones was not found, while resistance to gentamicin was evident in 1.3% of the serovar Kentucky isolates, 14.3% of the serovar Heidelberg isolates and in 10.5% of the serovar Typhimurium var-5 isolates from poultry in 2011 (NARMS-USDA, 2014).

Poultry meat and products therefore present a significant reservoir of resistant *Salmonella* all around the world. However, the resistance patterns differ markedly from continent to continent and among countries. In this respect, the highest concern is the resistance of serovars Kentucky and Infantis which become well established in poultry flocks and frequently develop a multidrug resistant phenotype.

## CONCLUDING REMARKS

Much effort has been put through to provide safe poultry meat and products worldwide. In spite of the fact that many biological, chemical products and vaccines have been invented and implemented in poultry production systems, it is still difficult to eliminate *Salmonella* spp. from the food chain. Different *Salmonella* serovars tend to take place

in commercially produced poultry, as soon as an ecological niche becomes vacant. In many developed countries, where measures, such as vaccination, were undertaken to eradicate certain *Salmonella* serovars, other less immunogenic serovars emerge and become dominant. *Salmonella* control programs in poultry industry has to cover all the segments of food production by implementing various procedures and strategies in integrated poultry production systems. It has to follow up new trends in raising free range chickens with respect to new challenges regarding food safety in upcoming years. In countries where comprehensive programs have been implemented to

eliminate *Salmonella* spp. from the food production chain, travelling and trade still pose and will continue to pose a substantial risk for infection of humans and efficient dissemination of *Salmonella* spp. globally.

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#### CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interests. ■

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