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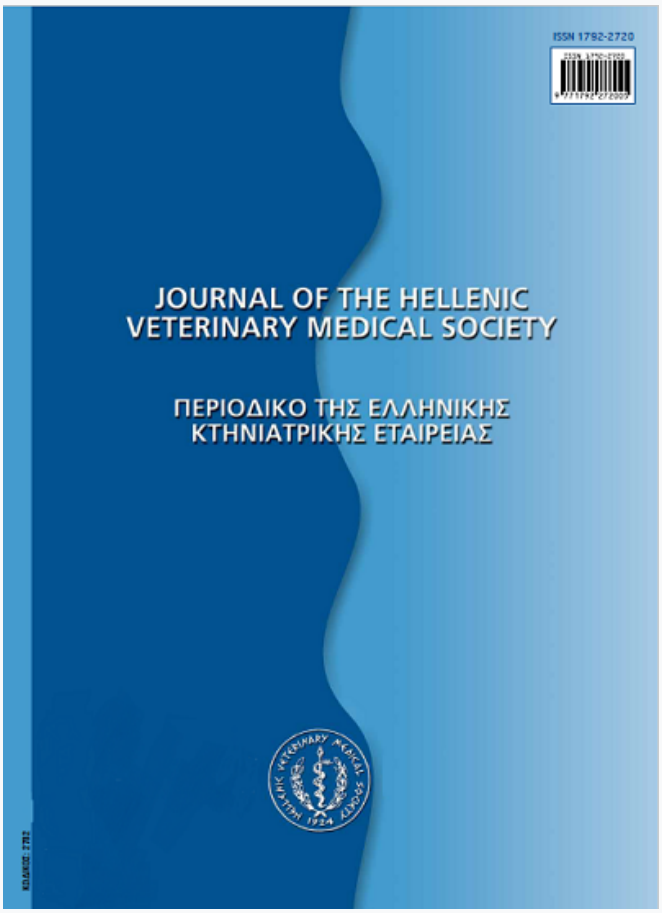

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M. VELHNER, D. MILANOV, G. KOZODEROVIĆ

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Review article
Ανασκόπηση

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

M. Velhner[1], D. Milanov[1], G. Kozoderović[2*]

[1] *Department of clinical microbiology, Scientific Veterinary Institute "Novi Sad", Novi Sad, Serbia*

[2] *Faculty of Education in Sombor, University of Novi Sad, Serbia*

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*

Corresponding Author:
Gordana Kozoderović
Faculty of Education in Sombor, University of Novi Sad,
Podgorička 4, Sombor 25000, Serbia
E-mail : gocakozoderovic@gmail.com

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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 ≤ 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 parenhymatous 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 CD8α+ 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 (Díez-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 *rpo*S activation (the main stress response regulator), which directly affects the activation of virulence genes such as the *inv*A, *hil*A and *sip*C (Aviles et al., 2013). However, experimental studies show that genes *inv*A and *hil*A 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 lactames 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 β-lactamase (ESBL) resistant *S*. Kentucky from poultry specimens (whole chicken, farm dust and chicken neck skin) in Ireland was attributed to $bla_{\text{SHV-12}}$ and $bla_{\text{CMY-2}}$ 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 β-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,* $bla_{\text{CMY-2}}$, *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 ≥ 1mg/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/sulfametoxazole (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 colisitin was 31.6% in *S*. Enteritidis while resistance to ciprofloxacin and nalidixic acid was 22.4%. No resistance toward colisitin 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, Typhimurum var-5 and Infantis (NARMS-USDA, 2014). Resistance to beta lactam/inhibitor combination and cephems 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.

REFERENCES

Andino A, Pendleton S, Zhang N, Chen W, Critzer F, Hanning I (2014) Survival of Salmonella enterica in poultry feed is strain dependent. Poult Sci 93:441–447.

Andino A, Hanning I (2015) Salmonella enterica: survival, colonization and

virulence differences among serovars. Scientific World J Article ID 520179. http://dx.doi.org/10.1155/2015/520179

Arnold JW, Yates IE (2009) Interventions for control of Salmonella: Clearance of microbial growth from rubber picker fingers. Poult Sci 88:1292−1298.

Aviles B, Klotz C, Smith T, Williams R, Ponder M (2013) Survival of Salmonella enterica serotype Tennessee during simulated gastric passage is improved by low water activity and high fat content. J Food Prot 76:333-337.

Aviv G, Tsyba K, Steck N, Salmon-Divon M, Cornelius A, Rahav G, Grassi GA, Gal-Mor O (2014) A unique megaplasmid contributes to stress tolerance and pathogenicity of an emergent Salmonella enterica serovar Infantis strain. Environ Microbiol 16:977-994.

Bailey JS, Cason JA, Cox NA (1998) Effect of Salmonella in young chicks on competitive exclusion treatment. Poult Sci 77:394-399.

Barrow PA, Freitas Neto OC (2011) Pullorum disease and fowl typhoid – new thoughts on old diseases: a review. Avian Pathol 40:1-13.

Bearson SM, Bearson BL, Rasmussen MA (2006) Identification of Salmonella enterica serovar Typhimurium genes important for survival in the swine gastric environment. Appl Environ Microbiol 72:2829–2836.

Berndt A, Wilhelm A, Jugert C, Pieper J, Sachse K, Methner U (2007) Chicken cecum immune response to Salmonella enterica serovars of different levels of invasiveness. Infect Immun 75:5993-6007.

Berndt A, Müller J, Borsi L, Kosmehl H, Methner U, Berndt A (2009) Reorganisation of the caecal extracellular matrix upon Salmonella infection-relation between bacterial invasiveness and expression of virulence genes. Vet Microbiol 133:123-137.

Blondel CJ, Yang HJ, Castro B, Chiang S, Toro CS, Zaldívar M,

Contreras I, Andrew-Polymenis HL, Santiviago CA (2010) Contribution of the type VI secretion system encoded in SPI-19 to chicken colonization by Salmonella enterica serotypes Gallinarum and Enteritidis. PLoS One. 5(7), e11724, doi:10.1371/journal.pone.0011724.

Boyle F, Morris D, O'Conor J, DeLappe N, Ward J, Cormican M (2010) First report of extended-spectrum-β-lactamase-producing Salmonella enterica serovar Kentucky isolated from poultry in Ireland. Antimicorb Agents Chemother 54:551-553.

Braukmann M, Methner U, and Brendt A (2015) Immune reaction and survivability of Salmonella Typhimurium and Salmonella Infantis after infection of primary avian macrophages. PLoS One. 10(3), e0122540, doi:10.1371/journal.pone.0122540.

Buhr RJ, Richardson LJ, Cason JA, Cox NA, Fairchild BD (2007) Comparison of four sampling methods for the detection of Salmonella in broiler litter. Poult Sci 86:21-25.

Carrique-Mas JJ, Davies RH (2008) Salmonella Enteritidis in commercial layer flocks in Europe: Legislative background, on farm sampling and main challenges. Braz J Poultry Sci 10:1-9.

Cheng Y, Pedroso AA, Porwollik S, McClelland M, Lee MD, Kwan T, Zamperini K, Soni V, Sellers HS, Russell SM, Maurer JJ (2015) rpoS-Regulated core genes involved in the competitive fitness of Salmonella enterica serovar Kentucky in the intestines of chickens. Appl Environ Microbiol 81:502-514.

Commission Regulation (EC) No 1177/2006: Implementing Regulation (EC) No 2016/2003 of the European Parliament and of the Council as regards requirements for the use of specific control methods in the framework of the national programmes for the control of salmonella in poultry.

Commission Regulation (EC) No 1168/2006: Implementing Regulation (EC) No 2160/2003 as regards a Community target for the reduction of the prevalence of certain salmonella serotypes in laying hens of Gallus gallus and amending Regulation (EC) No 1003/2005.

Commission Regulation (EC) No 584/2008: Implementing Regulation (EC) No 2160/2003 of the European Parliament and of the Council as regard a Community target for the reduction of the prevalence of Salmonella enteritidis and Salmonella Typhimurium in turkeys.

Commission Regulation (EC) No 200/2010: Implementing regulation (EC) No 2160/2003 of the European Parliament and of the Council as regards a Union target for the reduction of the prevalence of Salmonella serotypes in adult breeding flocks Gallus gallus.

Commission Regulation (EC) No 517/2011 implementing Regulation (EC) No 2160/2003 of the European Parliament and of the Council as regards a Union target for the reduction of the prevalence of certain Salmonella serotypes in laying hens of Gallus gallus and amending Regulation (EC) No 2160/2003 and Commission Regulation (EU) No 200/2010.

Cox JM, Pavic A (2010) Advances in enteropathogen control in poultry production. J Appl Microbiol 108:745-755.

Davies RH, Breslin M (2003) Observations on Salmonella contamination of commercial laying farms before and after cleaning and disinfection. Vet Res 152, 283-287.

De Buck J, Van Immerseel F, Haesebrouck F, Ducatelle R (2004) Effect of type 1 fimbriae of Salmonella enterica serotype Enteritidis on bacteraemia and reproductive tract infection in laying hens. Avian Pathol 33:314-320.

Derakhshandeh A, Firouzi R, and Khoshbakht R (2013) Association of three plasmid-encoded spv genes among different Salmonella serotypes isolated from different origins. Indian J Microbiol 53:106–110.

Dewaele I, Van Meirhaeghe, H, Rasschaert G, Vanrobaeys M, De Graef E, Herman L, Ducatelle R, Heyndrickx M, De Reu K (2012) Persistent Salmonella Enteritidis environmental contamination on layer farms in the context of an implemented national control program with obligatory vaccination. Poult Sci 91:282-291.

Díez-García M, Capita R, and Alonso-Calleja C (2012) Influence of serotype on the growth kinetics and the ability to form biofilms of Salmonella isolates from poultry. Food Microbiol 31:173-180.

Dionisi AM, Lucarelli C, Benedetti I, Owczarek S, Luzzi I (2011) Molecular characterization of multidrug-resistant Salmonella enterica serotype Infantis from humans, animals and the environment in Italy. Int J Antimicrob Agents 38:384-389.

Donlan RM (2002) Biofilms: microbial life on surfaces. Emerg Infect Dis 8:881–890.

Donlan RM, Costerton JW (2002) Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 15:167−193.

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2016a. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. EFSA Journal 2016 14(12):4634, p231, doi:10.2903/j.efsa.2016.4634

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2016b. EU Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2014. EFSA Journal 2016 14(2):4380, p60, doi:10.2903/j.efsa.2016.4380.

Foley SL, Nayak R, Hanning IB, Johnson TJ, Han J, Ricke SC (2011) Population dynamics of Salmonella enterica serotypes in commercial egg and poultry production. Appl Environ Microbiol 77:4273-4279.

Foley SL, Johnson TJ, Ricke SC, Nayak R, Danzeisen J (2013) Salmonella pathogenicity and host adaptation in chicken-associated serovars. Microbiol Mol Biol Rev 77:582-607.

Fonseca EL, Mykytczuk OL, Asensi MD, Reis EMF, Ferraz LR, Paula FL, Ng LK, Rodrigues DP (2006) Clonality and antimicrobial resistance gene profiles of multidrug-resistant Salmonella enterica serovar Infantis isolates from four public hospitals in Rio de Janeiro, Brazil. J Clin Microbiol 44:2767-2772.

Fricke WF, McDermott PF, Mammel MK, Zhao S, Johnson TJ, Rasko DA, Fedorka-Cray PJ, Pedroso A, Whichard JM, Leclerc JE, White DG, Cebula TA, Ravel J (2009) Antimicrobial resistance-conferring plasmids with similarity to virulence plasmids from avian pathogenic Escherichia coli strains in Salmonella enterica serovar Kentucky isolates from poultry. Appl Environ Microbiol 75:5963-5971.

Gal-Mor O, Valinsky L, Weinberger M, Guy S, Jaffe J, Schorr YI Raisfeld A, Agmon V, Nissan I (2010) Multidrug-resistant Salmonella enterica serovar Infantis, Israel. Emerg Infect Dis 16:1754-1757.

Garcia-Migura L, Hendriksen RS, Fraile L, Aarestrup FM (2014) Antimicrobial resistance of zoonotic and commensal bacteria in

Europe: the missing link between consumption and resistance in veterinary medicine. Vet Microbiol 170:1-9.

Gast RK, Guard-Bouldin J, and Holt PS (2004) Colonization of reproductive organs and internal contamination of eggs after experimental infection of laying hens with Salmonella heidelberg and Salmonella enteritidis. Avian Dis 48:863–869.

Gradel KO, Jørgensen JC, Andersen JS, Corry JE (2003) Laboratory heating studies with Salmonella spp. and Escherichia coli in organic matter, with a view to decontamination of poultry houses. J Appl Microbiol 94:919-928.

Gradel KO, Jørgensen JC, Andersen JS, Corry JE (2004) Monitoring the efficacy of steam and formaldehyde treatment of naturally Salmonella - infected layer houses. J. Appl Microbiol 96:613-622.

Guiney DG, Lesnick M (2005) Targeting of the actin cytoskeleton during infection by Salmonella strains. Clin Immunol 114:248–255.

Guiney DG, Fierer J (2011) The role of the spv genes in Salmonella pathogenesis. Front Microbiol 2:129. doi: 10.3389/fmicb.2011.00129

Guard-Petter J (2001) The chicken, the egg and Salmonella enteritidis. Environ Microbiol 3:421–430.

Hauser E, Tietze E, Helmuth R, Junker E, Prager R, Schroeter A, Rabsch W, Fruth A, Toboldt A, Malrony B (2012) Clonal dissemination of Salmonella enterica serovar Infantis in Germany. Foodborne Pathog Dis 9:352-360.

Hensel M (2000) Salmonella pathogenicity island 2. Mol Microbiol 36:1015-1023.

Joseph B, Otta SK, Karunasagar I, Karunasagar I (2001) Biofilm formation by Salmonella spp. on food contact surfaces and their sensitivity to sanitizers. Int J Food Microbiol 64:367–372.

Johnson TJ, Thorsness JL, Anderson CP, Lynne AM, Foley SL, Han J, Fricke WF, McDermott PF, White DG, Khatri M, Stell AL, Flores C, Singer RS (2010) Horizontal gene transfer of a ColV plasmid has resulted in a dominant avian clonal type of Salmonella enterica serovar Kentucky. PLoS One 2010; 5(12), e15524, doi:10.1371/journal.pone.0015524

Kubori T, Sukhan A, Aizawa SI, Galán JE (2000) Molecular characterization and assembly of the needle complex of the Salmonella typhimurium type III protein secretion system. Proc Natl Acad SciUSA 97:10225-10230.

Le Hello S, Harrois D, Bouchrif B, Sontag L, Elhani D, Guibert V, Zerouali K, Weill FX (2013) Highly drug-resistant Salmonella enterica serotype Kentucky ST198-X1: a microbiological study. Lancet Infect Dis 13:672–679.

Mah TFC, O'Toole GA (2001) Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol 9:34-39.

Marin C, Hernandiz A, Lainez M (2009) Biofilm development capacity of Salmonella strains isolated in poultry risk factors and their resistance against disinfectants. Poult Sci 88:424-431.

Marin C, Balasch S, Vega S, Lainez M (2011) Sources of Salmonella contamination during broiler production in Eastern Spain. Prev Vet Med 98:39-45.

McKee SR, Townsend JC, Bilgili SF (2008) Use of a scald additive to reduce levels of Salmonella typhimurium during poultry processing. Poult Sci 87:1672–1677.

Merino LA, Ronconi MC, Navia MM, Ruiz J, Sierra JM, Cech NB, Lodeiro NS, Vila J (2003) Analysis of the clonal relationship among clinical isolates of Salmonella enterica serovar Infantis by different typing methods. Rev Inst Med Trop S Paulo 45: 119-123.

Mølbak K (2005) Human health consequences of antimicrobial drug-resistant Salmonella and other foodborne pathogens. Clin Infect Dis 41:1613-1620.

National Antimicrobial Resistance Monitoring System-NARMS, Annual Animal Report (2011) https://www.ars.usda.gov/ARSUserFiles/60401020/NARMS

/NARMS2011/NARMS%20USDA%202011%20Report.pdf

Nógrády N, Tóth A, Kostyák A, Pászti J, Nagy B (2007) Emergence of multidrug-resistant of Salmonella Infantis in broiler chickens and humans in Hungary. J Antimicrob Chemother 60:645-648.

Oliveira K, Oliveira T, Teixeira P, Azeredo J, Oliveira R (2007) Adhesion of Salmonella Enteritidis to stainless steel surfaces. Braz J Microbiol 38:318–323.

Pezoa D, Yang HJ, Blondel CJ, Santiviago CA, Andrews-Polymenis HL, Contreras I (2013) The type VI secretion system encoded in SPI-6 plays a role in gastrointestinal colonization and systemic spread of Salmonella enterica serovar Typhimurum in the chicken. PloS One 8(5), e63917, doi:10.1371/ journal.pone.0063917

Prouty AM, Gunn JS (2003) Comparative analysis of Salmonella enterica serovar Typhimurium biofilm formation in gallstones and on glass. Infect Immun 70:2640–2649.

Prunić B, Milanov D, Velhner M, Pajić M, Pavlović Lj, Mišić D (2016) Clonal persistence of Salmonella enterica serovars Montevideo, Tennessee and Infantis in feed factories. J Infect Dev Ctries 10:662-666.

Rabsch W, Hargis B.M, Tsolis RM, Kingsley RA, Hinz KH, Tschäpe H, Bäumler AJ (2000) Competitive exclusion of Salmonella Enteritidis by Salmonella Gallinarum in poultry. Emerg Infect Dis 6:443-448.

Ramesh N, Joseph SW, Carr LE, Douglass LW, Wheaton FW (2002) Evaluation of chemical disinfectants for the elimination of Salmonella biofilms from poultry transport containers. Poult Sci 81:904-910.

Rašeta M, Teodorović V, Bunčić O, Katić V, Branković-Lazić I, Polaček V, Vidanović D (2014) Antibiotic resistance and molecular studies on Salmonella enterica subspecies enterica serovar Infantis isolated in human cases and broiler carcasses. Acta Veterinaria 64:257-268.

Rose N, Beaudeau F, Drouin P, Toux J. Y, Rose V, Colin P (2000) Risk factors for Salmonella persistence after cleansing and disinfection in French broiler-chicken houses. Prev Vet Med 44:9–20.

Schonewille E, Nesse LL, Hauck R, Windhorst D, Hafez MH, Vestby LK (2012) Biofilm building capacity of Salmonella enterica strains from the poultry farm environment. FEMS Immunol. Med Microbiol 65:360-365.

Shahada F, Chuma T, Tobata T, Okamoto K, Sueyoshi M, Takase K (2006) Molecular epidemiology of antimicrobial resistance among Salmonella enterica serovar Infantis from poultry in Kagoshima, Japan. Int J Antimicrob Agents 28:302-307.

Solano C, Garcia B, Valle J, Berasain C, Ghigo JM, Gamazo C, Lasa I (2002) Genetic analysis of Salmonella enteritidis biofilm formation: critical role of cellulose. Mol Microbiol 43:793–808.

Solomon EB, Niemira BA, Sapers GM, Annous BA (2005) Biofilm

formation, cellulose production, and curli biosynthesis by Salmonella originating from produce, animal and clinical sources, J Food Protect 68:906-912.

Steenackers H, Hermans K, Vanderleyden J, De Keersmaecker SCJ (2012) Salmonella biofilms: An overview on occurrence, structure, regulation and eradication. Food Res Int 45:502-531.

Stepanović S, Ćirković I, Ranin L, Svabić-Vlahović M (2004) Biofilm formation by Salmonella spp. and Listeria monocytogenes on plastic surface. Lett Appl Microbiol 38:428–432.

Velge P, Cloeckaert A, Barrow P (2005) Emergence of Salmonella epidemics: The problems related to Salmonella enterica serotype Enteritidis and multiple antibiotic resistance in other major serotypes. Vet Res 36:267-288.

Velhner M, Kozoderović G, Grego E, Galić N, Stojanov I, Jelesić Z, Kehrenberg C (2014) 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 61:364-370.

Vestby LK, Møretrø T, Langsrud S, Heir E, Nesse LL (2009) Biofilm forming abilities of Salmonella are correlated with persistence in fish meal- and feed factories. BMC Vet Res 5, 20, doi:10.1186/1746-6148-5-20

Wales A, Breslin M, Carter B, Sayers R, Davies R (2007) A longitudinal study of environmental Salmonella contamination in caged and free-range layer flocks. Avian Pathol 36:187-197.

White AP, Surette MG (2006) Comparative Genetics of the rdar morphotype in Salmonella. J Bacteriol 188:8395–8406.

Wisner A, Desin T, White A, Potter A, Köster W (2012) The Salmonella pathogenicity island-1 and -2 encoded type III secretion systems. In: Salmonella - a diversified superbug. InTech: pp 469-494. doi: 10.5772/2471