Review article **UDC: 579.842:616.981.49**

SALMONELLA ENTERICA SUBSPECIES ENTERICA SEROVAR ENTERITIDIS – ACTUALITIES AND IMPORTANCE

Biljana Miljković-Selimović[1,2], Tatjana Babić[1,3] and Predrag Stojanović[1,2]

Salmonella enterica subspecies enterica serovar Enteritidis (S. Enteritidis) has been recently recognized as a prevalent cause of alimentary toxi-infection worldwide. Its widespread presence could be explained by intensification and globalization of traffic, global trade, and the rest of socioeconomic processes. However, no matter to global spreading of S. Enteritidis, there is unequal distribution of certain phage types (PT) where PT 4 and 8 are predominant. Salmonella is considered as a cause of various diseases from acute enterocolitis to typhoid fever. All bacteria from this species have numerous virulence factors such as: adhesins, toxins, virulence plasmids, and cell wall lipopolysaccharides (LPS). Similar to other salmonella serotypes, S. Enteritidis has a virulence plasmid. It allows a bacterium to persist inside the reticuloendothelial cells, while strains without it are eliminated quickly. In the last few years several virulent S. Enteritidis strains of PT 4 were described and considered to be of the same origin. The domination of PT 4 is probably subjected to the resistance of certain strains to nitrofurantoin which is used in poultry rising. The increased significance of S. Enteritidis refers not only to its association with pandemic problems but to frequent reports about extraintestinal infectious processes caused by this bacterium. Taking into consideration that eggs are very important source of infection besides poultry meat, the advised efficient preventive measures, among others, should be some changes in poultry meat preparation, investigation of outbreak-related flocks and devastation of infected ones, as well as egg pasteurization. *Acta Medica Medianae 2010;49(3):71-75.*

Key words: *salmonella enteritidis, infection, virulence factors*

National Referent Laboratory for Campylobacter and Helicobacter[1]
Institute of Microbiology and Immunology, Faculty of Medicine in Niš[2]
Center for Microbiology, Public Health Institute Niš[3]

Contact: Biljana Miljković-Selimović
Institut za mikrobiologiju i imunologiju
Medicinski fakultet Univerziteta u Nišu
Bul. dr Z. Đinđića 81, 18000, Niš,
Republika Srbija
E-mail: biljams@eunet.rs

Pandemic

The presence of different salmonella serotypes may vary by geographic areas. Though a large number of distinct serotypes could be isolated in a particular area, just a few of them are predominant. However, it is observed that this phenomenon is not static but constantly changes over time and more rapidly in the past few years. Due to the fact that *Salmonella enterica* subspecies *enterica* serovar Enteritidis (*S.* Enteritidis) is considered as the predominant cause of alimentary toxico-infections worldwide as a direct consequence of intensification and globalization of traffic, trading, and socio-economic processes, the question has arisen about the possibility of enterocolitis pandemic caused by *S.* Enteritidis.

An increase of the S. Enteritidis isolates in Europe was observed in the mid '70s, and, nowadays, it is considered as a predominant serotype (1). In 1979, *S.* Enteritidis was the most common isolate in Austria and Romania, while in 1987 this was the case in Argentina, Austria, Bulgaria, Finland, Hungary, Portugal, Spain, Sweden, and Scotland. According to incomplete data obtained in 1987, regarding the isolation rate, the Former Yugoslavia was at the fourth position behind Hungaria, Finland, and Sweden (2). By the end of the 80s' in the USA, *S.* Enteritidis was at the second place, behind *S.* Typhimurium (3). Recently, *S*. Enteritidis has become the most common *Salmonella* serotype in humans globally, especially in Europe, where it accounts for 85% of *Salmonella* cases followed by *S.* Typhimurium at the second place and *S*. Hadar, *S*. Virchow, and *S*. Infantis alternating from the third to the fifth position. Also, it represents the most frequently isolated serotype from human source in Asia (38%), and Latin America and Caribbean Isles (31%) followed by *S.* Typhimurium, *S*. Typhi, *S*. Montevideo, and *S*. Paratyphi B which are frequently isolated from human source, too. On the contrary, in 2002, in Oceania, it participated with only 9% in the human salmonella isolates.

That year, in Africa, both, *S*. Enteritidis and *S*. Typhimurium were evidenced in one quarter of human isolates. Furthermore, in the same year, in North America, *S*. Typhimurium isolates surpassed *S*. Enteritidis isolates among the strains obtained from the human source, though *S.* Newport and *S*. Heidelberg had a significant share. In Asia, between 2000 and 2002, Japan, Korea, and Thailand together reported *S*. Enteritidis as the most frequent human serotype. *S*. Weltevreden was the second most common serotype in 2000 and 2001, but dropped to the fourth position in 2002, when it was surpassed by *S*. Rissen and *S*. Typhimurium (4).

In Serbia, in 1984, S. Enteritidis was at the first place regarding the number of isolates (5), though, in 1982, in the district of Niš, it became the predominant serotype with 30 primo-isolates against 160 strains of different serotypes (6).

However, regardless of global spreading of *S*. Enteritidis, there is a nonuniform distribution of certain phagetypes (Phage Type – PT). PTs 4, 8, and 1 are predominant, and recently an increase in the frequency of isolation of PT 14b has been recorded (7). Even now, two mostly described phage types (PTs 4 and 8) in the last two decades are still predominant, being isolated in Hawaii (8) and South Africa (9).

Virulence factors

Salmonellae could cause different types of diseases, starting with acute enterocolitis to the typhoid fever. Once salmonella enters the ileum, it interacts with the mucosa above the mesenterial lymph node. Bacterium runs through the intestinal epithelium inside the vacuole by transcytosis. In order for microorganism to get inside the host cell, the increase of ribonucleic acid (RNA) synthesis starts as well as creation of bacterial outer membrane proteins essential for the invasion. As a part of a global regulatory network, these proteins are created through the induction in epithelial cells probably, and they are essential for intracellular bacteria survival. During the contact between salmonella and the top surfaces of epithelial cells, it causes the loss of apical, epithelial microvilli and a smaller disruption in intercellular connections partially due to bacterial cell wall lipopolysaccharide (10).

In some cases, salmonella could pass through the basal membrane of the intestinal tract mucosa. Once salmonella has reached mono-cytomacrophagic system, it survives, multiplies, and disseminates throughout other tissues, causing typhus fever. Intracellular survival and multiplying is possible due to salmonella ability to use purine and aromatic compounds, while the presence of the iron siderophor (enterochelin) does not seem to be necessary for the process (10). There are numerous salmonella virulence factors as adhesins, toxins, virulence plasmids, cell wall lipopolysaccharides.

Adhesins

The exact role of adherence factors (fimbriae type 1 and mannose-resistant hemagglutinin) and flagella still remains unknown. Flagella are not necessary in colonization of the gastrointestinal tract, but they are essential for growth and survival in the host spleen and liver. Flagella either prevent macrophages to destroy microorganisms or contribute to the intracellular multiplication in macrophages (10).

Toxins

It is considered that salmonella have the ability to produce at least three types of toxic substances. A thermolabile enterotoxin is one of these, and it binds to gangliosides, increases the level of intracellular cyclic adenosine monophosphate (cAMP), and intensifies the liquid secretion. The second one is cytotoxin, a non - lipopolysaccharidic component of the outer membrane, which inhibits the protein synthesis in eukaryotes leading to the elongation of tissue culture cells – CHO (Chinese Hamster Ovary) cells. Endotoxin, lipid A, a component of the cell wall lipopolysaccharide, activates macrophages and lymphocytes, and consequently triggers a series of biological effects: fever, leukocytosis, lowering of blood pressure (10).

Virulence plasmid

Most of the salmonella serotypes have the so-called virulence plasmid. It allows bacteria to persist in reticuloendothelial cells, while strains without plasmids are quickly eliminated (10). In case of *S.* Enteritidis, its size is about 38 megadaltons (MDa) which, in spite of its various origin and geographical distribution of strains, gives the same restriction fragments by the restriction enzyme digestion (eg. *EcoRI*, *Hind*III, and *BamH*I). Additionally, it is referred as species-specific plasmid, or plasmid virulent for mouse, or constitutive plasmid (11), and it could be found in 85% to 100% of examined strains. Often, in S. Enteritidis strains, only the presence of virulence plasmid is recorded (12), which significantly dimininshes discriminatory power of plasmid profile analysis as one of the typing methods (13).

Investigations of strains with 38MDa plasmid, strains with artificially removed plasmid, and strains without plasmids, revealed that the presence of this plasmid in mice plays a role in spreading of infection from small intestine to mesenterial lymph nodes, liver and spleen. The plasmid has no influence on *S.* Enteritidis resistance to bactericidal activity of animal serum (14) and its ability to bind iron (15). Strains without virulence plasmid and strains, whose plasmids were artificially removed, were found to be less virulent for mice. Examination of role of this plasmid revealed that it is essential for full virulence expression of *S*. Enteritidis in mice, while virulence may vary among different strains. It is regarded that this plasmid does not take a part in virulence of this serotype in poultry. Additionally, it is indicated that 38 MDa plasmid is not associated neither to drug resistance nor to basic biochemical properties of *S.* Enteritidis (11).

The virulence plasmid shows stability in all strains of *S.* Enteriditis for the period longer than two-and-a-half years at temperatures of 5, 22, 30, and –80°C. Also, its profile obtained with *Sma*I restriction enzyme analysis will not vary with temperature (16). Some surveys have revealed two main classes of virulence plasmid of *S.* Enteritidis. Furthermore, the existence of plasmid variation is confirmed, which is more similar to the serotype-specific plasmid of *S.* Typhimurium than serotype-specific plasmid of *S.* Enteritidis. This version has originated from strains of *S*. Enteritidis phagetype 9b, isolated from poultry meet (17).

DNA hybridization studies have shown that virulence plasmid contains a 6.4 kb fragment responsible for virulence. Additionally, four proteins coded by this fragment have been identified. These are proteins SpvR, SpvA, SpvB, and SpvC. It is pointed out that gene for *SpvC* protein could contribute to the virulence expression of *S.* Enteritidis (15). It appears that these virulence regions could be found in some other plasmids specific to *S.* Enteritidis. The analysis of plasmids with sizes 38, 45, 59, and 65 MDa was conducted for selected type strains of 26 of the *S.* Enteritidis phage types by restriction enzyme fingerprinting and by DNA-DNA hybridization with olygonucleotide probes for *Salmonella* plasmid virulence genes (*Spv* gene). An identical 3.5 kb fragment homologous to *SpvC* gene was conserved in Hind III digests of all 38 MDa and 59 MDa plasmids, while similar fragment was obtained by digestion of 45 MDa plasmid, too. In contrast to this finding, plasmid of 65 MDa found in the strain of PT 10 was not related to these three plasmids and did not carry the *SpvC* gene (18). Additionally, a connection between virulence of *S.* Enteritidis and presence of small plasmids was described. A 5.3 kb plasmid was isolated as a unique extrachromosomal DNA from the strain responsible for an outbreak with a high mortality rate (19).

Large plasmids of some *Salmonella* serotypes have common virulence regions. Plasmid content and presence of genes considered to be responsible for virulence in mice have been investigated in strains of *S.* Enteritidis and *S.* Typhimurium. Three plasmids of different sizes in *S.* Enteritidis and one plasmid in *S.* Typhimurium contained a 3.5 kb fragment which carried virulence locus *VirA*. The *VirB* virulence locus was located on a 2.7 kb fragment in *S.* Enteritidis and on a 2.5 kb fragment in *S.* Thphimurium (20). Amino acid sequences of each protein coded by the virulence region of *S.* Enteritidis' virulence plasmid show high level of similarity to corresponding sequences coded by the virulence region of *Salmonella* Choleraesuis', *S.* Dublin', and *S.* Typhimurium' virulence plasmids. Thus, it is considered that virulence plasmids of salmonellae, along with *S.* Enteritidis, contain common, fixed region responsible for the virulence (15).

Virulence plasmid of *S.* Enteritidis is similar to virulence plasmid of *S.* Dublin (21). It is shown that serotype-specific plasmids of *S.* Enteritidis, *S.* Dublin, and *S.* Typhimurium expressed 35% similarity based on Dice coefficient. There are ongoing surveys on phylogenetic relationship of these plasmids (22). There is a possibility of spontaneous disappearance of 38 and 59 MDa plasmids in *S.* Enteritidis, though rarely ($<10^{-6}$).

Emergence of virulent clone

In the last few years, more virulent *S.* Enteritidis PT4 strains have been described, and it is considered that these have the mutual clonal origin. The more *S.* Enteritidis PT4 strains are frequently isolated the more reviews of outbreaks caused by these strains are reported. One of the reasons for increased virulence of *S.* Enteritidis PT4 strains is their elevated iron affinity, which consequently increased their efficiency in uptaking iron from ovotransferrin. *In vivo*, this process takes place through enterobactin. Additionally, it has been confirmed that the presence of 38 MDa plasmid is not necessary for expression of outer membrane protein (OMP) responsible for iron intake.

The domination of PT4 is probably associated with nitrofurantoin resistance of some strains due to utilization of nitrofurantoin in poultry farming which cause selection of PT4 strains infectious for humans. Though still dominant in Europe, the number of this phage type isolates subsequently decreases, being replaced by less frequent phage types (23).

Importance of extraintestinal isolates

If it is accepted that extraintestinal isolation of *Salmonella enterica* is considered as potential virulence index, it turns out that group D is the most invasive especially in patients older than one year. The importance of *S.* Enteritidis nowadays is increased not only for its pandemic-related problems, but due to more frequent reports related to extraintestinal localization of infections processes caused by those bacteria. Post-enterocolitis complications are more frequently reported in older population, immuno-compromized patients and pregnant women, and could lead to septicaemia and death. In Africa, the situation is additionally complicated by AIDS. 52% of hospitalized patients are HIV-positive and 10% of those have bacteremia caused by *S.* Typhimurium and *S.* Enteritidis (24).

Sources of infection

Dramatic increasing in *S.* Enteritidis isolates from outbreaks and sporadic salmonelloses cases in people has started an international debate on sources and spreading pathways of this micro-organism. Although poultry meat represents the main infection source of *S.* Enteritidis, epidemiologic data suggest that eggs are the source of infection of great importance (25). Salmonellae survive very easily in eggs if there are some irregularities in preparing, cooking and storing the food made of these ingredients, and the scale of infection spreading is amplified in case of centralized food preparation.. The international poultry meat trading, particularly contributes to spreading of infections caused by *S.* Enteritidis due to the fact that it allows introducing of virulence clones into new geographical area.

The presence of *S.* Enteritidis in class A eggs is linked with infection of chickens, in the ovaries and oviducts of which bacteria can be found responsible for contamination of eggs before eggshell is formed. Chickens could be infected by contaminated food of animal origin, while the contributing factor is stress, caused by irregular intake of food and water. *S.* Enteritidis could be spread by vertical transmission as well as through the contact with rodents, insects, wild birds, domestic animals, people, and waste material. Often, *S.* Enteritidis could be isolated from contaminated environment of infected flocks. This type of salmonella has been evidenced in Canada in 1%, and in 2.7% of environmental samples (26). Identification of infected poultry is a special issue due to difficulties in salmonella identification by cultivation of cloacal swab, and determination of serum antibodies in birds is followed by technical difficulties in particular.

Prevention of infection

Preventive measures based on elimination of broken eggs or washing of eggshell, are not sufficient in cases where eggs are contaminated before the formation of eggshell which wraps yolk and albumen. Also, a measure like prohibition of egg consumption from symptomatically infected hens is not completely efficient, because infection in hens could proceed asymptomatically (2).

Efficient preventive measures would be the change in poultry meat preparation, investigation of flocks related with outbreaks and their extermination and eggs pasteurization. Additionally, it is important to store eggs in the refrigerator as well as to avoid pooling the eggs prior to meal preparation. Note that persons particularly susceptible to *S.* Enteritidis infections (children, elderly, pregnant women, immunocompromised, as well as HIV-positive individuals) should avoid the consumption of raw eggs and insufficiently thermally treated meals containing eggs. For these risk subgroups, it is strongly recommended to consume pasteurized eggs and pasteurized eggs products.

References

1. Guard-Petter J. The chicken, the egg and Salmonella enteritidis. Environ Microbiol 2001;3(7): 421–30.
2. Rodrigue DC, Tauxe RV, Rowe B. International increase in Salmonella enteritidis: A new pandemic? Epidemiol Infect 1990;105(1):21-7.
3. Braden CR. Salmonella enterica serotype Enteritidis and eggs: a national epidemic in the United States. Clin Infect Dis 2006;43(4):512-7.
4. Galanis E, Wong DMALF, Patrick ME, Binsztein N, Cieslik A, Chalermchaikit T, et al. Web-based Surveillance and Global Salmonella Distribution, 2000–2002. Emerg Infect Dis 2006;12(3):381-8.
5. Republički zavod za zaštitu zdravlja "Milan Jovanović-Batut". Referentna laboratorija za crevne zaraze. Godišnji izveštaj o kretanju primoizolata salmonela u Srbiji, 1984. (Articles in Serbian)
6. Republički zavod za zaštitu zdravlja "Milan Jovanović-Batut". Referentna laboratorija za crevne zaraze. Godišnji izveštaj o kretanju primoizolata salmonela u Srbiji, 1982. (Articles in Serbian)
7. Nygård K, de Jong B, Guerin PJ, Andersson Y, Olsson A, Giesecke J. Emergence of new Salmonella Enteritidis phage types in Europe? Surveillance of infections in returning travellers. BMC Med 2004;2:32.
8. Burr R, Effler P, Kanenaka R, Nakata M, Holland B, Angulo FJ. Emergence of Salmonella serotype Enteritidis phage type 4 in Hawaii traced to locally-produced eggs. Int J Infect Dis 2005;9(6):340-6.
9. Maré L, Dick LM, van der Walt ML. Characterization of south african isolates of Salmonella enteritidis by phage typing, numerical analysis of RAPD-PCR banding patterns and plasmid profiles. Int J Food Microbiol 2001;64(3):237-45.
10. Ohl ME, Miller SI. Salmonella: a model for bacterial pathogenesis. Annu Rev Med 2001;52:259-74.
11. Nakamura M, Sato S, Ohya T, Suzki S, Ikeda S. Possible relationship of a 36-megadalton Salmonella enteritidis plasmid to virulence in mice. Infect Immun 1985;47(3):831-3.
12. Miljković-Selimović B, Babić T, Kocić B, Stojanović P, Ristić Lj, Dinić M. Plasmid profile analysis of salmonella enterica serotype enteritidis. Acta Medica Medianae 2008;47(2):54-7.
13. Miljkovic-Selimovic B, Kocic B, Babic T, Ristic L. Salmonella Enteritidis – Phenotypic and Genotypic Identification. Acta Medica Medianae 2009;48(3): 31-4.
14. Helmuth R, Stephen R, Bunge C, Hoog B, Steinbeck A, Bulling E. Epidemiology of virulence-associated plasmids and outer membrane protein patterns within seven common Salmonella serotypes. Infect Immun 1985;48(1):175-82.
15. Suzuki S, Ohishi K, Takahashi T, Tamura Y, Muramatsu M, Nakamura M, et al. The role of 36 megadalton plasmid of Salmonella enteritidis for the pathogenesis in mice. J Vet Med Sci 1992;54(5): 845-50.

16. Olsen JE, Brown DJ, Baggesen DL, Bisgaard M. Stability of plasmids in five strains of Salmonella maintained in stab culture at different temperatures. J Appl Bacteriol 1994;77(2):155-9.
17. Rankin SC, Benson CE, Platt DJ. The distribution of serotype-specific plasmids among different subgroups of strains of Salmonella enterica serotype Enteritidis: characterization of molecular variants by restriction enzyme fragmentation patterns. Epidemiol Infect 1995;114(1):25-40.
18. Brown DJ, Threlfall EJ, Hampton MD, Rowe B. Molecular characterization of plasmids in Salmonella enteritidis phage typhes. Epidemiol Infect 1993;110(2); 209-16.
19. Ibanez M, Rotger R. Characterization of a small cryptic plasmid from Salmonella enteritidis that affects the growth of Escherichia coli. FEMS Microbiol Lett 1993; 109(2-3):225-9.
20. Jones CS, Osborne DJ. Identification of contemporary plasmid virulence genes in ancestral isolates of Salmonella enteritidis and Salmonella typhimurium. FEMS Microbiol Lett 1991;64(1):7-11.
21. Hong SF, Chiu CH, Chu C, Feng Y, Ou JT. Complete nucleotide sequence of a virulence plasmid of Salmonella enterica serovar Dublin and its phylogenetic relationship to the virulence plasmids of serovars Choleraesuis, Enteritidis and Typhimurium. FEMS Microbiol Lett 2008;282(1):39-43.
22. Platt DJ, Taggart J, Heraght KA. Molecular divergence of the serotype-specific plasmid (pSLT) among strains of Salmonella typhimurium of human and veterinary origin and comparison of pSLT with the serotype specific plasmids of S. enteritidis and S. dublin. J Med Microbiol 1988;27(4):277-84.
23. Fisher IS; Enter-net participants. Dramatic shift in the epidemiology of Salmonella enterica serotype Enteritidis phage types in Western Europe, 1998-2003--results from the Enter-net international salmonella database. Euro Surveill 2004;9(11):43-5.
24. Aubry P, Niyongabo T, Nizigiye J, Muhirwa G, Kamanfu G, Ndahiragije A, et al. Bacteremia caused by non-typhoid Salmonellas during an infection by the human immunodeficiency virus (HIV) in the African adults. Med Trop Mars 1992;52(4):447-50. French.
25. Rabsch W, Prager R, Braun P, Methner U. Salmonella in poultry flocks and humans--S. enterica subspecies enterica serovar Enteritidis in the past. Berl Munch Tierarztl Wochenschr 2007; 120(7-8):328-33.
26. Poppe C, Irwin RJ, Forsberg CM, Clarke RC, Oggel J. The prevalence of Salmonella enteritidis and other Salmonella spp. among Canadian registered commercial layer flocks. Epidemiol Infect 1991; 106(2):259-70.

SALMONELLA ENTERICA SUBSPECIES ENTERICA SEROTIP ENTERITIDIS – AKTUELNOST I ZNAČAJ

Biljana Miljković-Selimović[1,2], Tatjana Babić[1,3] i Predrag Stojanović[1,2]

U poslednje vreme kao uzročnik alimentarnih toksiinfekcija u mnogim delovima sveta dominira Salmonella enterica subspecies enterica serotip Enteritidis (S. Enteritidis). Njenom širenju doprinose intenziviranje i globalizacija saobraćaja, trgovine i ostalih društveno-ekonomskih procesa. Međutim, bez obzira na globalno širenje S. Enteritidis, postoji nejednaka rasprostranjenost pojedinih fagotipova (Phage Type - PT) uz dominaciju PT 4 i 8. Salmonele mogu izazivati oboljenja, počev od akutnih enterokolitisa do tifusne groznice. Sve bakterije ovog roda poseduju brojne faktore virulencije u koje spadaju: athezini, toksini, plazmidi virulencije, lipopolisaharid ćelijskog zida. Kao i drugi serotipovi salmonela, S. Enteritidis poseduje plazmid virulencije. On omogućava bakteriji da perzistira unutar retikuloendotelnih ćelija, dok se sojevi bez plazmida brzo eliminišu. Poslednjih godina se opisuju virulentniji sojevi S. Enteritidis koji pripadaju PT 4 za koje se smatra da imaju zajedničko poreklo. Dominacija PT 4 uslovljena je, verovatno, i otpornošću nekih sojeva na nitrofurantoin koji se primenjuje u živinarstvu. Značaj koji S. Enteritidis ima povećava se ne samo zbog problema vezanih za pandemiju, već i zbog sve češćih izveštaja o ekstraintestinalnim lokalizacijama infektivnih procesa izazvanih ovom bakterijom. Imajući u vidu da su pored živinskog mesa jaja veoma važan izvor infekcije, efikasne mere prevencije bile bi, između ostalog, promene u načinu pripreme živinskog mesa i ispitivanje jata povezanih sa epidemijama, uništavanje inficiranih jata ili podvrgavanje jaja pasterizaciji. *Acta Medica Medianae 2010;49(3):71-75.*

Ključne reči: *salmonella enteritidis, infekcija, faktori virulencije*