Chromosomal and plasmidic virulence determinants of *Salmonella* Infantis in broiler chicks

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Introduction

Salmonella Infantis has been reported to be predominant among broiler chicks in Hungary and in several other countries in and outside Europe. Recently the chicken clone B2, carrying a large multiresistant (MDR) plasmid became also prevalent in the human population in some of these countries (1, 2, 3). The plasmid pSI 54/04 represents the prime difference when compared genomes of the recent and earlier clones B2 and A1 (4). It is however uncertain whether and how much of this spreading can be related to virulence determinants residing on Salmonella pathogenicity islands (SPIs) or to the MDR plasmid.

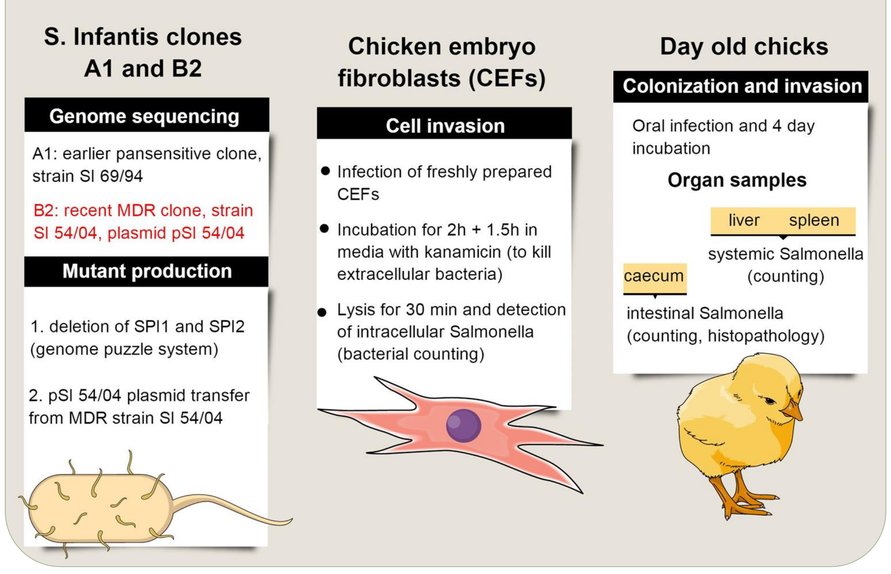
Hypothesis

The SPI1 and SPI2 or the large MDR plasmid pSI 54/04 may influence the colonization and spread of the *S*. Infantis clone B2 in broiler chicks.

Objectives

- to test the *in vitro* and *in vivo* pathogenicity of chromosomal mutants (ΔSPI) on chicken embryo fibroblast (CEF) cells and on orally infected day old chicks
- to test the impact of pSI 54/04 plasmid carriage on the *in vitro* and *in vivo* pathogenicity of the strains
- to provide the molecular characterization of the MDR plasmid pSI 54/04

Materials and methods

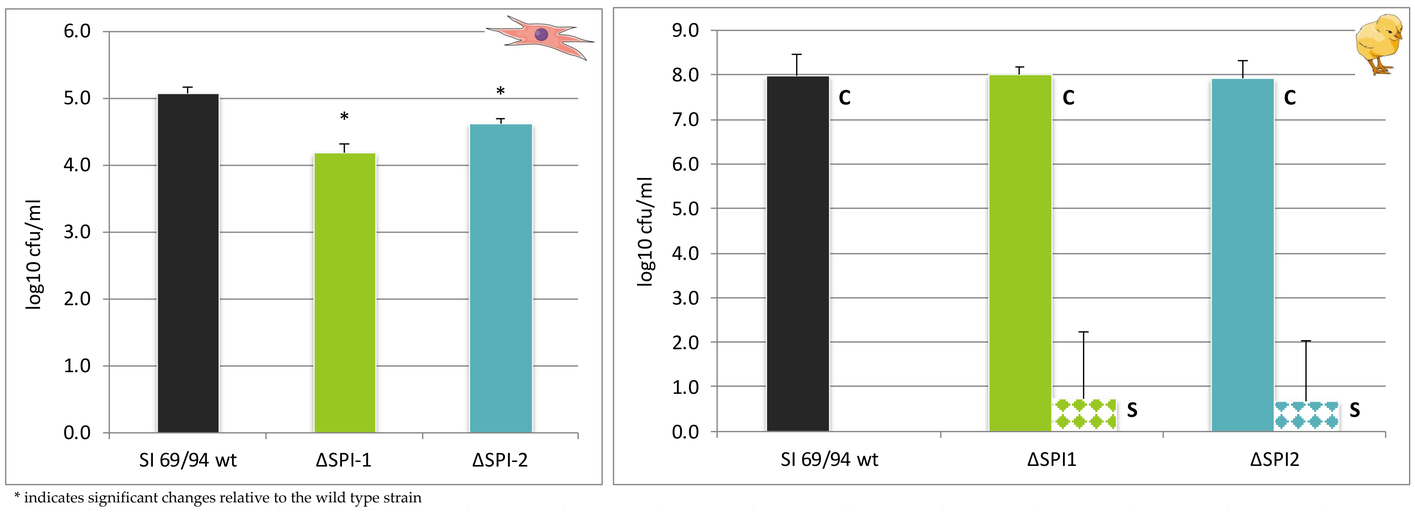


Results on chromosomal virulence regions (SPIs)

Deletion of Salmonella pathogencity islands SPI1 and SPI2

- The deletion of SPIs resulted in a significantly reduced invasiveness for CEFs, with a more pronounced effect for SPI1 (**Fig 1**).
- Chicken infection resulted in no significant difference between the wild type strain SI 69/94 and its ΔSPI1 and ΔSPI2 mutants regarding caecum and spleen (**Fig 1**).
- Histopathology of the infected caecum has only shown an increased lymphoid infiltration and thickening of the caecal submucosa.

Figure 1. The impact of SPI-deletion to CEF invasion and colonization of the chicken caecum (C) and spleen (S)



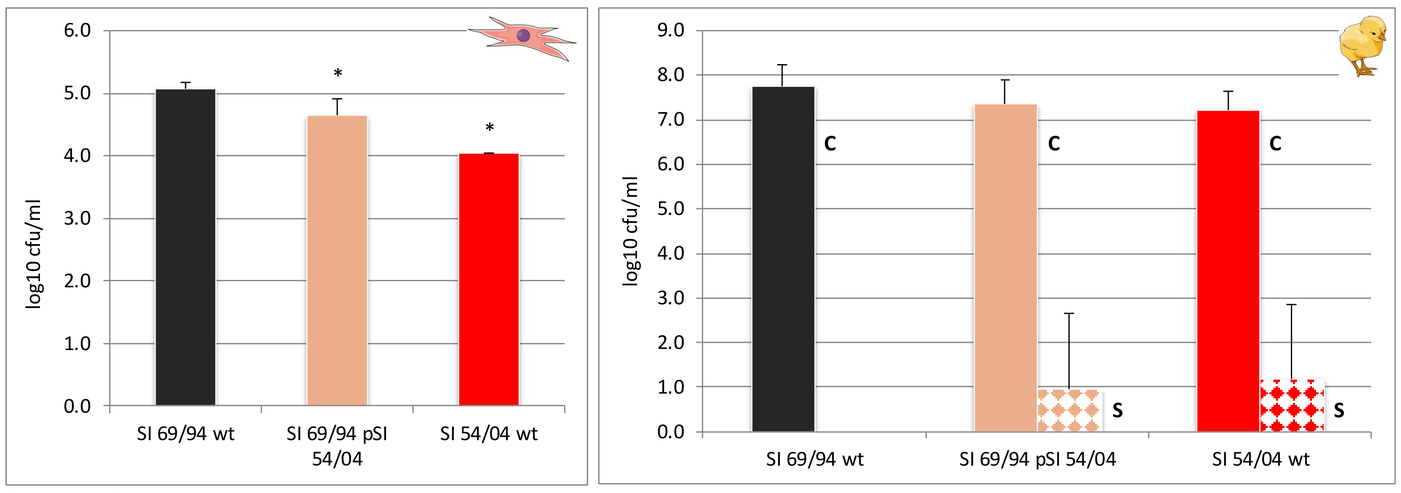
* indicates significant changes relative to the wild type strain

Results on plasmid pathogenicity

1. *In vitro* and *in vivo* pathogenic potential of MDR plasmid pSI 54/04

- The plasmidic strain SI 54/04 wt (clone B2) proved to be significantly less invasive than SI 69/94 wt (clone A1) for CEF cells.
- The pSI 54/04 plasmid transformant of SI 69/94 was also less invasive *in vitro* and showed reduced colonization of the chicken caeca as well, however *in vivo* differences were not significant (**Fig 2**).
- Invasion of the spleen was detected in only one animal/group, therefore can not be considered as characteristic to strains SI 54/04 wt and plasmidic SI 69/94. *Salmonella* was not detectable in the liver of infected chicks.

Figure 2. The impact of pSI 54/04 carriage to CEF invasion and colonization of the chicken caecum (C) and spleen (S)



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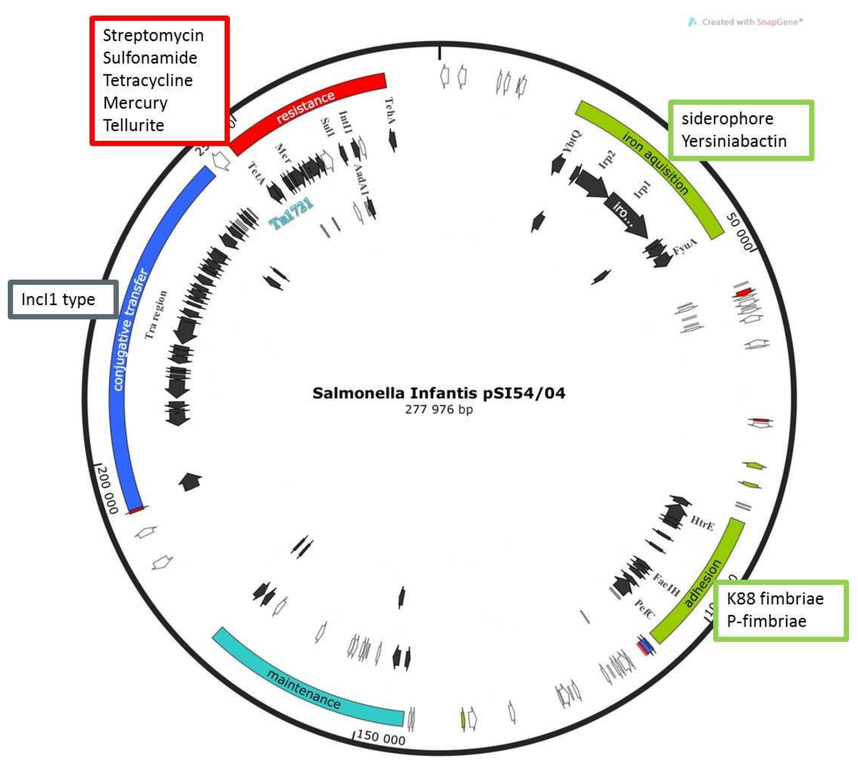
2. Draft genome of the large ~277 kb MDR plasmid pSI 54/04

- We provide the molecular characterization of the plasmid pSI 54/04, which can be considered as the first Hungarian reference plasmid of S. Infantis.
- This IncI type plasmid is containing a mosaic of resistance- and virulence regions.
- The coexistence of antimicrobial resistance and virulence genes promote survival not only in the host organism (siderophore Yersiniabactin, fimbriae) but also in the environment (tellurite, mercury resistance) (**Fig. 3**).

Figure 3. Physical map of the large MDR plasmid pSI 54/04



Conclusions

In harmony with our earlier results, it seems that *S*. Infantis is poorly invasive for broiler chicks but it is colonizing well in their intestine. These traits of S. Infantis are not influenced by SPI1 or SPI2.

It seems that the MDR plasmid pSI 54/04 represents a disadvantage in the interaction with the host cells. Therefore the spread of plasmidic clone B2 is more likely due to its multiresistance, conferring survival in the poultry flocks.

References

1. Nógrády N, Tóth A, Kostyák A, Pászti J, Nagy B. Emergence of multidrug-resistant clones of Salmonella Infantis in broiler chickens and humans in Hungary. J Antimicrob Chemother. 2007.

2. Nógrády N, Király M, Davies R, Nagy B. Multidrug resistant clones of Salmonella Infantis of broiler origin in Europe. Int J Food Microbiol. 2012.

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

4. Olasz F, Nagy T, Szabó M, Kiss J, Szmolka A, Barta E, van Tonder A, Thomson N, Barrow P, Nagy B. Genome Sequences of Three Salmonella enterica subsp. enterica Serovar Infantis Strains from Healthy Broiler Chicks in Hungary and in the United Kingdom. Genome Announc. 2015.

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