Title: Adhesion and invasion in HeLa cells by *Salmonella spp.* isolated from a poultry slaughter facility.

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Abstract

Salmonella spp. pathogenes is involves surface adhesion on the host cell through specific fimbriae (*flgK, fljB* and *flgL* genes*)*. After adhesion, some strains may remain extracellularly or may be internalized by phagocytosis, including in non-phagocytic cells, by the mechanism known as invasion (*invA, sipA, sipB, sipD, sopB* e *sopD* genes*)*. We tested the potential of 40 *Salmonella* spp. strains for adhesion and invasion through the presence of genes associated with these events in HeLa cells. The strains, previously isolated from mats in a poultry slaughtering facility, had their DNA extracted to be used for PCR reaction, visualized on 1.5% agarose gel, stained by SYBR Safe. HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) for the adhesion and invasion tests. Cells were grown in 24 well microplates and the 40 bacterial cultures grown at 37°C/24h in brain and hearth infusion broth (BHI) were diluted in DMEM to obtain a bacterial suspension containing 1.5 x 10^6 CFU/mL of which 1 ml was added, in duplicate, in the wells and the plate was incubated at 37°C/3h in 5% CO_2. Washes were performed with phosphate buffered saline (PBS) and adhered cells were detached from the plate by adding trypsin. The invasion and adhesion tests occurred simultaneously, and after incubation's plates at 37°C/3h, 1 mL of DMEM with 5% FBS and 300 µg/mL gentamicin were added to each well. Triton X-100 0.1% was added to lyse cells and release invading bacteria. The invasion rate was determined by the recovery and the counting colonies number, in relation to the percentage of the value determined in the adhesion test. The 40 strains analyzed showed some genes involved in the adhesion process to the target cells (*flgK*, *fljB* and *flgL*). Regarding to the invasion mediators, we found 100% positivity for *invA, sipB*, and *sipD*. The *sopB* and *sipA* genes were observed in 92.5% strains and *sopD* in 90%. All strains adhered and invaded HeLa cells, with invasion index varying from 1.46 to 73.86%. We have not been able to establish a relation between genes absence/presence with the low/high invasion rate, pointing out possibly the presence of other genes and mechanisms involved in the adhesion and invasion process.

Keywords: adhesion, invasion, *Salmonella* virulence genes

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