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Gentamicin Susceptibility and Comparison of Adhesion and Invasion of Caco-2 and HD11 Cell Lines by *Salmonella enterica* Serotypes

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Abstract: Foodborne *Salmonella* serovars are important facultative intracellular pathogens that cause gastroenteritis in humans. Four strains from three of the more predominant *Salmonella* serovars in poultry were studied: Typhimurium, Enteritidis, and Heidelberg. Gentamicin susceptibility was determined using an agar disc diffusion test and minimum inhibitory concentration (MIC) assays for *S.* Typhimurium ATCC 14028 and *S.* Heidelberg ARI-14. Both strains were susceptible to gentamicin in disc diffusion. The MIC of gentamicin was approximately 125 mg/ml for all strains tested. These strains' adhesion and invasion abilities were determined with two different cell lines, a human intestinal epithelial cell line (Caco-2) as well as a chicken macrophage cell line (HD11). Attachment percentages for each *Salmonella* strain were greater than the strain's ability to invade cells. Similar attachment percentages to Caco-2 cells were observed for *S.* Typhimurium and *S.* Heidelberg. Attachment percentages were lower in HD11 cells than in Caco-2 cells, although *Salmonella* exhibited higher apparent HD11 invasion, likely from HD11 phagocytosis. *Salmonella* Enteritidis showed lower rates of adhesion and invasion in HD11 cells compared to *Salmonella* Typhimurium. Developing a better understanding of *Salmonella* virulence mechanisms is critical to reducing *Salmonella* infections.

Keywords: *Salmonella*; poultry; tissue cell adhesion; tissue cell invasion; gentamicin

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1. Introduction

Salmonella infections is considered one of the foremost causes of foodborne gastroenteritis in humans [1]. It is estimated that nearly a million Americans contract *Salmonella* annually, and yearly costs of *Salmonella* control efforts have been estimated to reach several billion dollars [2–4]. Foodborne *Salmonella* have been associated with poultry consumption [5]. *Salmonella* Enteritidis, *S.* Typhimurium, and *S.* Heidelberg originating from poultry have been identified as being three of the more frequent serovars recovered from humans [5–8].

Some genes necessary for the invasion of intestinal epithelial cells and initiation of intestinal secretory and inflammatory responses are contained within *Salmonella* Pathogenicity Island 1 (SPI-1) [9]. *Salmonella* Pathogenicity Island 2 (SPI-2) is necessary for systemic infection and establishment beyond the intestinal epithelium and encodes genes essential for intracellular replication [9–11]. Intestinal epithelial surface adhesion is the initial *Salmonella* pathogenesis step and is central to its colonization. After *Salmonella* attaches to the intestinal epithelium, a multiprotein complex known as Type 3 Secretion System (T3SS) containing virulence genes involved in adhesion, invasion, and toxicity is expressed, facilitating endothelial update and invasion [12–14]. Over the past few decades, the human

intestinal Caco-2 cell line originating from a human colon adenocarcinoma has been extensively employed as an intestinal barrier model. The cell line undergoes a spontaneous differentiation process, forming a monolayer of cells and expressing several morphological and functional characteristics specific to mature enterocytes [15]. HD11 cells, a chicken macrophage virus-transformed cell line was also included in the current study [16]. *Salmonella* serovar adhesion and invasion responses to Caco-2 and HD11 cell lines were compared. Several pathogens and host factors may be necessary in determining the mechanisms of different *Salmonella* responses within various cell types [13,17]. Characterizing the ability and mechanisms of this pathogen to attach and invade different cell lines may offer further insight for understanding *Salmonella* infections.

2. Materials and Methods

2.1. Bacterial Strains for Antibiotic Susceptibility Testing

One colony each of *S.* Typhimurium ATCC 14028, *S.* Heidelberg ARI-14 [18], and *E. coli* ATCC 25,922 (control) were inoculated into Luria–Bertani (5 mL, LB) broth (Alfa Aesar, Ward Hill, MA, USA) followed by incubation for 16 h at 37 °C with shaking at 190 revolutions per minute (rpm).

2.2. Antibiotic Susceptibility Testing

The susceptibility of *Salmonella* Typhimurium ATCC 14,028 and *S.* Heidelberg ARI-14 to gentamicin was determined via an agar disc diffusion test along with a modified tube dilution assay. During the agar disc diffusion test, a sterile cotton swab was inserted into the respective overnight bacterial culture and squeezed gently against each tube to remove excess fluid. Cotton swabs were subsequently streaked onto Mueller–Hinton agar plates (BD Biosciences, Franklin Lakes, NJ, USA) employing several angles to promote even growth. Gentamicin paper discs (6-mm, Becton Dickson, Sparks, MD, USA) were aseptically placed at the center of each agar plate, followed by incubation at 37 °C for 16 to 24 h. Inhibition diameter zones were measured for each respective plate, and averages were calculated. The plates were streaked in triplicate, and each experiment was repeated three times.

A modified tube dilution assay determined the minimum inhibitory concentration (MIC) for each *Salmonella* serovar. Serial dilutions were generated by adding nutrient broth (10 mL, EMD Millipore, Billerica, MA, USA) to the first tube and 5 mL to the remaining tubes, resulting in a total of five tubes. A total of 100 µL of gentamicin (Life Technologies, Grand Island, NY, USA) from a 500 µg/mL stock concentration was added to the first tube (10 mL), and serial dilutions were generated by a transfer of 5 mL of the solution to each subsequent tube. This procedure was repeated for all five dilutions. Five milliliters were removed and discarded from the final tube to ensure identical volumes (5 mL). Tubes were inoculated with 50 µL of the bacterial overnight culture, along with 50 µL of triphenyl tetrazolium chloride (TTC) to act as a growth indicator. Tubes were incubated for 24 h at 37 °C, with a color change from light yellow to pink/red being used as an indication of bacterial growth. The MIC had the lowest concentration of gentamicin, which showed no growth or color in the medium. After incubation for 24 h, 100 µL of each dilution for the respective bacterial serovar was inoculated onto Mueller–Hinton agar (BD Biosciences) plates to enumerate CFU/mL and for confirmation of the MIC results. Plates were conducted in triplicate, and each experiment was repeated three times.

2.3. Cell Cultures

Caco-2 and HD11 cells were sustained in D10F medium (Dulbecco's Modified Eagles Medium (MEM), High Glucose (Thermo Scientific, Logan, UT, USA) combined with 10% fetal bovine serum (FBS) and nonessential amino acids. Cells were subsequently grown in a 75 cm^2 flask at 37 °C held in an (5% CO_2 atmosphere) incubator (New Brunswick, Eppendorf, Enfield, CT, USA). Once the cells in the flask reached approximately 80% confluence, they were subjected to 0.25% trypsin–EDTA (Life Technologies) for the release

of attached cells, followed by the inoculation (104 cells per mL) of new stock cultures. To conduct the adhesion and invasion assays, 10^4 Caco-2 and HD11 cells per mL were inoculated into 24-well tissue culture plates (Greiner Bio-One, Monroe, NC, USA) followed by 37 °C incubation in an incubator (5% CO_2 atmosphere) until a semi-confluent monolayer was obtained.

2.4. Bacterial Cultures for Adhesion and Invasion Assays

The *Salmonella* isolates used for this study are listed in Table 1. One colony of each strain was inoculated into 8 mL of LB broth and subsequently placed in the incubator (16 to 18 h at 37 °C).

Table 1. *Salmonella* strains used in this study.

Adhesion and Invasion to Caco-2 Cells	Adhesion and Invasion to HD11 Cells
Salmonella Typhimurium ATCC 14028	*Salmonella* Typhimurium ATCC 14028
	Salmonella Typhimurium UK-1
Salmonella Heidelberg ARI-14	*Salmonella* Heidelberg ARI-14
	Salmonella Enteritidis ATCC 13076

2.4.1. Adhesion Assays

Bacterial enumeration in each of three representative wells was performed by trypsinizing each respective well with 0.3 mL of trypsin–EDTA, followed by incubation at 37 °C in an incubator (5% CO_2 atmosphere) for 5 min and the subsequent addition of 0.7 mL of D10F. Bacterial cultures grown overnight were subsequently washed 3 times with PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4). Bacterial cells were diluted to an MOI ratio of 10:1 (10^6 *Salmonella*: 10^5 HD11 or Caco-2 cells). Washed bacteria cells were subsequently diluted 10^{-6} with PBS followed by plating 100 µL on LB agar plates to enumerate CFU/mL. The diluted bacteria were added to the respective cell lines, Caco-2 or HD11, and plates were incubated at 37 °C in an incubator (5% CO_2 atmosphere, Thermo/Forma Scientific, Marietta, OH, USA) for 2 h. Cells were subsequently washed 3 times with cell PBS (137 mM NaCl, 5.4 mM KCl, 3.5 mM Na_2HPO_4, 4.4 mM NaH_2PO_4, 11 mM glucose, pH 7.2) followed by treatment with 1 mL of 0.1% Triton X-100 in cell PBS. Plates were incubated for 10 min at 37 °C in an incubator (5% CO_2 atmosphere). The disrupted cells were collected in duplicate, serially diluted, and plated on LB agar to determine the adhesion percentage. Plates were subsequently incubated for 16 h at 37 °C. To determine CFU/mL, all LB agar plates were inoculated for each duplicate. Each strain was subsequently tested in triplicate in three independent experiments.

2.4.2. Invasion Assays

The enumeration of cells in each of the three representative wells was performed by trypsinizing each respective well with 0.3 mL of trypsin–EDTA, incubating at 37 °C in an incubator (5% CO_2 atmosphere) for 5 min, and adding 0.7 mL of D10F. Overnight bacterial cultures were washed 3 times with PBS and subsequently diluted to obtain an MOI ratio of 10:1 (*Salmonella*: animal cells). Washed bacteria were diluted 10^{-6} with PBS, followed by plating 100 µL on LB agar plates for CFU/mL enumeration. The diluted bacteria were added to the cell lines, Caco-2 or HD11, and the plates were incubated at 37 °C in an incubator (5% CO_2 atmosphere) for two hours. The cells were washed 3 times with cell PBS followed by treatment with 1 mL of DMEM containing 100 µg/mL gentamicin per well in order to kill extracellular bacteria considered to be adherent. The plate was incubated for 2 h at 37 °C in an incubator (5% CO_2 atmosphere). The cells were subsequently washed 3 times with cell PBS and subjected to treatment with 1 mL of 0.1% Triton X-100 in cell PBS. The plate was incubated for 10 min at 37 °C in an incubator (5% CO_2 atmosphere). Serial dilutions of suspensions were conducted in PBS followed by inoculation onto LB agar plates, in duplicate, to determine the number of organisms that survived treatment

with gentamicin and, hence, had invaded the Caco-2 or HD11 cells. Plates were incubated at 37 °C for 16 h. Each strain was tested in triplicate in three independent experiments.

2.5. Statistical Analysis

The JMP Pro Software Version 11.0 (SAS Institute Inc., Cary, NC, USA) program was used to conduct all statistical analyses. Mean ± standard deviation was determined for each antibiotic susceptibility test. One-way ANOVA and the Tukey–Kramer HSD test were applied to each bacterial strain's adhesion and invasion percentages to Caco-2 and HD11 cells. Statistical significance was established at $p < 0.05$.

3. Results and Discussion

Gentamicin has been shown to inhibit bacterial protein synthesis and eliminate *Salmonella* tissue cell culture invasion [19]. This makes it a useful treatment for differentiating between external bacterial cells and internalized cells. All three strains were observed to be susceptible to gentamicin. Both *Salmonella* Heidelberg ARI-14 and *S.* Typhimurium ATCC 14,028 yielded average inhibition zones of 20.7 ± 1.1 and 20.7 ± 0.1, respectively. *Escherichia coli* strain 25922 was used as a control and exhibited a zone of inhibition of 19.0 ± 0. To further quantify susceptibility, the gentamycin was serially diluted in a growth medium, cells were added, and aliquots were plated for enumeration. Growth was detected on agar plates for all three organisms after the third dilution. The MIC for gentamicin was 125 µg/mL for all three strains. These results validated using gentamicin during invasion assays as a means to kill cell-adherent extracellular bacteria. Expected MIC ranges have been reported by Andrews [20] for determining the susceptibility of several bacteria to a wide selection of antibiotics, as well as a list of appropriate controls for inclusion when determining MIC responses. As a control, their suggested MIC range for *Enterobacteriaceae* susceptibility against gentamicin is 0.03 to 128 mg/L using *E. coli* 25,922 [20]. Our results are consistent with this range. When Shah et al. [21] determined the cell invasion responses of several poultry-associated *S.* Enteritidis isolates, they noted a gentamicin MIC of <0.125 µg for all the isolates and used 100 µg per mL for their Caco-2 invasion assays. Menashe et al. [22] examined the MIC of *S.* Typhimurium 14,028 and *S.* Virchow and reported MIC values of 125 µg per mL for both serovars.

In the current study, we examined the ability of *Salmonella* strains from different serovars to attach and invade two cell lines, namely, Caco-2 and HD11. We only evaluated two serovars for the Caco-2 study, the well-characterized *S.* Typhimurium serovar strain and the previously uncharacterized *S.* Heidelberg serovar. Our rationale for the Caco-2 cell study was to only compare the *S.* Heidelberg directly with the standard *S.* Typhimurium typically used in these types of tissue culture studies to assess whether the invasion response of the *S.* Heidelberg isolate was similar to *S.* Typhimurium. As expected, the percentage attachment for each respective *Salmonella* strain was greater than the ability of the respective strain to invade the cells. *Salmonella* Heidelberg and *S.* Typhimurium yielded percentages of adhesion of 28.8 ± 6.37 and 18.1 ± 6.25, respectively, to Caco-2 cells (Table 2). However, the Caco-2 invasion ability of these respective strains was lower, 1.37 ± 0.25 for *S.* Heidelberg and 1.52 ± 0.02 for *S.* Typhimurium (Table 3). The underlying detailed mechanisms that intracellular pathogens such as *Salmonella* use to penetrate the host epithelium continue to be investigated. Cultured mammalian cells as in vitro models have been used to study the interaction and internalization of *Salmonella* [23–25]. The invasion of cultured epithelial cells is commonly used to measure *Salmonella* pathogenicity [21,26,27]. Previous studies report that several environmental stimuli such as osmolarity [28,29], carbohydrate availability [30], and oxygen availability [31–33] influence *Salmonella* invasion of cultured mammalian cells. For example, Durant et al. [25] reported that *Salmonella* exposed high concentrations of short-chain fatty acids (SCFAs) at levels similar to the lower regions of the gastrointestinal tract, along with pH changes, could influence the association and invasion of Hep-2 cells. Shah et al. [21] reported that in cultured Caco-2 cells, isolates

with high invasiveness could invade and/or survive more extensively within chicken macrophage cells compared to low-invasive isolates.

Table 2. *Salmonella* Typhimurium and *S.* Heidelberg adhesion to human epithelial Caco-2 cells.

Bacterial Strain	Log Dilution	# of Colonies	# Bacteria Added	# Bacteria Adhering	% Adhesion
S. Typhimurium ATCC 14028	4	17.67 ± 2.31	$9.83 \times 10^6 \pm 0$	$1.78 \times 10^6 \pm 0.25$	18.1 ± 6.25
S. Heidelberg ARI-14	4	26.0 ± 2.83	$9.13 \times 10^6 \pm 0$	$2.63 \times 10^6 \pm 0.32$	28.8 ± 6.37

Adhesion between *S.* Heidelberg and Typhimurium was not significantly different ($p > 0.05$).

Table 3. *Salmonella* Typhimurium and *S.* Heidelberg adhesion and invasion to human epithelial Caco-2 cells.

Bacterial Strain	Log Dilution	# of Colonies	# Bacteria Added	# Bacteria Invading	% Invasion
S. Typhimurium ATCC 14028	2	124.75 ± 10.69	$8.26 \times 10^6 \pm 0$	$1.25 \times 10^5 \pm 0.01$	1.52 ± 0.02
S. Heidelberg ARI-14	2	63.17 ± 17.54	$5.24 \times 10^6 \pm 0$	$5.96 \times 10^4 \pm 1.42$	1.37 ± 0.25

Invasion between *S.* Heidelberg and Typhimurium was not significantly different ($p > 0.05$).

Three *Salmonella* serovars, *S.* Typhimurium (two strains, ATCC 14028, and UK-1), *S.* Enteritidis, and *S.* Heidelberg, were compared in HD11 cell culture experiments (Tables 4 and 5). For HD11 cells, attachment percentages were generally greater (Table 4) for *S.* Heidelberg and *S.* Typhimurium ATCC 14028 than those observed for these strains in Caco-2 cell cultures (Table 2) and exhibited higher rates for invasion, ranging from 2.9 to 17.6% (Table 5) compared to Caco cell invasion percentages (Table 3). *S.* Heidelberg, *S.* Typhimurium ATCC 14,028, and *S.* Typhimurium UK-1 exhibited the greatest levels of percent attachment ($p < 0.05$), with *S.* Enteritidis attachment being less than half the other serovars (18.6 ± 2.42%). *Salmonella* Typhimurium strain UK-1 invaded HD11 cells at higher percentages, 17.6 ± 3.29, likely due to the strain's high virulence phenotype. *Salmonella* Typhimurium UK-1 (abbreviated for "universal killer") is a highly virulent strain initially isolated in 1991 from an infected horse and passaged through a chicken [34]. *Salmonella* Typhimurium ATCC 14028 (9.3 ± 0.57) and UK-1 (17.6 ± 3.29)'s respective invasion levels of HD11 cells support that strain differences can occur within the same serovar. Luo et al. [34] conducted an extensive genome analysis comparing UK-1 and other *S.* Typhimurium strains. They reported that virulence factors from one strain may increase or decrease virulence from another [35]. The identification of polymorphic genomic regions of the strains and further analyses revealed that even highly similar strains of *S.* Typhimurium could be distinguished [35]. Bhomik et al. [35] reported that mutants of *S.* Typhimurium UK-1 were less invasive of HD11 cells than the wildtype strain.

Table 4. Adhesion of four *Salmonella* strains from different serovars to chicken macrophage HD11 cells.

Bacterial Strain	Log Dilution	# of Colonies	# Bacteria Added	# Bacteria Adhering	% Adhesion *
S. Heidelberg	4	59.6 ± 15.96	$1.3 \times 10^7 \pm 0.47$	$5.5 \times 10^6 \pm 1.98$	43.4 ± 7.46 [a]
S. Typhimurium ATCC 14028	4	61.8 ± 17.58	$1.6 \times 10^7 \pm 0.47$	$6.2 \times 10^6 \pm 1.15$	38.7 ± 4.57 [a]
S. Typhimurium UK-1	4	69.2 ± 19.85	$1.8 \times 10^7 \pm 0.71$	$7.0 \times 10^6 \pm 1.93$	38.9 ± 9.24 [a]
S. Enteritidis	4	39.2 ± 9.24	$2.1 \times 10^7 \pm 0$	$3.9 \times 10^6 \pm 0.50$	18.6 ± 2.42 [b]

* Percentages not connected by the same letter are significantly different between each assay ($p < 0.05$).

Table 5. Invasion of four *Salmonella* strains from different serovars to chicken macrophage HD11 cells.

Bacterial Strain	Log Dilution	# of Colonies	# Bacteria Added	# Bacteria Invading	% Invasion *
S. Heidelberg	4	33.2 ± 9.04	$6.91 \times 10^7 \pm 0$	$3.3 \times 10^6 \pm 0.50$	4.8 ± 0.73 [c]
S. Typhimurium ATCC 14028	4	37.3 ± 4.19	$4.0 \times 10^7 \pm 0.28$	$3.7 \times 10^6 \pm 0.08$	9.3 ± 0.57 [b]
S. Typhimurium UK-1	4	25.3 ± 4.99	$1.4 \times 10^7 \pm 0.15$	$2.5 \times 10^6 \pm 0.40$	17.6 ± 3.29 [a]
S. Enteritidis	4	4.05 ± 3.06	$1.7 \times 10^7 \pm 0.04$	$4.9 \times 10^5 \pm 2.30$	2.9 ± 1.48 [c]

* Percentages not connected by the same letter are significantly different between each assay ($p < 0.05$).

Salmonella Enteritidis exhibited the lowest adhesion (Table 4, 18.6 ± 2.42%) and invasion percentage (Table 5, 2.9 ± 1.48%) of all serovars compared in this study. When the *Salmonella* cell invasion and intracellular survival of five different poultry-associated serovars was compared by He et al. (*S.* Heidelberg, *S.* Enteritidis, *S.* Typhimurium, *S.* Senftenberg, and *S.* Kentucky), *S.* Enteritidis appeared to be more resistant than the other serovars to intracellular killing, leading them to hypothesize that the serovar's intracellular survival ability may be associated with specific systemic invasion capabilities in chickens [36]. Matulova et al. [37] reported lower invasion after pre-treating the HD11 cells with avidin before *S.* Enteritidis infection. Shah et al. [21] suggested that not every isolate of *S.* Enteritidis recovered from poultry may be equally pathogenic or have a similar potential to invade cells. Saeed et al. [38] reported that unlike *S.* Enteritidis isolates recovered from chicken ceca, isolates from chicken eggs or human clinical cases exhibited greater adherence and invasion of chicken ovarian granulosa cells.

4. Conclusions

Cell line type and different serovars appear to be factors that can lead to detectable differences in adhesion and invasion. However, further studies are necessary that involve more *Salmonella* serovars and strains to elucidate the properties that enable some serovars to be able to attach and invade tissue culture cells to a greater extent than others. Understanding the ability of different serovars of this pathogen to adhere and invade specific cell lines could be helpful for further understanding *Salmonella* infections.

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References

1. Chlebicz, A.; Śliżewska, K. Campylobacteriosis, salmonellosis, yersiniosis, and listeriosis as xoonotic foodborne diseases: A review. *Int. J. Environ. Res. Public Health* **2018**, *15*, 863. [CrossRef] [PubMed]
2. Scallan, E.; Hoekstra, R.M.; Angulo, F.J.; Tauxe, R.V.; Widdowson, M.-A.; Roy, S.L.; Jones, J.L.; Griffin, P.M. Foodborne illness acquired in the United States—Major pathogens. *Emerg. Infect. Dis.* **2011**, *17*, 7–15. [CrossRef] [PubMed]
3. Heithoff, D.M.; Shimp, W.R.; House, J.K.; Xie, Y.; Weimer, B.C.; Sinsheimer, R.L.; Mahan, M.J. Intraspecies variation in the emergence of hyperinfectious bacterial strains in nature. *PLoS Pathog.* **2012**, *8*, 1–17. [CrossRef] [PubMed]

4. White, A.E.; Tillman, A.R.; Hedberg, C.; Bruce, B.B.; Batz, M.; Seys, S.A.; Dewey-Mattia, D.; Bazaco, M.C.; Walter, E.S. Foodborne illness outbreaks reported to national surveillance, United States, 2009–2018. *Emerg. Infect. Dis.* **2022**, *28*, 1117–1128. [CrossRef] [PubMed]
5. Chai, S.J.; Cole, D.; Nisler, A.; Mahon, B.E. Poultry: The most common food in outbreaks with known pathogens, United States, 1998-2012. *Epidemiol. Infect.* **2017**, *145*, 316–325. [CrossRef] [PubMed]
6. Andino, A.; Hanning, I. *Salmonella enterica*: Survival, colonization, and virulence differences among serovars. *Sci. World J.* **2015**, *2015*, 520179. [CrossRef] [PubMed]
7. Holt, P.S.; Davies, R.H.; Dewulf, J.; Gast, R.K.; Huwe, J.K.; Jones, D.R.; Waltman, D.; Willian, K.R. The impact of different housing systems on egg safety and quality. *Poultry Sci.* **2011**, *90*, 251–262. [CrossRef] [PubMed]
8. Pulido-Landínez, M. Food safety-*Salmonella* update in broilers. *Anim. Feed Sci. Technol.* **2019**, *250*, 53–58. [CrossRef]
9. dos Santos, A.M.P.; Ferrari, R.G.; Conte-Junior, C.A. Virulence factors in *Salmonella* Typhimurium: The sagacity of a bacterium. *Curr. Microbiol.* **2019**, *76*, 762–773. [CrossRef]
10. Cirillo, D.M.; Valdivia, R.H.; Monack, D.M.; Falkow, S. Macrophage-dependent induction of the *Salmonella* pathogenicity island 2 type III secretions system and its role in intracellular survival. *Mol. Microbiol.* **1998**, *30*, 175–188. [CrossRef]
11. Ohl, M.E.; Miller, S.I. *Salmonella:* A model for bacterial pathogenesis. *Annu. Rev. Med.* **2001**, *52*, 259–274. [CrossRef]
12. Foley, S.L.; Lynne, A.M.; Nayak, R. *Salmonella* challenges: Prevalence in swine and poultry and potential pathogenicity of such isolates. *J. Anim. Sci.* **2008**, *86*, E149–E162. [CrossRef] [PubMed]
13. Foley, S.L.; Johnson, T.J.; Ricke, S.C.; Nayak, R.; Danzelsen, J. *Salmonella* pathogenicity and host adaptation in chicken-associated serovars. *Microbiol. Mol. Biol. Rev.* **2013**, *77*, 582–607. [CrossRef] [PubMed]
14. Winnen, B.; Schlumberger, M.C.; Sturm, A.; Schupbach, K.; Siebenmann, S.; Jenny, P.; Hardt, W.D. Hierarchical effector protein transport by the *Salmonella* Typhimurium SPI-1 type III secretion system. *PLoS ONE* **2008**, *3*, e2178. [CrossRef] [PubMed]
15. Sambuy, Y.; De Angelis, I.; Ranaldi, G.; Scarino, M.L.; Stammati, A.; Zucco, F. The Caco-2 cell line as a model of the intestinal barrier: Influence of cell and culture-related factors on Caco-2 cell functional characteristics. *Cell Biol. Toxicol.* **2005**, *21*, 1–26. [CrossRef] [PubMed]
16. Beug, H.; von Kirchbach, A.; Doderlein, G.; Conscience, J.F.; Graf, T. Chicken hematopoietic cells transformed by seven strains of defective avian leukemia viruses display three distinct phenotypes of differentiation. *Cell* **1979**, *18*, 375–390. [CrossRef] [PubMed]
17. Bueno, S.M.; Riquelme, S.; Riedel, C.A.; Kalergis, A.M. Mechanisms used by virulent *Salmonella* to impair dendritic cell function and evade adaptive immunity. *Immunology* **2012**, *137*, 28–36. [CrossRef]
18. Calo, J.R.; Park, S.H.; Baker, C.A.; Ricke, S.C. Specificity of *Salmonella* Typhimurium strain (ATCC 14028) growth responses to *Salmonella* serovar-generated spent media. *J. Environ. Sci. Health* **2015**, *B50*, 422–429. [CrossRef] [PubMed]
19. MacBeth, K.J.; Lee, C.A. Prolonged inhibition of bacterial protein synthesis abolishes *Salmonella* invasion. *Infect. Immun.* **1993**, *61*, 1544–1546. [CrossRef]
20. Andrews, J.M. Determination of minimum inhibitory concentrations. *J. Antimicrob. Chemother.* **2001**, *48*, 5–16. [CrossRef]
21. Shah, D.H.; Zhou, X.; Addwebi, T.; Davis, M.A.; Orfe, L.; Call, D.R.; Guard, J.; Besser, T.E. Cell invasion of poultry-associated *Salmonella enterica* serovar Enteritidis isolates is associated with pathogenicity, motility and proteins secreted by the type III secretion system. *Microbiology* **2011**, *157*, 1428–1445. [CrossRef] [PubMed]
22. Menashe, O.; Kaganskaya, E.; Baasov, T.; Yaron, S. Aminoglycosides affect intracellular *Salmonella enterica* serovars Typhimurium and Virchow. *Antimicrob. Agents Chemother.* **2008**, *52*, 920–926. [CrossRef] [PubMed]
23. Giannella, R.A.; Washington, O.; Gemski, P.; Formal, S.B. Invasion of HeLa cells by *Salmonella typhimurium*: A model for study of invasiveness of *Salmonella*. *J. Infect. Dis.* **1973**, *128*, 69–75. [CrossRef] [PubMed]
24. Finlay, B.B.; Gumbiner, B.; Falkow, S. Penetration of *Salmonella* through a polarized Madin-Darby canine kidney epithelial cell monolayer. *J. Cell Biol.* **1988**, *107*, 221–230. [CrossRef] [PubMed]
25. Durant, J.A.; Lowry, V.K.; Nisbet, D.J.; Stanker, L.H.; Corrier, D.E.; Ricke, S.C. Short-chain fatty acids affect cell-association and invasion of HEp-2 cells by *Salmonella typhimurium*. *J. Environ. Sci. Health* **1999**, *B34*, 1083–1099. [CrossRef]
26. Van Asten, F.J.; Hendriks, H.G.; Koninkx, J.F.; Van der Zeijst, B.A.; Gaastra, W. Inactivation of the flagellin gene of *Salmonella enterica* serotype Enteritidis strongly reduces invasion into differentiated Caco-2 cells. *FEMS Microbiol. Lett.* **2000**, *185*, 175–179. [CrossRef] [PubMed]
27. Van Asten, F.J.; Hendriks, H.G.; Koninkx, J.F.; van Dijk, J.E. Flagella-mediated bacterial motility accelerates but is not required for *Salmonella* serotype Enteritidis invasion of differentiated Caco-2 cells. *Int. J. Med. Microbiol.* **2004**, *294*, 395–399. [CrossRef]
28. Galán, J.E.; Curtis, R., III. Expression of *Salmonella typhimurium* genes required for invasion is regulated by changes in DNA supercoiling. *Infect. Immun.* **1990**, *58*, 1879–1885. [CrossRef]
29. Tartera, C.; Metcalf, E.S. Osmolarity and growth phase overlap in regulation of *Salmonella typhi* adherence to and invasion of human intestinal cells. *Infect. Immun.* **1993**, *61*, 3084–3089. [CrossRef]
30. Schiemann, D.A. Association with MDCK epithelial cells by *Salmonella typhimurium* is reduced during utilization of carbohydrates. *Infect. Immun.* **1995**, *63*, 1462–1467. [CrossRef]
31. Ernst, R.K.; Dombroski, D.M.; Merrick, J.M. Anaerobiosis, type 1 fimbriae, and growth phase are factors that affect invasion of HEP-2 cells by *Salmonella typhimurium*. *Infect. Immun.* **1990**, *58*, 2014–2016. [CrossRef] [PubMed]
32. Lee, C.A.; Jones, B.D.; Falkow, S. Identification of a *Salmonella typhimurium* invasion locus by selection for hyperinvasive mutants. *Proc. Natl. Acad. Sci. USA* **1990**, *89*, 1847–1851. [CrossRef] [PubMed]

33. Francis, C.L.; Starnbach, M.N.; Falkow, S. Morphological and cytoskeletal changes in epithelial cells occur immediately upon interaction with *Salmonella typhimurium* grow under low-oxygen conditions. *Mol. Microbiol.* **1992**, *6*, 3077–3087. [CrossRef] [PubMed]
34. Luo, Y.; Kong, Q.; Yang, J.; Mitra, A.; Golden, G.; Wanda, S.Y.; Roland, K.L.; Jensen, R.V.; Ernst, P.B.; Curtiss III, R. Comparative genome analysis of the high pathogenicity *Salmonella* Typhimurium strain UK-1. *PLoS ONE* **2012**, *7*, e40645.
35. Bhowmik, B.K.; Kumar, A.; Gangaiah, D. Transcriptomic analyses of chicken primary macrophages infected with attenuated *Salmonella* Typhiurium mutants. *Front. Microbiol.* **2022**, *13*, 857378. [CrossRef] [PubMed]
36. He, H.; Genovese, K.J.; Swaggerty, C.L.; Nisbet, D.J.; Kogut, M.H. A comparative study on invasion, survival, modulation of oxidative burst, and nitric oxide responses of macrophages (HD11), and systemic infection in chickens by prevalent poultry *Salmonella* serovars. *Foodborne Pathog. Dis.* **2012**, *9*, 1104–1110. [CrossRef] [PubMed]
37. Matulova, M.; Rajova, J.; Vlasatikova, L.; Volf, J.; Stepanova, H.; Havlickova, H.; Sisak, F.; Rychlik, I. Characterization of chicken spleen transcriptome after infection with *Salmonella enterica* serovar Enteritidis. *PLoS ONE* **2012**, *7*, e48101. [CrossRef]
38. Saeed, A.M.; Walk, S.T.; Arshad, M.; Whittam, T.S. Clonal structure and variation in virulence of *Salmonella Enteritidis* isolated from mice, chickens, and humans. *J. AOAC Int.* **2006**, *89*, 504–511. [CrossRef]