1 # Resistance mechanisms adopted by a bacteriophage insensitive *Salmonella* Typhimurium
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26 **ABSTRACT**

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28 Bacteriophages have key roles in regulating bacterial populations in most habitats. A
29 *Salmonella* Typhimurium mutant (N18) with impaired sensitivity to phage fmb-p1
30 was examined to establish the adsorption efficiency of fmb-p1 to N18 was reduced to
31 6%, compared to more than 97% for wild type *S.* Typhimurium CMCC50115.
32 Reduced adsorption was accompanied by a reduction of 90% in the LPS content
33 compared to wild type. Electron microscopy showed phage scattered around N18 with
34 minimal engagement, while the phage were efficiently adsorbed to the wild type with
35 tails oriented towards the bacterial surface. Evidence suggests fmb-p1 can
36 inefficiently infect N18 but this does not give rise to an increase of phage titer. RT-
37 qPCR data show that several *Salmonella* genes involved in lipopolysaccharide
38 synthesis and five virulence related genes were down-regulated upon exposure of N18
39 to phage fmb-p1. In contrast, phage resistance related genes such as the SOS response,
40 restriction-modification (RM), and the CRISPR/Cas1 cluster were up-regulated in
41 N18. These data suggest that although inefficient adsorption and entry is the primary
42 mechanism of resistance, transcriptional responses to phage exposure indicate
43 alternative resistance mechanisms against phage infection are also brought to bear,
44 including digestion of phage nucleic acids and activation of the SOS. These findings
45 may help develop strategies for biocontrol of *Salmonella* where multi-resistant
46 bacteria are encountered or emerge in applications for food production,
47 bioremediation or wastewater treatment.

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49 **Key word:** bacteriophage; *Salmonella*; lipopolysaccharide; O-antigen; gene
50 expression; virulence

51 **1. Introduction**

52 *Salmonella* is one of the most important pathogens that frequently causes serious
53 foodborne disease worldwide (CDC, 2017; EFSA, ECDC, 2017; Song et al., 2018).
54 Globally, *S.* Typhimurium frequently isolated non-typhoid *Salmonella* serovar found
55 in eggs and poultry meats (Li et al., 2017; Panzenhagen, et al., 2016), pigs and pork
56 (Boyen et al., 2008), and human infection (Boxstael et al., 2012). Decades of the
57 overuse of antimicrobial agents in food animal production is regarded as one of the
58 underlying reasons for the emergence of antimicrobial resistance in *Salmonella*
59 (Hvistendahl, 2012). Multidrug resistance and cross resistance phenotypes are often
60 observed in *Salmonella* isolated from food products (Cai et al., 2016; Zhu et al., 2017).
61 These resistant types can be transmitted to humans through foods of animal origin,
62 which pose a serious threat to public health.

63 As natural predators of bacteria, bacteriophage (phage) are viruses that
64 specifically kill target bacterial strains, and generally do not attack non-target
65 bacterial species. It is estimated that phages are at least tenfold more abundant than
66 their bacterial hosts (Casjens, 2008). Increasing antibiotic-resistance worldwide (Li et
67 al., 2013; Newell et al., 2010; Yang et al., 2016) has led to a re-evaluation of phage to
68 control these bacteria in food, medical and environmental applications (Endersen et
69 al., 2013; Akhtar et al., 2014; Mostafa et al., 2016). The global incidence of
70 *Salmonella* has provoked a number of studies using phage infecting members of the
71 genus, such as *Salmonella* phage P22 (*Podoviridae*) and *Salmonella* phage Felix-O1
72 (*Myoviridae*) (LeLièvre et al., 2018; Yeh et al., 2017). Phages with activity against
73 *Salmonella* present in many foods have been reported. These include dairy products

74 (Virginie et al., 2019), pork (Hooton et al., 2011; Wang et al., 2017a), chicken meat
75 (Duc et al., 2018), duck meat (Wang et al., 2017b), liquid eggs, drinks, milk (Zinno et
76 al., 2014), hot dogs, cooked and sliced turkey breast, mixed seafood, chocolate milk,
77 and egg yolk (Guenther et al., 2012). To date, more than 100 *Salmonella* phage
78 genomes have been completely sequenced (NCBI Database, 2018).

79 The interaction between phage and bacteria is understood for relatively few
80 phages but an exemplar *Salmonella* phage P22 (Baxa et al., 1996; Steinbacher et al.,
81 1997; Casjens and Thuman-Commike, 2011). The phage infection process begins
82 with the specific adsorption of the phage to a receptor on the host surface. The
83 repetitive O-antigen structure in *Salmonella* lipopolysaccharide (LPS) was found to be
84 the receptor of phage P22 (Baxa et al., 1996). Phage P22 binds to the receptor via six
85 homotrimeric tailspikes, which possess endoglycosidase activity, hydrolyzing the O-
86 antigen polysaccharide, before binding a secondary cell receptor. Thereafter, the
87 phage injects its genome into the target cell to direct host cell resources to complete
88 the life cycle of the phage, culminating in host cell lysis and the emergence of new
89 virions.

90 However, host bacteria develop antiphage strategies to prevent cell lysis, for
91 example *Salmonella* modify the structure of LPS to block the adsorption of phage P22
92 (Steinbacher et al., 1997). Bacterial strains can contain multiple antiphage barriers,
93 which are of concern for phage therapy applications. Phage resistance mechanisms in
94 bacteria have been reported at various stages in the phage life cycle, including the
95 prevention of phage adsorption, the prevention of phage DNA entry, targeted
96 cleavage of phage nucleic acids, abortive infection systems and prophage mediated
97 mechanisms that prevent super-infection (Labrie et al., 2010; Davis and Waldor,
98 2002). However, the impact of multiple mechanisms in the same bacterial cell has

99 rarely been assessed to date. Furthermore, the mechanism of interaction between a
100 *Siphoviridae* phage and *Salmonella* host are not well documented.
101 This study aimed to provide insights into the mechanisms of phage resistance that
102 occur in *Salmonella* Typhimurium when phage and *Salmonella* coexist. The
103 expression of LPS biosynthetic genes, recognized virulence genes of *S.* Typhimurium
104 in a phage-insensitive mutant have been determined in this work. The molecular
105 mechanisms and strategies of how *S.* Typhimurium becomes resistant to phage are
106 analyzed and discussed.
107

108 **2. Material and methods**

109 **2.1. Phage, bacterial strains and culture**

110 Phage fmb-p1 morphologically resembles the *Siphoviridae* exhibiting a virulent
111 life cycle (no evidence of lysogeny) that was isolated from sewage in 2014. The
112 genome of fmb-p1 is composed of a 43,327-bp double-stranded DNA molecule with
113 60 open reading frames. Phage fmb-p1 lyses seven serovars of *Salmonella* (*S.*
114 Typhimurium, *S.* Enteritidis, *S.* Anatum, *S.* Miami, *S.* Agona, *S.* Saintpaul and *S.*
115 Paratyphi-C), and remains stable over a range of temperatures (40-75 °C), pH (4-10)
116 and NaCl solutions (1-11%). The latent period of fmb-p1 was approximately 20 min,
117 and the burst size was 77 ± 4 PFU/cell (Wang et al., 2017b). Phage fmb-p1 was stored
118 in SM buffer (10 mM NaCl, 10 mM $MgSO_4$, 50 mM Tris•HCl, pH 7.5) at -20°C prior
119 to experiment.
120 *S.* Typhimurium CMCC50115 (wild type) and *S.* Typhimurium N18 (mutant)
121 were used in this study. *S.* Typhimurium N18 was isolated after infection of a *S.*
122 Typhimurium culture CMCC50115 (10^9 CFU/mL) in LB with phage (10^2 PFU/mL),
123 after 7 days at 25 °C. Ten single colonies were recovered from an aliquot of the

124 culture spread on LB agar at 37 °C for 24h. After purification of the presumptive
125 resistant strains on LB agar, the stability of the phage resistance phenotype was
126 verified by five consecutive sub-cultures in the presence of fmb-p1.

127 **2.2 Characterization of *S.* Typhimurium N18**

128 A fresh working culture of N18 was prepared by inoculating the stock into 50 mL
129 LB and incubated at 37 °C for 12 h with constant, gentle shaking (180 r/min) to obtain
130 cell concentrations of ~10^9 CFU/mL. The culture was used to inaculate LB and
131 *Salmonella* Chromogenic Medium plates and incubated at 37 °C for 24h. The
132 characteristics of N18 were tested using a *Salmonella* dehydration biochemical
133 identification kit according to the manufacturer's specifications (Luqiao, Beijing). The
134 phage sensitivity of N18 was tested by the spot test method as described by Wang et
135 al. (2017a). The genomic DNA (gDNA) of N18 was extracted and purified using a
136 bacterial gDNA extraction kit (Shenggong, Shanghai, China). The concentration of
137 gDNA was measured using a Nano Drop 2000 spectrophotometer (Thermo Fisher
138 Scientific, USA). DNA samples were stored at −20 °C for PCR. The gDNA of N18
139 was tested using a specific primer pair for *S.* Typhimurium gene STM4494 and a
140 specific primer pair for phage fmb-p1gene P28 (Table 1). The PCR amplification
141 mixtures contained 12.5 μL 2×Taq Master Mix (Vazyme Biotech, Nanjing, China),
142 1μL of each primer（10 mmol/L, 1 μL gDNA（50 ng/μL)) and sterile distilled water
143 up to 25 μL. A reaction mixture with sterile distilled water instead of template DNA
144 was used as a negative control, the gDNA of CMCC50115 and phage fmb-p1 were
145 used as positive controls. The PCR cycling program consisted of an initial
146 denaturation at 94 °C for 5 min, followed by 30 amplification cycles (94 °C for 30 s,
147 55 °C for 30 s, and 72 °C for 45 s), and a final extension step at 72 °C for 10 min. The

148 PCR products were separated on 1% agarose gel electrophoresis and visualized using
149 a UV transilluminator. All the experiments were performed in triplicate.

150 **2.3. Determination of phage adsorption**

151 Phage adsorption was determined according to the method of Kropinski (2009)
152 with minor modifications. Briefly, a mid-log phase bacterial culture was infected with
153 a phage suspension to achieve a multiplicity of infection (MOI) of 0.01. The mixture
154 was allowed to adsorb for 15 min at room temperature before centrifugation at 10,000
155 ×g for 2 min. The supernatant was used for the determination of the unabsorbed phage
156 titer using the agar overlay method (Hungaro et al., 2013). The phage adsorption
157 efficiency was calculated as following:

158 phage adsorption efficiency = (total phage titer- unadsorbed phage titer)/total phage
159 titer×100%

160 **2.4 Determination of phage adsorption using transmission electron microscopy**

161 Bacterial cultures (CMCC50115 or N18) wwere prepared by inoculating the stock
162 into 50 mL LB and incubated at 37 °C for 12 h with constant, gentle shaking (180
163 r/min) to obtain cell concentrations of ~10^9 CFU/mL. The culture was diluted to 10^7
164 CFU/mL with SM buffer in a 1.5 mL tube, then a 100μL dilution solution and 100μL
165 phage stock (10^{10} PFU/mL) were mixed together in a new tube and kept static for 10
166 min at room temperature. Negative staining method was used to image phage
167 adsorption structures (Kropinski, 2009). Phage/host morphologies were examined
168 using a H-7650 electron microscope (Hitachi, Japan) operated at 80 kV.

169 **2.5. Extraction, purification and determination of LPS**

170 *Salmonella* without phage were inoculated into 100 mL LB and incubated at
171 37 °C for 12 h with 180 r/min to obtain ~10^9 CFU/mL prior to experiment. The
172 culture was centrifuged with Eppendorf centrifuge at 8,000 g at 4 °C for 10min. The
173 pellet was washed twice with normal saline, washed once with ddH_2O, and finally re-

174 suspended in 10ml ddH_2O. The cell suspension was sonicated using a Scientz-IID
175 Ultrasonic unit (Ningboxinzhi, China) for 1h until the solution clarified. The treated
176 solution was added as the bulk volume with 90% phenol, churned at 68 °C for 30 min,
177 put on ice overnight and then centrifuged with 5,000 g at 4 °C for 20 min. The
178 supernatant was collected, and the phenol saturated with ddH_2O before centrifuging
179 again. The aqueous supernatants were collected and dialyzed with flow water for 24 h,
180 and then dialyzed with ddH_2O for 72 h (until no purple emerged using a $FeCl_3$
181 test).The solution was concentrated to a quarter of the original volume in a fume hood.
182 The concentrate contained the crude LPS extract.

183 DNase and RNase were added into the LPS crude extract to final concentrations
184 of 100 μg/mL, respectively. The suspension was then incubated at 37 °C for 4 h. After
185 this, proteinase K was added into the suspension to final concentrations of 100 μg/mL,
186 and then incubated at 37°C for 3 h. The suspension was then heated at 100°C for 10
187 minutes, centrifuged at 5,000 g for 30 min after cooling to room temperature. The
188 supernatant was added to two-fold volume of acetone and kept overnight. The
189 solution was centrifuged with 10,000 g for 10 min, and the precipitate dried in a fume
190 hood. The dried precipitate represents purified LPS.

191 The purified LPS was dissolved in ddH_2O for further detection. The concentration
192 of LPS solution was determined using phenol-sulfuric acid method (Gabriela et al.,
193 2003) with slight modifications as indicated below. The LPS solution (1.0 mL) and
194 the phenol solution (0.5 mL) were added to screw cap tubes (13×150 mm), which
195 were capped and vortex-stirred. Then 3.0 mL of concentrated sulfuric acid was added
196 slowly down the side of the tube. The tubes were then closed, vortex-stirred for 5 s
197 and incubated at 100°C for 20 min, and then cooled to room temperature with flow

198 water, before reading the absorbance at 490 nm using distilled water as blank in a
199 UV-2600 UV/Visible spectrophotometer (Shimadzu, Japan).

200 **2.6 LPS as a decoy for phage adsorption**

201 The extract solutions of *Salmonella* LPS were diluted to the same
202 concentration (400 μg/mL) with sterile distilled water prior to the experiment. An
203 overnight *Salmonella* culture of 100 μL (10^5 CFU/mL) was added into 6 sterilized
204 tubes with cap (20 mL). Then, either 100μL or 400μL of LPS solution from either
205 wild type (50115LPS) or mutant (N18LPS) were added to the first four of the tubes.
206 Fresh LB medium was added to each of the six tubes to a total volume of 9.9 mL.
207 Finally, 100 μL of phage (10^{11} PFU/mL) was added into 5 of the above tubes, and
208 100μL of SM buffer was added to the last tube as a negative control. All tubes were
209 shaken and gently mixed and incubated at room temperature for 15 minutes. The
210 *Salmonella* count of each test tube was determined after incubation at 37 °C for 4 h.
211 All experiments were performed in triplicate.

212 **2.7. Gene expression analysis**

213 To detect the gene expression of *Salmonella* exposed to phage, total RNA was
214 extracted immediately after phage addition, after 10 min and 24h of incubation.
215 *Salmonella* infection was initiated by adding 100 μL of phage (10^7 PFU/mL) and 100
216 μL of overnight cultures of *Salmonella* N18 and CMCC50115 (10^9CFU/mL) to 100
217 mL of LB broth at 37 °C. All extractions were performed in triplicate.

218 Total RNA was isolated from *Salmonella* cells using a Bacterial RNA Kit (Omega
219 Bio-tek, USA) according to the manufacturer's instructions, and then each RNA
220 sample was treated with recombinant DNase I (Takara, Japan). The DNase-treated
221 RNA was reverse-transcribed using the First strand cDNA synthesis kit (Vazyme,
222 China), according to manufacturer's specifications (5 min at 25 °C, 15 min at 50 °C, 5

223 min at 85 °C). The concentrations of cDNA were determined using a Nano Drop 2000
224 spectrophotometer (Thermo Fisher Scientific, USA). The cDNA samples were then
225 divided into small volumes and stored at -20 °C until use.

226 Real-time PCR assays were prepared as follows (20 μL final volume per sample):
227 10 μL of SYBR® Green Master Mix (High ROX Premixed, Vazyme), 0.4 μL of each
228 primer (10 mM, Table1), 2 μL of cDNA template (100 ng/uL), and 7.2 μL of RNase-
229 free water. A thermocycler (ABI StepOnePlus™ system) was programmed as follows:
230 initial denaturation at 95 °C for 5 min, followed by 40 cycles of 10 s at 95 °C, 30 s at
231 60 °C, with a single fluorescence measurement; a final melting curve program of 15 s
232 at 95 °C and 1 min at 60 °C, followed by 15 s at 95 °C. A cDNA template-free
233 negative control was included in each run to confirm that there was no background
234 contamination, and a housekeeping gene for 16S rRNA was used as an endogenous
235 control since it is constitutively expressed under a wide range of conditions. The mean
236 values of curve thresholds (Ct) were considered to calculate the relative expression of
237 target genes by the comparative method using the $2^{-\Delta\Delta CT}$ equation (Livak &
238 Schmittgen, 2001). PCR efficiency was determined and melting curve analysis was
239 performed to ensure that a single gene product was amplified for each target gene
240 prior to experiment.

241 **2.8. Statistical analysis**

242 Statistical significances were determined by analysis of variance (ANOVA) in
243 SAS, and Duncan's new-multiple range test of SPSS 13.0 (SPSS Inc, Chicago, IL,
244 USA). The level of statistical significance was $p < 0.05$.

245

246 **3. Results**

247 **3.1 Characterization of *S.* Typhimurium N18**

248 *S*. Typhimurium N18 was isolated and purified post-infection of *S*. Typhimurium 249 CMCC50115 with phage fmb-p1. Plaque assays indicated strain N18 could not be 250 lysed by phage fmb-p1 (see Fig. 1B compared to wild type 1A). Fig. 1D shows the 251 N18 mutant produces an atypical colony morphology with irregular borders on LB 252 solid medium compared to the regular round colonies of wild type *S.* Typhimurium 253 CMCC50115 (Fig. 1C). However, N18 retains the diagnostic purple color on 254 *Salmonella* Chromogenic Medium plate as *S.* Typhimurium CMCC50115 (Figs. 1E, 255 1F) and the same biochemical profile as the wild type. Genomic DNA of N18 could 256 be PCR amplified with specific primers designed on the *S.* Typhimurium 257 CMCC50115 strain but did not produce amplicons with fmb-p1 phage specific 258 primers (Fig. 1G). These results suggest that N18 is a phage insensitive non-lysogenic 259 derivative of *S*. Typhimurium CMCC50115.

260 **3.2 Differences in phage adsorption between N18 and CMCC50115**

261 Transmission electron microscopy showed that phages gathered in an orderly 262 fashion upon encountering *S.* Typhimurium CMCC50115 cells with phage tails 263 observed near the host cell (Fig. 2A, 2C). However, there were only a few non-264 adherent phage particles scattered around N18 cells, and even fewer adsorbed with 265 tails orientated towards the N18 cell (Figs. 2B, 2D). These images suggest that the 266 primary interaction of phage fmb-p1 with N18 has been compromised. The phage 267 adsorption rate to *S.* Typhimurium N18 was reduced in comparison to *S.* 268 Typhimurium CMCC50115, with only 6% of the applied titer adsorbed by N18 as 269 against 97% for CMCC50115 (Fig. 2E). Phage replication was not evident after 24h 270 incubation with N18, while the phage titer of wild type *S.* Typhimurium CMCC50115 271 increased by 4 $\log_{10}$ PFU/mL (Fig. 2F). As shown in Table 2, the LPS content of N18

272 was determined as only 10% of that for *S.* Typhimurium CMCC50115, which may
273 account for the lack of adsorption.

274 Fig. 3 shows the ability of LPS to act as decoy for phage fmb-p1 binding to *S.*
275 Typhimurium CMCC50115 to reduce phage infection the degree of the fall in the
276 viable count. In the absence of LPS the viable count of *S.* Typhimurium CMCC50115
277 falls from $\log_{10}$ 5.3 CFU/ml to undetectable levels post exposure to 10^{10} PFU fmb-p1.
278 LPS extracted from wild type *S.* Typhimurium acts an effective decoy reducing the
279 loss in viable count to 0.9 or 0.3 $\log_{10}$ CFU/mL from cultures containing respectively
280 low (100 µL) or high (400 µL) concentrations of 50115LPS. The N18 LPS extract
281 was not as effective with reductions of 2.1 or 1.5 $\log_{10}$ CFU/mL in the *Salmonella*
282 count containing respectively either low (100 µL) or high (400 µL) concentrations of
283 N18LPS.

284 **3.3 Effect of phage on *Salmonella* LPS synthesis gene expression**

285 The biosynthetic pathway and export mechanisms of LPS are common to most
286 Gram-negative bacteria (Wang & Quinn, 2010). The expression of the majority of
287 LPS-related synthesis genes in the absence of phage was not significantly different
288 between the wild type strain and phage-insensitive isolate N18 in LB broth. However,
289 there were four key exceptions to this: *lpxA, rfaL, pagP* and *wzzB* that show reduced
290 expression in N18 (Fig. 4). Fig 5A shows 13 LPS synthesis genes of *S.* Typhimurium
291 N18 were significantly down-regulated ($p < 0.01$) upon exposure to phage with most
292 showing down-regulation greater than 3 $\log_2$ fold, compared to the control. Genes
293 *lpxA*, *lpxB*, *pagP*, and *msbA* have key functions in lipid A biosynthesis. These four
294 genes of N18 were down-regulated (3.9-, 3.7-, 3.4-, and 3.8- $\log_2$ fold, respectively),
295 suggesting that the lipid A biosynthesis was reduced in phage-exposed *Salmonella*.
296 Genes *rfaG*, *rfaI*, *rfaJ*, *rfaK*, and *rfaL* play an important role in core oligosaccharide

297 biosynthesis. These five genes of N18 strain were also down-regulated by phage (3.7-,
298 3.6-, 3.8-, 3.5-, and 3.7- $\log_2$ fold, respectively), indicating that the core
299 oligosaccharides biosynthesis was similarly affected. Genes *rfbA*, *kdtA*, *wzzB*, *lpdT*
300 are the major genes responsible for O-antigen biosynthesis. These four genes of N18
301 strain were down-regulated by phage (3.8-, 3.8-, 3.6-, and 3.4- $\log_2$ fold, respectively),
302 indicating that the O-antigens biosynthesis were modified in *Salmonella*. These results
303 were consistent with the reduction of LPS content observed for N18 (Table 2).
304 However, seven genes of *S.* Typhimurium CMCC50115 were down-regulated by
305 phage fmb-p1 exposure. Under these circumstances (10 minutes post-infection at
306 MOI=1) *S.* Typhimurium CMCC50115 is phage infected with demonstrable
307 transcription of the representative phage genes *p1, p11* and *p28*. In contrast, phage
308 gene transcription in the phage insensitive mutant N18 at 10 minutes was undetectable
309 but was evident by 24 h; accordingly, the differences recorded for *S.* Typhimurium
310 CMCC50115 were significantly less than that determined for N18.

311 **3.4 Effect of phage on the expression of *Salmonella* virulence genes**

312 The virulence of *Salmonella* is of general concern as it represents a serious threat
313 to the health of domestic animals and humans worldwide. In the present study,
314 expression of five host virulence genes (*invA, sseL, mgtC, sopB* and *spvC*) was tested
315 during phage infection. Fig. 5B shows the expression of five virulence genes to be
316 significantly down-regulated by phage in *S.* Typhimurium CMCC50115 and N18.

317 **3.5 Effect of phage on gene expression of phage resistance in *Salmonella***

318 Phage infection up-regulated gene expression of *lexA, recA,* and *CRISPR/cas1* in
319 *S.* Typhimurium CMCC50115 by 1.5, 1.3 and 2.9-$\log_2$ fold, respectively (Fig 6A).
320 These changes were greater than the corresponding values observed for the phage
321 treatment of N18 (0.9, 0.7, and 1.4-$\log_2$ fold,).

322 As shown in Fig. 6B, genes *hsdR, hsdM* and *hsdS* comprising the type I RM
323 system were up-regulated by phage in strain N18 (1.7, 1.1, and 1.4-$\log_2$ fold,
324 respectively). In contrast, *hsdM* was up-regulated by phage in *S.* Typhimurium
325 CMCC50115, and *hsdS* was slightly down-regulated after phage infection. Two genes
326 representing the type III RM system were significantly up-regulated ($p < 0.05$) by
327 phage in both *S.* Typhimurium CMCC50115 and N18 (Fig. 6C).
328

329 4. Discussion

330 Recently, there has been renewed interest in phage-bacteria interactions because
331 phages have the potential to treat multi-drug resistant bacteria in in medicine and
332 agriculture. However, there are still obstacles to phage application, notably phage
333 resistance. Phage resistance mechanisms include the inhibition of phage attachment to
334 cell surface receptors, cleavage of the invading phage genome, replication
335 interference exerted by bacterial CRISPR–Cas systems and even the inductive
336 abortion of phage infection (Labrie et al., 2010; Samson et al., 2013).
337 The *S.* Typhimurium CMCC50115 derivative N18 was largely but not
338 completely insensitive to infection by bacteriophage fmb-p1. Phage gene transcription
339 was detected in N18 was detected 24 h post treatment albeit at low levels. Low-level
340 transcription may have arisen as a consequence of mutant instability permitting phage
341 access to a subpopulation of bacteria, or that a genetic subpopulation of phage can
342 achieve entry and initiate transcription or simply that sufficient phage host
343 interactions can give rise to infection by stochastic process. These interactions do not
344 give rise to an increase in phage titer either because the replication does not complete
345 or that the subpopulations supporting phage infection are insufficient to increase
346 phage titer against phage loss due to inactivation.

347 The first barrier to phage replication in N18 is poor adsorption. Transmission
348 electron microscopy confirmed that adsorption of fmb-p1 to the surface of N18
349 bacteria is disordered compared to the regular engagement observed for the wild type
350 strain. LPS is a significant structure in this process as demonstrated by the
351 concentration dependent decoy effect of LPS extracts to reduce phage infection of
352 sensitive *S.* Typhimurium CMCC50115. *S.* Typhimurium N18 yielded only 10% of
353 the extractable LPS of wild type. *S.* Typhimurium cells may decrease the density of
354 LPS or alter the structure of the receptor critical for phage adsorption. This
355 mechanism could be conducive for the survival of bacteria along with phage present
356 in the same habitat. LPS extracts of wild type *S.* Typhimurium and phage- insensitive
357 mutant can interfere with the infection and lysis of *Salmonella* by phage. The LPS of
358 N18 is not as effective as wild type LPS in preventing phage adsorption but is still
359 capable of interfering with phage adsorption and lysis suggesting the essential
360 structures for LPS phage interaction are present but are limited due low overall LPS
361 content and low specificity in the phage-insensitive *Salmonella*. We also observed
362 down-regulation of the LPS synthesis genes upon exposure to phage. These data
363 imply a host adaptive response to phage infection that was most notable in the phage
364 insensitive *S.* Typhimurium, likely because the host was not wholly committed to
365 phage transcription and the shifts in host metabolism that support replication. These
366 data do not rule out external sensor signaling as a result of phage host surface
367 interactions or early diffusible lysis products.

368 Bacteria have evolved a range of barriers to prevent phage adsorption, such as the
369 blocking of phage receptors, the production of extracellular matrix and the production
370 of competitive inhibitors. For example, *Escherichia coli* phage T5, produces a
371 lipoprotein (Llp) that blocks its own receptor, ferrichrome-iron receptor (FhuA)

372 (Pedruzzi et al., 1998). Phage ΦV10 possesses an O-acetyltransferase that modifies
373 the O157 antigen of *E. coli* O157:H7 to block adsorption of ΦV10 and phages with
374 similar specificities (Perry et al., 2009). *Salmonella* phage P22, which recognizes the
375 O antigen, has also evolved to specifically recognize polysaccharides such as O
376 antigens (Steinbacher et al., 1997). The antimicrobial molecule microcin J25 uses
377 FhuA as a receptor and can outcompete phage T5 for binding to FhuA (Destoumieux-
378 Garzón et al., 2005). The changes we observed in LPS of *Salmonella* CMCC50115
379 upon phage infection show broad similarity to mechanisms adopted to evade phage
380 infection.

381 High concentrations of LPS can induce fever, increase heart rate, and lead to
382 septic shock and death following organ failure (Wang & Quinn, 2010). The content of
383 LPS in N18 was much lower than that of the wild type *S.* Typhimurium strain. The
384 selection of phage insensitive types with reduced LPS content could also reduce the
385 risk associated with endotoxicity of *Salmonella*, and given that LPS is a common
386 feature of many Gram-negative bacteria this may be true of other phage therapy
387 targets (Tran & Whitfield, 2009). Attenuated virulence has been observed for phage
388 resistant *Salmonella* that lack the O-polysaccharide chain from LPS (Santander and
389 Robeson, 2007). However, this is in contrast to reports of temperate phage that can
390 add to the pathogenicity or virulence of their hosts, such as *Vibrio cholera* phage
391 CTXϕ and *E.* coli phages STX (Waldor & Mekalanos, 1996; O'Brien et al., 1984).

392 The virulence *Salmonella* requires the expression of *Salmonella* pathogenicity
393 islands (SPIs), 16 variants of which are currently described (Sterzenbach et al., 2013).
394 Some of these SPIs are conserved throughout the genus, and others serovar specific
395 (Hensel, 2004). The *invA* gene is essential for *Salmonella* to enter cultured epithelial
396 cells and belongs to SPI-1 (Galán et al., 1992), whereas *sseL* encodes a *Salmonella*

397 deubiquitinase required for macrophage killing and virulence which belongs to SPI-2
398 (Rytkönen et al., 2007). SPI-1 and SPI-2 encode effectors of the type III secretion
399 systems (T3SS) that confer the major virulence traits of *Samonella* enterica (*S.*
400 enterica), such as invasion, intracellular survival and proliferation, and
401 enteropathogenesis (Burkinshaw & Strynadka, 2014). The *mgtC* gene encodes a
402 virulence factor of *Salmonella* in response to low Mg^{2+} and pH levels and belongs to
403 SPI-3. *Salmonella* require *mgtC* to adapt to the nutritional limitations of the
404 intraphagosomal habitat, and down regulation would adversely affect phagosomal
405 survival (Retamal et al., 2009). Gene *sopB* encodes proteins to hydrolyze inositol
406 phosphatase and belongs to SPI-5 (Norris et al., 1998). Down-regulation of *sopB*
407 could reduce the action of inositol phosphatase involved in triggering fluid secretion
408 and diarrhoeal symptoms. Gene *spvC* is present on the virulence plasmid of
409 *Salmonella* and is a T3SS effector, and which is closely related to bacterial adhesion,
410 colonization and serum resistance factors (Mazurkiewicz et al., 2008). Down-
411 regulation of *spvC* would reduce the capacity of *Salmonella* to cause disease. A phage
412 resistant *Salmonella* strain called Salp572φ1R lacks the O-polysaccharide from LPS,
413 and expression of several genes related to virulence was under-expressed. Moreover,
414 Salp572φ1R was avirulent in mice. These examples suggest that modifications in LPS
415 can produce phage resistance and impair virulence (Capparelli et al., 2010). In this
416 study, five virulent genes were shown to be down-regulated by phage in *S.*
417 Typhimurium N18.

418 When the first defensive line is broken, bacteria maintain an arsenal of defense
419 mechanisms against the phage. Gene *lexA* encodes protein binding to its specific
420 recognition motif in the promoter region of SOS genes. Gene *recA* encodes RecA
421 protein that promotes the autocatalytic cleavage of LexA repressor (Little, 1991). The

422 relative gene expression level of *recA* has clearly increased in both *Salmonella* strains
423 by phage. The SOS genes of *Salmonella* were induced by gene *recA* encoding
424 products. This mechanism enables cell survival in the presence of extensive DNA
425 damage. Gene *CRISPR/Cas1* encodes CRISPR/Cas system-associated protein Cas1,
426 which targets invasive nucleic acid in the host cell for degradation (Touchon & Rocha,
427 2012). The increase of gene expression level of *CRISPR/Cas1* indicated that
428 *Salmonella* cell increased its capacity to degrade of exogenous nucleic acids during
429 phage infection. The type I restriction-modification (RM) systems consisting of the
430 gene products *hsdR*, *hsdM* and *hsdS* cleaves nonmethylated DNA randomly at a
431 remote site from the recognition sequence determined by the specificity subunit. This
432 could enable the bacterium to distinguish between its own (methylated) DNA and
433 incoming non-methylated DNA (Murray, 2000). The type III RM systems, consisting
434 of the gene products Res and Mod, hemimethylate the DNA and cleave DNA at
435 specific sites (Rao et al., 2014). Higher gene expression of type I RM and type III RM
436 systems in phage treated strains could be part of a stress response or a general
437 adaptation to phage interaction.
438

439 **5. Conclusion**

440 While bacterial strains often contain multiple antiphage barriers, the conjunction
441 of these mechanisms in a single host have rarely been assessed. This study has shown
442 that several phage resistance mechanisms of *S*. Typhimurium can function together in
443 a single strain, including the prevention of phage adsorption, the prevention phage
444 DNA entry, awakening of the SOS system, and the targeting of phage nucleic acids.
445 Furthermore, this study found that virulent phage could decrease the content of LPS
446 and the potential toxicity of *Salmonella*. These findings will assist in the development

447 of phage biocontrol strategies for *Salmonella* when responses to multi-resistant
448 bacteria are required.

449

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451

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460 **References**

461 Akhtar M, Viazis S, Diez-Gonzalez F. (2014). Isolation, identification and characterization of lytic,
462 wide host range bacteriophages from waste effluents against *Salmonella enterica, serovars*.
463 Food Control, 38(1), 67–74.

464 Baxa U, Steinbacher S, Miller S, Weintraub A, Huber R, Seckler R. (1996). Interactions of phage
465 P22 tails with their cellular receptor, *Salmonella* O-antigen polysaccharide. Biophysical
466 Journal, 71(4), 2040-2048.

467 Boxstael SV, Dierick K, Huffel XV, Uyttendaele M, Berkvens D, Herman L, et al. (2012).
468 Comparison of antimicrobial resistance patterns and phage types of *Salmonella*
469 Typhimurium isolated from pigs, pork and humans in Belgium between 2001 and 2006.
470 Food Research International, 45(2), 913-918.

471 Boyen, F., Haesebrouck, F., Maes, D., Van, I. F., Ducatelle, R., & Pasmans, F. (2008). Non-
472 typhoidal *Salmonella* infections in pigs: a closer look at epidemiology, pathogenesis and
473 control. Veterinary Microbiology, 130(1–2), 1-19.

474 Burkinshaw B. J. & Strynadka N. C. (2014). Assembly and structure of the T3SS. Biochimica et
475 Biophysica Acta (BBA)-Molecular Cell Research, 1843(8), 1649-1663.
476 Cai, Y., Tao, J., Jiao, Y., Fei, X., Zhou, L., & Wang, Y., et al. (2016). Phenotypic characteristics
477 and genotypic correlation between *Salmonella* isolates from a slaughterhouse and retail
478 markets in Yangzhou, China. International Journal of Food Microbiology, 222, 56-64.
479 Casjens S R. (2008). Diversity among the tailed-bacteriophages that infect the Enterobacteriaceae.
480 Research in Microbiology, 159(5), 340-348.
481 Casjens, SR, Thuman-Commike, PA. (2011). Evolution of mosaically related tailed bacteriophage
482 genomes seen through the lens of phage P22 virion assembly. Virology, 411(2), 393-415.
483 Capparelli R., Nocerino N., Iannaccone M., Ercolini D., Parlato M., Chiara M., et al. (2010).
484 Bacteriophage therapy of *Salmonella* enterica: a fresh appraisal of bacteriophage therapy.
485 Journal of Infectious Diseases, 201, 52–61.
486 Centers for Disease Control and Prevention (CDC). (2017). *Salmonella* and food.
487 https://www.cdc.gov/salmonella/braenderup-08-14/signs-symptoms.html (Accessed 5 July
488 2017).
489 Davis B M, Waldor M K. (2002). Mobile genetic elements and bacterial pathogenesis. In Mobile
490 DNA II (Eds), ASM Press, pp 1040–1059.
491 Destoumieux-Garzón D, Duquesne S, Peduzzi J, Goulard C, Desmadril M, Letellier L, et al.
492 (2005). The iron-siderophore transporter FhuA is the receptor for the antimicrobial peptide
493 microcin J25: role of the microcin Val11-Pro16 beta-hairpin region in the recognition
494 mechanism. Biochemical Journal, 389(3), 869-876.
495 Duc, H. M. , Son, H. M. , Honjoh, K. , & Miyamoto, T. (2018). Isolation and application of
496 bacteriophages to reduce *Salmonella* contamination in raw chicken meat. LWT, 91, 353–
497 360.
498 EFSA, ECDC. (2017). The European Union summary report on trends and sources of zoonoses,
499 zoonotic agents and food-borne outbreaks in 2016. EFSA Journal, 15, 1-288.
500 Ellen T. Arena, Sigrid D. Auweter, L. Caetano M. Antunes, A. Wayne Vogl, Jun Han, & Julian A.
501 Guttman, et al. (2011). The deubiquitinase activity of the *Salmonella* pathogenicity island 2

502 effector, ssel, prevents accumulation of cellular lipid droplets. Infection and immunity,
503 79(11), 4392-4400.
504 Endersen L, Coffey A, Neve H, Mcauliffe O, Ross RP, O'Mahony JM. (2013). Isolation and
505 characterisation of six novel mycobacteriophages and investigation of their antimicrobial
506 potential in milk. International Dairy Journal, 28(1), 8-14.
507 Gabriela C, Norma S, Bessio MI, Fernando F, Hugo M. (2003). Quantitative determination of
508 pneumococcal capsular polysaccharide serotype 14 using a modification of phenol-sulfuric
509 acid method. Journal of microbiological methods, 52(1), 69-73.
510 Galán J E, Ginocchio C, Costeas P. (1992). Molecular and functional characterization of the
511 *Salmonella* invasion gene *invA*: homology of InvA to members of a new protein family.
512 Journal of Bacteriology, 174(13), 4338-4349.
513 Guenther S, Herzig O, Fieseler L, Klumpp J, Loessner MJ. (2012). Biocontrol of *Salmonella*
514 Typhimurium in RTE foods with the virulent bacteriophage FO1-E2. International Journal
515 of Food Microbiology, 154(1-2), 66-72.
516 Hensel M. (2004). Evolution of pathogenicity islands of *Salmonella enterica*. International Journal
517 of Medical Microbiology, 294(2-3), 95-102.
518 Hooton SP, Atterbury RJ, Connerton IF. (2011). Application of a bacteriophage cocktail to reduce
519 *Salmonella Typhimurium* U288 contamination on pig skin. International Journal of Food
520 Microbiology 151, 157-163.
521 Hungaro HM, Mendonça RCS, Gouvêa DM, Vanetti MCD, Pinto CLDO. (2013). Use of
522 bacteriophages to reduce *Salmonella*, in chicken skin in comparison with chemical agents.
523 Food Research International, 52(1), 75-81.
524 Hvistendahl, M. (2012). China takes aim at rampant antibiotic resistance. Science, 336, 795.
525 Kropinski AM. (2009). In Bacteriophages: methods and protocols, vol 1. Editors: Clokie MRJ
526 and Kropinski AM. Humana Press.
527 Labrie S J, Samson J E, Moineau S. (2010). Bacteriophage resistance mechanisms. Nature
528 Reviews Microbiology, 8(5), 317-327.

529 LeLièvre V, Besnard A, Schlusselhuber M, Desmasures N, & Dalmasso M. (2018). Phages for
530 biocontrol in foods: What opportunities for *Salmonella* sp. control along the dairy food
531 chain?. Food microbiology. Volume 78, 89-98.
532 Li K, Ye S, AlaliWQ, Wang Y, Wang X, & Xia X.et al (2017). Antimicrobial susceptibility,
533 virulence gene and pulsed-field gel electrophoresis profiles of *Salmonella* enterica serovar
534 Typhimurium recovered from retail raw chickens, China. Food Control, 72, 36-42.
535 Li R, Lai J, Wang Y, Liu S, Li Y, Liu Ket al. (2013). Prevalence and characterization of
536 *Salmonella* species isolated from pigs, ducks and chickens in Sichuan Province, China.
537 International Journal of Food Microbiology, 163, 14-18.
538 Little JW. (1991). Mechanism of specific LexA cleavage: autodigestion and the role of RecA
539 coprotease. Biochimie, 73(4), 411-422.
540 Livak KJ, Schmittgen TD. (2001). Analysis of relative gene expression data using real-time
541 quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods, 25(4), 402-408.
542 Mazurkiewicz P., Thomas J., Thompson J. A., Liu M., Arbibe L., Sansonetti P. & Holden D. W.
543 (2008). SpvC is a *Salmonella* effector with phosphothreonine lyase activity on host mitogen
544 ‐activated protein kinases. Molecular microbiology, 67(6), 1371-1383.
545 Mostafa M M, Nassef M, Badr A. (2016). Computational determination of the effects of virulent
546 *Escherichia* coli and *Salmonella* bacteriophages on human gut. Computer Methods &
547 Programs in Biomedicine, 135, 27-35.
548 Murray NE. (2000). Type I restriction systems: sophisticated molecular machines, (a legacy of
549 bertani and weigle). Microbiology & Molecular Biology Reviews, 64(2), 412-434.
550 National Center for Biotechnology Information Database (NCBI).
551 https://www.ncbi.nlm.nih.gov/genome?term=salmonella+phage&cmd=DetailsSearch.
552 2017/6/22.
553 Newell DG, Koopmans M, Verhoef L, Duizer E, Aidara-Kane A, Sprong H, et al. (2010). Food-
554 borne diseases-the challenges of 20 years ago still persist while new ones continue to
555 emerge. International Journal of Food Microbiology, 139:S3-S15.

556 Norris FA, Wilson MP, Wallis TS, Galyov EE, Majerus PW. (1998). SopB, a protein required for
557 virulence of *Salmonella* dublin, is an inositol phosphate phosphatase. Proceedings of the
558 National Academy of Sciences of the United States of America, 95(24), 14057-14059.
559 O'Brien AD, Newland JW, Miller SF, Holmes RK, Smith HW, Formal SB. (1984). Shiga-like
560 toxin-converting phages from *Escherichia coli* strains that cause hemorrhagic colitis or
561 infantile diarrhea. Science, 226(4675), 694-696.
562 O'Flynn G, Coffey A, Fitzgerald G, Ross RP. (2007) *Salmonella enterica* phage-resistant mutant
563 colonies display an unusual phenotype in the presence of phage Felix 01. Letters in Applied
564 Microbiology 5:581-585
565 Oliveira A, Sillankorva S, Quinta R, Henriques A, Sereno R, Azeredo J. (2009). Isolation and
566 characterization of bacteriophages for avian pathogenic *E. coli*, strains. Journal of Applied
567 Microbiology, 106(6), 1919-1927.
568 Panzenhagen, PHN, Aguiar WS, Frasão B DS, Pereira V LDA, Abreu DL DC, & Rodrigues D. P,
569 et al. (2016). Prevalence and fluoroquinolones resistance of *Campylobacter*, and
570 *Salmonella*, isolates from poultry carcasses in rio de janeiro, brazil. Food Control, 61, 243-
571 247.
572 Pedruzzi I, Rosenbusch J P, Locher K P. (1998). Inactivation in vitro of the *Escherichia coli*, outer
573 membrane protein FhuA by a phage T5-encoded lipoprotein. FEMS Microbiology Letters,
574 168(1), 119-125.
575 Perry L L, Sanmiguel P, Minocha U, Terekhov AI, Shroyer ML, Farris LA, et al. (2009).
576 Sequence analysis of *Escherichia coli* O157:H7 bacteriophage ΦV10 and identification of a
577 phage-encoded immunity protein that modifies the O157 antigen. FEMS Microbiology
578 Letters, 292(2), 182-186.
579 Rao D N, Dryden D T, Bheemanaik S. (2014). Type III restriction-modification enzymes: a
580 historical perspective. Nucleic Acids Research, 42(1), 45-55.
581 Retamal P., Castillo-Ruiz M. & Mora G. C. (2009). Characterization of MgtC, a virulence factor
582 of *Salmonella* enterica Serovar Typhi. PLoS One, 4(5), e5551.

583 Rytkönen A., Poh, J., Garmendia J., Boyle C., Thompson A., Liu M. & Holden D. W. (2007).
584 SseL, a *Salmonella* deubiquitinase required for macrophage killing and virulence.
585 Proceedings of the National Academy of Sciences, 104(9), 3502-3507.
586 Samson J. E., Magadán A. H., Sabri M. & Moineau S. (2013). Revenge of the phages: defeating
587 bacterial defences. Nature Reviews Microbiology, 11(10), 675-687.
588 Santander J. & Robeson J. (2007). Phage-resistance of *Salmonella* enterica serovar Enteritidis and
589 pathogenesis in Caenorhabditis elegans is mediated by the lipopolysaccharide. Electronic
590 Journal of Biotechnology, 10, 627–632.
591 Song, Q., Xu, Z., Hong, G., & Zhang, D. (2018). Overview of the development of quinolone
592 resistance in *Salmonella* species in China, 2005–2016. Infection & Drug Resistance, 11,
593 267-274.
594 Steinbacher S, Miller S, Baxa U, Weintraub A, Seckler R. (1997). Interaction of *Salmonella* phage
595 P22 with its O-antigen receptor studied by X-ray crystallography. Biological Chemistry,
596 378(3-4), 337-343.
597 Sterzenbach T, Crawford RW, Winter SE, Ba¨umler AJ. (2013). *Salmonella* virulence
598 mechanisms and their genetic basis. In: Barrow, P.A., Methner, U. (Eds.), *Salmonella* in
599 Domestic Animals. CABI Publishing, Wallingford, UK.
600 Thompson JA. (2008). SpvC is a *Salmonella* effector with phosphothreonine lyase activity on host
601 mitogen-activated protein kinases. Molecular Microbiology, 67(6), 1371-1383
602 Touchon M, Rocha EP. (2012). The small, slow and specialized CRISPR and anti-CRISPR of
603 *Escherichia* and *Salmonell*a. Plos One, 5(6), 11126-11140.
604 Tran AX, Whitfield C. (2009). Lipopolysaccharides (Endotoxins). Encyclopedia of Microbiology,
605 71(1), 513-528.
606 Virginie LeLièvre, Alexandra Besnard, Margot Schlusselhuber, Nathalie Desmasures, Marion
607 Dalmasso. (2019). Phages for biocontrol in foods: What opportunities for Salmonella sp.
608 control along the dairy food chain? Food Microbiology, 78, 89-98.
609 Waldor MK, Mekalanos JJ. (1996). Lysogenic Conversion by a *Filamentous* Phage Encoding
610 Cholera Toxin. Science, 272(5270), 1910.

611 Wang C, Yang J, Zhu X, Lu Y, Xue Y, Lu Z. (2017a). Effects of *Salmonella*, bacteriophage, nisin
612 and potassium sorbate and their combination on safety and shelf life of fresh chilled pork.
613 Food Control, 73, 869-877.
614 Wang C, Chen Q, Zhang C, Yang J, Lu Z, Lu F, Bie X. (2017b). Characterization of a wide host
615 spectrum virulent *Salmonella* bacteriophage fmb-p1 and its application on duck meat. Virus
616 Research , 236, 14–23.
617 Wang X, Quinn PJ. (2010). Lipopolysaccharide: Biosynthetic pathway and structure modification.
618 Progress in Lipid Research, 49(2), 97-107.
619 Yang X, Huang J, Wu Q, Zhang J, Liu S, Guo W. (2016). Prevalence, antimicrobial resistance
620 and genetic diversity of *Salmonella*, isolated from retail ready-to-eat foods in China. Food
621 Control, 60, 50-56.
622 Yeh Y, Purushothaman P, Gupta N, Ragnone M, Verma SC, & De Mello AS. (2017).
623 Bacteriophage application on red meats and poultry: Effects on *Salmonella* population in
624 final ground products. Meat science, 127, 30-34.
625 Zhai LG. (2015). Molecular rapid detection method research of *Salmonella* and pathogenic
626 serotypes in food. Nanjing：Nanjing Agricultural University.
627 Zheng J, Tian F, Cui S, Song J, Zhao S, Brown EW., et al. (2011). Differential gene expression by
628 RamA in ciprofloxacin-resistant *Salmonella Typhimurium*. PLOS One, 7, 22161-22169.
629 Zhu Y, Lai H, Zou L, Yin S, Wang C, & Han X. et al.(2017). Antimicrobial resistance and
630 resistance genes in *Salmonella* strains isolated from broiler chickens along the slaughtering
631 process in China. International Journal of Food Microbiology, 259, 43-51.
632 Zinno P, Devirgiliis C, Ercolini D, Ongeng D, Mauriello G. (2014). Bacteriophage P22 to
633 challenge *Salmonella* in foods. International Journal of Food Microbiology, 191C(1), 69-74.
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647 **Table 1 The primers of PCR assay in this study**

Target genes	Coding protein	Sequence of primers (5'-3')	Fragment sizes(bp)	Reference
16s rRNA	16S ribosomal RNA	GTTACCCGCAGAAGAAGCAC	123	Zheng et al., (2011)
		CACATCCGACTTGACAGACC		
lpxA	UDP-N-acetylglucosamine acyltransferase	AAGCGTCACCATTCATCGTG	200	This study
		GATGAACTGCCGTCATACCG		
lpxB	lipid-A-disaccharide synthase	TGGGCATTGTTGAAGTGCTC	188	This study
		AACGGACGGGCTGACATAAT		
lpdT	LPS-assembly protein LptD	AACGTCTGGGAAGGCGATTA	165	This study
		GATGACTTCACTCCCCACCA		
rfaG	alpha-1,3-glucosyltransferase	GATGACTGAACCATTCCGCC	152	This study
		TAATATCCGCGGCCTTCTCC		
rfaI	lipopolysaccharide 1,3-galactosyltransferase	CTGAAGGCGAGTTGGAATGG	173	This study
		GCGCTGTACTACCTCTGGAT		
rfaJ	alpha-1,2-glucosyltransferase	GCTGCGGTCGTAAAAGATGT	176	This study
		TCAGCCTCTTTACCTGCCAA		
rfaK	lipopolysaccharide 1,2-N-acetylglucosaminetransferase	CACCGTTCTCTTGTATGCCG	231	This study
		ATCTGGTCAGGAGATTGCCC		
rfaL	O-antigen ligase	ATGGCGCTATCATCAGGGAA	172	This study
		GCCAGCAGAAAACCGGTAAT		
rfbA	glucose-1-phosphate thymidylyltransferase	ACGGTGTGGTTGAGTTTGAC	221	This study

kdtA	3-deoxy-D-manno-octulosonic-acid transferase	CCCCATCATAGCGACAGACA ATTCCGGTACTGATGGGTCC	195	This study
msbA	lipid A export ATP-binding /permease	CGCGCCCTGATTTTGATACA CTGGTTGTTTTAGCGCCGAT	237	This study
pagP	lipid A palmitoyltransferase PagP	TGACGAGGCAGAGACCATTT AATTTTCGCCTTGGACTGGG	175	This study
wzzB	polysaccharide antigen chain length regulator	GCATCCAGGCGAAATAGACG ACAGTTATGGCGTGGGAAGA	160	This study
lexA	LexA repressor	GTTGAGCGCGTTGGTATAGG CGCGAGGTATCCGTCTGTTA	221	This study
recA	recombination protein RecA	CGTTTTATGTACCGCCAGCA GATATCCGTCGTATTGGCGC	215	This study
mgtC	Mg(2+) transport ATPase protein C (Retamal et al., 2009)	CCGTTGTAGCTGTACCATGC TTCTGAGCTCCATGACGACA AGAATAATGATCGTCGCCGC	217	This study
invA	attachment/invasion protein (Galán et al., 1992)	ACCGTGGTCCAGTTTATCGT GCTTTCCCTTTCCAGTACGC	177	This study
sopB	Type III secretion system effector protein (Norris et al., 1998)	GCTCGCCCGGAAATTATTGT GGTCCGCTTTAACTTTGGCT	208	This study
spvC	*Salmonella* plasmid virulence protein (Mazurkiewicz et al., 2008)	ATTTGCCGGTGACAAGTTCC GGAGAAACGACGCACTGTAC	224	This study
sseL	Type III secretion system effector protein, deubiquitinase (Rytkönen et al., 2007)	GCCCCTTCCAGATTACTTTATATG TGCTTAATATATTTTCTTTGGTGG	268	This study
Cas1	CRISPR/Cas system-associated protein Cas1	GCAAAGCTGGCGTTAGATGA GATCCTTCAATACCGCGCAG	122	This study
hsdM	type I restriction enzyme methylase	TGTATGACCTGCGGACCAAT AAGCTCCACTCCCCTTCTTC	136	This study
hsdR	endonuclease R	AGCGGTAGATAAGCAGGTCC ATTGAAAATCTCGGCGCTCC	124	This study

hsdS	type I restriction enzyme specificity protein	GTGTTCCTGTCCCACCTCTT TGGGATTTGCTCAAGACGTG	104	This study
mod	type III restriction-modification system methyltransferase	CCGAAACCGACCGCATTATT TTCATTCAGCGCCATTACCG	120	This study
res	type III restriction-modification system DNA helicase	ATTCGTGACAATAAGCCCGC CTACGCTCGAATTGCAGCAT	119	This study
STM4494	sugar/spermidine/putrescine ABC transporter ATPase	CAACTAAAAGAGAAATACCCACAGG TACCGTAAACAGCATAATCAGCAC	534	Zhai (2015)
P1	amidase	ACATCAAGAGGGAGGAACGG TGCGCTCAATCATCCGACTA	193	This study
P11	putative endolysin	TGCCAAACCGAAACATCAGT GCCGTAGTGCCCATAACCTA	125	This study
P28	tailspike protein	GTTTCGGCATGGATGGGAAA AGTGAATACCGTTTCGTGCG	101	This study

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670 Table 2 LPS yields from *S*. Typhimurium CMCC50115 and N18

LPS	Sample volume	LPS mass/mg
50115LPS	100mL(5.0×10^9CFU/mL)	4.67 ±0.26
N18LPS	100mL(5.0×10^9 CFU/mL)	0.40 ±0.12

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