

Advance Publication

## The Journal of Veterinary Medical Science

Accepted Date: 19 February 2023

J-STAGE Advance Published Date: 3 March 2023

©2023 The Japanese Society of Veterinary Science

Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

1 Public Health

2 Full Paper

3

4 **Antimicrobial resistance profiles of *Campylobacter jejuni* and *Salmonella* spp.**  
5 **isolated from enteritis patients in Japan**

6

7 Yoshimasa SASAKI<sup>1,2)\*</sup>, Tetsuya IKEDA<sup>3)</sup>, Kenzo YONEMITSU<sup>4)</sup>, Makoto  
8 KURODA<sup>5)</sup>, Miho OGAWA<sup>6)</sup>, Ryuji SAKATA<sup>6)</sup>, Masashi UEMA<sup>1)</sup>, Yoshika MOMOSE<sup>1)</sup>,  
9 Kenji OHYA<sup>7)</sup>, Maiko WATANABE<sup>7)</sup>, Yukiko HARA-KUDO<sup>7)</sup>, Masashi OKAMURA<sup>8)</sup>,  
10 Tetsuo ASAI<sup>2)</sup>

11

12 <sup>1)</sup>Division of Biomedical Food Research, National Institute of Health Sciences, 3-25-26,  
13 Tonomachi, Kawasaki-ku, Kawasaki, Kanagawa 210-9501, Japan

14 <sup>2)</sup>Department of Applied Veterinary Science, The United Graduate School of Veterinary  
15 Science, Gifu University, 1-1, Yanagido, Gifu 501-1193, Japan

16 <sup>3)</sup>Department of Infectious Diseases, Hokkaido Institute of Public Health, Kita19 Nishi  
17 12, Kita-ku Sapporo, Hokkaido 060-0819, Japan

18 <sup>4)</sup>Murayama Branch, National Institute of Infectious Diseases. 4-7-1, Gakuen,  
19 Musashimurayama, Tokyo 208-0011, Japan

20 <sup>5)</sup>Pathogen Genomics Center, National Institute of Infectious Diseases, 1-23-1 Toyama,  
21 Shinjuku-ku, Tokyo 162-8640, Japan

22 <sup>6)</sup>Department of Bacteriology, BML Inc., 1361-1, Matoba, Kawagoe, Saitama 350-1101,  
23 Japan

24 <sup>7)</sup>Division of Microbiology, National Institute of Health Sciences, 3-25-26, Tonomachi,

25 Kawasaki-ku, Kawasaki, Kanagawa 210-9501, Japan

26 <sup>8)</sup>Division of Veterinary Science, Department of Veterinary Medicine, Obihiro  
27 University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-  
28 8555, Japan

29

30 **\*Correspondence:** Yoshimasa Sasaki

31 Division of Biomedical Food Research, National Institute of Health Sciences, 3-25-26,  
32 Tonomachi, Kawasaki-ku, Kawasaki, Kanagawa 210-9501, Japan

33 Present address: Division of Veterinary Science, Department of Veterinary Medicine,  
34 Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro,  
35 Hokkaido 080-8555, Japan

36 E-Mail: [ysasaki@obihiro.ac.jp](mailto:ysasaki@obihiro.ac.jp); Tel.: +81-155-49-5387

37

38 **Running head:** *Campylobacter* and *Salmonella* from Humans

39

40 **ABSTRACT**

41 Understanding the antimicrobial resistance of *Campylobacter jejuni* and  
42 *Salmonella* spp. isolated from patients with enteritis will aid in therapeutic decision-  
43 making. This study aimed to characterize *C. jejuni* and *Salmonella* spp. isolates from  
44 patients with enteritis. For *C. jejuni*, the resistance rates against ampicillin, tetracycline,  
45 and ciprofloxacin were 17.2%, 23.8%, and 46.4%, respectively. All the *C. jejuni* isolates  
46 were susceptible to erythromycin, which is recommended as a first-choice antimicrobial  
47 if *Campylobacter* enteritis is strongly suspected. *C. jejuni* was classified into 64  
48 sequence types (STs), and the five major STs were ST22, ST354, ST21, ST918, and  
49 ST50. The ciprofloxacin-resistance rate of ST22 was 85.7%. For *Salmonella*, the  
50 resistance rates against ampicillin, cefotaxime, streptomycin, kanamycin, tetracycline,  
51 and nalidixic acid were 14.7%, 2.0%, 57.8%, 10.8%, 16.7%, and 11.8%, respectively.  
52 All the *Salmonella* spp. isolates were susceptible to ciprofloxacin. Therefore,  
53 fluoroquinolones are the recommended antimicrobials against *Salmonella* enteritis. *S.*  
54 Thompson, *S. Enteritidis*, and *S. Schwarzengrund* were the three most prevalent  
55 serotypes. The two cefotaxime-resistant isolates were serotyped as *S. Typhimurium* and  
56 were found to harbor *bla*<sub>CMY-2</sub>. The results of this study would help select antimicrobials  
57 for treating patients with *Campylobacter* and *Salmonella* enteritis.

58

59 **KEYWORDS:** antimicrobial resistance, *Campylobacter*, enteritis, *Salmonella*

60

## 61 INTRODUCTION

62 *Campylobacter* spp. and non-typhoidal *Salmonella* spp. are bacterial pathogens  
63 causing foodborne illnesses globally. The World Health Organization has stated that  
64 these two genera are among the four key global causative agents of diarrhea [40, 42].  
65 Although several *Campylobacter* spp. have been identified as pathogens in human  
66 campylobacteriosis, *Campylobacter jejuni* infections account for more than 80% of  
67 human campylobacteriosis [4, 8, 22, 46]. The annual number of food poisoning  
68 outbreaks in Japan caused by *Campylobacter* spp. was higher than 200 between 2014  
69 and 2018 [44]. In Japan, chicken and beef are considered major sources of foodborne  
70 campylobacteriosis [4, 8]. *Salmonella* spp. are serotyped into more than 2,600 serotypes  
71 based on three structures, somatic (O), flagellar, and capsular surface antigens. Four O-  
72 serogroups (O:4, O:7, O:8, and O:9) are major contributors to human non-typhoidal  
73 salmonellosis [12, 19, 21, 27]. Following the Infectious Agents Surveillance Report  
74 (<https://kansen-levelmap.mhlw.go.jp/Byogentai/Pdf/data81j.pdf>), the top seven  
75 frequent *Salmonella* serotypes in patients in 2021 were *S. Typhimurium*, *S.*  
76 *Schwarzengrund*, *S. Infantis*, *S. Thompson*, *S. Enteritidis*, *S. Braenderup* and *S. Corvallis*.  
77 Although the annual number of food poisoning outbreaks in Japan caused by *Salmonella*  
78 spp. was less than 50 since 2011, the average number of patients per outbreak is 38 [44].  
79 Eggs, vegetables, and chicken are considered major sources of foodborne salmonellosis  
80 in Japan [18].

81 *Campylobacter* and *Salmonella* infections typically cause acute self-limiting  
82 enteritis. Although antimicrobial therapy is not recommended in various cases, it may  
83 be lifesaving in patients with severe symptoms and health risk groups such as infants,  
84 the older population, and immunocompromised patients [16, 40, 42]. Fluoroquinolones,

85 such as ciprofloxacin and levofloxacin, and third-generation cephalosporins (TGCs),  
86 such as cefotaxime and ceftriaxone, have been classified as “critically important  
87 antimicrobials for human medicine” by the World Health Organization [41]. These  
88 antimicrobials, along with penicillins, tetracyclines, aminoglycosides, sulfonamides,  
89 and macrolides, are also used to treat bacterial infections in food-producing animals in  
90 Japan [24]. Since numerous Japanese studies have reported multidrug-resistant *C. jejuni*  
91 and *Salmonella* spp. isolated from broilers, cattle, and pigs [14, 15, 17, 28, 30, 33],  
92 humans can get infected with these multidrug-resistant species by consuming various  
93 foods derived from these animals. Furthermore, there are Japanese reports on the  
94 isolation of fluoroquinolone-resistant *Campylobacter* spp. and TGC-resistant  
95 *Salmonella* spp. from enteritis patients [26, 27]. Therefore, antimicrobial resistance in  
96 the two genera is an important issue in the chemotherapeutic treatment of patients with  
97 enteritis. This study aimed to determine the antimicrobial resistance profiles of the two  
98 genera isolated from patients with enteritis. Moreover, we characterized these isolates  
99 genotypically and serologically. The results of this study would help characterize  
100 *Campylobacter jejuni* and *Salmonella* spp. isolated from patients with enteritis and  
101 select antimicrobials for treating enteritis patients.

102

## 103 **MATERIALS AND METHODS**

### 104 *Isolates*

105 This study used 151 clinical isolates of *C. jejuni* and 102 clinical isolates of  
106 *Salmonella* spp. belonging to O:4 (36 isolates), O:7 (40 isolates), O:8 (7 isolates), and  
107 O:9 (19 isolates) serogroups. These isolates were obtained from the stool specimens of  
108 253 different patients with enteritis between December 2019 and April 2022 at BML,

109 Inc., in Saitama, Japan. The stool specimens for *C. jejuni* and *Salmonella* spp. were  
110 obtained from hospitals in 29 (61.7%) and 24 (51.1%) of the 47 prefectures in Japan,  
111 respectively. For the isolation of *C. jejuni*, each specimen was streaked on a modified  
112 charcoal cefoperazone deoxycholate agar plates containing a chromogenic substrate  
113 (BD<sup>TM</sup> mCCDA Clear-HT; Nippon Becton Dickenson Company Ltd, Tokyo, Japan) and  
114 incubated microaerobically at 42 °C for 48 hr using AnaeroPack-Microaero (Mitsubishi  
115 Gas Chemicals, Tokyo, Japan). For the isolation of *Salmonella* spp. each specimen was  
116 streaked onto a modified Salmonella–Shigella agar plate (Eiken Chemical Co., Tokyo,  
117 Japan) and incubated at 37 °C for 24 hr. In this study, no human participants were  
118 directly involved. Hence, clearance of human ethics is not required. We used isolates  
119 routinely cultured from clinical specimens from hospitals. At the laboratory, these  
120 isolates were collected in Microbank<sup>TM</sup> vials (Pro-Lab Diagnostics Inc., Round Rock,  
121 TX, USA) and stored at –80 °C. To characterize these isolates, *C. jejuni* isolates were  
122 grown on brain heart infusion agar containing 5% horse blood (Oxoid Ltd., Hampshire,  
123 UK) microaerobically at 42 °C using AnaeroPack-Microaero (Mitsubishi Gas  
124 Chemicals), while *Salmonella* spp. isolates were grown on brain heart infusion agar  
125 (Oxoid) aerobically at 37 °C.

#### 126 *Antimicrobial susceptibility testing*

127 Antimicrobial susceptibility testing of *C. jejuni* and *Salmonella* spp. isolates  
128 was conducted using the broth microdilution method using dried plates (Eiken  
129 Chemical). *C. jejuni* ATCC 33560 and *Escherichia coli* ATCC 25922 were used as  
130 quality control strains for *C. jejuni* and *Salmonella* spp., respectively.

131 Antimicrobial susceptibility testing of *C. jejuni* isolates was conducted against  
132 ampicillin (0.12–256 mg/L), streptomycin (0.12–128 mg/L), tetracycline (0.12–128

133 mg/L), chloramphenicol (0.12–256 mg/L), nalidixic acid (0.12–128 mg/L),  
134 ciprofloxacin (0.03–64 mg/L), erythromycin (0.12–128 mg/L), and gentamicin (0.12–  
135 256 mg/L). The breakpoints for ampicillin (32 mg/L), streptomycin (32 mg/L),  
136 erythromycin (32 mg/L), tetracycline (16 mg/L), nalidixic acid (32 mg/L), ciprofloxacin  
137 (4 mg/L), and chloramphenicol (16 mg/L) were adopted from the Clinical and  
138 Laboratory Standards Institute (CLSI) [6] and Japanese Veterinary Antimicrobial  
139 Resistance Monitoring (JVARM) system [24]. The breakpoint for gentamicin (2 mg/L)  
140 was specified by the Danish Integrated Antimicrobial Resistance Monitoring and  
141 Research Programme [9].

142 Antimicrobial susceptibility testing for *Salmonella* spp. isolates was conducted  
143 against ampicillin (1–128 mg/L), cefazolin (1–128 mg/L), cefotaxime (0.5–64 mg/L),  
144 streptomycin (1–128 mg/L), kanamycin (1–128 mg/L), tetracycline (0.5–64 mg/L),  
145 nalidixic acid (1–128 mg/L), ciprofloxacin (0.03–4 mg/L), colistin (0.12–16 mg/L),  
146 chloramphenicol (1–128 mg/L), gentamicin (0.5–64 mg/L), and trimethoprim (0.25–16  
147 mg/L). The breakpoints for ampicillin (32 mg/L), cefazolin (8 mg/L), cefotaxime (4  
148 mg/L), streptomycin (32 mg/L), kanamycin (64 mg/L), tetracycline (16 mg/L), nalidixic  
149 acid (32 mg/L), ciprofloxacin (1 mg/L), colistin (4 mg/L), chloramphenicol (32 mg/L),  
150 gentamicin (16 mg/L), and trimethoprim (16 mg/L) were adopted from the CLSI [7] and  
151 JVARM system [24].

#### 152 *Multilocus sequence typing of C. jejuni isolates*

153 Multilocus sequence typing of *C. jejuni* isolates was performed following the  
154 seven-locus scheme for *Campylobacter*, employing the primer sets and experimental  
155 conditions suggested by the *Campylobacter* Multilocus sequence typing (MLST)  
156 database (<http://pubmlst.org/campylobacter/>).



157 *Sequencing of partial gyrA genes in ciprofloxacin-resistant C. jejuni*

158 In one *C. jejuni* isolate per sequence type determined by MLST, partial *gyrA*  
159 genes of the isolates were amplified using polymerase chain reaction (PCR) [47], and  
160 the PCR products were directly sequenced.

161 *Serotyping of Salmonella spp. isolates*

162 Somatic antigens of *Salmonella* spp. isolates were confirmed by slide  
163 agglutination using O antisera (Denka Co., Tokyo, Japan). *Salmonella* isolates were  
164 further tested for flagella antigens via tube agglutination using H antisera (Denka).  
165 Serovars were determined based on the combinations of O and H group antigens  
166 following the Kauffmann–White scheme [13]. Isolates agglutinated with anti-O:4 and  
167 anti-H:i serum but not anti-H:1 or anti-H:2 serum were confirmed as monophasic  
168 variants of *S. Typhimurium* using a previously reported PCR method [11].

169 *Determination of antimicrobial resistance genes and sequence types based on MLST in*  
170 *cefotaxime-resistant Salmonella spp. isolates using whole-genome sequence analysis*

171 DNA was extracted from cefotaxime-resistant strains using the DNeasy<sup>®</sup>  
172 UltraClean<sup>®</sup> Microbial Kit (Qiagen GmbH, Hilden, Germany). Whole-genome  
173 sequence analysis was performed as previously described [34]. Sequencing libraries for  
174 each isolate were prepared using the QIAseq FX Library Kit (Qiagen) to obtain paired-  
175 end sequences (300 bp × 2) using the Illumina Miseq platform. The draft genome  
176 sequence was assembled using A5-miseq with only Illumina short-read data. Gene  
177 annotation was performed using DFAST version 1.2.3 with the following databases:  
178 DFAST default database [39], ResFinder database [45], and Bacterial Antimicrobial  
179 Resistance Reference Gene database (PRJNA313047). MLST was performed using the  
180 “mlst” program version 2.16.2 (<https://github.com/tseemann/mlst>) with the PubMLST

181 database (<https://pubmlst.org/>).

182

## 183 RESULTS

184 For *C. jejuni*, the resistance rates against ampicillin, streptomycin, tetracycline,  
185 nalidixic acid, and ciprofloxacin were 17.2%, 2.6%, 23.8%, 47.0%, and 46.4%,  
186 respectively (Table 1). All the *C. jejuni* isolates were susceptible to erythromycin,  
187 gentamicin, and chloramphenicol. In addition, *C. jejuni* was classified into 64 sequence  
188 types (STs) using MLST (Table 2). The five major STs were ST22 (14 isolates), ST354  
189 (12 isolates), ST21 (9 isolates), ST918 (9 isolates), and ST50 (8 isolates). The  
190 ciprofloxacin resistance rates of ST22, ST354, ST21, ST918, and ST50 were 85.7%  
191 (12/14), 25.0% (3/12), 100.0% (9/9), 33.3% (3/9), and 0.0% (0/8), respectively.  
192 Ciprofloxacin resistance was observed in 32 (50.0%) of these 64 STs. Among the 32  
193 STs, the Thr86Ile substitution (mediated by the C257T mutation in the *gyrA* genes) was  
194 detected in 31 STs. The remaining one ST (ST11491) had the Thr86Lys substitution  
195 (mediated by the C257A mutation). Moreover, ST8071 and ST10424 had the Asp90Asn  
196 substitution (mediated by the G268A mutation) and the Val149Ile substitution (mediated  
197 by the G508A mutation), respectively, in addition to the Thr86Ile substitution.

198 For *Salmonella* spp., the resistance rates against ampicillin, cefazolin,  
199 cefotaxime, streptomycin, gentamicin, kanamycin, tetracycline, nalidixic acid, colistin,  
200 chloramphenicol, and trimethoprim were 14.7%, 2.0%, 2.0%, 57.8%, 1.0%, 10.8%,  
201 16.7%, 11.8%, 7.8%, 2.9%, and 9.8%, respectively. All the isolates were susceptible to  
202 ciprofloxacin. In the O:4 serogroup (36 isolates), *S. Schwarzengrund* was the most  
203 prevalent serovar (13 isolates), and nine (69.2%) isolates were resistant to kanamycin  
204 (Table 3). The *S. Typhimurium* monophasic variant was the second most prevalent

205 serovar (nine isolates), and five (55.6%) isolates were resistant to ampicillin and  
206 streptomycin. The two cefotaxime-resistant isolates obtained from two different  
207 prefectures were serotyped as *S. Typhimurium*, and they harbored the AmpC-type  $\beta$ -  
208 lactamase gene of *bla*<sub>CMY-2</sub> (Table 4). These two cefotaxime-resistant isolates also had  
209 seven genes encoding resistance to aminoglycoside (*aac(6')-Iaa*, *ant(3'')-Ib*, and *aph(6)-*  
210 *Id*), phenicol (*floR*), quinolone (*qnrB19*), sulfonamide (*sul2*), and tetracycline (*tet(A)*).  
211 In the O:7 serogroup (40 isolates), *S. Thompson* was the most prevalent serovar (22  
212 isolates). Among these 40 isolates, 39 (97.5%) did not show multidrug resistance. In the  
213 O:8 serogroup (seven isolates), *S. Manhattan* was the most prevalent serovar, and two  
214 isolates were resistant to streptomycin and tetracycline. In the O:9 serogroup (19  
215 isolates), *S. Enteritidis* was the most prevalent serovar (15 isolates), and seven (46.7%)  
216 isolates were resistant to ampicillin, streptomycin, and nalidixic acid.

217

## 218 **DISCUSSION**

219 In Japan, fluoroquinolones are recommended as first-choice antimicrobials for  
220 empiric therapy of patients with diarrhea and *Salmonella* enteritis [16]. Moreover,  
221 macrolides are recommended as first-choice antimicrobials if *Campylobacter* enteritis  
222 is strongly suspected or patients have been exposed to regions where quinolone-resistant  
223 *Campylobacter* spp. is prevalent [16]. Ohishi *et al.* [26] reported that the ciprofloxacin-  
224 resistance rate in *C. jejuni* isolated from patients between 2007 and 2014 is 44.3%.  
225 Moreover, Yamada *et al.* [43] reported that the ciprofloxacin-resistance rate in *C. jejuni*  
226 isolated from patients between 2009 and 2017 was 41.9%, and the Thr86Ile substitution  
227 in GyrA was observed in 93.9% of ciprofloxacin-resistant *C. jejuni* isolates. The  
228 Thr86Ile point mutation in GyrA has been identified as the predominant mutation and

229 is associated with increased quinolone resistance [20]. In the present study, the  
230 ciprofloxacin-resistance rate in *C. jejuni* was 46.4%, and the Thr86Ile substitution in  
231 GyrA was observed in 96.9% of ciprofloxacin-resistant *C. jejuni* isolates, suggesting  
232 that the fluoroquinolone-resistance rate has consistently been more than 40% in the last  
233 decade, and macrolides must ideally be the first-choice antimicrobials if *Campylobacter*  
234 enteritis is strongly suspected, given that erythromycin resistance was not observed in  
235 *C. jejuni* isolates. The top five STs were ST22, ST354, ST21, ST918, and ST50 in the  
236 present study. Among these, ST21 is one of the predominant STs in *C. jejuni* isolated  
237 from cattle [33], and the remaining 4 STs are less abundant in *C. jejuni* isolated from  
238 cattle and poultry [2, 26, 33]. We reported that seven (77.8%) of the nine ST21 isolates  
239 from cattle are resistant to tetracycline, nalidixic acid, and ciprofloxacin [33]. Of the  
240 nine ST21 isolates obtained in the present study, six (66.7%) were also resistant to these  
241 three antimicrobials. Moreover, we reported that the two most abundant STs in *C. jejuni*  
242 isolated from Japanese layer flocks are ST4389 (eight isolates) and ST6704 (seven  
243 isolates), and 93.3% (14/15) of them are ampicillin-resistant [32]. In that study, two  
244 ST354 and two ST918 isolates were obtained from layer flocks, of which 75.5% (3/4)  
245 were susceptible to all the tested antimicrobials. These results suggest that some of the  
246 STs adapted to cattle and poultry are likely to be pathogenic to humans, and the  
247 causative foods of human *C. jejuni* infection can be identified using a combination of  
248 MLST and antimicrobial resistance profiles of *C. jejuni* isolates. Asakura *et al.* [3]  
249 reported ST22 as the most prevalent ST in *C. jejuni* isolated from human  
250 campylobacteriosis cases in Osaka Prefecture, Japan, between 2010 and 2011. In  
251 contrast, ST22 *C. jejuni* is less abundant in cattle and poultry [2, 30, 32, 33]. Thus,  
252 humans might exhibit enteritis easily when infected with ST22, compared to that with

253 other STs. This may be the reason ST22 is the predominant ST isolated from stool  
254 specimens of campylobacteriosis patients. Moreover, Takahashi *et al.* [38] reported that  
255 serotype HS:19 isolates in Japan accounted for 67 out of 102 (65.7%) *C. jejuni* isolates  
256 obtained from patients with Guillain-Barré syndrome (GBS), a severe post-infection  
257 autoimmune disease. Akase *et al.* [1] reported 98.9% (87/88) of serotype HS:19 isolated  
258 from patients with GBS, sporadic diarrheal patients, and poultry meat samples to be  
259 ST22. To understand the relationship between ST22 isolates from humans and livestock,  
260 further characterization of these ST22 isolates is needed.

261 To the best of our knowledge, there are no reports about the isolation of  
262 fluoroquinolone-resistant *Salmonella* spp. from enteritis patients in Japan this decade.  
263 In this study, ciprofloxacin resistance was not observed in *Salmonella* isolates.  
264 Therefore, fluoroquinolones are the ideal first-choice antimicrobials against *Salmonella*  
265 enteritis. In addition, ceftriaxone is recommended as a second-choice antimicrobial for  
266 *Salmonella* enteritis [16]. Although the prevalence of TGC-resistant *Salmonella* spp. in  
267 chicken products and broilers has increased since 2005 [5, 10, 25, 28], the prevalence  
268 has decreased after withdrawing the use of TGC in broiler production in 2012 [5, 35].  
269 We recently reported that 1.3% (4/309) of *Salmonella* isolates (three *S. Infantis* and one  
270 *S. Manhattan* strains) isolated from chicken products sampled between January 2018  
271 and October 2021 were resistant to cefotaxime and harbored *bla*<sub>CMY-2</sub> or *bla*<sub>TEM-52B</sub> [31].  
272 In the present study, cefotaxime resistance was very low (2.0%, 2/102), and both the  
273 TGC-resistant *Salmonella* isolates were *S. Typhimurium* harboring *bla*<sub>CMY-2</sub>. Although  
274 TGC-resistant *S. Typhimurium* has never been isolated from broilers, layers, or pigs in  
275 Japan [5, 28, 29, 35, 36], it has been isolated from cattle, and all of them have been  
276 found to harbor *bla*<sub>CMY-2</sub> [17]. Shimojima *et al.* [37] investigated the presence of

277 *Salmonella* in 993 imported meat products (281 chicken, 393 pork, and 319 beef  
278 products) between 2009 and 2017, but no TGC-resistant *S. Typhimurium* was isolated.

279 *Salmonella* Thompson, *S. Schwarzengrund*, *S. Infantis*, and *S. Braenderup* were  
280 the prevalent serotypes in this study. Most of the *S. Schwarzengrund* serotypes were  
281 multidrug resistant; however, *S. Thompson*, *S. Infantis*, and *S. Braenderup* were not. *S.*  
282 *Schwarzengrund* and *S. Infantis* are the two most prevalent serotypes in chicken meat  
283 in Japan, and more than 65% of them are multidrug-resistant [23, 31, 37]. *S. Thompson*,  
284 *S. Infatnis*, and *S. Braenderup* are the prevalent serotypes in layer breeding chains in  
285 Japan, and most of them are not multidrug-resistant [29, 36]. Meanwhile, in beef and  
286 pork, *S. Typhimurium* and its monophasic variant are the two most prevalent serotypes,  
287 while *S. Thompson*, *S. Schwarzengrund*, *S. Infantis*, and *S. Braenderup* are barely  
288 isolated [37].

289 In conclusion, the characteristics of human *C. jejuni* and *Salmonella* spp.  
290 isolates could represent the characteristics of these two bacterial isolates originating  
291 from contaminated food. Monitoring the antimicrobial resistance of *C. jejuni* and  
292 *Salmonella* spp. isolated from food-producing animals and food would thus aid in the  
293 selection of antimicrobials for treating *Campylobacter* and *Salmonella* enteritis patients.

294

## 295 **CONFLICT OF INTEREST**

296 The authors declare no conflict of interest.

297

## 298 **ACKNOWLEDGMENTS**

299 This study was supported by grants from the Japan Agency for Medical  
300 Research and the Development Research Program on Emerging and Re-emerging

301 Infectious Diseases (JP21fk0108103). The authors wish to acknowledge BML, Inc., for  
302 providing the clinical isolates for this study.

303

## 304 REFERENCES

- 305 1. Akase A, Yokoyama K, Obata H, Monma C, Konishi N, Hatakeyama H, Saiki D,  
306 Maeda M, Asayama C, Suzuki J, Sadamas K. 2022. Multi-locus sequence typing and  
307 lipopligosaccharide class analysis of *Campylobacter jejuni* HS:19 isolated in Japan.  
308 *Jpn J Infect Dis* **75**: 199–201.
- 309 2. Asakura H, Brüggemann H, Sheppard SK, Ekawa T, Meyer TF, Yamamoto S, Igimi  
310 S. 2012. Molecular evidence for the thriving of *Campylobacter jejuni* ST-4526 in  
311 Japan. *PLoS One* **7**: e48394.
- 312 3. Asakura H, Taguchi M, Ekawa T, Yamamoto S, Igimi S. 2013. Continued widespread  
313 dissemination and increased poultry host fitness of *Campylobacter jejuni* ST-4526  
314 and ST-4253 in Japan. *J Appl Microbiol* **114**: 1529–1538.
- 315 4. Asakura H, Sakata J, Nakamura H, Yamamoto S, Murakami S. 2019. Phylogenetic  
316 diversity and antimicrobial resistance of *Campylobacter coli* from humans and  
317 animals in Japan. *Microbes Environ* **34**: 146–154.
- 318 5. Chuma T, Miyasako D, Dahshan H, Takayama T, Nakamoto Y, Shahada F, Akiba M,  
319 Okamoto K. 2013. Chronological change of resistance to  $\beta$ -lactams in *Salmonella*  
320 *enterica* serovar Infantis isolated from broilers in Japan. *Front Microbiol* **4**: 113.
- 321 6. Clinical and Laboratory Standards Institute. 2013. Performance standards for  
322 antimicrobial disk and dilution susceptibility tests for bacteria isolated from  
323 animals; approved standard, 4th ed. CLSI document VET01-A4. Clinical and  
324 Laboratory Standards Institute, Wayne, PA.

- 325 7. Clinical and Laboratory Standards Institute. 2016. Methods for antimicrobial  
326 dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria,  
327 3rd ed. CLSI guideline M45. Clinical and Laboratory Standards Institute, Wayne,  
328 PA.
- 329 8. Cody AJ, McCarthy NM, Winmalarathna HL, Colles FM, Clark L, Bowler ICJW,  
330 Maiden MCJ, Dingle KE. 2012. A longitudinal 6-year study of the molecular  
331 epidemiology of clinical *Campylobacter* isolates in Oxfordshire, United Kingdom.  
332 *J Clin Microbiol* **50**: 3193–3201.
- 333 9. DANMAP 2020 – Use of antimicrobial agents and occurrence of antimicrobial  
334 resistance in bacteria from food animals, foods and humans in Denmark. ISSN 1600-  
335 2032. Available at: <https://www.danmap.org/reports/2020> (accessed on 21 Feb 2022).
- 336 10. Duc VM, Kakiuchi R, Muneyasu H, Toyofuku H, Obi T, Chuma T. 2022. Decreasing  
337 trend of  $\beta$ -lactam resistance in *Salmonella* isolates from broiler chickens due to the  
338 cessation of ceftiofur *in ovo* administration. *Vet Anim Sci* **16**: 100248.
- 339 11. Echeita MA, Herrera S, Usera MA. 2001. Atypical, *fljB*-negative *Salmonella*  
340 *enterica* subsp. *enterica* strain of serovar 4,5,12:i:- appears to be a monophasic  
341 variant of serovar Typhimurium. *J Clin Microbiol* **39**: 2981–2983.
- 342 12. Frasson I, Bettanello S, De Canale E, Richter SN, Palù, G. 2016. Serotype  
343 epidemiology and multidrug resistance patterns of *Salmonella enterica* infecting  
344 humans in Italy. *Gut Pathog* **8**: 26.
- 345 13. Grimont PAD, Weil F. 2007. Antigenic Formulas of the Salmonella Serovars, 9th ed.;  
346 WHO Collaborating Centre for Reference and Research on Salmonella: Paris,  
347 France; Institute Pasteur: Paris, France.
- 348 14. Haruna M, Sasaki Y, Murakami M, Ikeda A, Kusukawa M, Tsujiyama Y, Ito K, Asai



- 349 T, Yamada, Y. 2012. Prevalence and antimicrobial susceptibility of *Campylobacter*  
350 in broiler flocks in Japan. *Zoonoses Public Health* **59**: 241–245.
- 351 15. Haruna M, Sasaki Y, Murakami M, Mori T, Asai T, Ito T, Yamada Y. 2013. Prevalence  
352 and antimicrobial resistance *Campylobacter* isolates from beef cattle and pigs in  
353 Japan. *J Vet Med Sci* **75**: 625–628.
- 354 16. Japanese Association for Infectious Disease/Japanese Society of Chemotherapy;  
355 JAID/JSC Guide to Clinical Management of Infectious Disease/Guideline-preparing  
356 Committee; Intestinal Infections Working Group; Ohnishi K; Ainoda Y; Imamura A;  
357 Iwabuchi S; Okuda M; Nakano T. 2018. JAID/JSC guidelines for infection treatment  
358 2015 – Intestinal infections. *J Infect Chemother* **24**: 1–17.
- 359 17. Kijima M, Shirakawa T, Uchiyama M, Kawanishi M, Ozawa M, Koike R. 2019.  
360 Trends in the serovar and antimicrobial resistance in clinical isolates of *Salmonella*  
361 *enterica* from cattle and pigs between 2002 and 2016 in Japan. *J Appl Microbiol* **127**:  
362 1869–1875.
- 363 18. Kumagai Y, Pires SM, Kubota K, Asakura H. 2020. Attributing human foodborne  
364 diseases to food sources and water in Japan using analysis of outbreak surveillance  
365 data. *J Food Prot* **83**: 2087–2094.
- 366 19. Li X, Singh N, Beshearse E, Blanton JL, DeMent J, Havelaar AH. 2021. Spatial  
367 epidemiology of salmonellosis in Florida, 2009–2018. *Front Public health* **8**: Article  
368 603005.
- 369 20. Lovine NM. 2013. Resistance mechanisms in *Campylobacter jejuni*. *Virulence* **4**:  
370 230–240.
- 371 21. Mascaro V, Pilegg C, Crinò M, Proroga YTR, Carullo MR, Graziani C, Arigori F,  
372 Turno P, Pavia M. 2017. Non-typhoidal *Salmonella* in Calabria, Italy: A laboratory

- 373 and patient-based survey. *BMJ Open* **7**: e017037.
- 374 22. Metreveli M, Bulia S, Shalamberidze I, Tevzadze L, Tsanova S, Goenaga JC, Stingl  
375 K, Imnadze P. 2022. Campylobacteriosis, shigellosis and salmonellosis in  
376 hospitalized children with acute inflammatory diarrhea in Georgia. *Pathogens* **11**:  
377 232.
- 378 23. Mori T, Okamura N, Kishino K, Wada S, Zou B, Nanba T, Ito T. 2017. Prevalence  
379 and antimicrobial resistance of *Salmonella* serotypes isolated from poultry meat in  
380 Japan. *Food Safety* **6**: 126–129.
- 381 24. National Veterinary Assay Laboratory of Japan. 2020. Report on the Japanese  
382 Veterinary Antimicrobial Resistance Monitoring System 2016–2017; National  
383 Veterinary Assay Laboratory, Ministry of Agriculture, Forestry and Fisheries: Tokyo,  
384 Japan.
- 385 25. Noda T, Murakami K, Etoh Y, Okamoto F, Yatsuyanagi J, Sera N, Furuta M, Onozuka  
386 D, Oda T, Asai T, Fujimoto S. 2015. Increase in resistance to extended-spectrum  
387 cephalosporins in *Salmonella* isolated from retail chicken products in Japan. *PLoS*  
388 *One* **10**: e0116927.
- 389 26. Ohishi T, Aoki K, Ishii Y, Usui M, Tamura Y, Kawanishi M, Ohnishi K, Tareda K.  
390 2017. Molecular epidemiological analysis of human- and chicken-derived isolates  
391 of *Campylobacter jejuni* in Japan using next-generation sequencing. *J Infect*  
392 *Chemother* **23**: 165–172.
- 393 27. Saito S, Koori Y, Ohsaki Y, Osaka S, Oana K, Nagano Y, Arakawa Y, Nagano N.  
394 2017. Third-generation cephalosporin-resistant non-typhoidal *Salmonella* isolated  
395 from human feces in Japan. *Jpn J Infect Dis* **70**: 301–304.
- 396 28. Sasaki Y, Ikeda A, Ishikawa K, Murakami M, Kusukawa M, Asai T, Yamada Y. 2012.

- 397 Prevalence and antimicrobial susceptibility of *Salmonella* in Japanese broiler flocks.  
398 *Epidemiol Infect* **140**: 2074–2081.
- 399 29. Sasaki Y, Yonemitsu K, Uema M, Igimi S, Asakura H. 2019. *Salmonella* prevalence  
400 in laying hen farms and estimation of effective tools for controlling *Salmonella*  
401 infections. *J Jpn Soc Poult Dis* **55**: 159–163 (in Japanese, with an English summary).
- 402 30. Sasaki Y, Iwata Y, Uema M, Asakura H. 2020. Prevalence and characterization of  
403 *Campylobacter* in bile from bovine gallbladders. *Shokuhin Eiseigaku Zasshi* **61**:  
404 126–131 (in Japanese with an English summary).
- 405 31. Sasaki Y, Kakizawa H, Baba Y, Ito T, Haremaki Y, Yonemichi M, Ikeda T, Kuroda  
406 M, Ohya K, Hara-Kudo Y, Asai T, Asakura H. 2021. Antimicrobial resistance in  
407 *Salmonella* isolated from food workers and chicken products in Japan. *Antibiotics*  
408 **10**: 1541.
- 409 32. Sasaki Y, Iwata T, Uema M, Yonemitsu K, Igimi S, Asakura H. 2022. *Campylobacter*  
410 spp. prevalence and fluoroquinolone resistance in chicken layer farms. *J Vet Med Sci*  
411 **84**:743–746.
- 412 33. Sasaki Y, Asakura H, Asai T. 2022. Prevalence and fluoroquinolone resistance of  
413 *Campylobacter* spp. isolated from beef cattle in Japan. *Anim Dis* **2**: 15.
- 414 34. Sekizuka T, Yatsu K, Inamine Y, Segawa T, Nishio M, Kishi N, Kuroda M. 2018.  
415 Complete genome sequence of a *bla*<sub>KPC-2</sub>-positive *Klebsiella pneumoniae* strain  
416 isolated from the effluent of an urban sewage treatment plant in Japan. *mSphere* **3**:  
417 e00314-18.
- 418 35. Shigemura H, Matsui M, Sekizuka T, Sekizuka T, Onozuka D, Noda T, Yamashita A,  
419 Kuroda M, Suzuki S, Kimura H, Fujimoto S, Oishi K, Sera N, Inoshima Y, Murakami  
420 K. 2018 Decrease in the prevalence of extended-spectrum cephalosporin-resistant

- 421 *Salmonella* following cessation of ceftiofur use by the Japanese poultry industry. *Int*  
422 *J Food Microbiol* **274**: 45–51.
- 423 36. Shigemura H, Maeda T, Nakayama S, Ohishi A, Carle Y, Ookuma E, Etoh Y, Hirai  
424 S, Matsui M, Kimura H, Sekizuka T, Kuroda M, Sera N, Inoshima Y, Murakami K.  
425 2021. Transmission of extended-spectrum cephalosporin-resistant *Salmonella*  
426 harboring a *bla*<sub>CMY-2</sub>-carrying IncA/C<sub>2</sub> plasmid chromosomally integrated by *ISEcp1*  
427 or *IS26* in layer breeding chains in Japan. *J Vet Med Sci* **83**: 1345–1355.
- 428 37. Shimojima Y, Nishino Y, Fukui R, Kuroda S, Suzuki J, Sadamasu K. 2020.  
429 *Salmonella* serovars isolated from retail meats in Tokyo, Japan and their  
430 antimicrobial susceptibility. *Shokuhin Eiseigaku Zasshi* **61**: 211–217 (in Japanese,  
431 with an English summary).
- 432 38. Takahashi M, Koga M, Yokoyama K, Yuki N. 2005. Epidemiology of *Campylobacter*  
433 *jejuni* isolated from patients with Guillain-Barré and Fisher syndromes in Japan. *J*  
434 *Clin Microbiol* **43**: 335–339.
- 435 39. Tanizawa Y, Fujisawa T, Nakamura Y. 2018. DFAST: a flexible prokaryotic genome  
436 annotation pipeline for faster genome publication. *Bioinformatics* **34**: 1037–1039.
- 437 40. World Health Organization. 2018. Factsheet, *Salmonella* (non-typhoidal).  
438 ([https://www.who.int/news-room/fact-sheets/detail/salmonella-\(non-typhoidal\)](https://www.who.int/news-room/fact-sheets/detail/salmonella-(non-typhoidal)))
- 439 41. World Health Organization. Critically Important Antimicrobials for Human  
440 Medicine, 6th Revision; World Health Organization: Geneva, Switzerland, 2019.
- 441 42. World Health Organization. 2020. Factsheet, *Campylobacter*.  
442 (<https://www.who.int/news-room/fact-sheets/detail/campylobacter>) [accessed on  
443 January 6, 2023]
- 444 43. Yamada K, Saito R, Muto S, Sasaki M, Murakami H, Aoki K, Ishii Y, Tateda K. 2019.

445 Long-term observation of antimicrobial susceptibility and molecular  
446 characterisation of *Campylobacter jejuni* isolated in a Japanese general hospital  
447 2000-2017. *J Glob Antimicrob Res* **18**: 59–63.

448 44. Yoshikura H. 2020. Declining *Vibrio parahaemolyticus* and *Salmonella*, increasing  
449 *Campylobacter* and persisting Norovirus food poisonings: Inference derived from food  
450 poisoning statistics of Japan. *Jpn J Infect Dis* **73**: 102–110.

451 45. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup  
452 FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. *J*  
453 *Antimicrob Chemother* **67**: 2640–2644.

454 46. Zhang P, Zhang X, Liu Y, Jiang J, Shen Z, Chen Q, Ma X. 2020. Multilocus sequence  
455 types and antimicrobial resistance of *Campylobacter jejuni* and *C. coli* isolates of  
456 human patients from Beijing, China, 2017–2018. *Front Microbiol* **11**: 554784.

457 47. Zirnstein G, Li Y, Swaminathan B., Angulo F. 1999. Ciprofloxacin resistance in  
458 *Campylobacter jejuni* isolates: detection of *gyrA* resistance mutations by mismatch  
459 amplification mutation assay PCR and DNA sequence analysis. *J Clin Microbiol* **37**:  
460 3276–3280.

461

Table 1. Antimicrobial resistance rates of *Campylobacter jejuni* and *Salmonella* spp. isolates.

Antimicrobial	No. of resistant isolates (%)	
	<i>Campylobacter jejuni</i>	<i>Salmoella</i> spp.
Ampicillin	26 (17.2)	15 (14.7)
Cefazolin	NT	2 (2.0)
Cefotaxime	NT	2 (2.0)
Streptomycin	4 (2.6)	59 (57.8)
Erythromycin	0 (0.0)	NT
Gentamicin	0 (0.0)	1 (1.0)
Kanamycin	NT	11 (10.8)
Tetracycline	36 (23.8)	17 (16.7)
Nalidixic acid	71 (47.0)	12 (11.8)
Ciprofloxacin	70 (46.4)	0 (0.0)
Colistin	NT	8 (7.8)
Chloramphenicol	0 (0.0)	3 (2.9)
Trimethoprim	NT	10 (9.8)

462 48. NT: not tested.

Table 2. Antimicrobial resistance profiles of 151 *Campylobacter jejuni* isolates.

CC (No.)	ST	ARP	No.	CC (No.)	ST	ARP	No.		
21 (35)	19	NA+CPFX	5	354 (17)	354	ABPC+NA+CPFX	1		
		TC+NA+CPFX	6			TC+NA+CPFX	1		
		SM+NA+CPFX	1			NA+CPFX	1		
		NA+CPFX	2			ABPC	1		
	50	ABPC	2			susceptible	8		
		TC	2			1723	susceptible	1	
		susceptible	4			4091	susceptible	1	
	53	susceptible	2			5721	susceptible	1	
	806	TC+NA+CPFX	1			10010	susceptible	1	
		TC	1			10432	susceptible	1	
	883	susceptible	2			443 (6)	51	ABPC+NA+CPFX	1
	4253	susceptible	3				440	NA+CPFX	1
	4526	TC+NA+CPFX	2					NA	1
	5649	NA+CPFX	1					susceptible	2
	9776	NA+CPFX	1				1904	ABPC	1
	22 (14)	22	ABPC+NA+CPFX			2	464 (7)	4106	TC
TC+NA+CPFX			2	4389	ABPC	1			
NA+CPFX			8	5731	susceptible	1			
ABPC			1	6704	ABPC	3			
susceptible			1	10424	NA+CPFX	1			
42 (5)	42	TC+NA+CPFX	1	508 (1)	508	susceptible	1		
		susceptible	1	574 (1)	9996	susceptible	1		
447	NA+CPFX	2	607 (5)	607	NA+CPFX	3			
459	TC+NA+CPFX	1		4600	ABPC	1			
45 (7)	11	susceptible	1		10431	susceptible	1		
		45	ABPC+NA+CPFX	2	658 (4)	1044	susceptible	3	
	TC+NA+CPFX	2		10443		susceptible	1		
		ABPC	1	Unassigned (20)	468	susceptible	1		
		susceptible	1		922	ABPC+TC+NA+CPFX	2		
48 (9)	918	NA+CPFX	3			NA+CPFX	1		
		susceptible	6		2274	TC+NA+CPFX	1		
52 (3)	52	NA+CPFX	2			NA+CPFX	1		
		10440	susceptible	1	2535	ABPC+NA+CPFX	1		
61 (5)	61	susceptible	2		4325	NA+CPFX	1		
		628	susceptible	1		ABPC	2		
	1244	TC	1		4622	ABPC+TC	1		
	11491	TC+NA+CPFX	1		6609	TC+SM+NA+CPFX	1		
257 (4)	257	susceptible	2		8071	TC+NA+CPFX	1		
		824	susceptible	1		NA+CPFX	2		
		4022	ABPC+SM+TC	1		10006	NA+CPFX	1	
283 (3)	4063	susceptible	2		10437	SM+TC+NA+CPFX	1		
		10486	TC+NA+CPFX	1		11069	ABPC+TC+NA+CPFX	1	
353 (5)	400	NA+CPFX	1		11080	NA+CPFX	1		
		10425	TC	4		11081	ABPC+NA+CPFX	1	

Abbreviations: CC: clonal complex, ST: sequence type, ARP: antimicrobial resistance profile,

49. ABPC: ampicillin, SM: streptomycin, TC: tetracycline, NA: nalidixic acid, CPFX: ciprofloxacin.

463  
464

Table 3. Antimicrobial resistance profiles of 102 *Salmonella* isolates.

O serogroup (No.)	Serovar (No.)	ARP	No.		
O:4 (36)	Schwarzengrund (13)	SM+KM+TC+NA+TMP	1		
		SM+KM+TC+TMP	2		
		SM+KM+TMP	2		
		SM+KM+TC	1		
		SM+TC+TMP	1		
		KM+TMP	3		
		Susceptible	3		
		Typhimurium (5)	ABPC+CEZ+CTX+SM+TC+NA+CP	2	
			SM+GM+KM+TC+TMP	1	
			SM	1	
		Typhimurium monophasic variant (9)	Susceptible	1	
			ABPC+SM+KM+TC+CL+CP	1	
			ABPC+SM+TC	3	
	ABPC+SM		1		
	SM+TC		2		
	SM		1		
	TC		1		
	Agona (3)		SM	3	
	Saintpoul (4)		SM	1	
			Susceptible	3	
	O:7 (40)		Paratyphi B (1)	Susceptible	1
		Brandenburg (1)	Susceptible	1	
Thompson (22)		SM+NA	1		
		SM	10		
		Susceptible	11		
		Infantis (7)	SM	4	
			Susceptible	3	
			Braenderup (7)	SM	5
		Susceptible		2	
		Virchow (2)		SM	2
				Oranienburg (1)	SM
	Montevideo (1)	Susceptible	1		
O:8 (7)	Manhattan (3)	SM+TC	2		
		Susceptible	1		
	Corvallis (2)	Susceptible	2		
		Bovismorbificans (1)	SM	1	
	Untypable (1)	Susceptible	1		
	O:9 (19)	Enteritidis (15)	ABPC+SM+NA+CL	5	
			ABPC+SM+NA	2	
ABPC+NA			1		
CL			2		
Susceptible			5		
Panama (3)		SM	2		
		Susceptible	1		
		Durban (1)	SM	1	

Abbreviations: ARP: antimicrobial resistance profile, ABPC: ampicillin, SM: streptomycin, TC: tetracycline,

50. CL: colistin, CP: chloramphenicol, NA: nalidixic acid, TMP: trimetoprim.



Table 4. Antimicrobial resistance genes in cefotaxime-resistant isolates

Serovar	Isolate	ST	Antimicrobial resistance profile	Antimicrobial resistance genes
Typhimurium	202203	19	ABPC+CEZ+CTX+SM+TC+NA+CP	<i>aac(6')-Iaa</i> , <i>ant(3'')-Ib</i> , <i>bla<sub>CMY-2</sub></i> , <i>aph(6)-Id</i> , <i>floR</i> , <i>qnrB19</i> , <i>sul2</i> , <i>tet(A)</i>
Typhimurium	202204	19	ABPC+CEZ+CTX+SM+TC+NA+CP	<i>aac(6')-Iaa</i> , <i>ant(3'')-Ib</i> , <i>bla<sub>CMY-2</sub></i> , <i>aph(6)-Id</i> , <i>floR</i> , <i>qnrB19</i> , <i>sul2</i> , <i>tet(A)</i>

ST: sequence type.

467