Modeling Invasion of *Campylobacter jejuni* into Human Small Intestinal Epithelial-Like Cells by Bayesian Inference

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**ABSTRACT** Current approaches used for dose-response modeling of low-dose exposures of pathogens rely on assumptions and extrapolations. These models are important for quantitative microbial risk assessment of food. A mechanistic framework has been advocated as an alternative approach for evaluating dose-response relationships. The objectives of this study were to investigate the invasion behavior of *Campylobacter jejuni*, which could arise as a foodborne illness even if there are low counts of pathogens, into Caco-2 cells as a model of intestinal cells and to develop a mathematical model for invading cell counts to reveal a part of the infection dose-response mechanism. Monolayer-cultured Caco-2 cells and various concentrations of *C. jejuni* in culture were cocultured for up to 12 h. The numbers of *C. jejuni* bacteria invading Caco-2 cells were determined after coculture for different time periods. There appeared to be a maximum limit to the invading bacterial counts, which showed an asymptotic exponential increase. The invading bacterial counts were higher with higher exposure concentrations (maximum, 5.0 log CFU/cm²) than with lower exposure concentrations (minimum, 0.6 log CFU/cm²). In contrast, the ratio of invading bacteria (number of invading bacteria divided by the total number of bacteria exposed) showed a similar trend regardless of the exposure concentration. Invasion of *C. jejuni* into intestinal cells was successfully demonstrated and described by the developed differential equation model with Bayesian inference. The model accuracy showed that the 99% prediction band covered more than 97% of the observed values. These findings provide important information on mechanistic pathogen dose-response relationships and an alternative approach for dose-response modeling.

**IMPORTANCE** One of the infection processes of *C. jejuni*, the invasion behavior of the bacteria in intestinal epithelial cells, was revealed, and a mathematical model for prediction of the cell-invading pathogen counts was developed for the purpose of providing part of a dose-response model for *C. jejuni* based on the infection mechanism. The developed predictive model showed a high accuracy of more than 97% and successfully described the *C. jejuni* invading counts. The bacterial invasion predictive model of this study will be essential for the development of a dose-response model for *C. jejuni* based on the infection mechanism.

**KEYWORDS** dose-response model, key events, dose-response framework, quantitative microbial risk assessment, foodborne pathogen, Bayesian model, *Campylobacter jejuni*

*Campylobacter jejuni* is one of the most common foodborne pathogens worldwide, causing substantial health and economic losses to society. Thus, this food-, beverage (1)-, and waterborne pathogen has become an important focus of microbial risk assessment (2). Appropriate quantitative risk assessment of *C. jejuni* in foods should be carried out to predict the status of *C. jejuni* infection through foods.

Although dose-response models are important for quantitative microbial risk as-
essment (3), dose-response information on human infection with *C. jejuni* is limited. Only one human feeding study has been reported (4), but only high doses of *C. jejuni* populations were tested, resulting in high infection/illness rates. Although few epidemiological estimation data are available (5) on *C. jejuni* infection caused by unpasteurized milk on farms (6), the relationship between low-dose *C. jejuni* ingestion and the probability of infection is still not fully understood (7). In the case of pathogens such as *C. jejuni* which easily cause infection, information on relationships between a low dose and a response (i.e., low-dose–response relationships) is of great importance for maintaining public health.

An alternative approach to assessing low-dose–response relationships, named the key-events dose-response framework (KEDRF) (8, 9), has been proposed based on the mechanism of foodborne pathogen ingestion for causing an infection. The KEDRF is an analytical approach that facilitates the use of currently available data to gain insight into dose-response relationships. For example, the KEDRF approach for *Listeria* has been proposed and involves the following five major and necessary steps, termed key events: (i) survival of pathogens in the upper gastrointestinal tract, (ii) establishment in the intestine and attachment and uptake in epithelial cells, (iii) survival and escape from phagosomes in enterocytes and transfer of the pathogens to phagocytes, (iv) transfer of pathogens across the placenta, and (v) pathogen growth leading to fetal morbidity and mortality. Therefore, it is necessary to estimate the microbial behavior at each key event from oral ingestion to illness onset when implementing the KEDRF. A study has also been conducted to determine the dose-response relationship of *Listeria monocytogenes* based on the infection mechanism (10), and the establishment of a mechanism-based dose-response model is becoming more important for clarifying the appropriate dose-response relationship.

*Campylobacter* spp., including *C. jejuni*, invade host human tissues through complex putative mechanisms. *Campylobacter jejuni* bacteria reaching the intestinal tract in contaminated food attempt to migrate to upper intestinal epithelial cells because of their chemotaxis motility activated by mucus (11). The pathogen binds to intestinal cell matrix protein fibronectin by utilizing its putative adhesion or binding factors, such as the fibronectin-binding outer membrane protein CadF, the autotransporter CapA, the periplasmic binding protein PEB1, and the surface-exposed lipoprotein JlpA (12). Finally, *C. jejuni* bacteria enter intestinal cells via a microtubule-dependent and actin filament-independent invasion mechanism (11). The invasion process enables *C. jejuni* to enter the cell first with its tip followed by its flagellar end (13). After cell invasion, *C. jejuni* uses a transcytosis mechanism (transfer of molecules/materials from one side of a cell to another side in membrane-bound vesicles) to migrate to various body tissues and adversely affects vital functions in the host, such as intestine, blood vessel, and lymphatic fluid functions, by producing cytolethal distending toxin, which causes inflammation (14). Therefore, the pathogenic mechanism of *C. jejuni* involves the above-mentioned key event (key event iii). Quantitative modeling of the cell-invasive behavior of *C. jejuni* in intestinal cells is essential for estimating dose-response relationships using the KEDRF.

This study was conducted to experimentally investigate the invading counts of *C. jejuni* in intestinal epithelial cells using human colonic carcinoma (Caco-2) cells *in vitro*, which have functional properties characteristic of small bowel enterocytes and have been widely used as a model cell line for small intestinal epithelial-like cells (15), as one of the key events. Furthermore, we developed a mathematical model describing the invasion behavior of *C. jejuni* in Caco-2 cells, which is necessary for constructing a new dose-response model of *C. jejuni* based on the KEDRF concept.

RESULTS

Behaviors of *C. jejuni* bacteria invading Caco-2 cells. Figures 1, 2, and 3 show that invading *C. jejuni* counts in Caco-2 cells (CFU per square centimeter) showed an asymptotic increase (maximum of ~4.5 log CFU/cm²) over time in the three tested strains. Each point denotes the mean value from three iterations under each condition,
and the error bars denote the standard deviations under each condition. No intracellular invasion of bacteria was observed under some short-exposure-duration conditions, such as 10 s and 1.5 h (no plot is shown in Fig. 1, 2, and 3). Under high-dose conditions, such as 5.7 log CFU/ml, the invading *C. jejuni* counts reached a maximum and became almost constant for more than 3 h of coculture with Caco-2 cells. In contrast, invasion of *C. jejuni* proceeded slowly after low-dose exposure, such as <4.5 log CFU/ml. The number of invading *C. jejuni* bacteria showed an increasing trend with higher doses for the same exposure duration. Although no intracellular invasion was observed within 10 s of exposure at most concentrations, *C. jejuni* exhibited immediate intracellular invasion (<10 s) at higher concentrations (>6.6 log CFU/ml).

![RIMD 0366027](image1)

**FIG 1** Differences in the numbers of invading *Campylobacter jejuni* (RIMD 0366027) bacteria in monolayer-cultured Caco-2 cells (RCB0988). Each symbol and line describe the behaviors under each exposure concentration condition. The exposure concentrations (CFU per milliliter) of each *C. jejuni* strain were approximately 10^3 (circles), 10^4 (inverted triangles), 10^5 (triangles), 10^6 (squares), 10^7 (diamonds), and 10^8 (pentagons).

![RIMD 0366042](image2)

**FIG 2** Differences in the numbers of invading *Campylobacter jejuni* (RIMD 0366042) bacteria in monolayer-cultured Caco-2 cells (RCB0988). Each symbol and line describe the behaviors under each exposure concentration condition. The exposure concentrations (CFU per milliliter) of each *C. jejuni* strain were approximately 10^3 (circles), 10^4 (inverted triangles), 10^5 (triangles), 10^6 (squares), 10^7 (diamonds), and 10^8 (pentagons).
Comparison of the invasion counts and invasion ratios showed that the invasion ratio (invading bacterial count divided by the total exposed bacterial count) did not tend to depend on the exposure concentration, except for the highest dose (8.6 to 8.7 log CFU/ml) (Fig. 4, 5, and 6), although two-way analysis of variance (ANOVA) indicated a statistically significant influence of the concentration factors (\(P < 0.05\)). The invasion ratios for each \(C. jejuni\) strain were approximately \(-2.0\) (1.0%) for exposure.

**Bayesian inference for model parameters for invading \(C. jejuni\) bacteria.** The model parameters for invasion of \(C. jejuni\) were successfully estimated by Bayesian inference. The mean values and standard deviations for each parameter derived by the Bayesian estimation and the Gelman-Rubin convergence statistics (R-hat values) representing the convergence of the parameter distribution estimates are summarized in Table 1. The estimated posterior distributions converged successfully because the R-hat values for all parameters were lower than 1.1. Figures 7, 8, and 9 show the distributions of the estimated parameters, scatterplots, and correlation coefficient (\(R\)) values between parameters. There was a negative correlation between \(\log N_{\text{max}}\) (the maximum invading pathogen counts) and \(\log \mu\) (the cell-invading rate constant of the pathogen) (\(-0.79 \leq R \leq -0.84\)), whereas \(\sigma\) was correlated with neither factor (\(-0.04 \leq R \leq -0.04\)). Figures 10, 11, and 12 illustrate the changes in invading \(C. jejuni\) counts in Caco-2 cells over time based on the estimated parameters and experimentally observed values. The colored gradation ranges from dark to pale in Fig. 10, 11, and 12, representing 60%, 95%, and 99% prediction intervals in order from the median. Table 2 shows the root mean square errors of the median predicted values and percentages of observed values within the 99% prediction interval derived from the prediction model for each isolation.

**DISCUSSION**

**Advantages of the present model.** Our results revealing asymptotically increasing changes were similar to those reported in previous studies of the increasing behavior of adherent bacterial counts (16). Other studies on \(C. jejuni\) cell invasion (17, 18) also indicated saturation of invading bacterial counts. Therefore, it could be suggested that the present experiment reproduces general \(C. jejuni\) adhesion and invasion dynamics in Caco-2 cell layers. In this study, we clarified the temporal changes in the invasion behavior of \(Campylobacter\) in intestinal cells.

According to physiological studies, the mean time required for food to pass through the stomach is 3 to 4 h (19), and the mean time to reach the colon is 10 to 12 h (20). Therefore, the average retention time in the small intestine, which is a microaerobic environment that is most optimal for \(C. jejuni\), would be 6 to 9 h. Thus, the bacterial invasion behavior of intestinal epithelial cells observed in the present study can occur during the actual digestion process. As the ratio of \(C. jejuni\) invasion into intestinal epithelial cells tended to remain approximately constant under conditions of low exposure concentrations (3.6 to 7.7 log CFU/ml), bacterial invasion behavior can be predicted at low doses. In addition, the estimation range of the present mathematical model successfully and accurately described the invading \(C. jejuni\) counts. No studies have attempted to predict the time-dependent number of cell-invading \(C. jejuni\) bacteria using mathematical models; therefore, our results can be used to study dose-response relationships based on the KEDRF concept.

<table>
<thead>
<tr>
<th>Strain</th>
<th>(\log N_{\text{max}}) Mean</th>
<th>(\log N_{\text{max}}) SD</th>
<th>(\log \mu) Mean</th>
<th>(\log \mu) SD</th>
<th>(\sigma) Mean</th>
<th>(\sigma) SD</th>
<th>R-hat</th>
<th>R-hat</th>
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<tr>
<td>RIMD 0366027</td>
<td>4.9</td>
<td>0.13</td>
<td>1.0</td>
<td>0.16</td>
<td>0.55</td>
<td>0.05</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
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<td>4.7</td>
<td>0.10</td>
<td>1.0</td>
<td>0.13</td>
<td>0.49</td>
<td>0.04</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>RIMD 0366048</td>
<td>4.0</td>
<td>0.08</td>
<td>1.0</td>
<td>0.10</td>
<td>0.40</td>
<td>0.03</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>
The present model equation was derived from part of the one-compartment model that estimates chemical absorption behaviors (21). It was revealed that the rate of change in the *C. jejuni* invasion count depended on the extracellular concentration of dose material, which is the same as the nutritional or chemical absorption through human gastrointestinal tracts. Although it seems to be that the nutritional absorption originating from only the human digestion system is simpler than that of pathogenic invasion into epithelial intestinal cells, which is an interactive biophenomenon between human cells and *C. jejuni*, the potential that the absorption model could describe the invasion of *C. jejuni* into cells was indicated by the result. Additionally, because of the potential of the absorption kinetics model describing the cell-invading bacterial counts of *C. jejuni*, there is a possibility that the cell invasion behavior of other infectious food poisoning bacteria can also be represented using predictive models of chemical absorption.

**FIG 3** Differences in the numbers of invading *Campylobacter jejuni* (RIMD 0366048) bacteria in monolayer-cultured Caco-2 cells (RCB0988). Each symbol and line describe the behaviors under each exposure concentration condition. The exposure concentrations (CFU per milliliter) of each *C. jejuni* strain were approximately $10^3$ (circles), $10^4$ (inverted triangles), $10^5$ (triangles), $10^6$ (squares), $10^7$ (diamonds), and $10^8$ (pentagons).

**FIG 4** Differences in invading *Campylobacter jejuni* (RIMD 0366027) ratios in monolayer-cultured Caco-2 cells (RCB0988). Each symbol and line describe the behaviors under each exposure concentration condition, as described in the legend of Fig. 1.
The modeling procedure presented in this study based on Bayesian inference separately describes model uncertainty and variability in the parameter and prediction distributions. It has been suggested that prior distributions of the prediction describe experimental uncertainty (18, 19), and the distributions of kinetic parameters describe variability (22–24). In this study, by applying normal distribution as a posterior distribution to the prediction (equation 7), experimental uncertainty in the invading bacterial counts was described as mean values derived from the model equation and standard deviations ($\sigma$). In contrast, the parameter distributions describe the variabilities in biological characteristics. There were two biological variability sources in C. jejuni and Caco-2 cells in this study. However, these variability sources were not separately described as parameter distributions, and both variability sources were treated as a combined parameter distribution. The distribution of $\mu$ describes variability in the cellular chemotaxis of C. jejuni and the invasion signal receptor activity of Caco-2 cells. Furthermore, the distribution of $N_{\text{max}}$ describes the variability in the bacterial invasion capacity in Caco-2 cells. Considering both uncertainty and variability, the present model enables the determination of more realistic prediction ranges than those of predictions obtained by non-Bayesian modeling.

FIG 5 Differences in invading Campylobacter jejuni (RIMD 0366042) ratios in monolayer-cultured Caco-2 cells (RCB0988). Each symbol and line describe the behaviors under each exposure concentration condition, as described in the legend of Fig. 2.

FIG 6 Differences in invading Campylobacter jejuni (RIMD 0366048) ratios in monolayer-cultured Caco-2 cells (RCB0988). Each symbol and line describe the behaviors under each exposure concentration condition, as described in the legend of Fig. 3.
Prediction models based on Bayesian estimation can explain the variability and uncertainty of predictive models. The importance of these factors has been proposed in dose-response models (1), and modeling using Bayesian inference is necessary. Studies have suggested that the Bayesian approach is convenient for estimating the variability and uncertainty in both exposure assessment and dose-response quantitative microbiological risk assessment of food (25–27). Considering the efficacy of describing variability and uncertainty in predicting various bacterial behaviors by Bayesian modeling, Bayesian inference is important not only in dose-response models but also in each key-event model for the KEDRF.

**Limitations of the present model.** The predictive model in this study represents the invasion behavior of *C. jejuni* in Caco-2 cells; however, this is only an experimental
reproduction of the infection mechanism. This is common to all key-event models used in the KEDRF. Although several key-event models should be combined to estimate the dose-response relationship, and its validity should be assessed by comparison with human feeding test and epidemiological estimation data, the validity of human reproduction cannot be assessed only by the experimental methods used in the present study. Whether our in vitro experiments represent the actual intestinal environment remains unclear, and artificial organs can be used to reproduce the intestinal environment, or a conversion factor can be developed to correct for the difference.

Only the invasion behavior of C. jejuni in Caco-2 cells was predicted in this study, which does not directly estimate the dose-response relationship. To estimate this relationship, the predictive models for bacterial invasion into intestinal cells should be combined with other key-events models. By combining all the key models predicting C. jejuni dynamics in the human body, it could be possible to develop a dose-response model for C. jejuni.

**How to use the information obtained from the cell invasion model.** This estimation model can be used to determine the probability that one or more bacteria will invade cells, \( P_{\text{ invaders}} \). As at least one or more bacteria must invade intestinal cells to cause infection and disease development, \( P_{\text{ invaders}} \) can be one indicator of the infection strength. \( P_{\text{ invaders}} \) can be described as follows:

\[
P_{\text{ invaders}} = 1 - P_{\text{ not invaders}}
\]

\[
= 1 - (P_{\text{ not invaders}})^{N_{\text{ dose}}}
\]

\[
= 1 - (1 - P_{\text{ invading}})^{N_{\text{ dose}}}
\]

where \( P_{\text{ not invaders}} \) denotes the probability that all bacteria will not invade the cell, \( P_{\text{ invading}} \) denotes the probability that a single bacterium will not invade the cells, \( N_{\text{ dose}} \) denotes the dose bacterial count, and \( P_{\text{ invading}} \) denotes the probability that a single bacterium will invade the cells. The invasion ratio of C. jejuni for monolayer-cultured Caco-2 cells of 1 cm\(^2\) (Fig. 4 to 6) is essentially equal to the probability that a single bacterium will invade 1 cm\(^2\) of cells, \( r_{\text{ invading}} \). As in equation 1, \( P_{\text{ invading}} \) can be described using \( r_{\text{ invading}} \):

\[
P_{\text{ invading}} = 1 - (1 - r_{\text{ invading}})^{5}
\]

**FIG 9** Characteristics of estimated cell invasion model parameters of each bacterial isolate (RIMD 0366048) by Bayesian regression. The relationships of the posterior distribution of the parameters \( \log_{10} N_{\text{ max}}, \log_{10} \mu_t \), and \( \sigma \) (under the diagonal); corresponding histograms (on the diagonal); and correlation coefficients (over the diagonal) are shown.
where $S$ denotes the surface area of the human small intestine. Substituting equation 6 for the definition of $r_{\text{invading}}$, $r_{\text{invading}}$ can be described as follows:

$$
   r_{\text{invading}} = \frac{N_{\text{invading}}}{N_{\text{Dose}}} = \frac{N_{\text{max}}(1 - e^{-\frac{C_{\text{extracellular}}}{V} t})}{N_{\text{Dose}}} = N_{\text{max}} \left( \frac{1 - e^{-\frac{C_{\text{extracellular}}}{V} t}}{N_{\text{Dose}}} \right)
$$

where $V$ denotes the volume of the human small intestine; the extracellular concentration was used as the average concentration in the intestinal fluid, $C_{\text{extracellular}} = N_{\text{Dose}}/V$, and $t$ denotes the exposure time of pathogens to intestinal cells. Here, the relationship between $P_{p, \text{invading}}$ and $\log_{10} N_{\text{Dose}}$ was numerically derived using the model-estimated invading bacterial count and estimated data on the small intestine from previous studies: $S$ of 32 m$^2$ (28), $V$ of 319 ml (29), and $t$ of 7.5 h (19, 20). $P_{p, \text{invading}}$ as a function of the ingested dose of $C. \text{jejuni}$ exhibited the same trend as the reported dose-response relationships (5), as shown in Fig. 13; this figure also indicates nonsignificant differences in $P_{p, \text{invading}}$ among strains. A larger $\mu$ parameter for each strain was associated with a higher $P_{p, \text{invading}}$ value for each strain. This suggests that $P_{p, \text{invading}}$ depends on the $\mu$ parameter and can indicate the infectious potential of different strains and bacterial species. Although $P_{p, \text{invading}}$ was higher than the reported dose-response relationship of $C. \text{jejuni}$, other factors will inhibit infection besides invasion into intestinal cells. In this case, other key events in infection, such as the inactivation of $C. \text{jejuni}$ in the stomach or the immune system, will be influenced. $P_{p, \text{invading}}$ will be useful for deriving the dose-response relationship of an objective

**FIG 10** Estimated (curves and ranges) and observed (points) invading bacterial counts of $C. \text{jejuni}$ (RIMD 0366027) in monolayer-cultured Caco-2 cells at each bacterial exposure concentration. Colored ranges show 60%, 95%, and 99% prediction intervals, in order from the median.
pathogen by combining this factor with other key-event predictive models.

Conclusions. In this study, the cell-invading pathogen counts of *C. jejuni* in monolayer-cultured intestinal epithelial cells was evaluated *in vitro* and described using a mathematical model based on Bayesian inference, which is useful in constructing a dose-response model based on the KEDRF. Studies are needed to develop a mathematical model to represent the body dynamics of *C. jejuni* at any key event to validate the potential of the KEDRF. This procedure can be applied to other bacterial species to provide fundamental information for evaluating and developing dose-response models.

MATERIALS AND METHODS

Cell strain and culture conditions. Human colonic carcinoma Caco-2 cells (RCB0988) were obtained from the Riken Cell Bank (Tsukuba, Japan) and stored at −80°C in cryopreservation medium (Merck, Darmstadt, Germany). The cells were grown at 37°C in Dulbecco’s modified Eagle’s medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA), 2 mM L-glutamine (Sigma-Aldrich), 1% nonessential amino acids (Sigma-Aldrich), and a penicillin (10,000 U/liter)-streptomycin (10 mg/liter) antibiotic solution in a humidified atmosphere containing 5% CO₂ and 95% air. The cells were cultured for 12 days, with two medium exchanges and passaging once. The cultured cells were plated at a density of 8.2 × 10⁴ cells/well into 96-well tissue culture plates (Techno Plastic Products, Trasadingen, Switzerland) and grown for 2 days to confluence in the same medium but in the presence of an antibiotic solution (penicillin-streptomycin).

Bacterial strains and culture conditions. Three strains of *C. jejuni* (RIMD 0366027, RIMD 0366042, and RIMD 0366048) originating from patients and patient feces were acquired from the Research Institution for Microbial Disease (Osaka, Japan). According to the bacterial strain donor, strain RIMD
0366048 causes Guillain-Barré syndrome. The stock culture was maintained at ~80°C in Bolton broth (Oxoid, Basingstoke, UK) containing 10% glycerol. The strain was activated by incubation at 42°C for 48 h on Preston agar under microaerophilic conditions (6 to 12% O2, 5 to 8% CO2) with Anaero Pack MicroAero (Mitsubishi, Tokyo, Japan), followed by two incubations in Bolton broth under the same conditions. After three washing and centrifugation steps (3,000 g for 10 min) with supplemented DMEM without the antibiotic solution, the bacteria were resuspended in DMEM and used in the experiments.

**Invading cell count assay.** Caco-2 cell monolayers in DMEM with supplements were infected with cultured *C. jejuni* bacteria at 37°C under microaerophilic conditions (6 to 12% O2, 5 to 8% CO2). The assays were based on previous research (11). *C. jejuni* bacteria at concentrations of 10^n (n = 4 to 9) CFU per ml were used. Caco-2 cell monolayers were infected with *C. jejuni* for different exposure times (10 s and 1.5, 3.0, 4.5, 6.0, 7.5, 9.0, 10.5, and 12 h). It was confirmed in advance that there is no remarkable difference in the Caco-2 cells and the *C. jejuni* counts before and after 12 h of culturing in DMEM. The infected cells were washed three times with Dulbecco’s phosphate-buffered saline (DPBS) and then incubated with 100 μg/ml of gentamicin (Wako, Osaka, Japan) in DMEM-FBS at 37°C for 1 h to kill any extracellular organisms. The cells were washed again with DPBS and treated with 1% Triton X-100 (Sigma) in DPBS at 4°C for 30 min to release intracellular organisms. Dilutions from each well were plated on Preston agar and incubated at 42°C for 48 h on Preston agar under microaerophilic conditions. After incubation, the invading bacterial counts were determined as CFU. We previously determined that Triton X-100 did not inhibit the growth of *C. jejuni*. The results obtained at bacterial concentrations of 10^n (n = 5, 7, and 9) CFU

### TABLE 2  Accuracy of each isolation model for test validation

<table>
<thead>
<tr>
<th>Strain</th>
<th>Root mean square error (log CFU)</th>
<th>% accuracy of 99% prediction range (validation data count)</th>
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<tbody>
<tr>
<td>RIMD 0366027</td>
<td>0.53</td>
<td>100 (68)</td>
</tr>
<tr>
<td>RIMD 0366042</td>
<td>0.47</td>
<td>96 (75)</td>
</tr>
<tr>
<td>RIMD 0366048</td>
<td>0.38</td>
<td>100 (73)</td>
</tr>
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</table>
were used for model development; the results at bacterial concentrations of $10^n$ (n = 4, 6, and 8) CFU were used for model validation.

Data analysis and Bayesian modeling conditions. Changes in the invading bacterial counts were modeled using an absorption model in the intestinal tract of the one-compartment model used for pharmacokinetics analysis (21). The rate of absorption (here, the rate of bacterial invasion, $\frac{d}{dt}N_{\text{invading}}$) was determined as the product of the absorption rate constant, $\mu$, and the concentration of medicine (extracellular bacterial concentration $[C_{\text{extracellular}}]$ in this case) in the absorption section, with a spatial upper limit to the amount of medicine (upper limit of invading bacteria, $N_{\text{max}}$) absorbed. Therefore, total absorption in the absorption section (in this paper, the invading bacterial count) is defined by the following differential equation, equation 4, and if the initial number of invading bacteria is set to 0, equation 4 can be solved as equation 5:

$$\frac{d}{dt}N_{\text{invading}} = \mu C_{\text{extracellular}}(N_{\text{max}} - N_{\text{invading}})$$  \hspace{1cm} (4)

$$N_{\text{invading}} = N_{\text{max}}(1 - e^{-\mu C_{\text{extracellular}}t})$$  \hspace{1cm} (5)

This can be transformed into logarithmic form, as shown in the following equation:

$$\log_{10}N_{\text{invading}} = \log_{10}[N_{\text{max}}(1 - e^{-\mu C_{\text{extracellular}}t})]$$  \hspace{1cm} (6)

The model was fitted to the observed invading behavior of each isolate by Bayesian inference regression (parameters $\log_{10}N_{\text{max}}$ and $\log_{10}\mu$). Bayesian inference regression, which considers uncertainty and variability, was used to estimate the fit of the invasion model to the observed data. We adopted a normal distribution with the standard deviation, $\sigma$, as the prior of $\log_{10}N_{\text{invading}}$ and a uniform distribution as a noninformal prior distribution for each parameter. PyStan (version 2.19) was used for Bayesian parameter estimation; the No-U-Turn sampler was used for the Markov chain Monte Carlo (MCMC) sample method of posterior parameter distributions. The predictive model equation used for Bayesian parameter estimation is as follows:

$$\log_{10}N_{\text{invading}} \sim \text{normal}(\log_{10}[N_{\text{max}}(1 - e^{-\mu C_{\text{extracellular}}t})], \sigma)$$  \hspace{1cm} (7)

ACKNOWLEDGMENTS

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We have no conflicts of interest.

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