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Mathematical quantification of inactivation of *Vibrio parahaemolyticus* on two types of surface soiled with different substrates

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ABSTRACT

Survivability of foodborne pathogens on food-processing surfaces is an important factor in understanding and quantifying bacterial transfer to foods (i.e. cross-contamination). This study examined the survival of *Vibrio parahaemolyticus* on two different surfaces in a laboratory-based simulation. *V. parahaemolyticus* was inoculated onto both polypropylene and stainless steel surfaces following contamination with saline solution (SS), tryptone soya broth (TSB), or seafood purge. *V. parahaemolyticus* remained viable on polypropylene for 10 days, but was undetectable within 24 h on the stainless steel surface. The survivability was similar on polypropylene in the presence of all contaminating substrates, as shown by data from the Weibull and biphasic models (adjusted-$R^2 > 0.91$). However, for stainless steel, SS and TSB prolonged the survival of *V. parahaemolyticus* to 144 and 120 h, respectively. Survivability data revealed a shoulder period in the first 4 h and a slight tailing effect at the end of the survival curve in the presence of seafood purge, which suggested that the biphasic model might be appropriate (adjusted-$R^2 = 0.9442$). These results indicate that the biphasic model may accurately estimate pathogen survival for cross contamination exposure. Integrating the survivability model into quantitative studies will help understand cross contamination.

1. Introduction

Vibrio parahaemolyticus, a ubiquitous marine bacterium, is an important public health concern worldwide. Consumption of food contaminated with *V. parahaemolyticus* may lead to development of acute gastroenteritis, characterized by symptoms such as diarrhea, headache, vomiting, nausea, abdominal pain, and low-grade fever (Su & Liu, 2007). Official statistics in China indicated that *V. parahaemolyticus* is a leading cause of bacterial foodborne disease (Chen, Liu, Fan, & Wang, 2008, 2010; Liu, Chen, Fan, & Wang, 2006; Liu, Chen, Guo, & Wang, 2008), especially in coastal regions of the country (Lin, Ran, Ma, Wang, & Feng, 2010; Liu, Chen, Wang, & Ji, 2004). It is also proposed that authentic human and economic burdens of *V. parahaemolyticus* may be much higher than reported, as outbreaks may be overlooked and incidents may occur sporadically in multiple locations (Chen et al., 2013; Kubota et al., 2011; Scallan et al., 2011).

Epidemiological investigation showed that foodborne *V. parahaemolyticus* outbreaks show a seasonal pattern (Daniels et al., 2000; Lesmana et al., 2001; World Health Organization, 1999). These outbreaks, mostly in the warmer months (June–October) (Li et al., 2014), suggest that temperature is an important factor in the epidemiology of this pathogen. *V. parahaemolyticus* is commonly found in raw seafood products (Feldhusen, 2000), and has been isolated from shrimps, prawns, crabs, and oysters (Koralage et al., 2012; Rojas, Matté, Dropa, Silva, & Matté, 2011; Vongxay et al., 2008). Studies have demonstrated a connection between *V. parahaemolyticus* infection and consumption of seafood (Vongxay et al., 2008), with raw or partially-cooked seafood products usually the main vehicles of infection (Martinez-Urtaza et al., 2004).

Cross-contamination is a common route of bacterial transmission to food products (Kusumaningrum, van Asselt, Beumer, &

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Zwietering, 2004). As reported by Wu, Wen, Ma, Ma, and Chen (2014), up to 50% of *V. parahaemolyticus* outbreaks between 2003 and 2008 in China were attributed to cross-contamination. The study pinpointed contaminated contact surfaces of foods and equipment as a key vehicle of cross contamination (Legnani, Leoni, Berveglieri, Mirolo, & Alvaro, 2004; Todd, Greig, Bartleson, & Michaels, 2009). Survivability of microorganisms on food contact surfaces is vital, as cross-contamination events depend on the presence of viable microorganisms on surfaces in contact with foods (Perez-Rodriguez, Valero, Carrasco, Garcia, & Zurera, 2008). Kitchen cutting boards and food processing surfaces can easily be contaminated with *V. parahaemolyticus* during the preparation of raw seafood (Zhao, Zhao, Doyle, Rubino, & Meng, 1998). As a result, cross-contamination between raw and cooked foods during food preparation serves as a common factor for *V. parahaemolyticus* outbreaks in China (Liu, Wang, & Yang, 1999; Wu et al., 2014).

The risk of microbiological cross-contamination depends heavily on the number of viable bacterial cells on a surface. Incorporating survivability models into quantitative risk assessment analyses is therefore key to accurate identification of cross-contamination processes (Pérez-Rodríguez et al., 2011; Spector & Kenyon, 2012). To date, there have been very few studies reporting predictive models for describing bacterial survivability on food contact surfaces (e.g., polypropylene or stainless steel), especially in relation to *V. parahaemolyticus* cross-contamination. Therefore, this study aimed to i) evaluate the survival of *V. parahaemolyticus* on two different work surfaces (polypropylene and stainless steel); ii) assess whether artificial growth media may serve as a model of contamination to aid in bacterial survival on each type of surface; and iii) to study, based on observations, the feasibility of the log-linear, the Weibull, and biphasic models in describing bacterial survival over the period of observation.

2. Materials and methods

2.1. Inoculum preparation

V. parahaemolyticus strain CICC 21617, obtained from the China Center of Industrial Culture Collection (CICC; Beijing, China), was used in this study. Cryobead stocks of the strain were prepared in cryopreservatives, and then stored at −20 °C in cryovials (Microbank, Pro-Lab Diagnostics, Austin, TX, USA). To prepare cultures, a single bead was transferred to a tube containing 4.5 mL of tryptone soya broth (TSB, Land Bridge, Beijing, China) supplemented with 1.5% NaCl, and then incubated for 24 h at 37 °C. Three consecutive passes were then made by transferring 0.5 mL of fresh bacterial culture to a tube containing 4.5 mL of TSB. After the third pass, the tube was incubated at 37 °C for 18 h. The culture was adjusted to an optical density at 600 nm of 0.205–0.262 in 0.1% peptone water (Oxoid, Basingstoke, UK) using a MV2550 UV Spectrophotometer (Shimadzu, Japan), resulting in a final bacterial concentration of ~10^9 colony forming units (cfu)/mL. To determine initial concentration, a 1-mL aliquot was serially diluted and plated onto petrifilm aerobic count plates (PACP, 3M, USA), and the resulting colonies were counted.

2.2. Inoculation

Polypropylene and stainless steel (304) were used to represent common food contact surfaces in this study, and were purchased at a supermarket in Beijing. The surfaces were delimited into 2 × 5 cm (10 cm^2) sectors using a marker, washed with soapy water, dried in a 50 °C incubator, and then placed under UV light in a biological safety cabinet (Thermo, USA) overnight prior to use. To obtain sterile seafood purge, frozen shrimps were thawed at room temperature (26 °C). The liquid released from the shrimps during thawing (referred to as seafood purge) was collected, centrifuged (6000 rpm for 12 min), and then filter-sterilized (0.22 μm filter, Millipore, USA) prior to being stored at −20 °C. Prior to inoculation, saline solution (SS, 0.85% NaCl), TSB, and seafood purge were used to contaminate the two surfaces. SS was used to simulate clean conditions, while TSB and seafood purge simulated contamination. Each sector was then inoculated with 0.1 mL of bacterial suspension at a concentration of 10^9 cfu/mL, which was evenly spread to obtain a final concentration of ~10^7 cfu/cm^2.

2.3. Sampling of the inoculated surfaces

The inoculated surfaces were incubated at 30 °C and 50% relative humidity to imitate a typical scenario in a restaurant or canteen during the warm season. The inoculated surfaces were incubated in a constant temperature and humidity chamber (Yiheng, Shanghai, China). During the experimental period, individual sectors on the surfaces were sampled at different time points to assess the bacterial decay over time, and to examine whether the different substrates had any effect on the outcome. At each sampling point, two different sectors (i.e. replicates) were analyzed. Starting immediately after inoculation (minute 0), samples were collected from the inoculated surfaces using swabs (Xingnanfeng, Hainan, China) soaked in sterile SS, following the procedure of Pérez-Rodríguez, Posada-Izquierdo, Valero, García-Gimeno, and Zurera (2013). Swab tips were then cut off using sterilized scissors and aseptically placed in tubes containing 3 mL of 0.1% peptone water (Oxoid, UK). The tubes were vortexed for 1 min, incubated at room temperature for 1 min, and then vortexed again for 1 min to release any bacterial cells trapped in the swab tips. To estimate the number of viable cells removed from the inoculated surface, the peptone water samples were diluted in SS and then cultured on PACP. Following incubation at 37 °C for 48 h, characteristic colonies present on the plates were counted.

2.4. Sterility tests

To assess the effectiveness of the disinfection process, Plate Count Agar (PCA, Land Bridge, Beijing, China) was pressed onto polypropylene or stainless steel coupons and then incubated at 37 °C for 48 h. To act as a negative control, several sectors of each surface were left uninoculated (i.e. substrate added but no bacterial inoculum). These sectors were analyzed at time 0 and at the end of the experimental period to confirm sterility prior to and during the experiments. In addition, to confirm sterility of the seafood purge, aliquots were plated onto PCA and incubated at 37 °C for 48 h. The sampling procedure and microbiological analyses used for sterility tests were as described for the inoculated area.

2.5. Statistical analysis and data modeling

The recovery rate was calculated as the percentage of recovered cells (cfu/cm^2) with respect to the initial inoculum (cfu/cm^2) deposited on the surfaces. Recovery rate data were analyzed by ANOVA and Tukey's honest significant difference test ($p < 0.05$ was considered significant). To generate survival curves, mean counts of *V. parahaemolyticus*, expressed in log cfu/cm^2, were plotted with respect to sampling times (h) using Excel software (Redmond, Microsoft Corporation). Different mathematical models were then fitted to the experimental data. The log-linear model, the Weibull model, and the biphasic model (Table 1) were fitted to mean counts (log cfu/cm^2) vs. time (h) using the curve fitting toolbox provided by the Excel add-in, GInaFiT (Geeraerd, Valdramidis, & Van Impe, 2005).