Food Microbiology xxx (2014) 1–9

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Modeling number of bacteria per food unit in comparison to bacterial concentration in quantitative risk assessment: Impact on risk estimates

Régis Pouillot*, Yuhuan Chen, Karin Hoelzer

U.S. Food and Drug Administration / Center for Food Safety and Applied Nutrition (HFS-005), 5100 Paint Branch Parkway, College Park, MD 20740, USA

Keywords:
Quantitative microbial risk assessment
Bacterial concentration
Bacterial number
Modeling process pathway
Simulation study

A B S T R A C T

When developing quantitative risk assessment models, a fundamental consideration for risk assessors is to decide whether to evaluate changes in bacterial levels in terms of concentrations or in terms of bacterial numbers. Although modeling bacteria in terms of integer numbers may be regarded as a more intuitive and rigorous choice, modeling bacterial concentrations is more popular as it is generally less mathematically complex. We tested three different modeling approaches in a simulation study. The first approach considered bacterial concentrations; the second considered the number of bacteria in contaminated units, and the third considered the expected number of bacteria in contaminated units. Simulation results indicate that modeling concentrations tends to overestimate risk compared to modeling the number of bacteria. A sensitivity analysis using a regression tree suggests that processes which include drastic scenarios consisting of combinations of large bacterial inactivation followed by large bacterial growth frequently lead to a >10-fold overestimation of the average risk when modeling concentrations as opposed to bacterial numbers. Alternatively, the approach of modeling the expected number of bacteria in positive units generates results similar to the second method and is easier to use, thus potentially representing a promising compromise.

Published by Elsevier Ltd.

1. Introduction

Quantitative microbial risk assessment (QMRA) models typically evaluate the dynamics of bacterial[1] populations in food along a food-production pathway to determine contamination at consumption, which is integrated with a dose-response curve to estimate risk to the consumer. Usually, the production pathway is described by using a number of discrete steps along the "farm-to-fork" continuum, and these steps may be grouped into various distinct modules (Cassin et al., 1998). At each process step, bacteria may be introduced, can increase or decrease, or may be completely eliminated from certain food units. These steps can be modeled as a set of well-characterized basic processes that may impact bacterial prevalence and/or levels. Various different basic processes have been defined in the literature including, for example, growth, inactivation, food partitioning, mixing, removal, and cross-contamination (Nauta, 2008). FDA-iRISK®, a web-based quantitative risk assessment tool, defines a set of slightly different basic processes: increase by growth, increase by addition, decrease, redistribution, food pooling, food partitioning and food evaporation (Chen et al., 2013). Because of the diversity of food production, processing, and handling situations that may occur, there is no standardized way to model these processes, even though some general modeling frameworks have been developed (FDA-iRISK, 2012; Nauta, 2008, 2005).

When modeling these processes, a fundamental consideration for risk assessors is to decide whether to model changes in bacterial levels in terms of concentrations, defined on a continuous scale, usually expressed in cfu/g or in log cfu/g, or in terms of actual bacterial numbers, on a discrete scale, usually expressed in cfu per food unit. A generic distribution of concentrations was for example used as the starting step to characterize prevalence and levels of *Listeria monocytogenes* in 23 ready-to-eat foods in a QMRA conducted jointly by the U.S. Food and Drug Administration and the U.S. Department of Agriculture Food Safety and Inspection Service (FDA/FSIS, 2003). In some published QMRAs risk assessors chose to model changes in bacterial concentration, such as *Escherichia coli* O157:H7 concentrations in spinach (Danyluk and Schaffner, 2011)

* Corresponding author. Tel.: +1 240 402 2123.
E-mail address: Regis.Pouillot@fda.hhs.gov (R. Pouillot).

[1] We used the term "bacteria" throughout this manuscript, but the study is generalizable to other microorganisms of interest.

http://dx.doi.org/10.1016/j.fm.2014.05.008
0740-0020/Published by Elsevier Ltd.

Table 1
Characteristics of the three evaluation methods.

Characteristics		Method #1	Method #2	Method #3
Prevalence (P)	Definition	Prob ($C > 0$ cfu/g)	Prob ($N \geq 1$ cfu/food unit)	Prob ($N \geq 1$ cfu/food unit)
	Evaluation	Deterministic	Deterministic	Deterministic
Contamination	Definition	Concentration (C)	Number per contaminated unit (N)	Expected number per contaminated unit (E[N])
	Unit	$\log_{10}$ cfu/g	cfu in the food unit	cfu in the food unit
	System	Rational	Natural ≥ 1	Rational ≥ 1
	Evaluation	Deterministic	Stochastic	Deterministic
Parameters (*e.g.* log-reduction, growth, redistribution factor, size of unit after partition, size of units after mixing)		Variable	Variable	Variable
Integration of the model		Monte-Carlo	Monte-Carlo	Monte-Carlo

and *Salmonella* concentrations in almonds (Lambertini et al., 2012). In other QMRAs, risk assessors have modeled the number of bacteria per food unit, such as *Salmonella* Enteritidis cells in shell eggs (Schroeder et al., 2006), while in yet other QMRAs both were considered, depending on the basic process being modeled (Rigaux et al., 2014). While modeling integer numbers of bacteria may be a more intuitive choice, modeling bacterial concentrations is generally less mathematically complex. Some recent efforts have strived to describe the way in which contamination is handled mathematically (FDA-iRISK, 2012; Nauta, 2008).

The objective of this study was to evaluate the impact of specific modeling choices on risk estimates and model complexity in a QMRA. Specifically, we evaluated the impact of considering bacterial populations as concentration (per unit of volume or weight of food, e.g. cfu/g or log cfu/g) or as absolute numbers of bacteria per food unit (e.g. per batch, package, can or leaf, e.g. cfu/can).

We report the results of a specific comparison among three different approaches to model changes in bacterial prevalence and levels in contaminated food products. One method tracks the bacterial concentrations; a second method models changes in the number of bacteria in contaminated food units, and a third, novel method, evaluates the expected number of bacteria in contaminated food units. To evaluate the potential impact of the choice in modeling approach on risk estimates, a simulation study was performed, evaluating randomly assembled, but plausible, sets of food pathways which included five distinct process steps.

2. Methods

2.1. Description of the modeling approaches

We derived three distinct modeling approaches, designated henceforth as method #1, #2 and #3.

2.1.1. Method #1

This approach tracks bacterial concentrations in food units. At step j of the modeled food-production pathway, contamination of the food units is characterized by: i) prevalence P_j, defined as the proportion of food units with a mean bacterial concentration >0 cfu/g, and ii) mean bacterial level C_j in the contaminated food units, modeled as some rational number (e.g. −1.23, or 2.5; unit: $\log_{10}$ cfu/g). As an illustrative example, consider food units contaminated at a given step of the pathway with $P_j = 10\%$ and $C_j = -1.22$ $\log_{10}$ cfu/g (i.e., 6 cfu in 100 g). In this case, 90% of the food products have a concentration of exactly 0 cfu/g and the mean concentration in the other 10% units is -1.22 $\log_{10}$ cfu/g. Note that at a relatively low mean concentration, the actual number of bacteria in any given quantity of product may be 0 cfu even though the mean concentration is > 0 cfu/g. In this approach, changes in prevalence and concentration are evaluated deterministically for any of the basic processes, given a specified parameter (see next section and Table 1). As an illustrative example, consider bacterial growth of 3.2 $\log_{10}$ cfu in the food units. This would lead to no change in prevalence and an increase in the bacterial level in contaminated products $C_{j+1} = -1.22 + 3.2 = 1.98$ $\log_{10}$ cfu/g (corresponding to 9509.4 cfu in a 100 g food unit).

2.1.2. Method #2

This approach tracks prevalence and number of bacteria in contaminated food units. The prevalence is the proportion of food units that contains ≥1 cfu. As an illustrative example, consider food units contaminated with prevalence $P_j = 10\%$ and number of bacteria per contaminated unit $n_j \geq 1$, where n_j is a natural number. In this case, 90% of the food units contain exactly 0 cfu while the remaining 10% of food units are contaminated with some natural number of bacteria (e.g., 1, 34, or 514; unit: cfu/food unit). For any given process with a given parameter, the prevalence is evaluated deterministically as the probability, at the end of the process, that the food unit contains ≥ 1 bacteria. The number of bacteria per contaminated unit is evaluated stochastically, using one random draw from a discrete distribution per iteration. As an illustrative example, assume one 100 g food unit contains $n_j = 6$ cfu (i.e., a concentration of -1.22 $\log_{10}$ cfu/g). In this case, bacterial growth of 3.2 $\log_{10}$ cfu would lead to no change in prevalence, while the number of bacteria in the contaminated units would be determined through a random draw from a negative binomial distribution used to characterize growth (See next section), leading for instance to a value of $N_{j+1} = 9822$ cfu in the unit for one iteration. The expected number of bacteria[2] in positive units for this specific process would be 9509.4 cfu for a 100 g unit.

2.1.3. Method #3

The third approach considers the prevalence (same definition as in method #2) and the expected number of bacteria per contaminated unit (i.e., in food units containing ≥ 1 bacteria) at each step of the food pathway. The expected number of bacteria in contaminated units is a rational number ≥1 (e.g., 1.2 or 65.8; unit: cfu/food unit). For any given process defined by a given parameter, the prevalence is evaluated deterministically as the expected probability that the food unit contains ≥1 bacteria. The expected number of bacteria per contaminated unit is evaluated deterministically. As an illustrative example, consider food units contaminated with prevalence $P_j = 10\%$ and $\mathrm{E}[N_j] = 6$ cfu/100 g food unit at step j. Bacterial growth of 3.2 $\log_{10}$ would lead to no change in prevalence and an expected number of bacteria of $\mathrm{E}[N_{j+1}] = 6 \times 10^{3.2} = 9509.4$ cfu in the 100 g contaminated units.

[2] The expected value E[X] of random variable x is the weighted average of all possible values of x weighted by the probability that x assumes it.

Please cite this article in press as: Pouillot, R., et al., Modeling number of bacteria per food unit in comparison to bacterial concentration in quantitative risk assessment: Impact on risk estimates, Food Microbiology (2014), http://dx.doi.org/10.1016/j.fm.2014.05.008