Bayesian meta-analysis to synthesize decay rate constant estimates for common fecal indicator bacteria

Lauren E. Brooks, Katharine G. Field[*]

Oregon State University, Department of Microbiology, 226 Nash Hall, Oregon State University, Corvallis, OR, 97331, USA

ABSTRACT

For decades, fecal indicator bacteria have been used as proxies to quantitatively estimate fecal loading into water bodies. Widely used cultured indicators (e.g. *Escherichia coli* and *Enterococcus* spp.) and more recently developed genetic markers are well studied, but their decay in the environment is still poorly understood. We used Hierarchical Bayesian Linear Modeling to conduct a series of meta-analyses using published decay rate constant estimates, to synthesize findings into pooled estimates and identify gaps in the data preventing reliable estimates. In addition to the meta-analysis assuming all estimates come from the same population, meta-regressions including covariates believed to contribute to decay were fit and used to provided synthesized estimates for specific combinations of significant variables. Additionally, statements regarding the significance of variables across studies were made using the 95% confidence interval for meta-regression coefficients. These models were used to construct a mean decay rate constant estimate as well as credible intervals for the mean and the distribution of all likely data points. While synthesized estimates for each targeted indicator bacteria were developed, the amount of data available varied widely for each target, as did the predictive power of the models as determined by testing with additional data not included in the modeling. Temperature was found to be significant for all selected indicators, while light was found to be significant only for culturable indicators. Results from the models must be interpreted with caution, as they are based only on the data available, which may not be representative of decay in other scenarios.

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1. Introduction

Differences between the persistence of fecal indicator bacteria (FIB) used to detect the presence of fecal contamination and enteric pathogens in water could limit the ability to accurately predict public exposure and health risks. As a result, the public could be exposed to pathogens at higher levels than predicted using these measures, or, conversely, unwarranted precautions such as closures to the public could be implemented. Having an accurate range of plausible values for the decay of FIB in the environment is vital to the success of their use as proxies for assessing contamination.

In addition to cultured *Escherichia coli* and *Enterococcus* spp., genetic markers detected by quantitative Polymerase Chain Reaction (qPCR) are used with increasing frequency. Genetic markers provide advantages over culture-based enumeration, including a shorter period between sample collection and quantification (Dick and Field, 2004; Haugland et al., 2005). Additionally, host specific markers have been identified that are able to distinguish host species of origin for microbial source tracking (Bernhard and Field, 2000). As the use of genetic markers for microbial source tracking increases, it has become important to understand decay of these markers as related to traditional FIB and pathogens as well as to each other for source allocation (Wang et al., 2013).

Previous studies have investigated the decay of FIB in controlled environments (Anderson et al., 2005; Bae and Wuertz, 2009, 2015; Bell et al., 2009; Dick et al., 2010; Green et al., 2011). Additionally, specific environmental conditions, such as sunlight, salinity, temperature and predation, have been studied to determine their effect on decay of FIB and genetic markers (e.g. Bell et al., 2009; Korajkic et al., 2014; Okabe and Shimazu, 2007; Schulz and Childers, 2011; Walters and Field, 2009). While these studies have provided valuable information, major disagreements among both decay rate constant estimates (Fig. 1) and the significance of environmental conditions make it difficult to forecast how FIB will persist in the

* Corresponding author.
E-mail addresses: brooksla@onid.orst.edu (L.E. Brooks), kate.field@oregonstate.edu (K.G. Field).
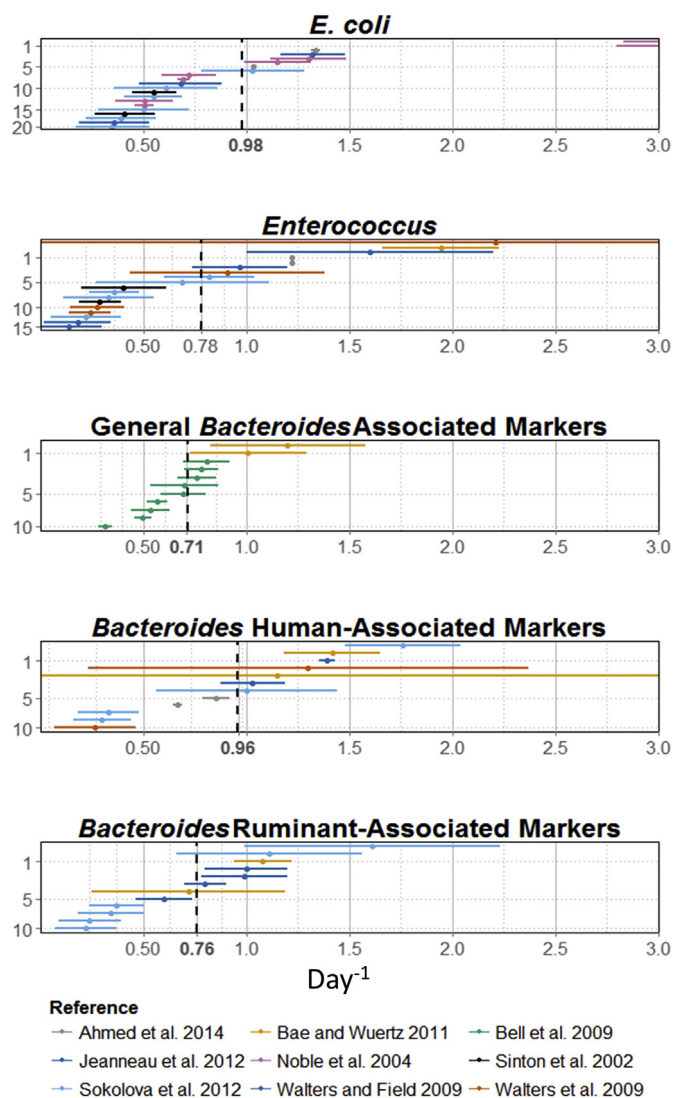
Fig. 1. First order decay rate estimates and standard errors from published papers for selected FIB reveal inconsistency in the literature among decay rate estimates.

environment.

Meta-analysis is a statistical approach for synthesizing prior studies estimating the same parameter (Sutton and Abrams, 2001). Meta-analyses can include defined study characteristics (fixed effects), unexplained variance (random effects), or a combination of the two (mixed effects). Fixed effects meta-analyses assume that studies to be synthesized not only estimate the same parameter, but that all studies are exchangeable as estimates of that parameter. Conversely, random effects models assume no exchangeability between studies. By using a mixed effects model, it is possible to assign variance in the data to predictor variables, leaving remaining variation accounted for by the undefined random effects.

The Bayesian approach to meta-analysis allows for, and can explicitly model, parameter uncertainty. Under the Bayesian paradigm, both the data and the parameters in the model are treated as unknowns with their own distributions. Using Bayes' Theorem, the likelihood function, which defines the plausibility of the data values given the model parameters, is combined with prior estimates and used to construct the posterior credible interval. Unlike Frequentist confidence intervals, direct probability statements about the posterior distribution can be made, allowing for easier interpretation

of the posterior credible interval (Thompson and Higgins, 2002).

This study used Bayesian hierarchical linear models to analyze and synthesize existing decay rate constant estimates for common FIB. We selected FIB targets of significance for water quality monitoring or microbial source tracking for which sufficient data were available for synthesis via meta-analysis. Fixed effects meta-analysis models for the general decay rate constant, excluding predictor variables, provided a synthesized estimate of decay rate constants for the selected indicators of fecal contamination. Mixed effects meta-regression models, including predictor variables provided in the description of each published study, were used to determine which variables included in the model were significant. Synthesized estimates for decay rate constants from combinations of significant variables were also generated. Our objectives were to provide synthesized decay rate constant estimates, improve our understanding of decay rates by determining what variables significantly alter decay rate estimates, and identify gaps in the current data that limit applications of FIB and molecular markers.

2. Methods

2.1. Literature search and data selection

Two classic indicators, cultivable *E. coli* and *Enterococcus* spp., were selected for this analysis, as they have been used for decades and have been the focus of many studies (e.g. Kay et al., 2005; Noble et al., 2004; Sinton et al., 2002). More recently, *Bacteroides* associated markers have become the focus of a number of studies, as both indicators of general contamination, and to distinguish sources of contamination (Ahmed et al., 2014; Green et al., 2011; Jeanneau et al., 2012; Tambalo et al., 2012; Walters and Field, 2009). To ensure enough data points were available, *Bacteroides* associated markers from the same general hosts (e.g. "*Bacteroides* ruminant-associated markers" or "*Bacteroides* human-associated markers") were grouped together for the meta-analyses, although they do not necessarily target the same phylogenetic groups or match the same coverage within these clades (see Table S2 for complete list of primers included).

Data from the literature were compiled for the selected FIB or genetic markers using databases available through the Web of Science citation indexing service. The literature search was conducted using a combination of the search terms "Fecal Indicator Bacteria" or "Microbial Source Tracking" combined with either "persistence", "decay", or "inactivation", and "water". This initial screening returned 411 results (Supplemental Table 1). To assess inclusion for the meta-analysis, titles and abstracts for all papers were reviewed and analyzed to determine if they included original data relevant to the decay of FIB or microbial source tracking markers in water bodies. Following this initial screening, full papers were read and screened for inclusion based on the following criteria:

2.1.1. Experimental design

Selected papers were screened based on several key features of the experimental design. As the purpose of this meta-analysis was to synthesize results for the decay rate constants of FIB as indicators of fecal contamination, we included studies that used fecal material, including sewage influent and effluent as well as raw fecal material, as a spike source and excluded studies that seeded waters using laboratory strains or isolated environmental strains. Additionally, studies that tested persistence under artificial pressures, such as chlorine, were excluded. Finally, the persistence data reported in a study must have been from a natural water body or from a microcosm that was constructed using natural waters. While microcosms that included sediment were included, data collected