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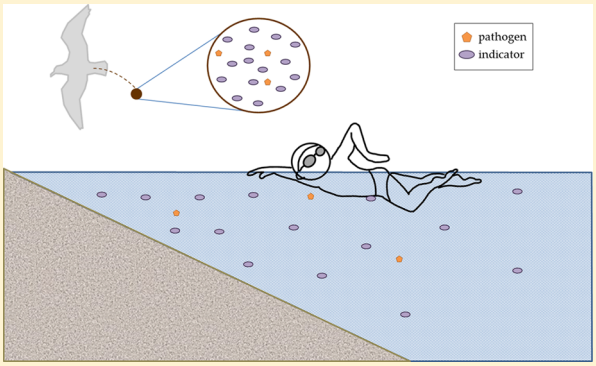
Risk-Based Threshold of Gull-Associated Fecal Marker Concentrations for Recreational Water

Kendra I. Brown, Katherine E. Graham, and Alexandria B. Boehm*

Department of Civil and Environmental Engineering, Stanford University, Stanford, California 94305-4020, United States

Supporting Information

ABSTRACT: A sensitive and specific marker of gull fecal contamination, *Catellicoccus* (CAT), has been used to conduct microbial source tracking in surface waters throughout the world, yet there are no guidelines for interpreting measured concentrations. Here, we use quantitative microbial risk assessment to evaluate CAT concentrations within a risk-based framework and develop a threshold at which the U.S. Environmental Protection Agency illness benchmark (~3 illnesses/100 swimmers) is exceeded. We modeled illness risk from exposure to different concentrations of CAT in bathing waters using a Monte Carlo approach that considered densities of CAT and infectious zoonotic pathogens *Salmonella* and *Campylobacter* in gull feces, volume of water ingested during bathing, and dose−response relationships. We measured CAT densities in 37 fresh gull fecal droppings from six California beaches. Log_{10} densities ranged from 4.6 to 9.8 log_{10} copies CAT/g of wet feces. When the level of CAT exceeds 4×10^6 copies/100 mL of water, the median predicted illness exceeds 3 illnesses/100 swimmers.



INTRODUCTION

Microbial source tracking (MST) has been employed at beaches around the world to determine sources of fecal pollution.[1−6] MST often utilizes molecular assays that target bacterial genes ("MST markers") found in the intestinal microflora of particular animal hosts. Identifying pollution sources is not only key to designing remediation strategies but also useful for gauging the health risks of swimming in recreational water. Feces from different animals may contain different pathogens with varying potentials for infecting humans.[7]

Many beaches host large gull populations, and beach managers often suspect that gulls are to blame for coastal water microbial contamination. Gull feces may contain *Salmonella* and *Campylobacter*,[8] and the presence of these zoonotic bacterial pathogens in coastal waters has been associated with the presence of gulls.[9] A limited number of epidemiological studies has sought to determine whether non-point source fecal contamination from birds is associated with increased risk of swimmer illness.[10,11] These studies found increased risk of mild illness in swimmers compared to nonswimmers in water believed to be contaminated by bird feces. However, establishing a clear link between the presence of animal feces and human illness using an epidemiology study can be difficult because of factors such as low expected rates of illness associated with exposure to zoonotic pathogens.[12]

In response to the need to identify gull-related contamination, several MST markers that are associated with gull feces (gull markers) have been developed.[13−15] Gull markers that target the 16S rRNA gene of *Catellicoccus marimammalium* (CAT)[16−18] have demonstrated sensitivity to (73−96%) and specificity for (86−96%) gull and pigeon feces in laboratory studies.[14,15] CAT has been measured in a variety of surface waters, and maximal concentrations from 10^4 to 10^6 copies/100 mL have been reported.[5,9,18−20] In some settings, CAT concentrations in bathing waters correlate to the presence of gulls along the shoreline.[9,18,19] However, interpreting the measured concentrations remains confusing as there is no threshold for comparison. This represents a major obstacle to the application and interpretation of not only CAT concentrations but also nearly all MST markers that have been developed to date.

This study uses a risk-based approach to establish a threshold value of CAT in coastal waters. A quantitative microbial risk assessment (QMRA)[21] is used to model the risk of illness from exposure to bathing waters contaminated with different levels of CAT. The QMRA utilizes Monte Carlo simulations[22−24] that sample from distributions including CAT concentrations in gull feces, pathogen concentrations in gull feces, and ingested water volumes.

QMRA has been used previously to model illness risk associated with swimming in gull feces-contaminated water,[7,8] but those studies related a traditional fecal indicator for marine

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water, culturable *Enterococcus* (ENT), to modeled risk rather than CAT. The QMRA approach we used has been recommended by the U.S. Environmental Protection Agency (EPA),[25,26] harmonized with an epidemiology study,[23] and applied to model risk from exposure to a range of bathing waters.[8,22,24,27,28]

METHODS

Collection of Feces. Thirty-seven gull (*Larus californicus* and *Larus occidentalis*) fecal samples were collected at six Californian beaches (Figure S1) using methods described in the Supporting Information. After individual fecal samples were weighed, each was added to 200 mL of deionized (DI) water and the water/feces mixture was shaken vigorously to create a slurry. Fecal slurries were filtered within 6 h of collection.

CAT Quantification. Between 10 and 200 mL (depending on turbidity) of the slurries was filtered through polycarbonate 0.4 μm pore size filters (EMD Millipore, Billerica, MA).[15] One filtration blank, consisting of sterile DI water, was filtered every 12 samples. Filters were stored at −80 °C (in a freezer or a cooler on dry ice) until DNA extraction was performed. DNA was extracted from filters using a DNA-EZ ST1 kit (Generite, North Brunswick, NJ), previously shown to have good DNA recovery and limited co-extraction of inhibitors.[29] One filterless extraction blank was processed alongside the sample extractions. Extracted DNA was stored for a maximum of 30 days at −20 °C before analysis.

CAT concentrations were quantified using quantitative polymerase chain reaction (qPCR) following the method described by Lee et al.,[18] with the modification that TaqmanEnvironmental Master Mix 2.0 (Applied Biosystems, Foster City, CA) was used to decrease the possibility of inhibition.[30] This assay was chosen as it was one of the best performing CAT assays in a multilaboratory method evaluation study.[15] Inhibition was tested using the spike-and-dilute method.[30] Information about primer and probe sequences, standard curves, and negative controls are given in the Supporting Information. CAT copies per reaction measured by qPCR were converted to copies per gram of wet feces. The concentrations of CAT in gull feces were $\log_{10}$-transformed, and a probability density function was fitted to the data using MATLAB (Natick, MA).

QMRA. QMRA was conducted to predict the probability of gastrointestinal illness from a single swimming event in recreational water with varying concentrations of CAT from gull feces. Using the concentration of CAT in the recreational water, the model calculates reference pathogen doses, and then the probabilities of infection and illness associated with those doses. MATLAB was used to run Monte Carlo simulations (n = 10000 trials for each CAT concentration). Each trial drew from distributions of the input variables to incorporate their inherent uncertainty and variability. It was assumed that (1) CAT comes from fresh gull feces and (2) only gulls, not pigeons or other animals, are the source of CAT.

Estimating Reference Pathogen Dose. The expected reference pathogen dose, μ_{rp}, from nondietary ingestion of gull-contaminated water was estimated by eq 1:[8]

$$\mu_{rp} = \frac{C_{CAT}}{F_{CAT}} \times R_{rp} p_{rp} V \quad (1)$$

where C_{CAT} is the concentration of CAT in ambient seawater (copies per 100 mL), F_{CAT} is the concentration of CAT in wet gull feces (copies per gram), R_{rp} is the concentration of pathogen species in wet gull feces [colony-forming units (CFU) per gram], p_{rp} is the fraction of human-infectious pathogenic species or serotypes in gull feces,[8] and V is the volume of seawater ingested (milliliters).

μ_{rp} was calculated for two reference pathogens, *Campylobacter* and *Salmonella*.[7] For each discrete order-of-magnitude value of C_{CAT} ranging from 10^3 to 10^7 copies/100 mL, a distribution of μ_{rp} was generated with Monte Carlo trials by drawing values for parameters in eq 1 (Table 1).

Table 1. Variable Distributions Used in Monte Carlo Simulations To Calculate the Reference Pathogen Dose, μ_{rp}, from Incidental Ingestion of Seawater[a]

variable	units	distribution parameters	ref
density of CAT in gull feces (F_{CAT})	copies/g of wet feces	A = 8.73, B = 8.26	this study
density of *Salmonella* in gull feces (R_S)	CFU/g of wet feces	a = 2.3, b = 9.0	41
density of *Campylobacter* in gull feces (R_C)	CFU/g of wet feces	a = 3.3, b = 6.0	41
human-infectious fraction of pathogen strains (p)	–	c = 0.01, d = 0.4	8, 42
volume of water ingested	mL	C = 2.92, D = 1.43	40

[a] A and B are the scale and shape parameters, respectively, of a Weibull distribution fit to $\log_{10}$-transformed F_{CAT} data. a and b are the upper and lower bounds, respectively, of a $\log_{10}$-uniform distribution for R_S and R_C. c and d are the upper and lower bounds, respectively, of a uniform distribution for p. C and D are the ln-mean and standard deviation, respectively, of a natural-log normal distribution for V. The medians of the distributions (as defined in the table) are as follows: 8.35 for F_{CAT}, 5.6 for R_S, 4.6 for R_C, 0.2 for p, and 2.92 for V.

The ratio $f = C_{CAT}/F_{CAT}$ represents the amount of gull feces present per volume of ambient water (gram of feces per 100 mL). An upper constraint of f = 10 g of feces/100 mL of seawater was applied as an upper limit, as this amount of contamination is extreme (~10% by mass). If during any particular trial the draw from the F_{CAT} distribution was low enough to result in a violation of that constraint, then a new value was drawn from the F_{CAT} distribution until f was <10 g/100 mL. The number of times F_{CAT} was redrawn per value of C_{CAT} is shown in Table S3.

Estimating the Probability of Illness. The probability of illness for one reference pathogen, $P_{ill,rp}$, as a function of μ_{rp} was calculated following the method described by Teunis et al.[31] This method estimates $P_{ill,rp}(\mu_{rp})$ with a series of two dose–response functions: the first estimates the probability of infection from one reference pathogen, $P_{inf,rp}$, and the second estimates the probability of illness given infection for one reference pathogen, $P_{illinf,rp}$. The choice of dose–response relationships is discussed in the Supporting Information.

Teunis et al.[31] and Teunis et al.[32] used pooled data from campylobacterosis and salmonellosis outbreaks, respectively, to develop hypergeometric $P_{inf,rp}$ dose–response relations. The hypergeometric equations arise from integrating over a distribution of ingested doses, as is necessary when only the mean dose ingested by a population is estimated or known. The corresponding conditional dose–response relationship that applies to cases, such as QMRA, in which the exact dose is calculated is given by eq 2:[33,34]

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Table 2. Dose−Response Parameters Used To Calculate $P_{inf,rp}$ and $P_{illinf,rp}$ for *Salmonella* and *Campylobacter*[a]

pathogen	α	β	η	ρ	ref
Salmonella	8.53×10^{-3}	3.14	69.0	8.23	32
Campylobacter	2.4×10^{-2}	1.1×10^{-2}	3.6×10^{-9}	2.4×10^{8}	31

[a] α and β are parameters for beta-distributed mean host sensitivities (eq 2), and η and ρ are parameters describing the distribution of the duration of infection (eq 3).

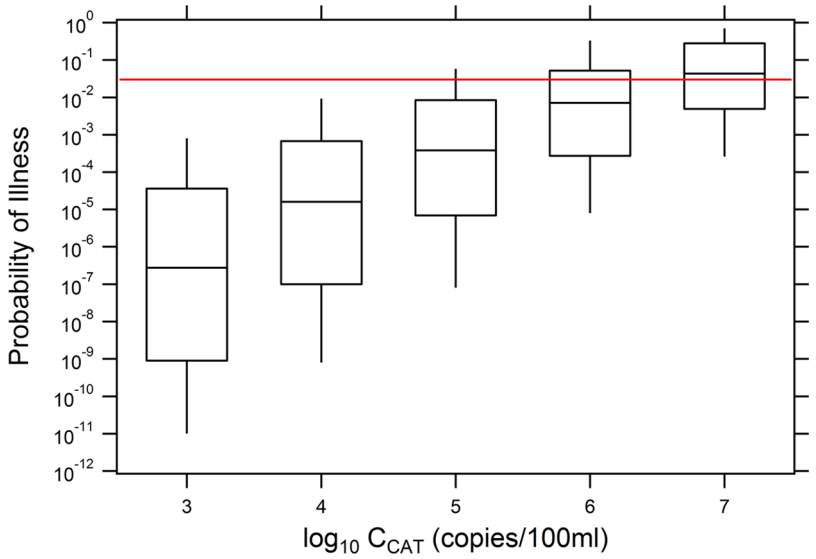

Figure 1. Probability of illness, P_{ill}, predicted when different concentrations of the gull marker, C_{CAT}, are present in ambient water. The midline of each box represents the median; the bottom and top of each box represent the first and third quartiles, respectively, and the bottom and top whiskers represent the 10th and 90th percentiles, respectively. The red line indicates the threshold of 3 cases of illness/100 swimmers.

$$P_{inf,rp}(\mu_{rp}) = 1 - \frac{B(\alpha, \beta + \mu_{rp})}{B(\alpha, \beta)} \quad (2)$$

where B is the standard beta function and α and β are parameters for beta-distributed mean host sensitivities.[33] The second dose−response function, for $P_{illinf,rp}$, is given by eq 3:

$$P_{illinf,rp}(\mu_{rp}) = 1 - (1 + \eta\mu_{rp})^{-\rho} \quad (3)$$

where η and ρ are parameters describing the distribution of the duration of infection.[32] The model parameters α, β, η, and ρ for *Salmonella*[32] and *Campylobacter*[8,31] are listed in Table 2. It is assumed that all exposed hosts are susceptible to illness.

The probability of illness for each reference pathogen is then calculated as $P_{ill,rp} = P_{inf,rp}P_{illinf,rp}$. Finally, the total probability of illness due to the presence of either pathogen, P_{ill}, is calculated using eq 4.[7] It is assumed that hosts are infected with only one pathogen at a time.

$$P_{ill} = 1 - \prod_{rp} (1 - P_{ill,rp}) \quad (4)$$

The final results of the Monte Carlo simulations were P_{ill} distributions. Distributions were compared to a threshold of 3 illnesses per 100 swimmers, the approximate illness threshold recommended by the EPA.[26]

Sensitivity Analysis. Sensitivity analyses were conducted following the method of Xue et al.[35] to test the effects of changing individual variables on P_{ill} (see the Supporting Information).

RESULTS AND DISCUSSION

Concentration Distributions. All positive and negative controls for the CAT assay resulted as expected. No PCR inhibition was observed. CAT concentrations in gull feces ranged from 10^2 and 10^{10} copies/g, with most concentrations between 10^8 and 10^9 copies/g (Table S4 and Figure S2). Data are described by a Weibull distribution with scale and shape parameters with 95% confidence intervals of 8.73 ± 0.180 for *a* and 8.26 ± 1.12 for *b*.

Probability of Illness. P_{ill} increases with C_{CAT} (Figure 1). There is a linear relationship between the $\log_{10}$-transformed median probability of illness and the $\log_{10}$-transformed CAT concentration: $\log_{10}$ median $P_{ill} = -10.2 + 1.3 \times \log_{10} C_{CAT}$, and $R^2 = 0.98$ (Figure S3).[36] On the basis of this regression, median P_{ill} equals 0.03 when $C_{CAT} = 4 \times 10^6$ copies/100 mL. For a C_{CAT} of 6×10^5 copies/100 mL and a C_{CAT} of 2×10^7 copies/100 mL, the 75th and 25th percentiles of the P_{ill} distribution are 0.03 (see the Supporting Information).

The relative contributions of *Campylobacter* and *Salmonella* to P_{ill} vary depending on C_{CAT} (Figure S4). For a C_{CAT} of 10^6–10^7 copies/100 mL, the probability of illness due to *Campylobacter* ($P_{ill,C}$) is greater than the probability of illness due to *Salmonella* ($P_{ill,S}$) by nearly an order of magnitude. CAT is a novel alternative indicator, so data on environmental concentrations are limited. A mean ambient CAT concentration as high as 2.8×10^6 copies/100 mL has been reported for a Lake Ontario beach with high observed gull impact.[19] At a Lake Erie beach, a maximal CAT concentration of 5.5×10^6 copies/100 mL was measured.[18] On the basis of the results of this study, at those concentrations, illness rates might exceed the threshold of 0.03.

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In a previous QMRA that considered gull fecal contamination, Schoen and Ashbolt[8] estimated P_{ill} from exposure to a seawater concentration of 35 ENT colony-forming units (CFU)/100 mL from a gull fecal source. The authors found the risk to adult swimmers from gull feces at that concentration ($\sim 10^{-4.5}$) is substantially less than a risk threshold of 0.01. Because we expect gull feces to contain 10−100 copies of CAT per CFU ENT,[14] a concentration of 35 ENT CFU/100 mL from gulls would correspond to a concentration of 350−3500 copies of CAT/100 mL. For that CAT concentration range, this study predicts a probability of illness much less than the threshold (at most $\sim 10^{-6}$), consistent with the previous results.

A previous study[36] estimated a risk-based threshold for human-specific fecal markers. They found a median risk of 0.03 when HF183 concentrations were $\sim 10^3$ copies/100 mL. The HF183 threshold is 3 orders of magnitude smaller than that CAT threshold, a a direct result of the diverging concentrations of MST markers and pathogens in human versus gull feces.

Sensitivity Analysis. The sensitivity analysis indicated the model is most sensitive to F_{CAT}, R_C, and V at concentrations of C_{CAT} near the threshold value of 10^6 copies/100 mL. P_{ill} estimates, therefore, could be improved by reducing uncertainty in the distributions of those variables. Because to date there have been few studies that characterize the F_{CAT} distribution, additional measurements would be particularly valuable. In contrast, although there is considerable uncertainty in p_{rp},[8] additional research to reduce that uncertainty is unlikely to improve estimates of P_{ill} in this model.

Study Limitations. An important consideration in estimating P_{ill} is the age of the gull feces, that is, the elapsed time between feces deposition and exposure. This QMRA study specifically estimates the risk from exposure to unaged gull feces deposited in recreational water. The concentrations of both CAT and pathogens will decay over time in environmental matrices,[37] and not necessarily at the same rates. The differential decay of CAT and pathogens therefore remains an important area for future research.

An additional consideration is that CAT has been detected not only in gull feces but also in pigeon feces,[14,15] and it may be present in other birds, as well.[13] Pigeon feces contain the same reference pathogens[25] as gull feces: *Campylobacter*[38] and *Salmonella*.[39] However, the concentrations and fractions of infective species may differ among bird feces, resulting in a different prediction of P_{ill}. Further limitations of using the dose−response models and the estimates for V Dufour et al.[40] are described in the Supporting Information.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.6b00473.

Additional information about methods as well as tables and figures (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: aboehm@stanford.edu. Phone: (650) 724-9128. Fax: (650) 725-3164.

ORCID

Alexandria B. Boehm: 0000-0002-8162-5090

Notes

The authors declare no competing financial interest.

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