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Quantitative risk analysis for potentially resistant *E. coli* in surface waters caused by antibiotic use in agricultural systems

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Antibiotics are frequently used in agricultural systems to promote livestock health and to control bacterial contaminants. Given the upsurge of the resistant fecal indicator bacteria (FIB) in the surface waters, a novel statistical method namely, microbial risk assessment (MRA) was performed, to evaluate the probability of infection by resistant FIB on populations exposed to recreational waters. Diarrheagenic *Escherichia coli*, except *E. coli* O157:H7, were selected for their prevalence in aquatic ecosystem. A comparative study between a typical *E. coli* pathway and a case scenario aggravated by antibiotic use has been performed via Crystal Ball® software in an effort to analyze a set of available inputs provided by the US institutions including *E. coli* concentrations in US Great Lakes through using random sampling and probability distributions. Results from forecasting a possible worst-case scenario dose-response, accounted for an approximate 50% chance for 20% of the exposed human populations to be infected by recreational water in the U.S. However, in a typical scenario, there is a 50% chance of infection for only 1% of the exposed human populations. The uncertain variable, *E. coli* concentration accounted for approximately 92.1% in a typical scenario as the major contributing factor of the dose-response model. Resistant FIB in recreational waters that are exacerbated by a low dose of antibiotic pollutants would increase the adverse health effects in exposed human populations by 10 fold.

Keywords: Agricultural system, antibiotic-resistance, risk analysis, FIB, *Escherichia coli*, probability of infection, surface waters

Introduction

Microbial contaminants have been of particular concern to bioethanol producers and farmers. The issue has led investors to use antibiotics in an effort to suppress potential bacterial contaminants and to promote livestock growth.[1] However, in 2008, the FDA detected antibiotic residues in 53% of 60 samples from dried distillers grains with solubles (DDGS) products collected from biofuel distilleries in the U.S. These samples included mostly erythromycin (27%), as well as virginiamycin (33%) and tylosin (11%), with some of these exceeding the concentration of 0.5 ppm.[2] This incidence has raised concern over the disproportionate use of antibiotics in the biofuel system, and created the need to set preventive and strict measures to protect public health.[2] Animals fed from DGs, including primarily beef cattle (40% of the feed ratio), as well as swine and poultry, carry in their gastrointestinal tract (GIT) commensal bacteria, some of which are fecal indicator bacteria (FIB), namely *E. faecium* and *E.coli*.

FIB could develop antibiotic resistance in the GIT,[3,4] and hence distribute it to the environment via the fecal route (i.e., manure or sewage) to surface or ground waters through agricultural runoff over a large area and long distances. *E. coli* has been shown to exhibit resistance to multiple antibiotics, including primarily, ampicillin, as well as amoxicillin with moderate resistance to third-generation cephalosporins.[5] Several studies have revealed resistance mediation via resistant gene acquisition (i.e., plasmid-encoded β-lactamase, SHV-1 or TEM1) or down regulation of the intrinsic wall porins (OmpF).[6]

In addition to its natural ability to acquire genes via a conjugation mechanism, *E. coli* was shown to easily undergo transformation that occurs from an uptake of a readily available extracellular DNA fragment in the environment or also through transduction, which is the case for *E. coli* K-12.[7] Courvalin [8] has reported that *E. coli* strains are able to transfer their genetic materials to other bacteria, and hence disseminate them easily in addition to their ability to acquire foreign DNA fragments from other bacteria or

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from the environment. It has been demonstrated that *E. coli* can transmit its genetic material to a wide range of microorganisms, namely, *Alcaligenes eutrophus* and *Enterococcus faecalis,* along with *Listeria monocytogenes*, *Staphylococcus aureus* and a considerable list of other microorganisms, including *Citrobacter freundii, Bacillus stearothermophilus* and *Streptococcus* spp. Thus far, Bailey et al.[9] have revealed that commensal *E. coli* could form a considerable reservoir for an extensive combination level of antibiotic resistance genes. Later studies by Gullberg et al.[10] have demonstrated that a low or sublethal dose of antibiotics will have to be considered, given the potential of generating antibiotic resistance.

The USDA Agricultural Research Service's National Animal Centers in Iowa and has reported that *E. coli* level that is initially around 10^9 CFU/g (10^{12} CFU/L) within the animal GIT [11] would undergo a drastic increase of *E. coli* by 20 to 100 fold in swine GIT exposed to antibiotic treatment or feed.[12] Furthermore, recent research studies implemented by the Proceedings of National Academy of Science (PNAS) have demonstrated a considerable increase of *Proteobacteria* in the medicated swine feces ranging from 1 to 11%.[13] Hamelin et al.[14] have reported the detection of a high level of pathogenic *E. coli* (29%) along with 8% unusual virulent ones in addition to 14% antibiotic resistance isolated from Great Lake beaches in the U.S.

Consequently, risk analysis has received more focus as a possible approach that would help antibiotic users to set preventive measures to ensure public health safety through assessing the probability of infection and severity of the disease on exposed human populations. This scientific-based method would offer a pragmatic insight to managers for taking effective actions. Currently, risk assessment is among the most promising scientific-based solutions upon which legislators rely to describe risk estimates from chemical or microbial contaminants in food products worldwide.[15–17] Generally, MRA method includes four major steps [18–20] namely, hazard identification followed by hazard characterization (dose-response assessment), exposure assessment and risk characterization (Fig. 1). MRA was also further developed to cope with the international standardizations.[8,15,16]

Hazard identification is the process that involves the collection and organization of data to further identify and evaluate the target pathogens responsible of the adverse health effects.[19] Identification of microbial pathogens is followed by hazard characterization, which involves mainly the correlation between target pathogens and the health adverse effects via response-dose assessment. It measures the microbial dose ingested that can cause a detectable harmful effects and its severity within the host.[20] Exposure assessment provides a qualitative and a quantitative estimation of foodborne intake from farm-to-consumer.[15,16] Once combined, data including response-dose and dose assessments associated with uncertainties provide a qualitative and quantitative risk estimate and final characterization (Fig. 1).[18,19]

The objective of this research study was to elucidate the potential outcomes of exposure to resistant FIB in surface waters originating from agricultural waste. It provides an assumption that antibiotic resistant *E. coli* from animals could potentially spread to surface waters that would then drain to the recreational US Great Lakes. In addition, this study compares two models: a typical pathway of *E. coli* flowing through an unmedicated agricultural zone, and a worst-case scenario pathway of *E. coli* flowing through an agricultural zone, intensified by antibiotic resistant *E. coli* and, the environment in this zone having adverse weather conditions. The specific focus of this study was to statistically estimate the probability of infection on exposed populations by resistant *E. coli*. A hypothetical MRA modeling was addressed to evaluate exposure assessment through a quantitative approach based on random available inputs and probability functions in an attempt to assess the adverse health effects from a disproportionate use of antibiotics in farm animals and adjacent industries.

Materials and methods

Hazard identification

Hazard characteristics, habitats and transmission route. In 2002, the EPA recommended that *E. coli* become the recreational fresh water indicator. Originating from animals or human intestinal tracts, *E. coli* can be spread in the manure on land. Generally, *E. coli* can be transmitted to humans via the faecal-oral route through consuming food or drinking water. Based on the available data in USDA-FSIS Microbial Laboratory Guidebook, *E. coli* comprises over 700 serotypes recognized through O, K and H antigens. The most prevalent pathogenic *E. coli* includes primarily, enterohaemorrhagic (*E. coli* O157: H 7 or EHEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), as well as enteroaggregative (EAEC) and enetroinvasive (EIEC). While most of enterovirulent *E. coli* are infective at a high dose with minimal infective doses of approximately 10^8 to 10^9 CFU/mL and 10^6 CFU/mL for ETEC and EPEC, respectively, EHEC requires only a dose of as few as 10 cells to cause infection as described by EPA, 2002.[21] However, parameters that have been selected for this risk assessment were related to most strains of enterovirulent *E. coli* except EHEC for their prevalence in surface waters and high potential to acquire resistance gene as described extensively by many agencies mainly by the EPA.

Outcomes and available detection methods. Typically based on USDA-FSIS and EPA guidebooks, the most common disease caused by pathogenic *E. coli* is gastroenteritis accompanied by a severe watery diarrhea through either toxin production or epithelial tissue damages. The very young children and elderly are the most sensitive population to pathogenic *E. coli*. Several detection methods have been

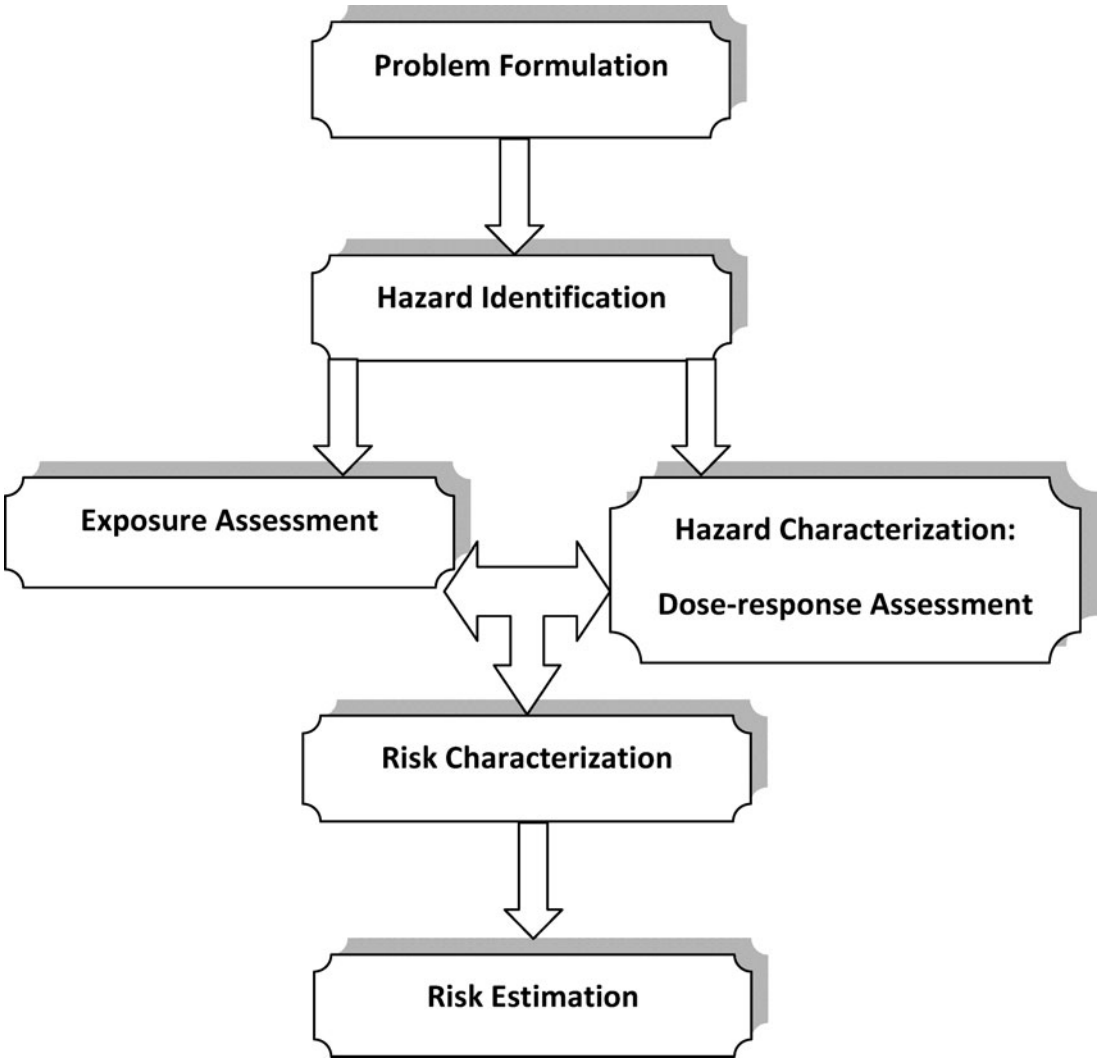

Fig. 1. General QMRA framework based on the classical concept.

established by the national agencies and institutions including primarily, Great Lakes Water Institute along with The Indiana Department of Environmental Management. The appropriate methods were tightly correlated to the laboratories' economics and possible investment. Generally, conventional standard methods along with serotyping and biochemical tests were often used in addition to molecular techniques involving primarily, real time polymerase chain reaction (RT-PCR). Pulsed field gel electrophoresis (PFGE) is the method of choice for the Center of Disease Control and Prevention (CDC).[22]

Statistical methods

Monte-Carlo simulation via probability distribution functions. The QMRA computational technique required essentially the establishment of a deterministic, probabilistic model (without point estimate) subsequent to a stochastic model. Once this model was established, the simulation was performed by Monte Carlo Method (MCM) using a software tool, namely, Crystal Ball® add-in to Microsoft Excel™ 2010 (Oracle Crystal Ball, Fusion Edition; Redmond, WA, USA) in an effort to analyze and quantify parameter uncertainties that entered the model. The construction of the deterministic model subsequent to a stochastic model involved one or more parameters called uncertain variables in the MCM framework. The Crystal Ball tool was used for its ability to select randomly from a set of inputs from several probability distributions, as shown in Table 1. This allows for obtaining output-values, as well as to evaluate multiple deterministic models and elucidate the uncertain variables through an extensive number of iterations (i.e., 10^4 trials). The uncertain variables that were selected for this model are the volume of water ingested from the source namely, recreational water and the *E. coli* concentration in the water source. A set of a standard normal probability distribution fitting each model was performed, including two log-normal distributions, as shown in Table 1.

Table 1. Probability distribution assignment to uncertain variables.

Parameters	*Distribution*	*Units*	
Resistant-*E. coli* in surface water			
Recreational pathway			
Volume ingested	Log Normal	Liters/cap/day	
Mean	0.085		
Std. dev.	0.101		[20]
Typical *E. coli* CONC in lakes	Log Normal	CFU/liter	[14]
(37% pathogens)	$3.30*10^5$	Assumption:	[23]a
Mean Std. dev.	$2.92*10^7$	(100-fold increase for the worst-case scenario)	[21]

[a]Dufour:[23] The ingested volume of water distribution was determined based on the outcomes for adults intake of 16 mL/ 45 min in recreational water.
Std. dev.: Standard deviation.

The general equation for the probability density function (PDF) $f(x)$ is described in Eq. (1)[24]

$$f(x;\mu,\sigma^2)=\frac{1}{\sigma\sqrt{2\pi}}e^{-\frac{(x-\mu)^2}{2\sigma^2}} \quad (1)$$

Typically, when the random variable x is within some range A and B, $f_{(x)}$ will become a non negative integrable function, as follows:

$$P[A<x<B]=\int_a^B f(x)dx,$$

where μ is the mean (location parameter) and σ is the standard deviation (the scale parameter) that defines the particular normal (or Gaussian) distribution. The horizontal axis gives the values of the uncertain variables and the vertical axis is the probability that the values of an uncertain variable will occur. Figure 2 is the plot of the continuous log normal distribution for both random variables, volume of water ingested and the 37% pathogenic *E. coli* concentrations picked randomly in water surfaces adjacent to Great Lake beaches (37% of *E. coli* are pathogenic in Great Lakes according to Hamelin et al.[14] The ingested volume of water distribution was determined based on the outcomes for adults intake in recreational water as described in Table 1.

In addition to the PDF, the cumulative density function (CDF) has been also used to convey a greater communication of the results including the continuous random variable and is expressed as in Eq. (2)[24]

$$F(x)=\int_{-\infty}^{x} f(t)\,dt. \quad (2)$$

Although CDF function is not ranging between two values, it provides the probability (P) of a value that are less than the all displayed values in x-axis (Fig. 3). and can still determine the range from A to B as follows: $P[A<x<B]=F(B)-F(A)$. While, the PDF only shows the probability of an exact value on the horizontal-axis.

The uncertain variables, termed assumption cells in Crystal Ball ® software, are assigned a probability distribution. These different distributions are subsequent to the determination of the forecast called deterministic model in Crystal Ball ® software. It provides the dose-response outputs via MCM attributed to the best-fit equation, which is beta-Poisson in this case. This equation is presented in Table 2. N50 is the dose at which approximately half the population (50%) is infected, and d presents the average dose administrated to the population. In this case, the median infectious dose (N50) as well as alpha parameter (the slope parameter of the equation) was selected based on Haas[20] model adapted to non-enterohaemorrhagic *E. coli.*

Exposure assessment

A schematic diagram illustrating the dynamic integration of the conceptual modules of bacterial hazard pathway in the modern agricultural system is shown in Fig. 4. It includes two different scenarios including the typical fluctuating level of *E. coli* in an unmedicated zone and the worst-case scenario that involves a medicated system that would generate antibiotic resistance in the animal GIT prior to its spread in water surfaces.

The hypothetical route exposure of the model from the source, surface water-to-consumption (i.e., lakes, rivers), is

Table 2. Best fit dose-response model adapted to non-enterohaemorrhagic strains of *E. coli*.

Dose-response model	*beta-Poisson*	
Equation	$p(\text{response})=1-\left(\left[1+\text{dose}\frac{(2^{\frac{1}{\alpha}}-1)}{N_{50}}\right]\right)^{-\alpha}$	[20]
Alpha (α)	0.175	
D	d1 × d2[b]	
N50	$2.55\ 10^6$	

[a]P(d) is the probability of illness or infection; d is the average dose administrated to population; α is the slope parameter of the equation adopted from Haas.[32]
[b]d1: Ingested volume of water; d2: Hazard concentration in the source (i.e., surface waters).

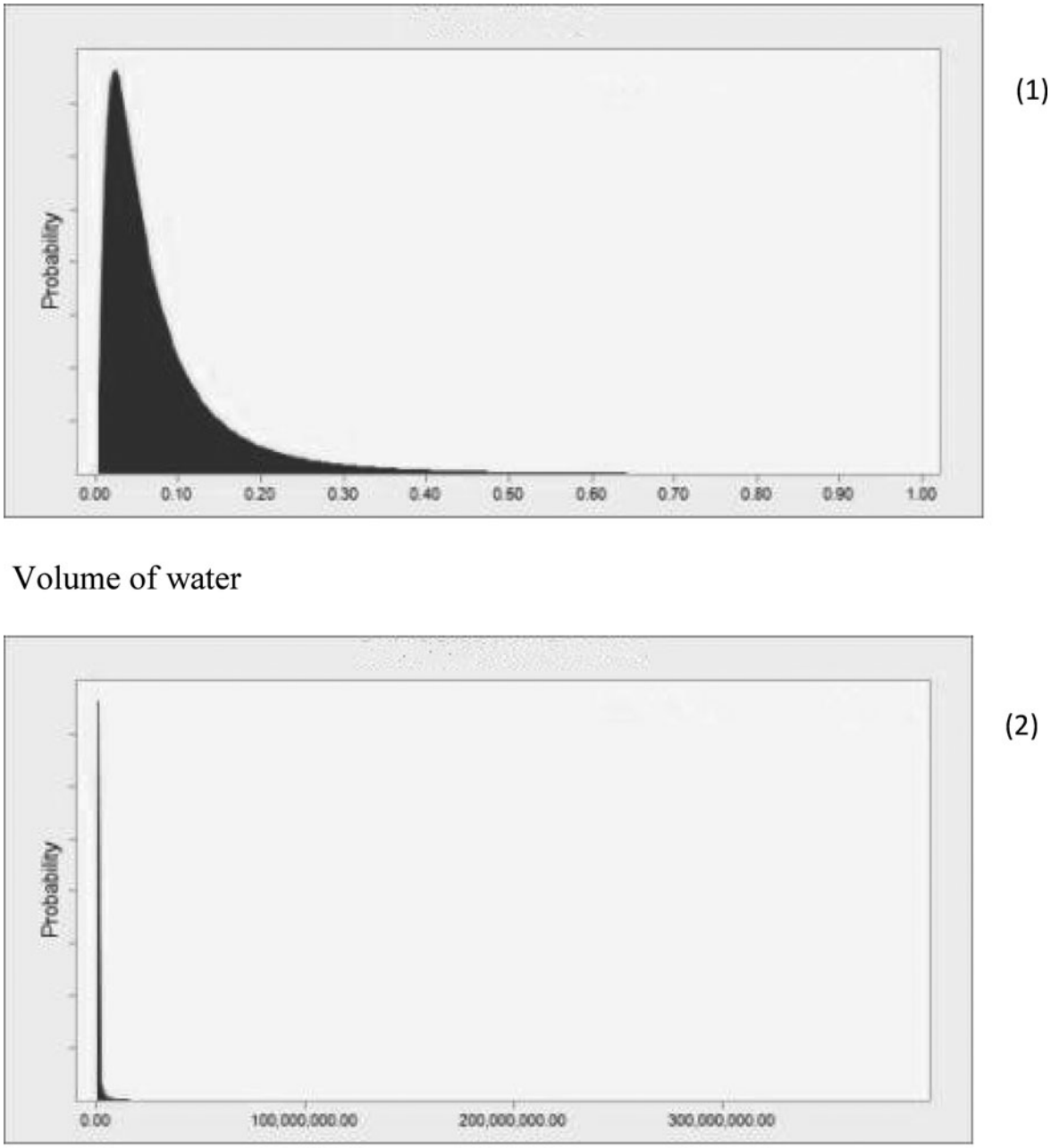

Fig. 2. The uncertain variable lognormal distribution; (1) volume of water ingested; (2) typical *E. coli* concentration in recreational lakes.

under the scope of this risk assessment, and is shown in Fig. 4. Although *E. coli* concentrations in water surfaces undergo a dramatic fluctuations that depend on several variables including primarily the location (i.e., beaches, north or south shorelines. weather and sanitation coverage), the likelihood level of *E. coli* that is typically detected in the shoreline adjacent to beaches along with creeks and rivers is mostly in the range of 10^3 to 10^7CFU/L and is shown in Table 3.

Results and discussion

Risk characterization

The probability of infection by antibiotic resistant *E. coli* is estimated through the outputs resulting from the combination and integration of all MRA components (i.e., hazard identification, exposure modules and dose-response assessment) that can be evaluated from different scales or units

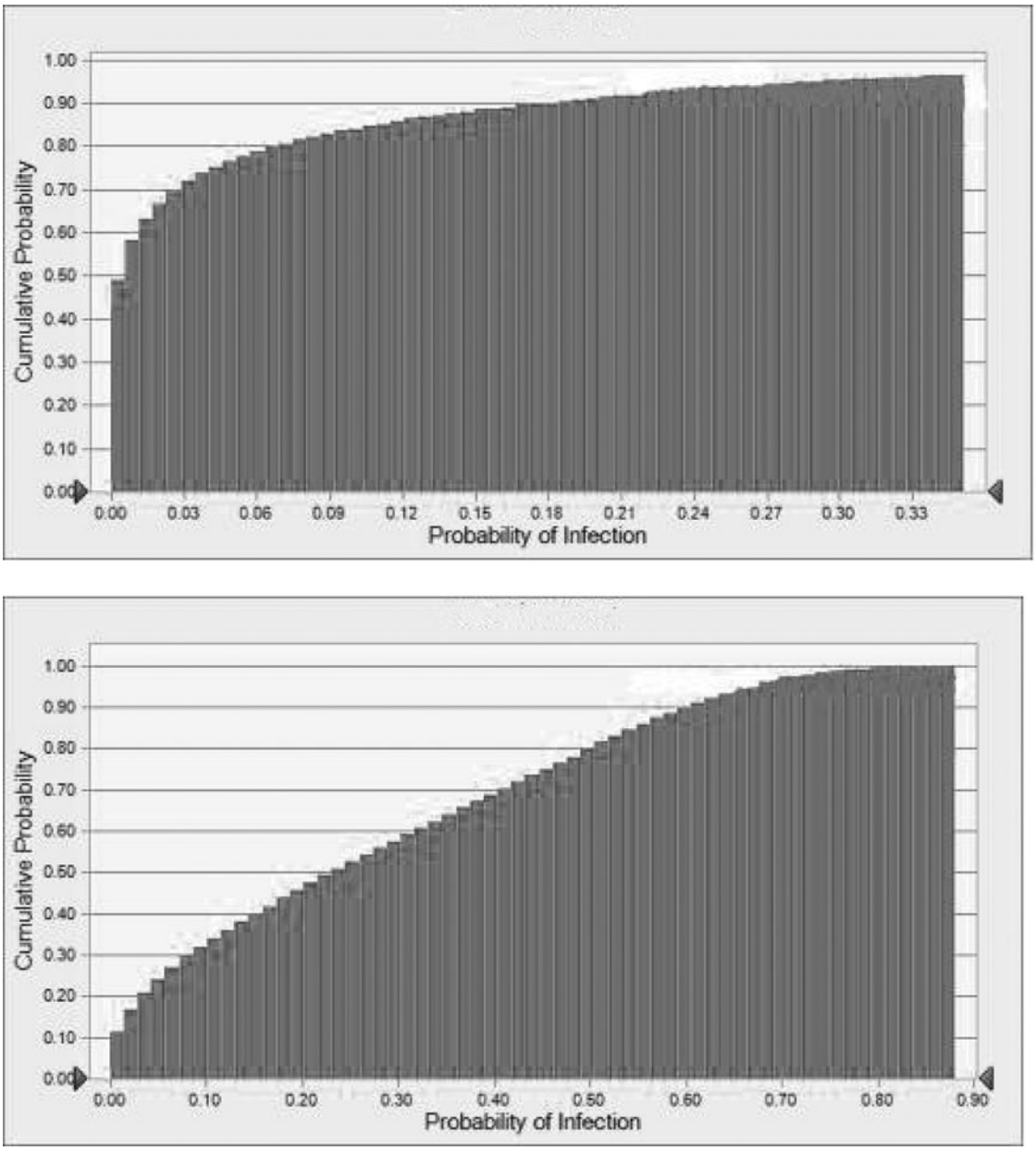

Fig. 3. Dose response simulation outputs of probability distribution via cumulative function; (1) unmedicated water surfaces; (2) the worst-case scenario.

(i.e., days, years). The outcomes from this research study cover possible adverse health effects evaluations from a typical and the worst-case scenario as well as the sensitivity and the scatter chart of the forecast models (dose or dose-response) to the uncertain variables (Figs. 3, 5 and 6). Detailed key findings, as well as data gaps and possible solutions with intervention scenarios, are also addressed in this risk estimation.

This study assumed a human population exposure for 4 h/ day on average. Therefore, the mean become 0.085 and the related standard deviation 0.101, $f(x, \mu = 0.085, \sigma = 0.101)$. However, the lognormal distribution associated to the pathogenic *E. coli* fluctuating concentrations in lake beaches was determined through a series of data chosen randomly by Crystal ball software based on Table 1 available data to fit a continuous probability distribution. The best fit for this random variable was the log normal $X = \log f(x)$ ranked by goodness-of-fit statistic, Kolmogorov–Smirnov (K-S) and using the empirical cumulative distribution formula of the numerical data. These results were fitted to a mean of 3.3×10^5 and a standard deviation of $\sigma = 2.\ 92 \times 10^7$ for atypical scenario, $f'(x, \mu = 3.3 \times 10^5\ 2.92 \times 10^7)$ and $f'(x, \mu = 3.3 \times 10^7\ 2.92 \times 10^9)$ for the worst-case scenario, respectively.

Table 3. The most likely range of *E. coli* level that is located in the areas adjacent to the beaches of the US recreational Great Lakes.

Major recreational lakes in the U.S.	E. coli *level detected*	
Lake Michigan (i.e., Wisconsin harbor)	From 10^3 up to 2.7×10^5 CFU/L	[25]
Indiana Lake	From 2 CFU/L to 8×10^6 CFU/L	[26]
Lake Erie (i.e., Ohio)	From 6500 to 7.1×10^5CFU/L in east end of the beach	[27]
Lake Huron	up to 1.6×10^7 CFU/L	[28,29]
Lake Ontario (i.e., Hamilton)	up to 1.14×10^5 CFU/g dry sand in wet foreshore	[30]
Milwaukee Harbor	Average E. coli 8.16×10^5CFU/L	[31]
	Range was $<10^3$ to 3.9×10^6CFU/L	[25]

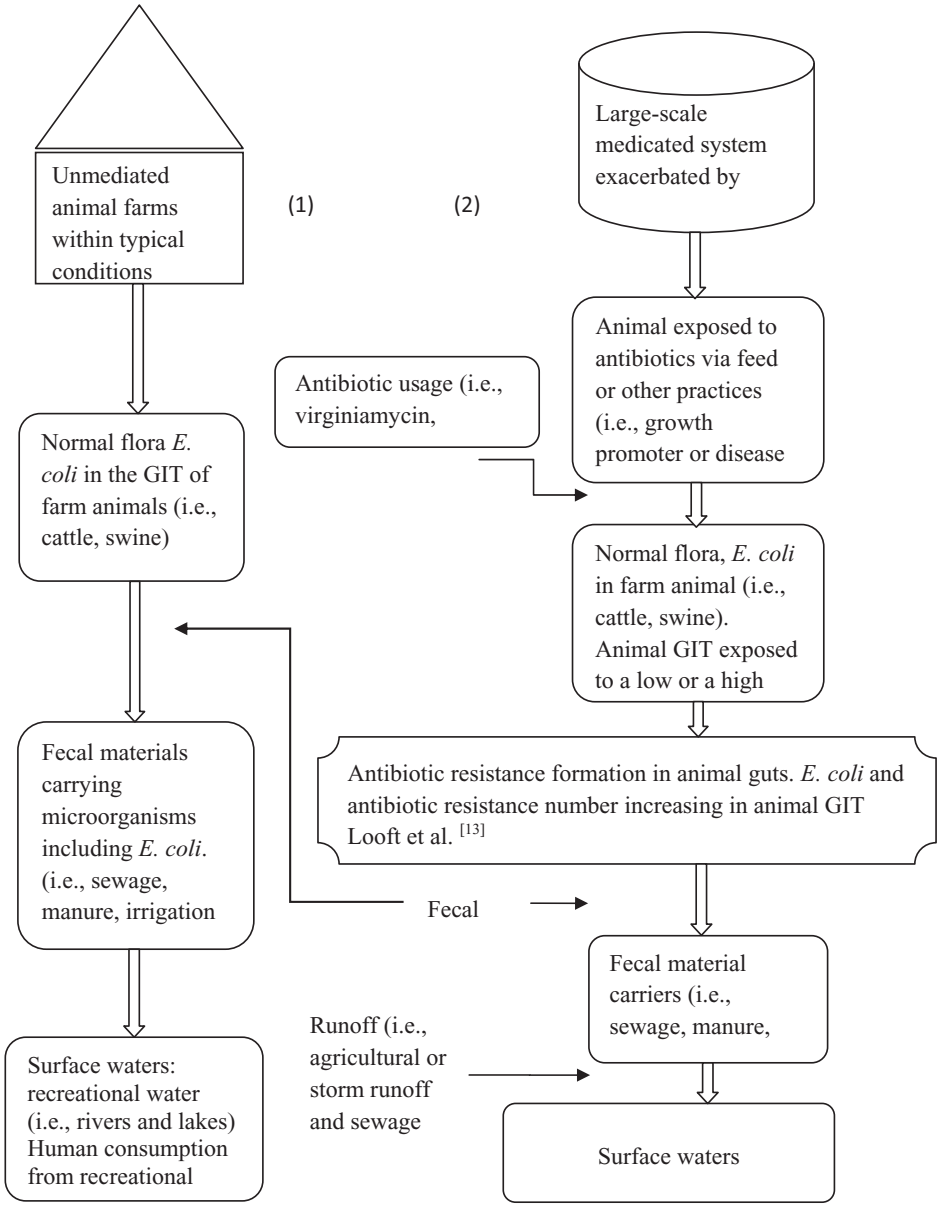

Fig. 4. Hypothetical model comparaison from harvest-to-consumption dynamic flow: (1) unmedicated farm animals; (2) worst-case scenario: farm animals exposed to antibiotic usage and aggravated by runoffs and sewage overflows.

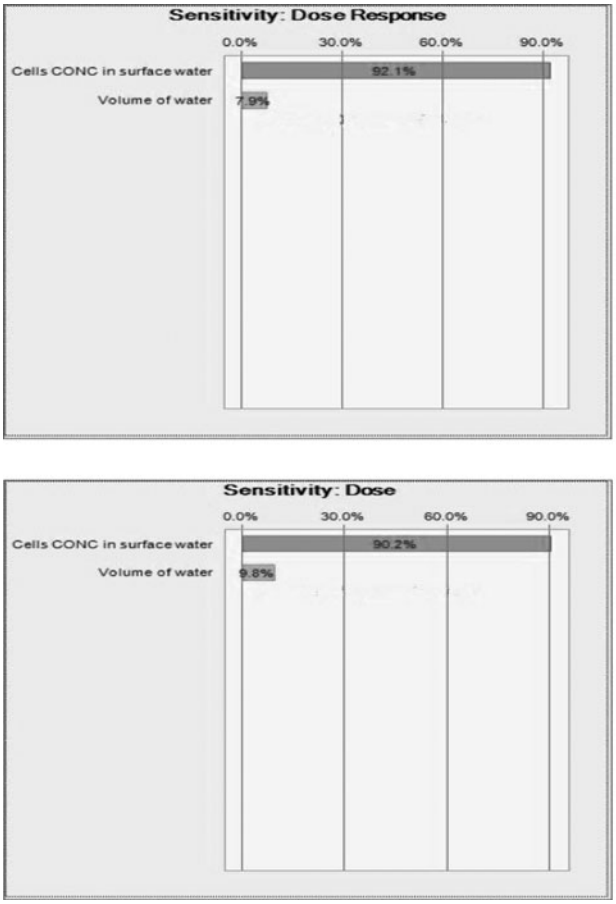

Fig. 5. Chart indicative of the unceratin variables contribution; (1) unmedicated system; (2) the worst-case scenario.

The output forecasting dose-responses in a typical scenario, indicate a risk of approximately 50% to a population of 1% to be infected, as shown in Fig. 3 related to (1) unmedicated water surfaces. However, in the worst-case scenario, there is 50% chance of infection for 20% of the exposed human populations. The proportion of the possible infected population could increase by approximately 10 fold or 2.10 fold if the agricultural field is exposed to antibiotic misuse. These levels of possible infection could be considered high, since there is no way to underestimate a minimal proportion in order to ensure a radical public health protection. Furthermore, the sensitivity chart indicates that the uncertain variable parameters, namely, the concentration of *E. coli* in water surface contributed the most to the dose-response model with 92.1% (typical scenario) and 90.2% (the worst-case scenario) over the volume of water ingested of 9.8% and 7.9%, respectively and 28.0% (Fig. 5). This chart was informative to, where the most variability in the dose or dose-response model was present. The strong positive correlation between the dose response forecast and the concentration of *E. coli* in surface waters of approximately 90.2% over the volume ingested, 9.8% has been confirmed by the scatter charts for the worst case scenario that indicate a correlation of approximately (0. 954) for the level of *E. coli* in surface waters and a ratio of (0.2651) for the volume of water ingested and is shown in Fig. 6.

Although this exploratory software offers a clearer vision of the impact of the uncertain variables on the forecast models, namely, dose and dose-responses, there are still several caveats and data gaps to consider in an attempt to offer a more complete insight. For instance, it would be a considerable extension of the model effort to determine antibiotic residue concentration in DDGS as well as their biological

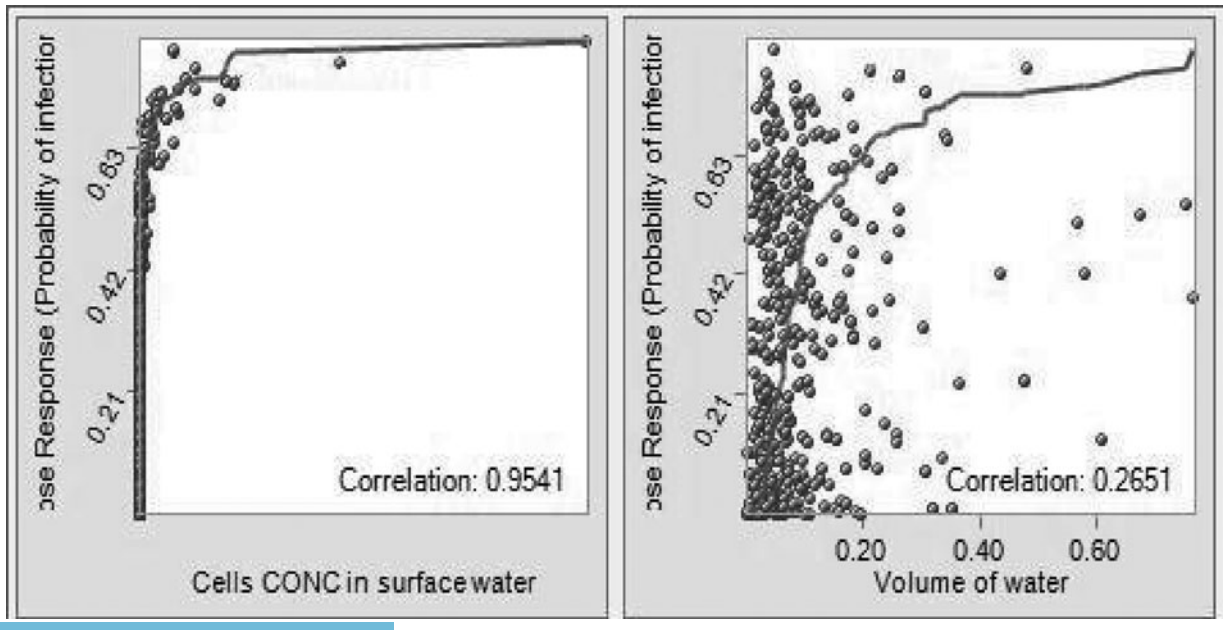

Fig. 6. The scatter chart verification: the worst-case scenario.

activity after heating. Therefore, a chemical risk assessment approach could be addressed to further clarify the minimal lethal dose of antibiotics that causes either adverse health effect or antibiotic resistance *in vivo.* However, the lack of a biological epidemiology method that would enable estimating the extent of antibiotic-resistance transfer from zoonotic bacteria *in vivo* could be a serious hindrance to the extension of the risk analysis. From the microbial hazard standpoint, there has been a wide range of information related to pathogenic microorganisms and their concentrations in different water sources. However, there is still a need to explore ways of incorporating fecal indicators in the MRA list, and establish a specific dose-response model for them. In addition to *E. coli,* it would be useful to explore other fecal indicators, such as *E. faecium,* an emergent antibiotic-resistant bacterium in the agricultural field. Further, the available beta-Poisson modeling considers only healthy adult volunteers, and does not involve primarily sensitive human or even animal populations.

Intervention scenario

While the real size of the problem remains incomplete to-date, there are several avenues and possible outputs that could be generated from the MRA approach to elucidate statistically the risk estimate, and offer pragmatic insights to risk managers for taking precautionary actions. Various intervention scenarios could be undertaken to limit antibiotic residues and possible resistance generation from biofuel system. Among the most emergent alternatives are bacteriophages or biological antimicrobial applications. These antimicrobials, including components from plant extracts (i.e., hope) or peptides (i.e., nisin), represent an effective and inexpensive alternate choice, and some have a practical broad spectrum to sufficiently ensure limitation of bacterial contamination of large-scale yeast fermentation systems, without compromising environment biosafety. A regular solar disinfection SODIS (Solar Water Disinfection) procedure created by Eawag (The Swiss Federal Institute for Environmental Science and Technology, 1991) would reduce the hazard at least by 2 logs. Viewed from another angle, surface waters and sewages surrounding biofuel distilleries will have to be treated adequately with the appropriate antimicrobial (i.e., chlorine or biological antimicrobials). It is quite possible to start tracking microbial contaminants from the source via rapid molecular methods to protect the ecosystem and environment pristine from bacterial resistance spread and prevent the environment from becoming a reservoir for resistant fecal indicators or other bacteria. This model could be amended based on the current available data. However, other uncertain variables, including primarily water sanitation coverage or solar disinfection, could possibly provide a more accurate output for this research project. The generation of more precise inputs would fulfill gaps to reach a "closer to reality" comprehensive model.

Conclusions

Given to the antibiotic resistant FIB increase in surface waters during the last decade, a preventive method namely, MRA have become essential in determining a comprehensive model to estimate the adverse health effects by resistant *E. coli* on the populations exposed to recreational water. It is quite possible that a low dose of antibiotics would maintain and enrich resistance in the microbial population as revealed by Gullberg et al.[10]. This low dose of antibiotic spread in the aquatic systems would also increase the probability of infection within the human populations. This risk estimation suggested that antibiotic resistant FIB in surface waters that were generated and amplified by a sublethal dose of antibiotic pollutants originating from an uncontrolled agricultural and industrial waste would increase the probability of infection by 10 to 2.10 fold on exposed human populations.

Acknowledgments

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