Quantitative Microbial Risk Assessment as support for bathing waters profiling

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Abstract

Profiling bathing waters supported by Quantitative Microbial Risk Assessment (QMRA) is key to the WHO's recommendations for the 2020/2021 revision of the European Bathing Water Directive. We developed an area-specific QMRA model on four pathogens, using fecal indicator concentrations (*E. coli*, enterococci) for calculating pathogen loads. The predominance of illness was found to be attributable to Human Adenovirus, followed by *Salmonella*, *Vibrio*, and Norovirus. Overall, the cumulative illness risk showed a median of around 1 case/10000 exposures. The risk estimates were

strongly influenced by the indicators that were used, suggesting the need for a more detailed investigation of the different sources of fecal contamination. Area-specific threshold values for fecal indicators were estimated on a risk-basis by modelling the cumulative risk against *E. coli* and enterococci concentrations. To improve bathing waters assessment, we suggest considering source apportionment, locally estimating of pathogen/indicator ratios, and calculating site-specific indicators thresholds based on risk assessment.

Keywords: Bathing waters; Quantitative Microbial Risk Assessment (QMRA); Fecal indicators; Virus; Salmonella; Vibrio

~~1.~~1 Introduction

The exposure to recreational waters is commonly associated with infections, which can lead mainly to gastrointestinal diseases, but also to acute febrile respiratory illnesses. Monitoring studies reveal that recreational waters can be a vehicle for numerous pathogens, including viruses (i.e. adenovirus, norovirus, hepatitis A virus), bacteria (i.e. *E. coli* O157:H7, *Campylobacter jejuni*, *Salmonella* spp., *Vibrio* spp.) and parasitic protozoa (i.e. *Giardia intestinalis*, *Cryptosporidium parvum*) (Fewtrell and Kay, 2015). However, microbial indicators are currently used to assess bathing water quality, because of the great variety of pathogens and their variable occurrence depending on the epidemiological scenario and environmental conditions. In Europe, the Bathing Water Directive (BWD) 2006/7/EC is based on both sanitary inspections and evaluation of fecal contamination (EU, 2006). The sanitary inspections consist of an on-site visual evaluation of any observable features or conditions at or near the bathing waters that might impair the quality of the bathing water. Fecal contamination is assessed through monitoring of two indicator bacteria (*Escherichia coli* and intestinal enterococci), and by comparing the detected concentrations with reference limits estimated on the basis of epidemiological studies, which found a relationship between indicator density and adverse health outcomes in swimmers. This relationship has been obtained for the first time by Kay et al. (1994) and Fleisher et al. (1998) and it has been used by both the current WHO guidelines on recreational waters and European BWD (WHO, 2003; EU, 2006). Later, other epidemiological studies supported the increasing risk of illness with increasing fecal indicator load, mainly in temperate freshwater (Wade et al., 2006, 2008; Wiedenmann et al., 2006; Marion et al., 2010; Colford et al., 2012).

For individual samples, bathing suitability is defined by compliance with the established limits, such as 500 CFU/100 ml for *E. coli* and 200 CFU/100 ml for enterococci for marine waters. For each bathing season, at least four samples need to be collected for every location so that the 4-year monitoring microbial data (at least 16) are analyzed in order to classify the bathing water quality according to four levels: “excellent” (*E. coli* ≤ 250 CFU/100 ml and enterococci ≤ 100 CFU/100 ml in at least 95% of samples), “good” (251 – 500 CFU/100 ml and 101 – 200 CFU/100 ml, respectively, in at least 95% of samples), “sufficient” (≤ 500 CFU/100 ml and ≤ 185 CFU/100 ml in at least 90% of samples) and “poor” for anything above the values of the “sufficient” level (EU, 2006; WHO, 2018).

BWD has been in operation for ~~more than~~ ≥10 years and has revealed some criticisms which have been highlighted by WHO (2018): the classification system based on both 95th (excellent, good) and 90th (sufficient) percentiles without any clear reason is a possible source of confusion; (ii) the number of mandatory samples (16) is too low for precise classification, and may induce possible errors of under- and over-estimation; (iii) the

percentile calculation is based on the assumption of $\log_{10}$-normality of the microbial dataset, without any verification.

To overcome these issues, WHO released recommendations for the planned revision of the directive in 2020 suggesting the uniform adoption of the 95th percentile across all of the classifications, along with increasing the number of samples for the classification (to at least 80), and verifying the $\log_{10}$-normality of microbial datasets (WHO, 2018).

The WHO also suggested improving the bathing water profiling procedure, which aims "*to provide a better understanding of risks as a basis for management measures*" (Annex III of EU, 2006, 2009). In fact, managing the quality of recreational waters is hampered by a series of issues regarding the analytical methods (i.e. length of time required for the bacterial indicator analyses) and the difficulties in understanding the complexity of the scenarios for fecal contamination, including the main pathogens involved and the types of pollution sources, both sewage and animal discharges. Given that the bathing water is classified simply on the basis of few data regarding the concentration of two bacterial indicators, this may hamper the interpretation of analytical results: for example, when the threshold levels are crossed by just one of the indicators this could nevertheless merit further studies on the pollution sources.

To improve the instruments available for beach profiling, the WHO proposed incorporating a Quantitative Microbial Risk Assessment (QMRA) into this process "*for ensuring that bathing water profiles accurately reflect the conditions of the bathing water*" (WHO, 2018). The QMRA methodology is a structured, systematic, science-based approach that quantitatively estimates the level of exposure to microbial hazards and the resulting risk to human health (Haas et al., 2014). In fact in 2003, WHO had suggested using a QMRA to explore via numerical simulations the potential efficacy of the control measures, and recommended it for the adoption of guidelines for bathing locations with different environmental conditions (e.g., water temperatures, sunlight) (WHO, 2003).

In the scientific literature, the QMRA has been applied to recreational waters with a great variety of purposes as recently reviewed by Federigi et al. (2019) and, in the majority of the papers, it was used so to understand the impact on bathers of the health risks in various contamination scenarios and to simulate the effect of management interventions. In some countries (Australia, USA, New Zealand) QMRA is also applied in bathing water regulations (NHMRC, 2008; MfE, 2017; US EPA, 2017).

In the framework of the planned 2020/2021 review of the European BWD, QMRA could be useful in order to improve the bathing water profiling and the classification of bathing areas as an aid for the development of a site-specific risk assessment.

We thus applied the QMRA to marine beaches in Italy which were studied within the project "Support for the implementation of risk analysis in potable and bathing waters and management of the water portal information system", financed by the Ministry of Health (CCM No. 2S62, 12/12/2017–11/12/2018). Our goal is to perform an area-specific risk assessment and to estimate risk-based microbial thresholds for *E. coli* and enterococci assuming an acceptable health risk of 5 gastrointestinal illnesses per 100 exposure events (WHO, 2003).

~~2.~~2 Materials and ~~M~~methods

~~2.1.~~2.1 Study areas and classification

The three study areas already described by Bonadonna et al. (2019) are situated on the shores of the Adriatic Sea (two sites) and of the Tyrrhenian Sea (one site). According to the institutional division of the bathing areas, they are defined as Foce Marano 50 mt Nord, Foce Marano 50 mt Sud, and 250 mt left Foce Fosso Rio Grande (hereafter named as Area No 1, Area No 2, and Area No 3, respectively). Fig. 1 shows the location of the bathing areas. In particular, they are located on urbanized coasts and are characterized by sandy beaches and by the presence of riverine discharges, which receive different pollution sources. In Area No 1 and Area No 2, the only sources of fecal pollution is attributable to the presence of a municipal wastewater treatment plant (>15.000 population equivalents) flowing into the terminal tract of the river. When the wastewater flow exceeds the capacity of the treatment plant (usually during rainfall), the overflow is discharged into the river waters, without adequate treatment. Area No 3 is affected by pollution sources of human origin as described for the other study areas, but also by animal fecal contamination, owing to the presence of agricultural activities and livestock farms. Information on the general description of the bathing waters and the pollution sources has been collected from the current bathing water profiles retrieved from the Italian Ministry of Health.

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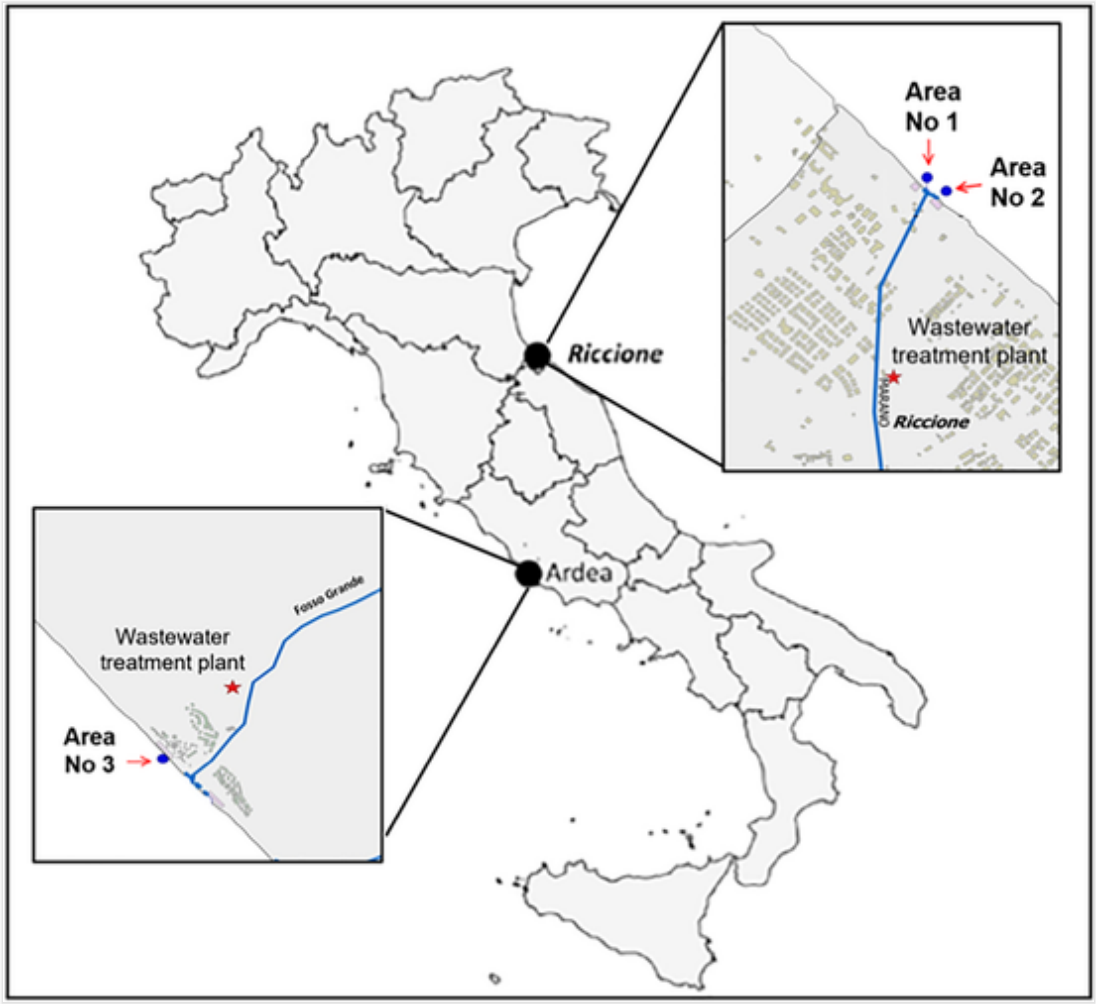
~~Figure 1~~Fig. 1



Location of the three study areas (modified from Bonadonna et al., 2019).

In 2019 these areas were classified as “good” (Area No 1) or “poor” (Area No 2 and Area No 3) on the basis of indicator data from 2015 to 2018 collected during routine monitoring. Taking into account the WHO recommendations for the EU BWD revision, in order to have the highest number of samples available, we re-classified the study areas on the basis of 8-year data (i.e. all the data available since BWD was first started in

Italy). After collecting ~~more than~~ ≥60 samples for each bathing area and testing the entire dataset for $\log_{10}$-normality (as explained in Section 2.3 Statistical analysis), the 95th percentile values were calculated and compared with 95th percentile EU BWD standards not only in terms of "excellent" and "good" water quality (EU, 2006) but also in terms of "sufficient" water quality as reported by WHO (WHO, 2018). In fact, WHO explicitly recommended the adoption of 95th values for the calculation of all the water quality categories, thus suggesting to replace the 90th values with the 95th ones for the "sufficient" class, corresponding to ≤993 *E. coli*/100 ml and ≤367 enterococci/100 ml (WHO, 2018).

~~2.2.~~2.2 QMRA methodology

We used a stochastic, static QMRA methodology in order to estimate the probability of gastrointestinal illness from pathogenic microorganisms through the ingestion of water during swimming. The QMRA methodology estimates the risk of illness, focusing on pathogen concentrations in the water matrix with health effects inferred using known mathematical dose-response relationships (Haas et al., 2014). This involves the four steps that are described below.

~~2.2.1.~~2.2.1 *Identification of pathogens and estimation of their concentrations*

~~2.2.1.1.~~2.2.1.1 *Selection of the reference pathogens*

Four reference pathogens (two bacteria and two viruses) were included in the QMRA model, based on their epidemiological relevance in bathing waters and their occurrence in the study areas (Bonadonna et al., 2019): norovirus (NoV), human adenovirus (HAdV), *Vibrio parahaemolyticus*, and *Salmonella* spp.

NoVs are gastrointestinal pathogens, responsible for waterborne infections due to exposure to contaminated drinking or recreational waters, and they were implied in many large outbreaks associated with bathing waters worldwide (Fewtrell and Kay, 2015). Moreover, NoV has been detected in 25% of the bathing water samples collected during monitoring by Bonadonna et al. (2019). HAdV includes several serotypes associated with a wide range of infections, affecting ocular, respiratory, urinary, and gastrointestinal tracts. These viruses have been widely detected in waters used for recreational purposes, not only natural bathing waters but also swimming pools, as a result of human fecal contamination (Bonadonna and La Rosa, 2019). Although in the study area HAdV was found only in non-bathing waters, it was included in the present QMRA model because previous European projects (VIROBATHE-EU FP6 513648; EPIBATHE-EU FW6 022618) demonstrated its validity as an index pathogen for recreational waters assessment. Vibrios are bacteria adapted to saltwater environments, which include potential pathogens species responsible for extraintestinal symptoms (wounds and skin infections) and gastrointestinal illness. *Vibrio*-associated diseases are expected to increase owing to climate change because temperature over 17–20 °C enhances *Vibrio* species replication (Schets et al., 2011). Thus, *V. parahaemolyticus* was chosen as one of the most representative species responsible for intestinal symptoms and because of its high detection rate (45%) in the study area (Bonadonna et al., 2019). *Salmonella* has been included in the model because several European monitoring studies have demonstrated its relatively high prevalence in coastal waters (Efstratiou and Tsirtsis, 2009; Mansilha et al., 2010) and outbreaks of *Salmonella* associated with recreational waters are reported throughout the world (Dale et al., 2010).

~~2.2.1.2.~~2.2.1.2 *Pathogen load estimation from bacterial indicators*

The microbial monitoring carried out during the CCM project (Bonadonna et al., 2019) provided data on fecal indicators (*E. coli*, enterococci, somatic coliphages) and some pathogens (*Vibrio* spp., norovirus, adenovirus, enterovirus, and aichivirus). Other monitoring studies carried out in the Mediterranean Sea (Efstratiou and

Tsirtsis, 2009; Bofill-Mas et al., 2010) were used in order to increase the amount of data. Nevertheless, the information on pathogen occurrence and concentrations were too scarce and discontinuous for a QMRA model. Consequently, the concentrations of pathogens were estimated through indicators (*E. coli* and enterococci), using conversion factors in accordance with WHO guidelines (WHO, 2016) and with some QMRA studies for recreational waters as summarized in a recent review (Federigi et al., 2019). For each pathogen, the conversion factor (hereinafter pathogen-to-indicator ratio) was calculated as the ratio between the pathogen and the bacterial indicator loads measured in the same sample. The pathogen-to-indicator specific ratios were determined, where possible, in samples collected in the study locations. Thus, for NoV and *Vibrio parahaemolyticus*, a conservative ratio was derived from the monitoring study by Bonadonna et al. (2019) considering only the samples in which the pathogens were detected (Le Roux et al., 2012), whereas for the HAdV and *Salmonella* the ratios were calculated from published studies on the microbiological monitoring of marine bathing waters with environmental conditions similar to our study areas. In particular, the HAdV-to-bacterial indicators ratio was calculated using data from the European project VIROBATHE-EU FP6 513,648, considering the results of samples collected in marine waters along the Tyrrhenian Sea (Bofill-Mas et al., 2010). For *Salmonella*, we used the rate of detection rather than the counts for ratio calculation because in European studies the gold standard approach is based on a presence/absence cultural assay (Efstratiou and Tsirtsis, 2009).

The variability of pathogens-to-indicators ratio was represented using uniform distributions between a minimum and a maximum value calculated from the datasets available (Table 1). This is in accordance with the US EPA (2010) approach, which suggests using uniform distributions when the datasets are too small to establish a rigorous statistical distribution. The selected monitoring studies measured viruses as genome copies/volume because molecular methods are commonly used for virus detection in the environment (McBride et al., 2013). Nevertheless, it is well known that genome copies count does not correspond to the amount of infective virus in water samples. Therefore, we applied a correction factor to the HAdV genome copies (0.07 according to McBride et al., 2013) to harmonize molecular data with infectivity data used in the dose-response relationship (Bambic et al., 2011; McBride et al., 2013). Instead, for NoV we considered directly genome copies because the available dose-response relationship for NoV has been derived from clinical trials in which NoV doses were expressed as genome (Teunis et al., 2008).

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Table 1

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Conversion ratios of pathogens-to-bacterial indicators.

Reference pathogens	Pathogen to *E. coli* ratio	Pathogen to enterococci ratio	Data sources
NoV	Uniform distribution Min = 1.52×10^{-4}; Max = 4.77×10^{-3}	Uniform distribution Min = 1.39×10^{-3}; Max = 8.75×10^{-3}	Bonadonna et al. (2019). Monitoring data from Adriatic and Tyrrhenian Sea (Italy) from a national CCM project
HAdV	Uniform distribution Min = 3.37×10^{-1};	Uniform distribution Min = 6.73×10^{-1}	Bofill-Mas et al. (2010). European monitoring data collected during VIROBATHE project, considering

	Max = 4.23×10^{0}	Max = 7.60×10^{0}	marine recreational waters in the Northern and Southern Tyrrhenian Sea
Vibrio parahaemolyticus	Uniform distribution Min = 4.76×10^{-3}; Max = 2.94×10^{-1}	Uniform distribution Min = 2.17×10^{-2}; Max = 1×10^{0}	Bonadonna et al. (2019). Monitoring data from Adriatic and Tyrrhenian Sea (Italy) from a national CCM project
Salmonella spp.	Uniform distribution Min = 3.24×10^{-5}; Max = 1.76×10^{-3}	Uniform distribution Min = 2.57×10^{-4}; Max = 3.51×10^{-3}	Efstratiou and Tsirtsis (2009) monitoring data from Saronic Gulf (Greece), considering high and moderate polluted marine recreational waters

To estimate the distribution of the concentrations of the pathogens, the specific ratios were applied to the levels of indicators reported from the institutional monitoring under the BWD. In order to increase the quantity of available data, they were extracted from an 8-year period (during the bathing seasons from 2011 to 2018 inclusive) reported by the online databases of the Environmental Protection Agencies of Emilia-Romagna (ARPAE, https://www.arpae.it/balneazione/) and Lazio (ARPAL, http://www.arpalazio.gov.it/). These data were then used so to generate theoretical concentration distributions of *E. coli* and enterococci in each study location.

~~2.2.2.~~2.2.2 *Exposure assessment*

The ingested dose for each pathogen was calculated as the product between the pathogen loads in bathing waters and the distribution of the volume of water swallowed by bathers (Eq~~uation~~. (1)).

$$\mathbf{Dose_{path} = C_{path} \times V_{ing}} \quad (1)$$

where C_{path} represents the pathogen concentration estimated as described above, and V_{ing} is the accidentally ingested volume of water during swimming fitted to a triangular distribution with a minimum, mode, and maximum of 20, 35, and 50 ml, respectively, based on the data reported by WHO (2016).

~~2.2.3.~~2.2.3 *Dose-response assessment*

Dose-response equations for each reference pathogen consist of mathematical functions that combine the dose (amount of pathogen ingested) derived from the exposure assessment in order to calculate the expected individual probability of infection (P_{inf}) per exposure event (bathing).

In this study, we used two-parameter functions (with parameters α and β) indicated in Table 2, which have frequently been used by authors when studying the risk associated with recreational waters (Federigi et al., 2019). A hypergeometric function was used for NoV and HAdV, while for *Salmonella* and *Vibrio* a beta-Poisson approximation was used (Teunis and Havelaar, 2000). The QMRA was conducted for illness as the endpoint (Bambic et al., 2011), therefore the probability of illness (P_{ill}) for each pathogen was estimated by multiplying P_{inf} (the probability of infection) and the pathogenicity, which represents the probability of developing illness given the infection (US EPA, 2014).

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Table 2

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Dose-response relationship used for the selected reference pathogens.

	Best-fit model and parameters	Pathogenicity	Ref.
NoV	$Pinf(dose;\alpha,\beta)=1-{}_1F_1(\alpha,\alpha+\beta,-dose)$ $\alpha,\beta=0.04,0.055$	$Pill \mid Pinf(dose\mid\eta,r)=1-(1+dose\,\eta)^{-r}$ $\eta, r=2.55\times10^{-3}, 0.086$	Teunis et al. (2008)
HAdV	$Pinf(dose;\alpha,\beta)=1-{}_1F_1(\alpha,\alpha+\beta,-dose)$ $\alpha,\beta=5.11,2.80$	$Pill \mid Pinf(dose\mid\eta,r)=1-(1+dose\,\eta)^{-r}$ $\eta, r=6.35\times10^{-1}, 0.41$	Teunis et al. (2016)
Vibrio parahaemolyticus	$Pinf(dose;\alpha,\beta)=1-\left(1+\frac{dose}{\beta}\right)^{-\alpha}$ $\alpha,\beta=0.6,1.3\times10^{6}$	NA	US FDA (2005)
Salmonella spp.	$Pinf(dose;\alpha,\beta)=1-\left(1+\frac{dose}{\beta}\right)^{-\alpha}$ $\alpha,\beta=0.33,139.9$	$Pill \mid Pinf=1$	Rose and Gerba (1991)

${}_1F_1$ is the hypergeometric function. α and β represent infection parameters specific for each microorganism. r and η are the illness parameters. NA = not applicable, because the dose-response curve for *V. parahaemolyticus* refers to illness directly.

For viruses, the dose-response models were developed from the data collected from clinical trials on oral ingestion of viral suspensions of norovirus GI.1 strain (Teunis et al., 2008) and of various types of adenovirus (AdV4, AdV7, AdV16) (Teunis et al., 2016). For *Salmonella*, the dose-response parameters were estimated by human feeding trials on the consumption of drinking waters contaminated with multiple non-typhoid strains of *Salmonella* (Rose and Gerba, 1991). The pathogenicity was set at a value of 1 (which means that all the infections result in illness), based on the proportions of illness exhibited during the trials (McBride et al., 2013). For *Vibrio*, the dose-response function is for illness, because it was derived from outbreak data for *V. parahaemolyticus* on seafood (US FDA, 2005).

~~2.2.4.~~ 2.2.4 *Risk characterization and sensitivity analysis*

The health risk for each pathogen per recreation event was obtained using a Monte Carlo simulation, running 10,000 iterations from the probability distribution functions of each input parameter (Vensim package, Ventana Systems, Inc., Harvard, MA, USA). These distributions represent the variability within the data used as an input parameter in the QMRA model (the fecal indicator concentrations in seawaters, the conversion ratios of pathogens to fecal indicators, the accidental ingestion volume of water while swimming). The four QMRA models were then unified according to Eq~~uation.~~ (2) (Sales-Ortells and Medema, 2014) in order to calculate the cumulative risk of illness from the exposure to all the four reference pathogens ($CumP_{ill}$), assuming that risks for different recreation events were equal and statistically independent.

$$\mathbf{Cum}P_{ill} = 1 - \left[(1 - P_{ill}\mathbf{HAdV}) \times (1 - P_{ill}\mathbf{NoV}) \times (1 - P_{ill}\mathbf{Vibrio}) \times (1 - P_{ill}\mathbf{Salmonella})\right] \quad (2)$$

where P_{ill}HAdV, P_{ill}NoV, P_{ill}*Vibrio*, and P_{ill}*Salmonella* are the probabilities of illness per recreation event for HAdV, NoV, *V. parahaemolyticus*, and *Salmonella*, respectively.

A sensitivity analysis was then carried out to test the relative importance of six stochastic variables that affect the model output ($CumP_{ill}$): concentration of each indicator (either $C_{E.\ coli}$ or $C_{Enterococci}$), ingestion volume (V_{ing}) and the four conversion factors from bacterial indicators to pathogens (conversion factor to HAdV, conversion factor to NoV, conversion factor to *Vibrio* and conversion factor to *Salmonella*). We performed a simple univariate sensitivity analysis, in which the value of each input parameter was varied, one at a time, within the variability range of that parameter in order to determine its effect on the final risk estimate (Gan et al., 2014; Carducci et al., 2018). For each of the indicators (*E. coli* and enterococci), we thus calculated the $CumP_{ill-i}$ set of values in the seven different conditions (described below). Each $CumP_{ill}$ set of values (denoted as $CumP_{ill-j}$ with j = 0, 1, 2, ..., 6) was obtained from a simulation of 10,000 steps, each step representing a single exposure. The simulation conditions produce the following sets of values:

$CumP_{ill-0}$ when all the parameters are set at their constant (average) values;

$CumP_{ill-1}$ when only the indicator concentration varies randomly within its own distribution;

$CumP_{ill-2}$ when only the volume of ingestion (V_{ing}) varies randomly within its own distribution;

$CumP_{ill-3}$ when only the conversion factor to HAdV varies randomly within its own distribution;

$CumP_{ill-4}$ when only the conversion factor to Norovirus varies randomly within its own distribution;

$CumP_{ill-5}$ when only the conversion factor to *Salmonella* varies randomly within its own distribution;

$CumP_{ill-6}$ when only the conversion factor to *V. parahaemolyticus* varies randomly within its own distribution.

We thus obtained seven arrays of 10,000 values each (one array for every $CumP_{ill-j}$ for each of the two indicators). In order to evaluate the relative importance of each of the above parameters on the final result, and thus on $CumP_{ill}$, we calculated the mean values of the pairwise differences (in absolute value), step by step, between the values of $CumP_{ill-0}$ and each of the other $CumP_{ill-j}$ in turn.

We evaluated the composite function *mean value* (*absolute value*($CumP_{ill-0}$ - $CumP_{ill-j}$)) with j = 1, ~~..~~ ..., 6. We used the absolute value to avoid compensations between positive and negative differences in the overall value so that in this way we evaluate the distances in a sort of mean deviation. We were thus able to assess the mean distance of the various values of the probability in all those cases where one of the parameters varies and the others are held at a constant value.

~~2.3.~~2.3 Statistical analysis

E. coli and enterococci concentrations were $\log_{10}$-transformed before the statistical analysis. Therefore, the results were expressed in terms of the geometric mean of microbial load (Wymer and Wade, 2007). The values of 95th percentiles were calculated according to the parametric approach as described by WHO in guidelines for bathing waters (WHO, 2003, 2009). Parametric calculations of 95th percentiles were chosen after ~~more than~~ ≥60 samples had been collected for each bathing area and the entire datasets (8-years data) had been tested for $\log_{10}$-normality. The best probability distribution function for the microbial concentrations (*E. coli* and enterococci) was thus chosen from three theoretical distributions (Lognormal, Weibull, Gamma), which are commonly used to approximate microbiological data (Pouillot and Delignette-Muller, 2010). These distributions were tested with the maximum likelihood estimation (MLE) and the best were selected using the Akaike (AIC) criterion. The analysis was done in R-Language with the *fitdistrplus* package (Pouillot and Delignette-Muller, 2010; R Core

Team, 2018). The best-fit distribution functions were also used in the QMRA model. In addition, to check the accuracy of the model fitting, a *t*-test was performed between the collected microbial data and the simulated data based on theoretical probability distributions, separately for each bathing area and for each fecal indicator (when *P* values were ~~less than~~ ≤0.05, the results were considered to be statistically significant). To analyze the differences in risk estimation according to the type of fecal indicator, in each bathing area, a *t*-test was used between $CumP_{ill}$ obtained from *E. coli* and the one obtained from enterococci. For each bathing area, the simulated data of $CumP_{ill}$ were used to develop a regression model for the bathers~~'~~ health risk based on each microbial concentration. The probability of illness, separately for each pathogen and cumulatively, was also described in terms of the interquartile range (IQR), considering the first and the third quartile of the simulated data. Statistical analyses were performed and figures were generated with R-Language (R Core Team, 2018).

~~3.~~3 Results

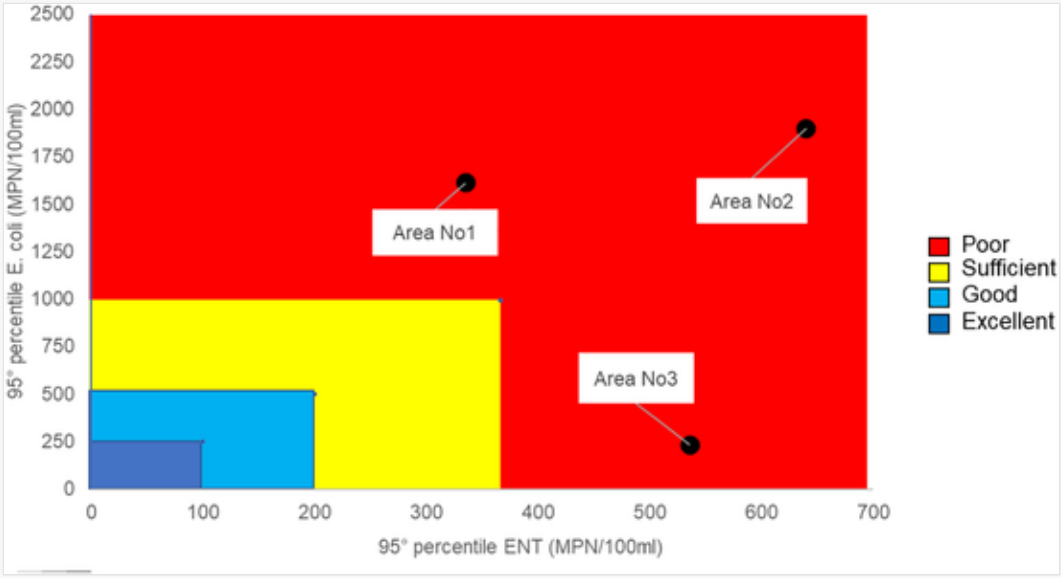
~~3.1.~~3.1 Microbial data and water quality classification

Considering an 8-year period of environmental agency monitoring, a total of 73, 82, and 63 samples were recorded for Area No 1, Area No 2, and Area No 3, respectively.

The log-normal distributions showed the best-fit with the collected data for all the areas and both indicators. This is in line with previous papers demonstrating how microbiological counts are represented by positive right-skewed data, such as a lognormal distribution (Limpert et al., 2001). On the basis of these data, and following the approach described in Section 2.1 (i.e. using 8-years dataset and 95th thresholds for all the classification levels, including the class "sufficient"), all the three bathing areas should be classified as "poor", but for different combinations of data: Area No 2 had both *E. coli* and enterococci values above the 95th thresholds (640 enterococci/100 ml and 1899 *E. coli*/100 ml). Area No 1, had only the *E. coli* concentration over the limit (335 enterococci/100 ml and 1611 *E. coli*/100 ml) and Area No 3 only the enterococci level (536 enterococci/100 ml and 234 *E. coli*/100 ml). Fig. 2 shows the bathing water classification based on 95th values.

alt-text: Fig. 2

~~Figure 2~~Fig. 2



Bathing water classification based on EU directive 2006/7/EC (EU, 2006) and the WHO recommendation (WHO, 2018) based on 8-years dataset.

~~3.2.~~3.2 Fitted distribution for bacterial indicators and pathogen doses derived from bacterial indicators

The best-fit parameters for log-normal distributions of indicators in the three study areas are reported in Table 3. The best-fit models simulated the observed data with reasonable accuracy (t-test, $P < 0.05$), as reported in the Supplementary information, separately for the frequency distribution of the *E. coli* concentration (Fig~~ure.~~ S1) and enterococci concentration (Fig~~ure.~~ S2). Other descriptive statistics on microbial concentrations are reported in Tables S1 and S2 of the Supplementary material, for a comparison of the collected and simulated data.

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Table 3

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Theoretical distributions of *E. coli* and enterococci.

Bathing areas	Theoretical distribution of *E. coli*	Theoretical distribution of enterococci
Area No 1	Lognormal distribution Meanlog = -0.8497859 Sdlog = 2.1750954	Lognormal distribution Meanlog = -1.810284 Sdlog = 1.824445
Area No 2	Lognormal distribution Meanlog = -0.6874391 Sdlog = 2.1856214	Lognormal distribution Meanlog = -1.546898 Sdlog = 2.056872
Area No 3	Lognormal distribution Meanlog = -2.712498 Sdlog = 2.149094	Lognormal distribution Meanlog = -1.446682 Sdlog = 1.885637

The pathogen doses estimated from the theoretical distributions reported above are shown in Table 4. As expected, the dose calculation from *E. coli* produced different values for each pathogen compared to that based on enterococci, and these were in relation with the concentrations of the indicators in the considered area.

alt-text: Table 4

Table 4

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Arithmetic means and standard deviations of pathogen doses calculated for HAdV, NoV, Salmonella, and *Vibrio* ~~parahemoliticus~~ *parahaemolyticus* based on *E. coli* and enterococci. The results are expressed as the most probable number (MPN).

	Bacterial indicator for dose calculation	Pathogen doses (mean ± standard deviation)		
		Area No 1	Area No 2	Area No 3
HAdV	*E. coli*	0.49 ± 3.12	0.65 ± 5.69	0.09 ± 0.76
	Enterococci	0.18 ± 0.96	0.37 ± 2.53	0.30 ± 1.75
NoV	*E. coli*	0.37 ± 2.27	0.53 ± 5.43	0.06 ± 0.50
	Enterococci	0.15 ± 0.90	0.28 ± 1.62	0.22 ± 1,32
Vibrio parahaemolyticus	*E. coli*	20.96 ± 110.29	32.30 ± 366.50	4.01 ± 33.31
	Enterococci	16.17 ± 103.06	33.11 ± 271.58	26.12 ± 158.26
Salmonella	*E. coli*	0.14 ± 0.99	0.19 ± 2.01	0.02 ± 0.19
	Enterococci	0.06 ± 0.38	0.12 ± 0.75	0.09 ± 0.48

The doses were calculated as the product between the pathogen concentrations (expressed as MPN/100 ml because they are derived from fecal indicator concentrations) and the volume of ingestion (expressed as ml).

~~3.3.~~3.3 Results of QMRA simulation

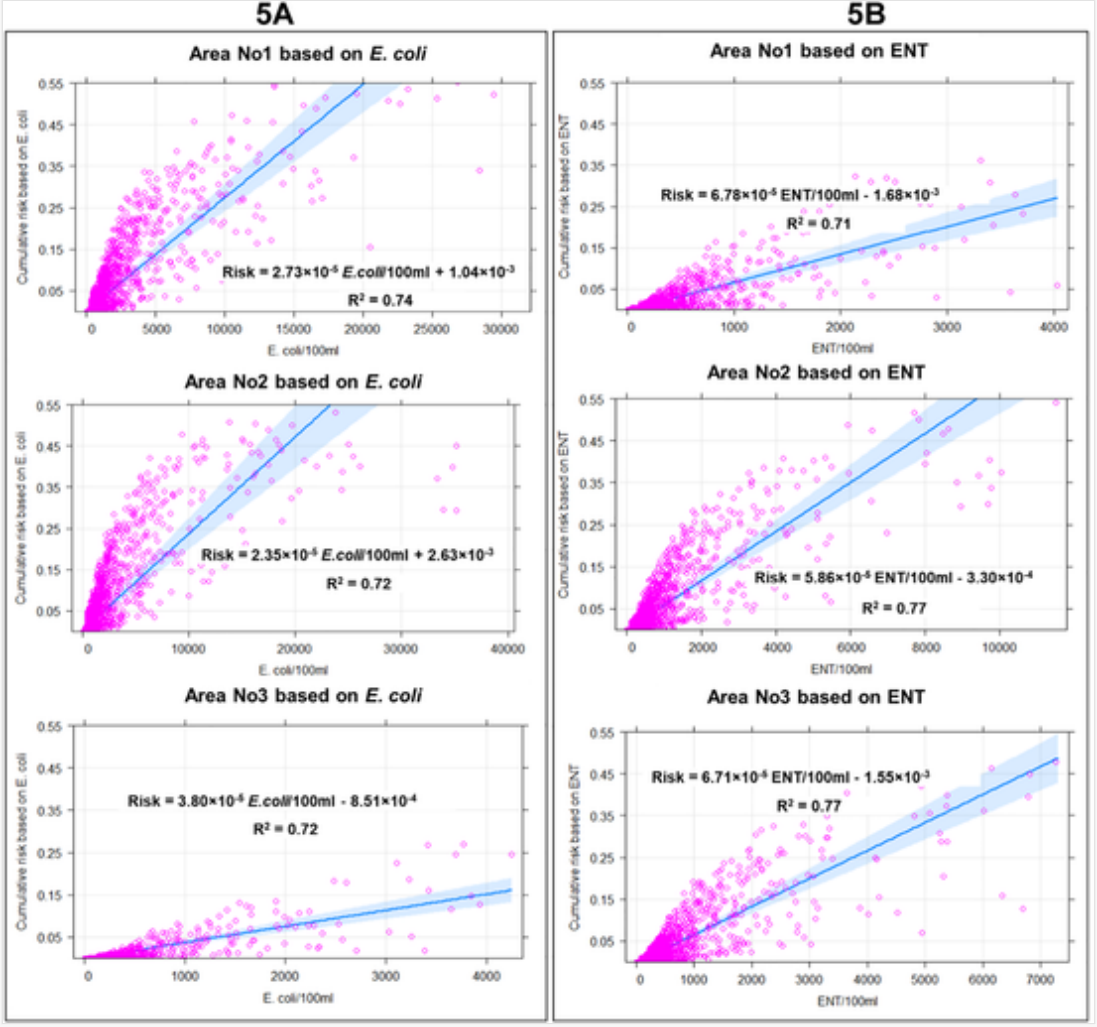
For each bathing area (separately for *E. coli* and enterococci), the health risk for each pathogen was calculated through simulations from the specific dose-response relationship.

~~3.3.1.~~3.3.1 *Probability of illness*

Fig. 3 reports the QMRA results for the total probability of illness in the three bathing areas under investigation. The $CumP_{ill}$ from the Monte Carlo simulations (10,000 iterations) were plotted against the bacterial indicator concentrations (x-axis), separately for *E. coli* and enterococci. The simulated $CumP_{ill}$ data were then modelled using a linear equation that represents the data with reasonable accuracy (in all the bathing sites, $R^2 > 0.70$ for QMRA results based on *E. coli* and enterococci).

alt-text: Fig. 3

~~Figure 3~~Fig. 3



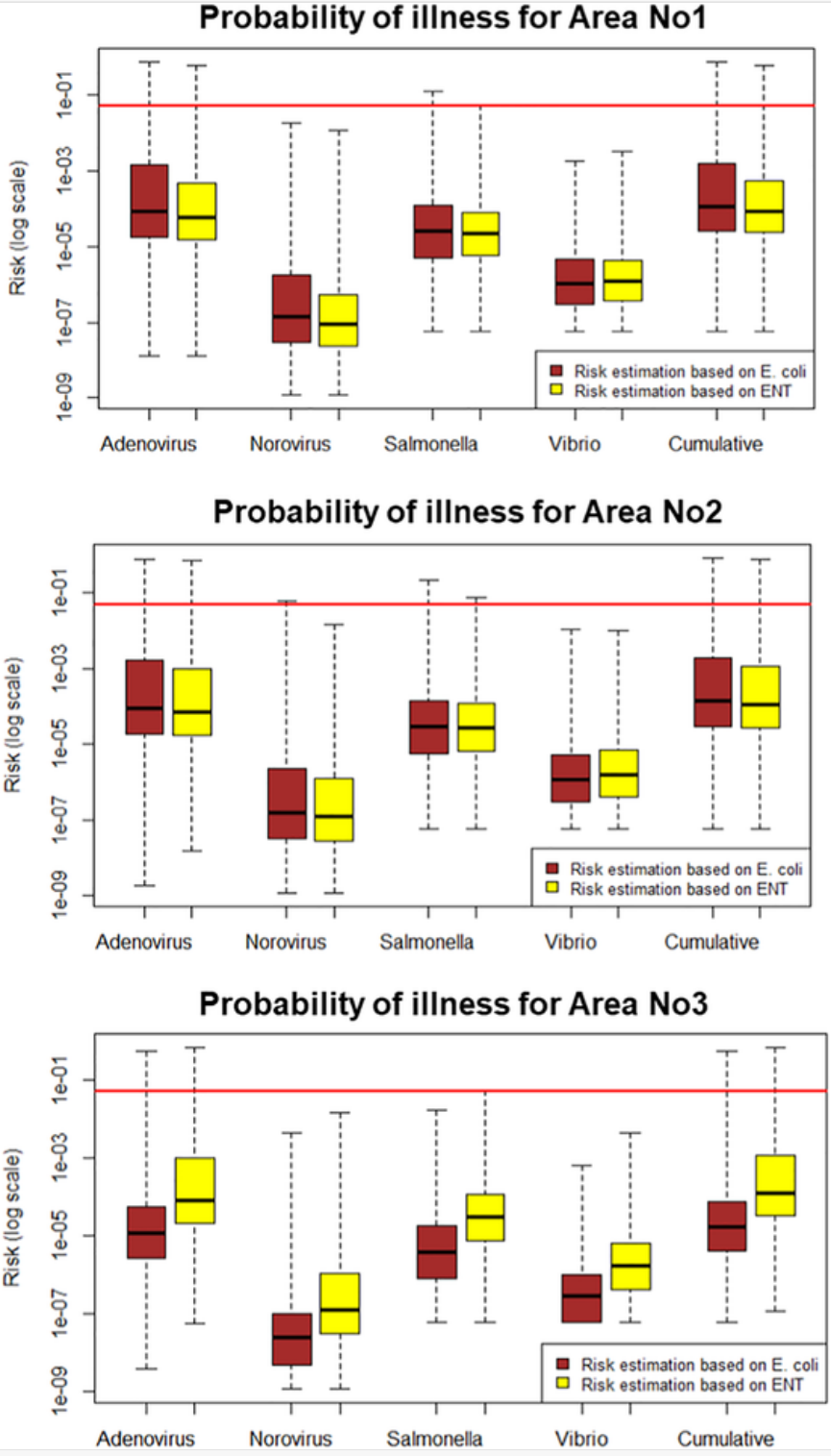
Bacterial indicator concentrations related to the cumulative health risk for *E. coli* on the left (5A), and enterococci (ENT) on the right (5B). Magenta circles represent 10,000 simulations, and blue lines are the linear model for the simulations (the light halo represents the 95% confidence interval). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In Fig. 4, the median probability of illness and the interquartile ranges (IQR) are plotted for the reference pathogens (either alone or in combination) for each bathing area and separately for the estimates based on *E. coli* and enterococci concentrations. These values are compared with the WHO recreational water benchmark of 5 gastrointestinal illnesses per 100 recreation events (Fig. 4). Considering each pathogen separately, the results show a predominance of illness due to HAdV, followed by *Salmonella*, *V. parahaemolyticus,* and finally NoV. Considering the $CumP_{ill}$, in both areas No 1 and No 2 the risk estimation based on *E. coli* is greater than that based on enterococci, but this difference is not statistically significant (*t*-test, $P > 0.05$). Conversely, for Area No

3, the risk estimation based on enterococci is significantly higher compared to the estimation based on *E. coli* (*t*-test, $P < 0.05$).

alt-text: Fig. 4

~~Figure 4~~Fig. 4



Probability of illness (median, first and third quartiles, minimum and maximum) by each reference pathogen (adenovirus, norovirus, *V. parahaemolyticus*, and *Salmonella*) and by a combination of all four based on *E. coli* (dark red boxplot) and enterococci (ENT, yellow boxplot).The red horizontal lines represent the WHO thresholds for health risk for a single recreation event (5%). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Considering the $CumP_{ill}$ based on *E. coli*, Area No 2 exhibits the highest risk with a median of 14 cases/10^5 exposures, ranging in IQR of 29 cases/10^6 exposures (25th percentile) and 19 cases/10^4 exposures (75th

percentile). Similar values were obtained for Area No 1, with a median of 12 cases/10^5 exposures (IQR = 26×10^{-6} – 16×10^{-4}). In Area No 3 the risk based on *E. coli* was very low (median = 17×10^{-6}, IQR = 40×10^{-7} – 77×10^{-6}). Accordingly, the risk exceeded the WHO threshold (5 cases/10^2 exposures) in 6% of the recreation events in areas No 1 and No 2, and 1% in Area No 3.

The risk scenario was different considering the $CumP_{ill}$ based on enterococci. The QMRA results show the highest risk in Area No 3, with a median of 12 cases/10^5 exposures (IQR = 33×10^{-6} – 11×10^{-4}), followed by Area No 2 with a median of 11 cases/10^5 exposures (IQR = 27×10^{-6} – 12×10^{-4}), and Area No 1 with a median of 87 cases/10^6 exposures (IQR = 24×10^{-6} – 56×10^{-5}). According to these estimates, the risk exceeded the WHO threshold of 5 cases/10^2 exposures in 5%, 4%, and 2% of the recreation event in Area No 2, Area No 3, and Area No 1, respectively.

The results of the QMRA simulation (see Fig. 3) were also used to estimate the thresholds for bacterial indicators corresponding to the tolerable health risk of gastrointestinal diseases, namely 5%, according to the WHO guidelines (WHO, 2003). In Table 5, these thresholds are reported with their 95% confidence interval (CI).

alt-text: Table 5

Table 5

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Site-specific thresholds for bacterial indicators corresponding to a 5% health risk (tolerable according to WHO, 2003).

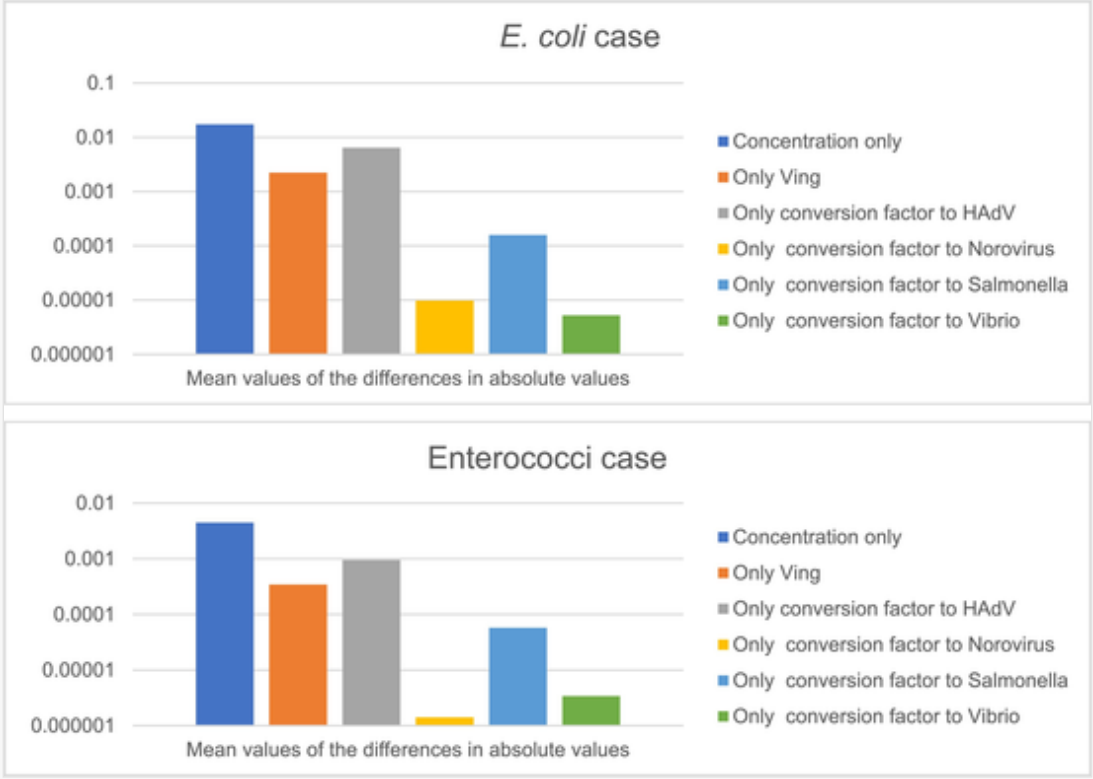
Bathing areas	Thresholds for *E. coli* (MPN/100 ml)		Thresholds enterococci (MPN/100 ml)	
	Value	95% CI	Value	95% CI
Area No 1	1792	1757–1828	762	750–775
Area No 2	2016	1969–2064	859	844–875
Area No 3	1337	1319–1357	768	756–781

~~3.3.2.~~3.3.2 *Sensitivity analysis*

The sensitivity analysis was executed for both *E. coli* and enterococci. Fig. 5 reports the results separately for the two indicators.

alt-text: Fig. 5

~~Figure 5~~Fig. 5



Sensitivity analysis results for the QMRA model based on *E. coli* (above) and enterococci (below).

For the QMRA model based on *E. coli*, the ordering of the relative importance of the parameters on the outcome of the simulations is:

$C_{E.coli}$>Conversion factor to HAdV>V_{ing}>Conversion factor to Salmonella>*Conversion factor to NoV*>*Conversion factor*

For the model based on enterococci, the relative importance of the parameters on the outcome of the simulations is:

$C_{Enterococci}$>Conversion factor to HAdV>V_{ing}>Conversion factor to Salmonella>*Conversion factor to Vibrio*>*Conversion f*

The inversion of the order of importance (in italics in the previous orderings) between two of the parameters from *E. coli* case to enterococci case is caused by the different values of the conversion ratios of the bacterial indicators to *Vibrio* (see Table 1).

~~4.~~4 Discussion

The European BWD will be re-examined in 2020/2021. According to WHO recommendations, the only aspects to be retained are the two current parameters (*E. coli* and enterococci) and the four levels within the classification system. Concerning bathing profiling, the WHO suggested improving this procedure by including a quantitative risk assessment-based approach (QMRA).

At an international level, some governments have already included QMRA in water quality guidelines for recreation with different regulatory purposes.

In New Zealand, the Ministry for the Environment used QMRA for setting the *E. coli* threshold limits in freshwaters, based on *Campylobacter* infection risk, because *Campylobacteriosis* accounts for more than half of New Zealand's burden of notifiable diseases (MfE, 2003, 2017). In particular, QMRA was performed by linking the monitoring data on *E. coli* and *Campylobacter* concentrations in bathing waters with the epidemiological findings on *Campylobacter* infections. These data were used to develop a statistical model for predicting the level of *Campylobacter* infection risk for a range of different *E. coli* concentrations. Then, considering a 5% tolerable infection risk, a limit of 540 *E. coli* MPN/100 ml was derived and used as a reference value for water quality classification.

In the USA, the US Environmental Protection Agency (US EPA) introduced QMRA to develop site-specific water monitoring criteria, particularly for beaches impacted by agricultural animal sources of fecal contamination, which is the pollution derived from cattle, swine, and chicken (US EPA, 2010, 2012, 2017). In fact, the ongoing fecal indicator levels for water quality are derived from the epidemiological evidence referring to beaches impacted by sewage-sources of pollution, where the contribution of wastewater flows to the human pathogen load is relatively continuous (with possible, but predictable, increases in untreated or poorly treated sewage during rain events). Therefore, these bathing safety criteria are not suitable to infer the health risks for swimmers of beaches impacted by non-sewage sources, because of the heterogeneity of these types of pollution, with different concentration ranges of pathogens of animal origin, which differ also with regard to the pathogenicity to humans (Fewtrell and Kay, 2015; US EPA, 2017). In general, the risks posed by feces from seagulls, chickens, and pigs are lower than for human fecal waste, while the main concern is for bovine cattle (Soller et al., 2010). Thus, US EPA used QMRA to explore the relative contribution of different fecal sources to the health risk in recreational waters, taking into account the relative occurrences of pathogens in different fecal sources and the infectivity and probability of illness associated with those pathogens.

In Australia, the National Health and Medical Research Council (NHMRC) recommended using the QMRA during general screening-level risk assessments (NHMRC, 2008). In this case, a QMRA should be performed for index pathogens, which are not necessarily the main etiological agents but are representatives of the likely pathogens from each microbial group (bacteria, protozoa, and viruses) and for which a dose-response relationship is available. These results could be used for calculating the health risk under different scenarios and for simulating the potential efficacy of control measures in reducing the health risks at different recreational sites.

Our study applied the QMRA to three bathing areas differently impacted by fecal pollution: in 2019 two of them were classified as “poor”, one as “good” according to the BWD. An analysis of 8-year historical data revealed that all three areas could be classified as “poor”, but for different combinations of *E. coli* and enterococci: in fact, only Area No 2 was above the limits for both indicators (see Fig. 2). Instead, considering the classification based on the current BWD (4-years data) for the entire observation period, Area No 1 was classified as “poor” for one year, Area No 2 for three years and Area No 3 for six years: so the Area No 3 could be defined the worst one (classification data available at the website of the Italian Ministry of Health). On the contrary, the risk of enteric illnesses from index pathogens as obtained from QMRA was the lowest for Area No 3. The model simulation

results showed little correspondence with the present classification: this could be due to the use of 8-year rather than 4-year data which are needed to have a sufficient amount of data in order to estimate the bacterial distributions. For the EU BWD revision, the WHO recommends increasing the number of samples to at least 80 in four years, instead of 16. We thus used all the data available since the first application of the Directive in Italy (Ministerial Decree of 30 March 2010 implementing the Legislative Decree 116/2008).

Our results also highlight that the risk estimates are strongly influenced by the indicator that we used, in particular when only one of the two is high. For example, of the three areas, Area No 3 had the highest risk based on enterococci and the lowest based on *E. coli*. The difference between the levels of the two indicators suggests that the impact of the various sources of fecal contamination should be investigated, as indicated by US EPA, and that the QMRA should be applied taking into account the different infective risks posed by pollution of different origins.

We believe that the site-specific threshold limits that we calculated for the three areas could be useful for assessing the efficacy of the measures used to reduce fecal pollution, also from a cost-benefit point of view. For example, if the level of enterococci pollution of Area No 3 was reduced below the limit, this would rapidly change the site-related risk and classification.

~~4.1.~~4.1 Limitations of the study

Despite the advantages provided by a quantitative approach, QMRA has a series of limitations. Unfortunately, the sources of variability and uncertainty in the available data are numerous including the pathogen and indicator measures, the ways and amount of exposure, and the dose-response relationships. In the context of pathogen dose calculation, we faced with the scarcity of pathogen data due to the difficulties and costs of regular monitoring for contamination from pathogens for a large number of bathing sites, thus we estimated pathogen doses using indicator data and specific pathogen-to-indicator ratios. To have enough data for probability distribution functions for fecal indicators, we collected monitoring data from an 8-years period, but we should consider that this could introduce further variability in such input parameters because during such wide time span variations in contamination and control measure may have occurred.

Then, we used ratios of pathogens-to-indicators calculated from Mediterranean seawater samples in order to avoid possible changes in the relationship between pathogens and indicators due to the characteristics of the water matrix (i.e., salinity, turbidity, temperature, sunlight) (Verani et al., 2019). Various studies reported the possible low reliability of indicators in relation to pathogens (Wyn-Jones et al., 2011; Love et al., 2014; Bonadonna et al., 2019). However, recent epidemiological findings support the consistency of the relationship between health and bacterial indicators, especially for enterococci at sewage-impacted marine water sites (King et al., 2015; WHO, 2018). The complexity of this issue is reported in Korajkic et al. (2018) who reviewed the microbial indicators and pathogens in recreational waters. They found significant relationships between fecal indicators and pathogens in the studies on waters impacted by human fecal contamination and with a high number of bathers, but this relationship weakens in the case of bathing waters impacted by multiple sources of fecal contamination. Therefore, we decided to follow conservative assumptions for risk estimation and in the case of *V. parahaemolyticus* and NoV, the pathogen-to-indicator ratio was calculated from the samples in which the pathogens were detected, thus not considering the samples in which the pathogens were below the detection limit of the analytical methods. This could determine a possible overestimation of the risk. A further limitation of this study derives from the fact that dose-response relationships for viruses available from literature refer to surrogate organisms, namely HAdV 4, 7, 16 for HAdV and NoV GI for NoV. Thus, we assume that the HAdV enteric serotype (40, 41) and NoV GII has the same infectivity as their surrogates (Viau et al., 2011).

The scarcity of data for quantifying the model inputs, the difficulties in quantifying the uncertainties and in incorporating them into the risk outcomes and the validity of the default assumptions, have been outlined by WHO (2016) and recently reviewed by Federigi et al. (2019) for the specific implementation of QMRA to recreational waters.

~~5.~~5 Conclusions

Modelling pathogens using a QMRA framework represents a valuable tool for recreational waters assessment and management, provided that there is a sufficient amount of good quality data. The present work shows the possibility of performing QMRA based on site-specific pathogen-to-indicator ratios, coming from a dedicated study. This approach would imply the monitoring of waters for both pathogens and indicators (at least for one bathing season) in order to provide more precise data, but it would require additional cost due to pathogen analysis. To improve the assessment and the classification of bathing waters, we can propose some suggestions, mainly useful for areas with pollution problems:

- Considering the source apportionment;
- For a limited number of samples, carrying out pathogen monitoring in parallel with indicators;
- Performing QMRA using site-specific data to calculate site-specific thresholds for acceptable risks and simulating the effects of interventions also in terms of cost/benefit.

In the present study, the QMRA model has been used to calculate the health risk per recreation event with the aim of using the model results in the context of bathing water classification. However, the QMRA model could be used also to characterize daily or annual risk by including an additional parameter in the model, represented by the number of bathing events per day or year. This parameter should be carefully investigated for each study location since the climatic features influence both the duration of the bathing season and the bather's behavior. Further investigations will be planned to include exposure frequency in the model.

CRediT authorship contribution statement

Ileana Federigi: Conceptualization, Methodology, Formal analysis, Writing - original draft. **Lucia Bonadonna:** Writing - review & editing. **Giusy Bonanno Ferraro:** Writing - review & editing. **Rossella Briancesco:** Writing - review & editing. **Lorenzo Cioni:** Methodology, Software, Formal analysis, Writing - review & editing. **Anna Maria Coccia:** Writing - review & editing. **Simonetta Della Libera:** Writing - review & editing. **Emanuele Ferretti:** Writing - review & editing. **Liana Gramaccioni:** Writing - review & editing. **Marcello Iaconelli:** Writing - review & editing. **Giuseppina La Rosa:** Writing - original draft, Writing - review & editing. **Luca Lucentini:** Writing - review & editing, Funding acquisition. **Pamela Mancini:** Writing - review & editing. **Elisabetta Suffredini:** Writing - original draft, Writing - review & editing. **Teresa Vicenza:** Writing - review & editing. **Carolina Veneri:** Writing - review & editing. **Marco Verani:** Writing - review & editing. **Annalaura Carducci:** Conceptualization, Methodology, Writing - original draft, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We wish to thank English for Academics (e4ac.com) for editing and proofreading the manuscript.

Funding

This work was supported by CCM project No. 2S62 “support for the implementation of risk analysis in potable and bathing waters and management of the water portal information system” (12/12/2017–11/12/2018), with the financial support of the Ministry of Health – CCM (Centro Nazionale per la Prevenzione e il Controllo delle Malattie). The specific objective of this project was “pilot study using Quantitative Microbial Risk Assessment (QMRA) according to WHO guidelines, applied to bathing water”.

~~Appendix A.~~Appendix A Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2020.111318.

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The corrections made in this section will be reviewed and approved by a journal production editor. The newly added/removed references and its citations will be reordered and rearranged by the production team.

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Highlights

- Development of QMRA model to improve bathing water profiling
- Use of fecal indicators monitoring data for pathogens load estimation
- Calculation of fecal indicator threshold limits on a risk-basis

~~Appendix A.~~Appendix A Supplementary data

Multimedia Component 1

Supplementary material

alt-text: Image 1

Queries and Answers

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Figure S1 – Frequency distribution of E. coli concentrations from simulated (yellow histograms) and collected (blue histograms) data Figure S2 – Frequency distribution of Enterococci concentrations from simulated data (yellow histograms) and collected (blue histograms) data. ENT stands for EnterococciTable S1 – Descriptive statistics for collected and simulated dataset in each bathing area, considering E. coli concentrations. E. coli threshold for bathing suitability is 500 MPN/100ml according to Bathing Water Directive 2006/7/ECTable S2 – Descriptive statistics for collected and simulated dataset in each bathing area, considering Enterococci concentrations. Enterococci threshold for bathing suitability is 200 MPN/100ml according to Bathing Water Directive 2006/7/EC. ENT stands for Enterococci