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Human exposure to antibiotic resistant-*Escherichia coli* through irrigated lettuce

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ABSTRACT

Antibiotic resistant bacteria (ARB) have been found on fresh fruit and vegetables globally. These types of ARB infections are spreading rapidly and are a major human health threat. A quantitative human exposure assessment model was created using scenario analysis to investigate the potential human exposure to antibiotic resistant *Escherichia coli* (AR-*E. coli*) through the consumption of lettuce irrigated with surface water. Scientific literature and site specific data were collected to model each process from farm to fork to calculate the concentration of AR-*E. coli* on the lettuce at the point of human consumption. The processes examined were the adhesion, colonisation and viability of bacteria on the lettuce; the effect of different post-harvest cleaning processes; the effect of consuming the lettuce before, on or after the expiry date; and the effect of the consumer washing the lettuce. The results show the mean human exposure levels ranged between 1.00×10^{-2} and 1.35×10^{6} colony forming units (CFU) of AR-*E. coli* per 100 g of surface water irrigated lettuce for the different scenarios investigated. The mean probability of illness from consuming 100 g of lettuce contaminated with potential pathogenic antibiotic-sensitive *E. coli* was between 1.46×10^{-9} to 1.88×10^{-2}. A back calculation revealed that in order for the EC No 1441/2007 regulation to be exceeded ($\geq$1000 CFU/g of *E. coli* on lettuce at the manufacturing stage), the mean contamination levels required in the irrigation water would need to be 2.7, 3.1 or 4.8 log CFU/ml using the post-harvest treatments of washing with water, rapid cooling with water and washing with a chlorine solution respectively. The information generated from this model could help to set guidelines for producers on maximum permissible AR-*E. coli* contamination levels in irrigation water and provides recommendations on the best post-harvest treatment to use.

1. Introduction

Microbial contamination is the biggest threat to food safety and it is vital to ensure human exposure to harmful bacteria through this pathway is limited (Van Boxstael et al., 2013). Leafy greens, including ready-to-eat (RTE) packaged salads, are consumed raw with no heat treatment prior to human consumption, increasing the risk of human exposure to pathogenic bacteria through this food source (Holvoet et al., 2013). There have been a number of outbreaks reported globally that have been associated with the consumption of leafy greens contaminated by pathogenic strains of *E. coli* (Söderström et al., 2008; CDCP, 2010, 2012, 2018; Edelstein et al., 2014; Newitt et al., 2016). When *E. coli* infections are resistant to antibiotics (AR-*E. coli*) and in particular resistant to multiple antibiotics (i.e. multi drug resistant, MDR), serious complications may arise with the treatment of patients (Sabaté et al., 2008). Additionally, even if AR-*E. coli* are ingested, they may not have an immediate or obvious health outcome on an individual, but they still might transfer antibiotic resistant genes (ARGs) to other bacterial species present in the gut microbiota (Allen et al., 2010) and thus increasing the risk of future difficulties in antibiotic treatment regimes.

There is strong evidence showing the presence of ARB and ARGs on leafy greens, this could be a potential threat to human health, especially for immunocompromised individuals (Holvoet et al., 2013; Walia et al., 2013; Rasheed et al., 2014; Njage and Buys, 2015; Wang et al., 2015; Faour-Klingbeil et al., 2016; Araújo et al., 2017; Zhu et al., 2017). Contamination of leafy greens can occur through many pathways (e.g. through soil, animal manures, farming equipment, human handling, post-harvest cleaning processes and others; Holvoet et al., 2014; Weller et al., 2017) but irrigation water is considered the most important source of crop contamination (Holvoet et al., 2013; Jung et al., 2014; Araújo et al., 2017; O'Flaherty and Cummins, 2017). Unfortunately, the

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Table 1
The probability distributions used to represent the initial *E. coli* concentrations (EC_{water}) found at the three different surface water sites (CFU/ml).

Symbol	Description	Selected best fit model based on monitored data
$SAR_{Lake\text{-}rural}$	Single antibiotic resistant *E. coli* (i.e. resistant to at least one antibiotic) sampled at a rural lake	Triangular (min 0, most likely 0, max 1)
$SAR_{River\text{-}rural}$	Single antibiotic resistant *E. coli* sampled at a rural river	Triangular (min 1.40, most likely 1.40, max 297.54)
$SAR_{River\text{-}urban}$	Single antibiotic resistant *E. coli* sampled at an urban river	Triangular (min 12.61, most likely 12.61, max 495.67)
$MDR_{Lake\text{-}rural}$	Multiple antibiotic resistant *E. coli* (i.e. resistant to three or more antibiotics) sampled at a rural lake	Triangular (min 0, most likely 0.07, max 0.32)
$MDR_{River\text{-}rural}$	Multiple antibiotic resistant *E. coli* sampled at a rural river	Triangular (min 0.32, most likely 0.32, max 97.28)
$MDR_{River\text{-}urban}$	Multiple antibiotic resistant *E. coli* sampled at an urban river	Triangular (min 3.92, most likely 3.92, max 299.86)
$PAR_{Lake\text{-}rural}$	AR pathogenic *E. coli* strains (i.e. tested positive for either AR EHEC, EPEC or EAEC) at a rural lake	Triangular (min 0, most likely 0, max 0.30)
$PAR_{River\text{-}rural}$	AR pathogenic *E. coli* strains at a rural river	Triangular (min 0.62, most likely 0.62, max 98.17)
$PAR_{River\text{-}urban}$	AR pathogenic *E. coli* strains at an urban river	Triangular (min 0, most likely 0, max 144.13)
$PAS_{Lake\text{-}rural}$	Antibiotic sensitive pathogenic *E. coli* strains (i.e. tested positive for either EHEC, EPEC or EAEC) at a rural lake	Triangular (min 0, most likely 0, max 1.20)
$PAS_{River\text{-}rural}$	Pathogenic *E. coli* strains at a rural river	Triangular (min 1.72, most likely 1.72, max 188.76)
$PAS_{River\text{-}urban}$	Pathogenic *E. coli* strains at an urban river	Triangular (min 0, most likely 0, max 244.29)

quantity of ARB contamination resulting from irrigation water is unknown as well as its role in the transmission of pathogenic strains of AR-*E. coli* (Njage and Buys, 2015; Araújo et al., 2017).

Human exposure assessment models are a valuable tool in estimating the levels of harmful bacteria that humans are exposed to through a particular food source (Allende et al., 2017; Pang et al., 2017). These types of models can identify areas within a food production process that could be improving or deteriorating the safety of a particular food product. In this study, a quantitative human exposure assessment model was created to investigate the potential concentrations of AR-*E. coli* (including potentially pathogenic strains) humans are exposed to through the consumption of RTE salad, assuming the use of untreated lake and river water for irrigation. Each step involved from farm to fork was considered and a scenario analysis was used to investigate the influence of different parameters within the production process of RTE salad. The effect of different post-harvest cleaning treatments and the effect of consuming the lettuce before, on or after the recommended expiry date were examined. This study also examines the quantity of AR-*E. coli* concentration required in the irrigation water for the EC No 1441/2007 regulation to be exceeded ($\geq$1000 CFU/g of *E. coli* on lettuce at the manufacturing stage).

2. Material and methods

2.1. AR-E. coli concentration in irrigation water

Three replicate 1 l water samples were taken at five sites on three different dates (total = 45) during the growing season of lettuce (April to September) from surface waters used to irrigate local crops in Central Italy. Two sites were located along a small river (mean annual discharge < 1 m^3/s) draining a rural area (Arrone river, River-rural), two sites in the last section of a large river (mean annual discharge = 240 m^3/s) draining a large urban area (Tiber river, River-urban) and one site was located at a lake located in a rural area with sparse urbanisation (Bracciano lake, Lake-rural) (Supplementary data, Table 1). The sites were also chosen to examine if there were any AR-*E. coli* concentration differences between water located in rural areas in comparison to urban areas. Samples were analysed in the lab for the isolation, identification and enumeration of AR-*E. coli* after cultivation on Tryptone Bile X-Glucuronide Medium (TBX, Oxoid, Cambridge, UK) and overnight incubation at 37 °C. The isolates were then confirmed as *E. coli* with the API-20 E system (Biomérieux, France). Phenotypic resistance to antibiotics on randomly selected *E. coli* colonies (N = 221) were tested through the Kirby-Bauer disk diffusion technique (Bauer et al., 1966; EUCAST, 2017). Selected antibiotics and concentrations were: Tetracycline (16 µg/ml), Imipenem (10 µg/ml), Chloramphenicol (30 µg/ml), Ciprofloxacin (5 µg/ml), Trimethoprim-Sulfamethoxazole (64 µg/ml), Amoxicillin (2 µg/ml), AUG2 (Amoxicillin/clavulanic acid; 20/10 µg/ml), Gentamicin (10 µg/ml), Cefotaxime (5 µg/ml). Additionally, recovered *E. coli* isolates were tested for the presence of selected virulence genes associated with *E. coli* pathotypes (i.e.: *stx-1*; *stx-2*; *ehxA*; *eaeA*; *bfp*; *cdtB*; *Lt*; *St*; *aggR*; *east1*; *ipaH*) by standard PCR assay. The sequences of the primers of the respective virulence genes (VGs), previously searched in the literature, were then compared with the sequences present in the NCBI database (National Center for Biotechnology Information, 2018), in order to verify the specificity of the pairing of primers couples with the selected virulence genes (Sidhu et al., 2013). Tested genes were used as indicators for the potential presence of the following *E. coli* pathotypes: Enterotoxigenic (ETEC), Enteropathogenic (EPEC), Enteroinvasive (EIEC), Enterohaemorrhagic (EHEC) and Enteroaggregative (EAEC). No ETEC or EIEC virulence factors were found in any isolate, therefore, were not further considered. The sampling results were arranged into three categories to examine single antibiotic resistant *E. coli* (SAR) (resistant to at least one antibiotic), MDR *E. coli* (resistant to 3 or more antibiotics) and potentially pathogenic antibiotic-resistant *E. coli* (PAR) (resistant to at least one antibiotic and tested positive for one virulence factor of EHEC, EPEC or EAEC pathotypes). The best fit probability distributions applied to the measured concentrations of AR-*E. coli* are shown in Table 1 and Fig. 2A/B/C (using the Anderson Darling statistical test).

2.2. Model structure and development

A quantitative model was created to analyse the human exposure to SAR *E. coli*, MDR *E. coli* and PAR *E. coli* through the consumption of lettuce that was irrigated with untreated surface water. Fig. 1 shows the main steps involved in the development of the model for this study and highlights the data inputs required for each step. Scenario analysis was used to investigate the influence of different parameters on the RTE salad production process (Supplementary data, Table 2). The first step of the model was the input of the AR-*E. coli* concentrations measured at the various sampling sites (Table 1). Secondly, adhesion, colonisation and viability of AR-*E. coli* on the field lettuce after sprinkler irrigation were modelled through the collection of data from relevant scientific literature (see below in Section 2.3). Thirdly, previously collected data on *E. coli* contamination during the RTE life cycle (from harvest to fork) through local production practices were used to predict AR-*E. coli* concentrations at each production step. Three post-harvest treatment scenarios were modelled, including washing the lettuce with water (*P1*), washing with a chlorine solution (*P2*) and a rapid cooling process using water (*P3*). Additionally, the consumption time was included by modelling AR-*E. coli* concentrations on RTE salad (assuming proper storage of sealed packaged lettuce at 4 °C) before the expiry date, on the expiry date and two days after the expiry date. The next step examined was the effect of washing the RTE lettuce with tap water prior to consumption. The human exposure was estimated by multiplying a standard amount of lettuce (100 g) consumed by the concentration of AR-*E. coli* predicted at different RTE lettuce consumption time points

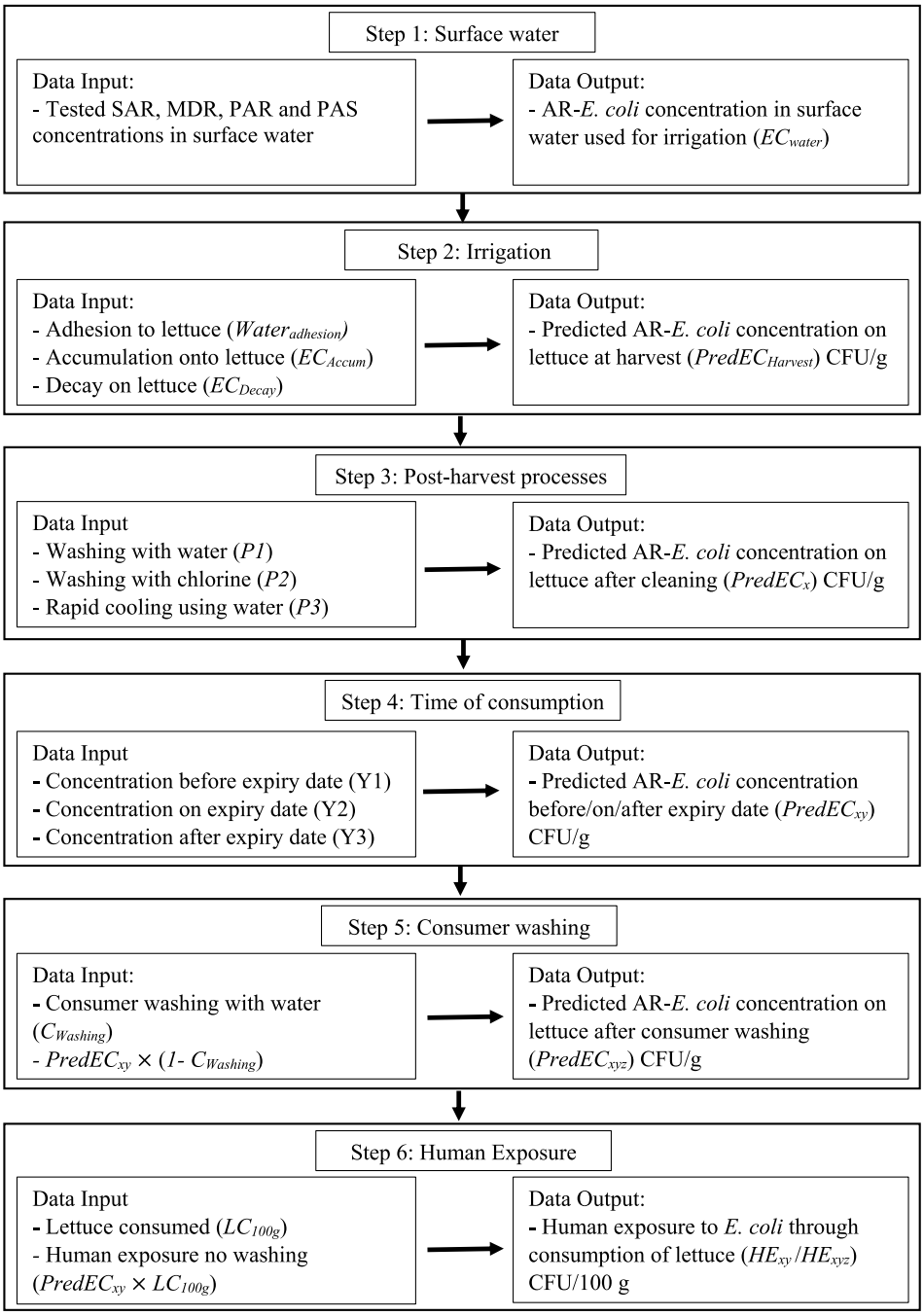

Fig. 1. Framework showing the steps and data inputs required to create the model.

(Shuval et al., 1997; Petterson et al., 2001; Ottoson et al., 2011). Finally, a dose response model was performed using the antibiotic sensitive pathogenic *E. coli* data (PAS, Table 1) for validation purposes, as no dose response models are available on AR-*E. coli* (Manaia, 2017).

2.3. Irrigation: adhesion, colonisation and viability of E. coli on field lettuce

Sprinkler irrigation is the most commonly used irrigation system for commercial lettuce production in the study area. It is well known that the "leaf-wetting" is a variable of great importance in plant pathology (Středa et al., 2013; Rowlandson et al., 2015). The presence of a veil of water on the surface of the leaves, benefits the bacterial viability and their penetration into the host's tissues (Rowlandson et al., 2015). The knowledge of this variable, therefore, becomes fundamental for disease prediction and crop protection. There have been a number of studies on the quantity of water and bacteria that can adhere to lettuce as a consequence of irrigation (Shuval et al., 1997; Hamilton et al., 2006; Oliveira et al., 2012; Mok and Hamilton, 2014). For the model created for this study, data from Mok and Hamilton (2014) were used to model the attachment of water onto lettuce after sprinkler irrigation as it provided the most accurate representation of this parameter. The R software (version 3.0.1) was used to create a Lognormal3 (-4.75, 0.50, 0.006) distribution, this distribution represents the quantity of water attached to the lettuce after 30 min of sprinkler irrigation ($Water_{attach}$, Fig. 2C) (Mok and Hamilton, 2014). It was assumed that all AR-*E. coli* bacteria in the irrigation water adhered to the lettuce (Hamilton et al., 2006; Mok and Hamilton, 2014). Eq. (1) was used to estimate the bacterial concentration adhered to the lettuce ($EC_{adhesion}$) for each

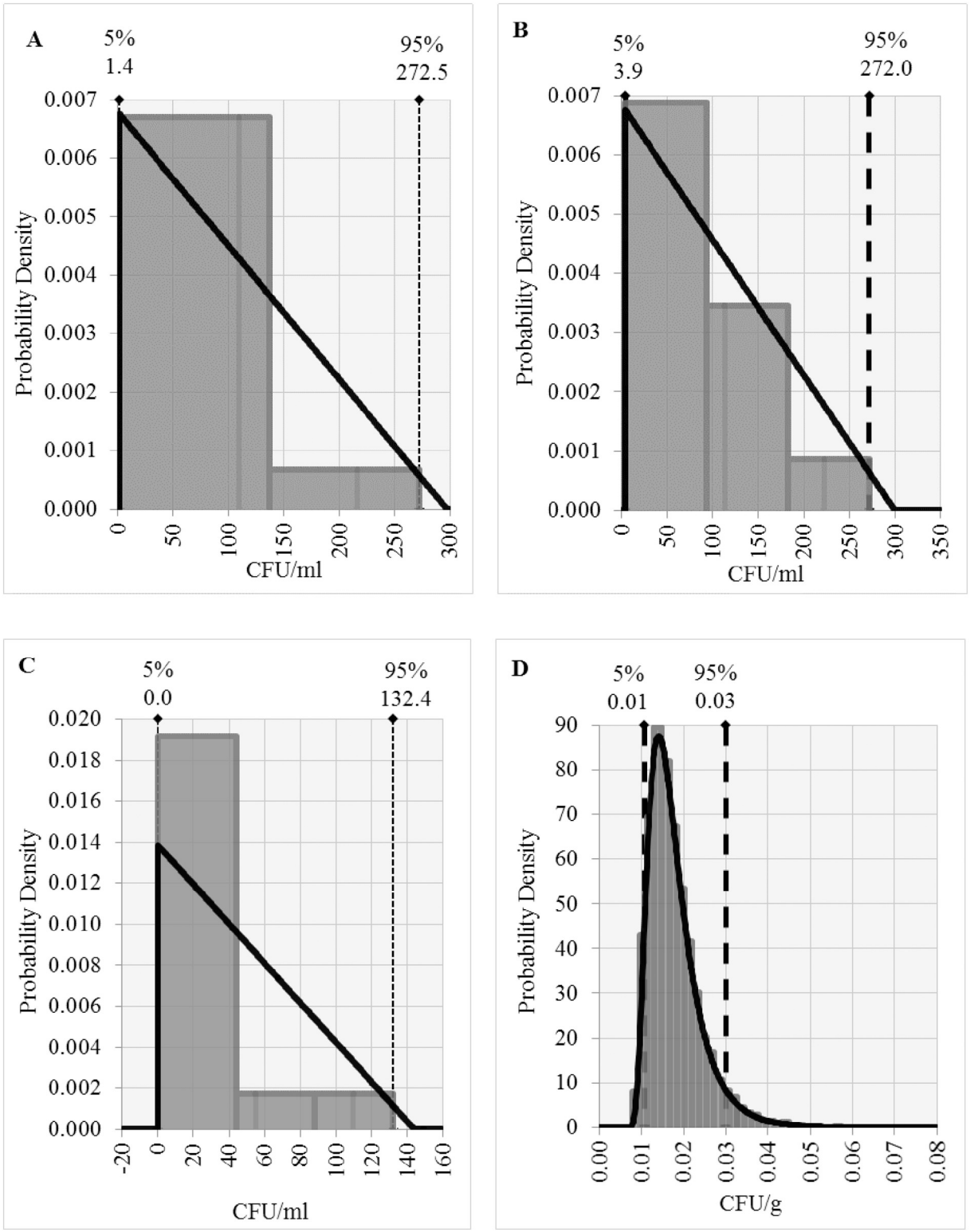

Fig. 2. The best fit probability density distributions representing initial levels of SAR *E. coli* in the rural river water (A), initial levels of MDR *E. coli* in the urban river water (B), initial levels of PAR in the urban river (C) and the attachment of water onto lettuce after sprinkler irrigation (D) (the gray bars represent the data and the black lines represent the best fit distribution).

category of AR-*E. coli* contamination found in the surface water used for irrigation (EC_{water}, Table 2).

$$EC_{adhesion} = EC_{water} \times Water_{attach} \quad (1)$$

It is important to model the decay of the bacteria on the field lettuce to help predict the final concentration of bacteria found when the lettuce is harvested. There are several studies on the survival of *E. coli* on lettuce (Stine et al., 2005; Erickson, 2010; Weller et al., 2017). WHO (2006) suggest a die-off of between 0.5 and 2 log units per day to model bacteria die off after wastewater irrigation. Currently, there is no model on the decay of AR-*E. coli* on lettuce available, therefore, an antibiotic sensitive *E. coli* model was used. Other studies have used a similar

Table 2
Parameters and description of inputs used in model and outputs produced by the model. Where *x* is either P1, P2 or P3 (P1 washed with water, P2 washed the chlorine solution and P3 rapidly cooled with water), *y* is either before, on or after the expiry date and *z* is consumer washing.

Symbol	Description and references	Model/equation	Units
Model inputs			
EC_{water}	Concentration of bacteria at surface water site (Table 1 for individual *E. coli* concentrations)	Table 1	CFU/ml
$Water_{attach}$	Attachment of water onto lettuce after sprinkler irrigation (Mok and Hamilton, 2014)	Lognormal3 (mean log −4.75, SD log 0.50, threshold 0.006)	ml/g
$EC_{adhesion}$	Adhesion of bacteria onto lettuce after daily sprinkler irrigation (Mok and Hamilton, 2014)	Eq. (1)	CFU/g
EC_{decay}	Decay of bacteria on lettuce in field (McKellar et al., 2014)	Eq. (2)	d^{-1}
EC_{accum}	Accumulation of bacteria on lettuce from daily irrigation	14	Days
$ObEC_{Harvest\text{-}x}$	Observed probability of *E. coli* concentrations after treatment *x* (i.e. *P1*, *P2* or *P3*) (De Giusti et al., 2010) (Supplementary data, Table 3)	General (0, X_{max}, X_{range}, P_{range})	CFU/g
$ObEC_{xy}$	Observed probability of *E. coli* concentrations after treatment *x* and on consumption date *y* (i.e. before, on or after the expiry date) (De Giusti et al., 2010) (Supplementary data, Table 3)	General (0, X_{max}, X_{range}, P_{range})	CFU/g
$Diff_{xy}$	Factor difference between $ObEC_{Harvest\text{-}x}$ and $ObEC_{xy}$ after treatment *x* and on consumption date *y*	$ObEC_{xy}/ObEC_{Harvest\text{-}x}$	Factor
$C_{Washing}$	Reduction of contamination by consumer washing with water	Triangular (min 0.65, most likely 0.99, max 0.99)	Decimal reduction
LC_{100g}	Lettuce consumption	100 g	100 g
Model outputs			
$PredEC_{Harvest}$	Predicted concentrations of bacteria when lettuce is harvested after 14 days of daily irrigation (Fig. 3)	$\Sigma[(EC_{attach} + EC_{decay}) \times EC_{Accum}]$	CFU/g
$PredEC_{xy}$	Predicted concentration of *E. coli* after treatment *x* and on consumption date *y*	$PredEC_{Harvest} \times Diff_{xy}$	CFU/g
$PredEC_{xyz}$	Predicted concentration of *E. coli* after treatment *x*, consumption date *y* and washing process z (i.e. consumer washing)	$PredEC_{XY} \times (1 - C_{Washing})$	CFU/g
HE_{xy}	Human exposure to *E. coli* after treatment *x* and on consumption date *y* (Table 4)	Eq. (3)	CFU/100 g
HE_{xyz}	Human exposure to *E. coli* after treatment *x*, on consumption date *y* and with washing process *z* (Table 4)	Eq. (4)	CFU/100 g
P_{ill}	Probability of illness as a result of human exposure to pathogenic *E. coli* (Table 5)	Eq. (5)	$100\,g^{-1}$

approach when research gaps are found when modelling ARB (Schijven et al., 2015; Njage and Buys, 2017; O'Flaherty et al., 2018). McKellar et al. (2014) analysed numerous scientific papers to create a meta-analysis study investigating the decay of *E. coli* on field lettuce (pre-harvest). This study only included studies that used real field conditions and were not carried out under controlled laboratory conditions. McKellar et al. (2014) found that a biphasic model (Cerf's equation) was the most appropriate model to represent the decay of *E. coli* on the field lettuce because this model provides more accurate information about the decay rate in comparison to the Weibull model. Eq. (2) was used in the model created for this study to estimate the decay of *E. coli* on field lettuce.

$$LogR = \log[f \times \exp(-k_1 \times t) + (1 - f) \times \exp(-k_2 \times t)] \quad (2)$$

LogR is the log reduction of the bacteria; *f* is the proportion of cells that have a fast decay (0.00007642); k_1 is the rate constant for the cells with a fast decay ($4.45\,d^{-1}$); k_2 is the rate constant for the cells with a slow decay ($0.06981\,d^{-1}$) and *t* is the time (day) (McKellar et al., 2014).

Sprinkler irrigation is typically performed daily up until the day of harvest to ensure lettuce hydration in the warm dry climate of the study region. Therefore, it is possible *E. coli* could accumulate on the lettuce through the daily application of irrigation water. Allende et al. (2017) modelled the accumulation of *E. coli* onto spinach taking into consideration the daily decay and daily contamination of *E. coli* from irrigation prior to the crop being harvested, a similar approach was used in this model. Given the rapid decay of *E. coli* on field lettuce (Eq. (2)), 14 days of daily bacterial accumulation and decay were considered prior to harvest for this study. This timeframe was used because the decay of the bacteria is rapid and from examining the decay over longer periods of time (using the Cerf's model from McKellar et al., 2014) there would not be a significant survival of the bacteria after this period of time. To calculate the accumulation of bacteria on the lettuce per day, the daily decay (*LogR*, Eq. (2)) was applied to the daily irrigation contamination level ($EC_{adhesion}$) over 14 days (EC_{Accum}). The final concentration of bacteria after each irrigation application over 14 days (taking into account the daily decay) were summed to calculate the total bacteria on the lettuce at harvest ($PredEC_{Harvest}$) (Table 2). The predicted range of AR-*E. coli* concentrations on the lettuce at harvest were validated using data presented by De Giusti et al. (2014) who tested RTE salads for *E. coli* concentrations at harvest time in the study region used for this model.

2.4. Post-harvest processing

It is vital that commercial post-harvest cleaning practices remove harmful bacteria from lettuce, in particular when the product is marketed as 'prewashed and RTE' (Gonzalez et al., 2004). The effect of post-harvest treatments and techniques used for commercial lettuce production was modelled using data from De Giusti et al. (2010) (Supplementary data, Table 3). This study investigated the effect of three different commercial post-harvest treatment processes performed by local producers of RTE salads. De Giusti et al. (2010) tested the lettuce by taking 25 g of lettuce, diluted it in 225 ml of Modified Tryptone Soya broth and novobiocin, homogenized it and then incubated it for 12–18 h at 42C, according to the standard culture method (ISO 16 654:2001). Post-harvest treatment process P1 washed the lettuce with water, P2 washed the lettuce with a 2% chlorine solution and P3 used water and rapidly cooled the lettuce at 4 °C (De Giusti et al., 2010). The general distribution function was used to represent the variability in the *E. coli* concentrations reported before and after each post-harvest treatment (Supplementary data, Table 3). The *E. coli* levels after each treatment were divided by the contamination level before treatment (at harvest) in order to calculate the factor difference as a result of the process. The factor difference was then multiplied by the predicted concentration of bacteria found on the lettuce at harvest to calculate the predicted concentration of *E. coli* after each process ($PredEC_{xy}$, Table 2).

2.5. Human consumption on, before or after expiry date

Data from De Giusti et al. (2010) were used to investigate the effect of when the packaged lettuce was consumed (Supplementary data, Table 3). This paper examined *E. coli* concentrations on lettuce at harvest time, 24 h after packaging (before the expiry date), on expiry date and two days after the expiry date (De Giusti et al., 2010). Leaf samples were taken from sealed packages of lettuce stored at 4 °C during the experiment (De Giusti et al., 2010). To calculate the *E. coli* concentrations on the lettuce before, on and after the expiry date, the differences between the levels found on the lettuce at harvest and before, on and after expiry were used to calculate the factor increase/decrease (Table 2). The predicted contamination concentrations were calculated for before, on and after the expiry date for each post-harvest treatment ($PredEC_{xy}$, Table 2).

2.6. Consumer washing

It is recommended that consumers wash lettuce before consumption to help reduce bacterial contamination, however, not all consumers perform this safety procedure (Beuchat et al., 1998). Surveys carried out on consumer washing habitats reveal that 46% in the US, 50% in Belgium and 67% in Spain do not wash pre-packaged lettuce (Verrill et al., 2012; Jacxsens et al., 2015). The effect of the consumer washing the lettuce with water was modelled to see the significance of this parameter. Research shows some varied results on the effect of household washing of lettuce with water. Beuchat et al. (1998) simulated household level washing of lettuce with water and found a 1.25 log reduction of *E. coli*. Jensen et al. (2015) examined a log-reduction of *E. coli* between 2 and 2.5 after washing with water for between 30 s and 5 min. Uhlig et al. (2017) found between 0.3 and 0.9 log reduction of *E. coli* from consumers washing lettuce with water. A triangular probability density distribution was used to represent the uncertainty and variability (using the Anderson Darling best fit test) in the data reported from the scientific literature on consumer washing of lettuce with water (Table 2).

2.7. Human consumption and exposure

A standard 100 g of lettuce was used in this model to estimate the human exposure to AR-*E. coli* through lettuce consumption (LC_{100g}), this quantity has been used as a standard measure in several other lettuce risk assessment studies (Shuval et al., 1997; Petterson et al., 2001; Ottoson et al., 2011).

$$HE_{xy} = LC_{100\,g} \times PredEC_{xy} \quad (3)$$

$$HE_{xyz} = LC_{100\,g} \times PredEC_{xyz} \quad (4)$$

The human exposure concentrations without consumer washing (HE_{xy}) were calculated by multiplying 100 g of consumed lettuce (LC_{100g}) by the level of contamination predicted on the lettuce at the different times of consumption for each post-harvest treatment ($PredEC_{xy}$, Eq. (3)). The human exposure concentrations with consumer washing (HE_{xyz}) were calculated by multiplying 100 g of consumed lettuce (LC_{100g}) by the level of contamination predicted on the lettuce at the different times of consumption for each post-harvest treatment with consumer washing ($PredEC_{xyz}$, Eq. (4)) (Table 2).

2.8. Dose-response

In this study no dose response was conducted for the AR-*E. coli* given the lack of dose response models currently available for AR-*E. coli*. This is in agreement with other studies examining human exposure to AR-*E. coli* through environmental pathways. (Depoorter et al., 2012; Harris et al., 2014; Leonard et al., 2015; Schijven et al., 2015; Njage and Buys, 2017). Antibiotic sensitive *E. coli* dose response models have been used in the past but it is recognised that they do not represent AR infections. AR infections could have a much more severe health outcome due to the difficulty in treating these types of infections (Ashbolt et al., 2013; Manaia, 2017; O'Flaherty et al., 2018). However, they can be used to characterise the probability of illness from antibiotic sensitive pathogenic *E. coli*. Hence in this paper, for validation and comparison purposes, it was decided that antibiotic sensitive pathogenic *E. coli* (PAS) data would be used to perform a dose response model to examine the probability of illness as a result of human exposure to only the PAS *E. coli* through RTE salad.

Powell et al. (2000) developed a Beta-Poisson dose response model for EPEC *E. coli*, this model was used in this study because this pathogenic strain was tested for (among others) in the collected water samples (Eq. (5)).

$$P_{ill} = 1 - \left[1 + D\frac{\left(2^{\frac{1}{\alpha}} - 1\right)}{N_{50}}\right] \quad (5)$$

P_{ill} is the probability of illness, D is the dose (HE_{xy} or HE_{xyz}, CFU/100 g), N_{50} (6.85×10^7) is the dose infecting 50% of the population with *E. coli*, and α is a coefficient (2.21×10^{-1}) (Powell et al., 2000).

2.9. Model run and software

Microsoft Excel 2013 with the @ Risk 7.5.1 add-on (Palisade Corporation, Newfield, NY) was used to create the human exposure model. To characterise uncertainty and variability in the model input data, Monte Carlo Simulation was used and the model was run for 10,000 iterations. A back calculation was performed using the Goal seek function in @Risk to calculate the concentration of *E. coli* required in the irrigation water in order for the concentration of *E. coli* to exceed the maximum acceptable level found on lettuce after post-harvest treatment (EC regulation No 1441/2007, ≥1000 CFU/g). This was calculated by setting the "goal cell" to the AR-*E. coli* concentration after each post-harvest treatment, the statistic value was set to the 95th percentile, the "goal value" was set to 1001 CFU/g (i.e. exceeding the EC regulation) and the "by changing" value was set to EC_{water} (Table 2). A sensitivity analysis was also performed to investigate the influence of the model input parameters on the final output data, this was done using Spearman's rank order correlation.

3. Results and discussion

3.1. Predicted concentrations of E. coli at harvest and after post-harvest treatment

Fig. 3 shows the mean predicted levels of *E. coli* found on the lettuce at harvest in comparison to the results found by De Giusti et al. (2014), the results show the predicted and observed values are comparable. The observed values (before 0 log) will always be lower than the predicted values because the detection method will not be sensitive enough to detect levels below the limit of detection. The results show the model has higher sensitivity than the cultural technique. Table 3 shows the mean predicted levels of bacteria on lettuce after the post-harvest treatments. According to the EC regulation on microbiological criteria for foodstuffs (EC No 1441/2007), the predicted results after post-harvest treatment are below the maximum *E. coli* limit recommended for RTE fruit and vegetables (≤1000 CFU/g of *E. coli* on lettuce) and are considered satisfactory (≤100 CFU/g *E. coli*). The results also show that the largest reduction of AR-*E. coli* concentrations were achieved when P2 (chlorine solution) was used as a post-harvest treatment. It has been reported in the scientific literature that chlorine is a better treatment for cleaning lettuce in comparison to using a water only cleaning process (Lang et al., 2004; Zhang et al., 2009). The results show there was a mean increase of bacterial levels after P1 and P3 post-harvest

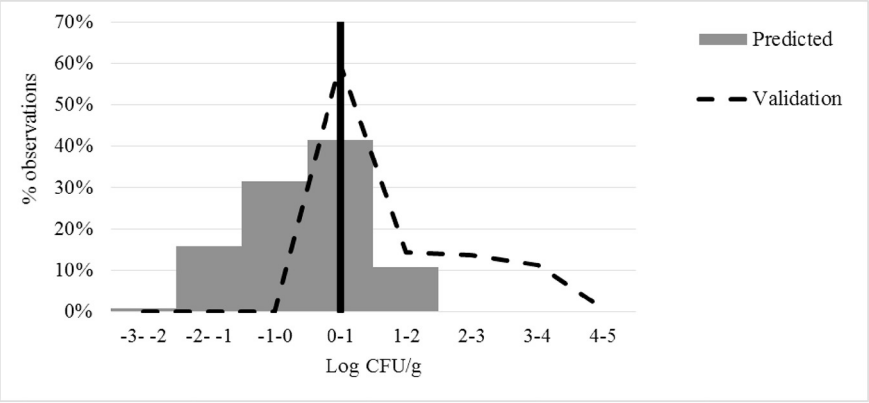

Fig. 3. Mean predicted concentrations of bacteria on lettuce at harvest (gray), observed validation data (De Giusti et al., 2014) (dashed black line) and the detection limit (≥0 log) (solid black line).

Table 3
Mean predicted concentrations of bacteria on lettuce after different post-harvest treatments (CFU/g). P1, P2 and P3 refers to the three different post-harvest cleaning processes (i.e. P1 washed with water, P2 washed the chlorine solution and P3 rapidly cooled with water).

Surface water site	Post-harvest treatment	SAR (CFU/g)	MAR (CFU/g)	PAR (CFU/g)
Lake-rural	P1	1.60E−01	6.42E−02	4.74E−02
River-rural	P1	4.90E+01	1.53E+01	1.49E+01
River-urban	P1	8.77E+01	5.05E+01	2.47E+01
Lake-rural	P2	4.10E−03	2.13E−03	1.16E−03
River-rural	P2	1.73E+00	4.53E−01	6.96E−01
River-urban	P2	2.23E+00	3.82E+00	1.38E+00
Lake-rural	P3	1.36E−01	3.86E−02	2.82E−02
River-rural	P3	3.02E+01	1.24E+01	9.49E+00
River-urban	P3	4.97E+01	3.85E+01	1.32E+01

treatments. Increasing bacterial concentrations after processing could be caused by the type of water used to clean the lettuce. According to a study by Holvoet et al. (2014) most farmers could not guarantee the quality of water used in rinsing lettuce after harvest, this could be a crucial step in contributing additional contamination to the crop. During commercial production of fresh fruit and vegetables, water used for cleaning is often reused and recycled and this can result in cross contamination (Ruiz-Cruz et al., 2007; Luo et al., 2011). It is also important to consider the possibility of delicate lettuce plants becoming physically damaged through commercial processing. The damage and openings on the plant may provide areas of protection for the bacteria when exposed to cleaning procedures and this may reduce cleaning processes efficiency and provide areas on the plant for bacterial growth (Gleeson and O'Beirne, 2005; WHO, 2008). Therefore, the increase of *E. coli* concentrations after P1 and P3 post-harvest treatments could have been a result of cross contamination and damage caused to the lettuce

Table 4
Mean human exposure to AR-*E. coli* through the consumption of lettuce irrigated with water for the different scenarios (CFU/100 g). P1, P2 and P3 refers to the three different post-harvest cleaning processes (i.e. P1 washed with water, P2 washed the chlorine solution and P3 rapidly cooled with water).

Location	Expiry date	Post-harvest treatment	Consumer washing			No washing		
			SAR CFU/g	MDR CFU/g	PAR CFU/g	SAR CFU/g	MDR CFU/g	PAR CFU/g
Lake-rural	Before	P1	1.82E+00	7.10E−01	5.10E−01	1.54E+01	6.04E+00	4.36E+00
		P2	5.00E−02	3.00E−02	2.00E−02	3.50E−01	1.80E−01	1.60E−01
		P3	1.66E+00	4.10E−01	3.90E−01	1.19E+01	3.32E+00	3.04E+00
	On	P1	1.07E+02	4.19E+01	3.17E+01	1.02E+03	3.92E+02	2.95E+02
		P2	2.00E−02	1.00E−02	3.00E−03	1.00E−01	7.00E−02	4.00E−02
		P3	3.39E+02	1.35E+02	9.35E+01	2.72E+03	1.04E+03	7.79E+02
	After	P1	1.02E+02	4.12E+01	3.02E+01	9.11E+02	3.40E+02	2.74E+02
		P2	4.00E−02	2.00E−02	1.59E−02	3.30E−01	1.50E−01	1.10E−01
		P3	6.60E+01	2.48E+01	1.87E+01	5.41E+02	2.12E+02	1.62E+02
River-rural	Before	P1	5.72E+02	1.71E+02	1.85E+02	4.57E+03	1.41E+03	1.49E+03
		P2	3.00E+01	7.15E+00	7.20E+00	1.87E+02	5.30E+01	5.21E+01
		P3	7.48E+02	1.16E+02	1.11E+02	4.70E+03	8.74E+02	8.76E+02
	On	P1	3.46E+04	1.07E+04	1.00E+04	3.11E+05	9.20E+04	8.74E+04
		P2	6.44E+00	1.32E+00	2.12E+00	4.75E+01	1.12E+01	1.43E+01
		P3	9.12E+04	2.70E+04	3.27E+04	7.46E+05	2.31E+05	2.54E+05
	After	P1	3.03E+04	1.02E+04	9.89E+03	2.53E+05	8.20E+04	8.39E+04
		P2	2.25E+01	3.48E+00	9.08E+00	2.63E+02	2.89E+01	1.15E+02
		P3	2.16E+04	7.22E+03	7.92E+03	1.70E+05	5.79E+04	6.01E+04
River-urban	Before	P1	9.10E+02	5.72E+02	2.59E+02	7.54E+03	4.84E+03	2.22E+03
		P2	2.78E+01	1.34E+01	1.04E+01	2.24E+02	1.06E+02	7.22E+01
		P3	8.35E+02	6.15E+02	2.54E+02	6.14E+03	4.17E+03	1.71E+03
	On	P1	5.78E+04	3.28E+04	1.49E+04	5.27E+05	2.90E+05	1.26E+05
		P2	5.04E+00	2.77E+00	1.25E+00	4.01E+01	2.22E+01	9.97E+00
		P3	1.70E+05	9.16E+04	5.11E+04	1.35E+06	7.28E+05	3.84E+05
	After	P1	5.20E+04	3.08E+04	1.49E+04	4.29E+05	2.58E+05	1.22E+05
		P2	2.69E+01	1.11E+01	1.36E+01	2.48E+02	9.68E+01	1.79E+02
		P3	4.12E+04	2.43E+04	1.09E+04	3.13E+05	1.89E+05	9.03E+04

Table 5
Probability of illness (P_{ill}) from exposure to pathogenic antibiotic sensitive *E. coli* (PAS) for the different scenarios (100 g^{-1}). P1, P2 and P3 refers to the three different post-harvest cleaning processes (i.e. P1 washed with water, P2 washed the chlorine solution and P3 rapidly cooled with water).

Y	Before expiry date						On expiry date		
$C_{washing}$	Washing			No washing			Washing		
x	P1	P2	P3	P1	P2	P3	P1	P2	P3
Lake-rural	1.97E−07	4.90E−09	9.47E−08	1.80E−06	4.29E−08	7.02E−07	8.71E−06	1.46E−09	2.43E−05
River-rural	3.24E−05	6.30E−07	1.35E−05	2.52E−04	4.72E−06	1.05E−04	1.24E−03	2.91E−07	2.82E−03
River-urban	3.25E−05	4.90E−07	1.85E−05	2.71E−04	4.00E−06	1.42E−04	1.63E−03	3.83E−07	3.30E−03
Y	On expiry date			After expiry date					
$C_{washing}$	No washing			Washing			No washing		
x	P1	P2	P3	P1	P2	P3	P1	P2	P3
Lake-rural	7.41E−05	9.64E−09	2.04E−04	9.29E−06	6.30E−09	6.90E−06	7.51E−05	5.04E−08	5.36E−05
River-rural	8.85E−03	1.84E−06	1.64E−02	1.35E−03	6.52E−07	8.80E−04	9.50E−03	8.14E−06	5.45E−03
River-urban	1.13E−02	2.55E−06	1.88E−02	1.67E−03	1.56E−06	1.04E−03	1.15E−02	1.03E−05	6.43E−03

through the production process.

3.2. Human exposure and dose response

The overall predicted mean human exposure levels to SAR, MDR and PAR ranged between 3.00×10^{-3} and 1.35×10^{6} CFU/100 g of lettuce consumed (Table 4). The mean probability of illness from exposure to PAS *E. coli* ranged from 1.46×10^{-9} to 1.88×10^{-2} per 100 g (Table 5). In order for 1 person to become ill out of 10,000 people, *E. coli* concentrations would need to be 3 log CFU/100 g on the lettuce. Pang et al. (2017) investigated the probability of illness from the human consumption of lettuce contaminated with the pathogenic *E. coli* O157:H7 that can cause severe illness in humans. The study examined various scenarios and found that when the lettuce was cleaned with chlorine the mean probability of illness per 85 g serving was 7.8×10^{-9} (Pang et al., 2017). In comparison to the results found by this study where the mean probability of illness levels from the consumption of lettuce (100 g) treated with chlorine and contaminated with pathogenic *E. coli*, levels ranged between 4.29×10^{-8} to 4.72×10^{-6} (varied as a result of the irrigation water used). The lowest human exposure levels and probability of illness resulted when lettuce was irrigated with rural lake water, cleaned with a chlorine solution after harvest, consumed on the expiry date with additional consumer washing. The reducing capabilities of using chlorine as a post-harvest treatment and storing the lettuce at 4 °C (from after post-harvest treatment to the point of consumption) can be examined from the results. The AR-*E. coli* concentrations reduced further after the cleaning process and are lower on the expiry date compared to before the expiry date of the lettuce. Similar results were reported by Doering et al. (2009), who reported a 75% further reduction of *E. coli* after using a chlorine treatment and storing the lettuce at 4 °C for 5 days. The storage temperature of lettuce after treatment can have a significant effect on contamination levels, therefore, it is important to maintain a maximum storage temperature of 5 °C to prevent bacterial growth on the lettuce after post-harvest cleaning treatments (FDA, 2010). The mean highest human exposure levels were experienced for the scenario when lettuce was irrigated with the urban river water, washed with water and rapid cooling process, consumed on the expiry date with no consumer washing. The water from the urban river had the highest microbial contamination levels and this highlights the importance of using a clean water supply for irrigation. Njage and Buys (2017) examined the human exposure to extended spectrum beta lactamase (ESBL) *E. coli* through the consumption of lettuce in South Africa. It was found that the most effective way to reduce human exposure to ESBL *E. coli* was by reducing the contamination level in the irrigation water. It may be necessary to use a water treatment at particular source water sites, this could significantly reduce the human exposure to AR-*E. coli* through the consumption of lettuce. AR-*E. coli* concentrations could be reduced by approximately 70% using sand filtration, 97% using chlorination and 99% using a UV system (O'Flaherty et al., 2018).

3.3. Sensitivity analysis

A sensitivity analysis was performed to investigate the effect of different input parameters on the final output data. As shown in Fig. 4, the sensitivity analysis examined human exposure to SAR *E. coli* irrigated with water from an urban river, cleaned with a chlorine solution, consumed before the expiry date with consumer washing. A positive correlation coefficient was seen for the concentration of SAR *E. coli* found at the surface water site ($SAR_{river\text{-}urban}$, 0.35) and the water attachment to the lettuce ($Water_{attach}$, 0.13), again showing the importance of using a clean water supply for irrigation. The positive correlation coefficient value for water attachment show that the sprinkler irrigation systems used at the study sites could be contributing contamination to the lettuce. There is evidence to suggest that sprinkler irrigation contributes more bacterial contamination to lettuce in

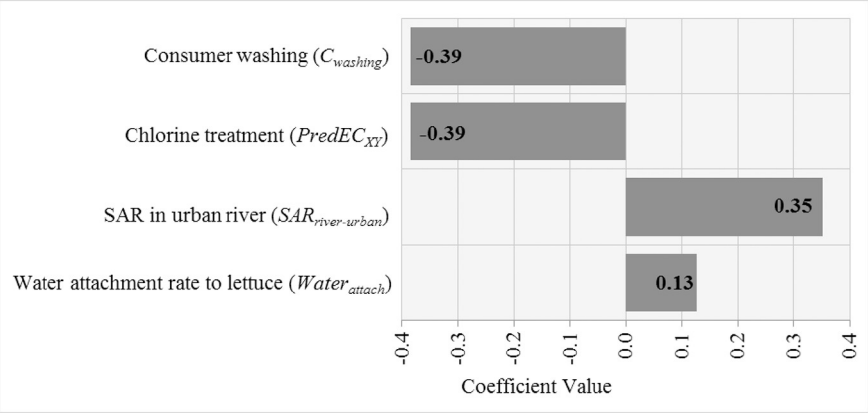

Fig. 4. Sensitivity analysis of human exposure to SAR *E. coli* through lettuce irrigated with urban river water, washed with a chlorine solution (post-harvest treatment) and additional washing by the consumer.

comparison to furrow and drip irrigation systems (Fonseca et al., 2011). Using an irrigation system that provides sufficient hydration to the lettuce but minimises the contact of water with the upper eatable part of the lettuce will help to reduce contamination. It is also important to mention the distribution system from where the water is abstracted from to where the water is released for irrigation purposes may have an effect on bacterial concentrations. Research has shown there can be an influence from distribution systems on bacterial concentrations (Bai et al., 2015; Garner et al., 2018; Liu et al., 2018), however, there was not enough quantifiable data on AR-*E. coli* to include this in the model.

In this study only the adhesion of bacteria onto the lettuce was included. However, it has also been suggested that there could be a risk to human health through the consumption of crops that can absorb ARB and ARGs through soil and water (Christou et al., 2017). Internalisation of bacteria into lettuce tissue is a concern and there is evidence showing it is possible for *E. coli* to be internalised into lettuce tissue (Solomon et al., 2002; Franz et al., 2007; Hirneisen et al., 2012; Wright et al., 2013). When *E. coli* is internalised by lettuce this makes sanitizers or washing techniques ineffective at removing them (Seo and Frank, 1999; Franz et al., 2007). There is very little research done on the quantity of bacteria that could be absorbed by the lettuce plant, however, it was examined by Wright et al. (2013) who found that 0.5% of the total *E. coli* exposed to the lettuce could be internalised within the lettuce tissue. The *E. coli* absorption potential was not included in the model for the this study due to the lack of research done in this area and even with the low *E. coli* absorption quantity reported by Wright et al. (2013), this quantity would not have had a significant effect on the final human exposure results. The negative correlation values for the chlorine post-harvest treatment ($PredEC_{xy}$, −0.39) and consumer washing ($C_{washing}$, −0.39) show these parameters can significantly reduce the bacterial contamination level on lettuce. As mentioned previously the chlorine post-harvest treatment provided the largest reduction of *E. coli* on lettuce in comparison to the other cleaning treatments. Also a simple but effective step such as cleaning the lettuce at home with water before consumption could significantly reduce the human exposure to AR-*E. coli*.

3.4. Back calculation

As highlighted from the human exposure and sensitivity analysis results, the contamination concentration in the irrigation water has a significant effect on the quantity of AR-*E. coli* humans can be exposed to through lettuce consumption. A back calculation was performed to investigate the quantity of contamination required in the irrigation water for the EC No 1441/2007 regulation to be exceeded (i.e. 1001 CFU/g of *E. coli* on the lettuce after post-harvest treatment). The results show P2 (chlorine solution) treatment required the largest amount of *E. coli* contamination (4.8 log CFU/ml) in the irrigation water to exceed the regulation. This was followed by P3 (3.1 log CFU/ml) and P1 (2.7 log CFU/ml). Again the results show P2 is the preferred post-harvest treatment for lettuce as high levels of contamination are required in the irrigation water. WHO recommend performing local risk assessments in order to define safety guidelines for irrigation water quality (WHO, 2006). The results found through this study could contribute to defining maximum permissible guidelines based on the post-harvest treatments used by producers.

4. Conclusion

A quantitative human exposure assessment model was created to examine the exposure to AR-*E. coli* through the consumption of lettuce irrigated with surface water. The results found from this study highlight several actions that can be taken to reduce the risk of human exposure to AR-*E. coli* through the consumption of this food source. For example, using a water treatment to reduce bacterial concentrations in irrigation water, using an irrigation system that minimises water contact with the eatable part of the lettuce, using a chlorine based post-harvest treatment and encouraging consumers to wash lettuce as an extra precaution. Considering the whole RTE salad production process, it is important to implement guidelines that are based on different treatment scenarios so that producers are aware of what processes ensure the highest safety of the final product, especially if the water supply used for irrigation is potentially contaminated. Within each step of the lettuce production process improvements can be made to help reduce human exposure to PAR *E. coli* and help prevent long term health implications associated with the human exposure to ARB. The information generated from this model could help set guidelines for producers on maximum permissible contamination levels in irrigation water. Additionally, the model provides recommendations on the best post-harvest practices to use and also the benefits of additional household washing prior to lettuce consumption. This work contributes to filling in the current research gap on the possible human exposure to AR-*E. coli*, and in general, to ARB through environmental sources.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2018.11.022.

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