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Integrated approach for legionellosis risk analysis in touristic-recreational facilities

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ABSTRACT

Legionellosis is a severe pneumonia caused by the inhalation of aerosols containing *Legionella*, Gram-negative bacteria present in the water systems of touristic-recreational facilities. The purpose of this study was to develop a scoring tool to predict the risk of both environmental contamination and Legionnaires' disease cases in such facilities in the Apulia region of southern Italy. We analyzed 47 structural and management parameters/risk factors related to the buildings, water systems, and air conditioning at the facilities. A Poisson regression model was used to compute an overall risk score for each facility with respect to three outcomes: water samples positive for *Legionella* (risk score range: 7–54), water samples positive for *Legionella* with an average load exceeding 1000 colony-forming units per liter (CFU/L) (risk score range: 22–179,871), and clinical cases of Legionnaire's disease (risk score range: 6–31). The cut-off values for three outcomes were determined by receiver operating characteristic curves (first outcome, samples positive for *Legionella* in a touristic-recreational facility: 19; second outcome, samples positive for *Legionella* in a touristic-recreational facility with an average load exceeding 1000 CFU/L: 2062; third outcome, clinical cases of Legionnaire's disease in a touristic-recreational facility: 22). Above these values, there was a significant probability of observing the outcome.

We constructed this predictive model using 70% of a large dataset (18 years of clinical and environmental surveillance) and tested the model on the remaining 30% of the dataset to demonstrate its reliability. Our model enables the assessment of risk for a touristic facility and the creation of a conceptual framework to link the risk analysis with prevention measures.

1. Introduction

Legionellosis can develop after inhalation of aerosols containing *Legionella*, Gram-negative bacteria that colonize natural (e.g., rivers, lakes, and ponds) and artificial water environments (e.g., potable water systems, taps, faucets, showers, cooling towers, fountains) (Cassell et al., 2018; De Giglio et al., 2020). The different clinical forms of legionellosis range from severe pneumonia known as Legionnaires' disease (LD), to a flu-like illness, Pontiac fever. Among waterborne diseases, LD is a major public health concern because of its severity and poorly resolutive interventions (Felice et al., 2019). Studies have demonstrated the wide diffusion of *Legionella* in the water systems of touristic recreational facilities and subsequent cases of legionellosis in tourists and visitors (Bonetta et al., 2010; Borella et al., 2005; Erdogan and Arslan, 2007; Mouchtouri et al., 2007). In Italy, 2964 cases of legionellosis, primarily LD, were reported in 2018, representing 48.9 cases per 1 million inhabitants; of these, 298 cases (10%) were associated with travel and hotel stay (Notiziario ISS, 2019). Although a possible person-to-person

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Abbreviations: CFU, colony-forming unit.

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Table 1

Risk scores attributed to structural features of touristic-recreational facilities.

Code	Item risk factors	Response	Risk score	Brief description	References
A	Facility type	Hotel, residence Guest houses, resorts, bed and breakfast	2 1	*Legionella* is present more frequently in larger buildings because of the complexity of water system	Notiziario ISS, (2019) D'Alò et al. (2019) De Filippis et al., (2017)
B	Activity period	Year-round Seasonal	1 2	The seasonal use of the facilities promotes the proliferation of *Legionella* due to water stagnation	Völker et al., 2016 Bartram et al., (2007)
C	Facility classification	0–1 star 2–3 stars 4–5 stars	1 2 3	The number of stars assigned to the facilities is directly proportional to the size of buildings and therefore, to the extention of the water network. This leads to greater variability in water temperatures and the formation of biofilms	Borella et al., (2005) Papadakis et al., (2018) Decreto 21 ottobre, 2008
D	Facility age	<10 years 10–20 years >20 years	1 2 3	Aged plumbing can promote the proliferation of *Legionella* due to biofilm and dead legs	Ghanizadeh et al., (2016) Orsi et al., (2014)
E	Time since last renovation	<10 years 10–20 years >20 years	1 2 3	Aged plumbing can promote the proliferation of *Legionella* due to biofilm and dead legs	Borella et al., (2005)
F	Number of floors	ground floor 2–5 >5	1 2 3	The number of floors is directly proportional to the size of the buildings and, therefore, to the distribution of the water network on several levels. This can lead to variable distribution of the water temperature and the formation of biofilms	D'Alò et al., (2019) De Filippis et al., (2017)
G	Number of rooms per floor	<3 4–10 >10	1 2 3	Larger structures show greater contamination, because the hydraulic system is more complex and has a high number of water supply points	D'Alò et al. (2019) De Filippis et al., (2017) Papadakis et al., (2018)
H	Number of rooms with showers	<10% 10%–40% >40%	1 2 3	Aerosol from showers represents a potential exposure to *Legionella* if the pipes are colonized	Proctor et al., (2018) De Filippis et al., (2017)
I	Presence of wellness water services (whirlpool, sauna, swimming pool)	Yes No	2 0	Recreational use of contaminated aerosolized water can be a source of legionellosis	Leoni et al., (2018) Papadakis et al., (2018)

Table 2
Risk scores attributed to water systems of touristic-recreational facilities.

Code	Item risk factors	Response	Risk score	Brief description	References
L	Source of water supply	Deep Mixed Superficial	1 2 3	The groundwater quality is preserved by soil filtration processes, compared to superficial source	Cassell et al., (2018) De Giglio et al., (2019a)
M	Distribution network	With recirculation Without recirculation	0 3	The absence of recirculation promotes biofilm, obstruction, stagnation of the water flow	Orsi et al., (2014)
N	Recirculation type	Total Partial	1 2	The absence of a proper water recirculation system limits the treatment options adoptable in an emergency condition	Orsi et al., (2014)
O	Use of water softener	Yes No	1 2	The hardness and calcium concentration are positively correlated to *Legionella*	Kyritsi et al., (2018)
P	Use of cold water storage tanks	No Yes	1 2	The water storage or stagnation can encourage the proliferation of *Legionella*	Peter and Routledge, (2018)
Q	Number of cold water storage tanks	1–3 4–8 >8	1 2 3	Excessive water storage or stagnation can encourage the proliferation of *Legionella*	Peter and Routledge, (2018)
R	Use of covers for the cold water storage tanks	Yes No	1 2	The covers prevent the heating of the cold water which favors the proliferation of *Legionella*	De Giglio et al., (2019b)
S	Water temperature at the point of use	<20°– >50 °C 20°–50 °C	0 2	The optimal growth temperature for *Legionella* is 20°–50 °C	De Giglio et al., (2021) Orsi et al., (2014)
T	Presence of treatment systems upstream of water heaters	Yes No	1 2	Water heaters can be colonized by *Legionella* when the water temperature is optimal for its growth	Lecointe et al., 2019 Orsi et al., (2014)
U	Use of hot water storage tanks	No Yes	1 2	*Legionella* probably concentrates on the bottom of the tank, where the temperature is a little lower	Wadowsky et al., (1982)
V	Means of heating water	Storage tanks Other	2 1	Water stagnation favorable to the proliferation of *Legionella* may be present in the storage tanks	Wadowsky et al., (1982) HSE, (2014a)
Z	Use of good practices to prevent legionellosis	Yes No	0 5	Good general hygiene practices and interventions to minimize exposure to specific risks are the foundation of all the prevention activities	De Filippis et al., (2017)
A1	Use of cleaning and sanitizing protocol	Yes No	0 5	Cleaning and sanitizing of the water network reduce the risk factors for legionellosis	Garrison et al., (2016) HSE, (2014a)
A2	Periodic inspection, cleaning, and disinfection of storage tanks	Yes No	0 2	The risk of *Legionella* contamination is foreseeable in the storage tanks of the water system if there are deposits that promote bacterial growth, such as rust, mud, scale and organic matter	Garrison et al., (2016) HSE, (2014a)
A3	Frequency of inspection, cleaning, and disinfection of storage tanks	0–3 months 4–6 months > months	0 1 2	More frequent are the preventive actions, lowest is the risk of legionellosis	Garrison et al., (2016) HSE, (2014a)
A4	Use of a register to record actions involving storage tanks	Yes No	0 2	Compiling the register allows to track the interventions of control and prevention of legionellosis over time	Garrison et al., (2016) HSE, (2014a)
A5	Use of a calendar of actions involving storage tanks	Yes No	0 2	A timely and correct planning of actions decrease the risk of contracting legionellosis	Garrison et al., (2016) HSE, (2014a)
A6	Use of a checklist of maintenance operations on storage tanks	Yes No	0 2	A timely and correct planning of actions decrease the risk of contracting legionellosis	Garrison et al., (2016) HSE, (2014a)

(*continued on next page*)

Table 2 (*continued*)

Code	Item risk factors	Response	Risk score	Brief description	References
A7	Periodic replacement of shower heads and the jet fringes of water faucets	Yes No	0 2	The biofilm on surfaces of hydraulic system can create a biological niche for *Legionella*	Schoen and Ashbolt, (2011) De Filippis et al., (2017)
A8	Frequency of replacing shower heads and the jet fringes of water faucets	0–3 months 4–6 months >6 months	0 1 2	Increasing the frequency of management of water networks, the favorable conditions for the proliferation of *Legionella* would be avoided	Schoen and Ashbolt, (2011) De Filippis et al., (2017)
A9	Microbiological monitoring of shower heads and the jet fringes of water faucets	Yes No	0 2	Microbiological monitoring allows to control the livel of bacterial proliferation	Montagna et al., (2018) De Giglio et al. (2021)
A10	Inclusion of *Legionella* detection in microbiological monitoring	Yes No	0 1	Legionella detection is one of the key elements to identify potential sources of infection	Garrison et al. (2016) Montagna et al., (2018)
A11	Use of a register of actions involving shower heads and the jet fringes of water faucets	Yes No	0 2	Compiling the register allows to track the interventions of control and prevention of legionellosis over time	Proctor et al., (2018)
A12	Use of a calendar of actions involving shower heads and the jet fringes of water faucets	Yes No	0 2	A timely and correct planning of actions decrease the risk of contracting legionellosis	Proctor et al., (2018)
A13	Use of a checklist of maintenance operations involving shower heads and the jet fringes of water faucets	Yes No	0 2	A timely and correct planning of actions decrease the risk of contracting legionellosis	Proctor et al., (2018)

transmission has been reported (Correia et al., 2016), environmental exposure is the main source of infection (Montagna et al., 2016; Proctor et al., 2018; Nocker et al., 2020).

Recently, the new European Drinking Water Directive introduces also *Legionella* among the microbiological parameters to be detected in the water network of health and community facilities (Direttiva [EU] 2020/2184, 2020). In Italy, the national guidelines for the control of legionellosis (Linee Guida per la Prevenzione e) recommend an environmental investigation to identify the source of the infection and disinfection of the water supply by the contamination threshold and/or the percentage of positive samples.

However, the scientific literature shows that counts of colony forming units per liter (CFU/L) do not provide the true estimate of the risk of infection, as the concentration of *Legionella* in the water network is not constant over time and for the lack of a reliable dose–response model (Napoli et al., 2009; Völker et al., 2016). The variability of *Legionella* load can depend by various factors, as the presence of viable cells but non culturable suggesting that the water network is a highly dynamic system (De Giglio et al., 2019a; Montagna et al., 2018; Völker et al., 2016).

Therefore, an accurate risk assessment should integrate microbiological evidence and antibiotic resistance studies to manage *Legionella* contamination in water systems (De Giglio et al., 2015, 2021). Such an assessment has been shown to rapidly identify the main environmental risk factors and to predict positive findings of *Legionella* (Hadjichristodoulou et al., 2006; Napoli et al., 2010; Papadakis et al., 2018). It is known that *Legionella* is dependent on various characteristics of the facilities (e.g. activity period and number of floors and/or rooms), and of water supply as stagnation, temperature between 25 and 55 °C, increased biofilm formation, age and pipe materials (Borella et al., 2005; D'Alò et al., 2019). Among the air conditioning systems, cooling tower systems represent an important role in the spread of *Legionella* because they have a large volume of water open to the atmosphere, with ideal conditions for microbial growth (elevate temperature, continuous aeration, and sunlight) (Iervolino et al., 2017).

As there is currently no standardized tool for such a risk assessment, the purpose of the present study was to design and validate a scoring tool using clinical and environmental surveillance data to predict whether a facility would be at risk of both environmental contamination and legionellosis.

2. Materials and methods

The study was conducted in the Apulia region of southern Italy, where the Regional Epidemiology Center has been tasked since 2001 with collecting clinical and environmental data relating to legionellosis into a specific database.

2.1. Study design

A census was conducted at the beginning of the study to establish the number of municipalities in the region that were equipped with touristic-recreational facilities between 2001 and 2018. Overall, 257 municipalities were identified over an area of 19,347 km^2; among these, 68 were located along a coastline of over 300 km and hosted 627 touristic-recreational facilities. In the same period, these 627 facilities accounted for 75% of all tourist arrivals in the Apulia region (Portale dell'Agenzia Regionale del Turismo Puglia Promozione, 2021). Assuming a response rate of 50%, 95% confidence level, and 5% margin of error, a sample of at least 238 facilities was required to be considered representative in the study.

Forty seven parameters were selected for analysis from evaluations conducted by public health professionals, hygienists, epidemiologists, and engineers and from previous studies carried out in the same geographical area (Napoli et al., 2010; Montagna et al., 2018). These consisted of nine structural parameters, 25 factors related to water

Table 3
Risk scores attributed to air conditioning systems of touristic-recreational facilities.

Code	Item risk factors	Response	Risk score	Brief description	References
A14	Presence of humidification section	Yes No	2 1	*Legionella* may proliferate in stagnant water within water humidifier followed by the generation of contaminated aerosols by this device.	Moran-Gilad et al., (2012)
A15	Humidifier type	Vapor Other	1 2	The vapor humidifier produces modest water droplets such as not to be a vehicle for *Legionella*	Moran-Gilad et al., (2012)
A16	Use of antibacterial substances	Yes No	0 2	The regular reclamation prevent legionellosis risk	Moran-Gilad et al., (2012)
A17	Use of droplet separator	Yes No	0 2	The extent of released aerosol depends on the efficiency of droplet separation and other technical traits aiming at droplets retention	Nocker et al., (2020)
A18	Periodic reclamation of air conditioning system	Yes No	0 2	Disinfection operations must be determined by an assessment of the fouling potential favoring *Legionella* contamination.	HSE, (2014b)
A19	Use of split systems	No Yes	0 2	Split systems can accumulate dust and bacteria	European Agency for Safety and Health at Work, (2011)
A20	Periodic inspection, cleaning, and disinfection of splits	Yes No	0 2	Regular and punctual maintenance of the split systems reduce the legionellosis risk	European Agency for Safety and Health at Work, (2011)
A21	Frequency of inspecting, cleaning, and disinfecting splits	<3 months 3–6 months >6 months	0 1 2	Regular and punctual maintenance of the split systems reduce the legionellosis risk	European Agency for Safety and Health at Work, (2011)
A22	Use of cooling towers	No Yes	0 2	Cooling towers provide a favorable environment for the proliferation and spread of *Legionella*	Iervolino et al., (2017) Llewellyn et al., (2017) McCormick et al., (2012) Carducci et al., (2010)
A23	Type of circuit in cooling towers	Closed Open	0 2	Closed cooling towers do not put process water in contact with air because they have a closed system, clean and free from possible contamination	
A24	Use of antibacterial substances in cooling towers	Yes No	0 2	The biocides used to decontaminate the cooling towers are effective in controlling *Legionella* pollution	Carducci et al., (2010) Iervolino et al., (2017)
A25	Microbiological monitoring of air conditioning system	Yes No	0 2	Microbiological monitoring of air allows to control the livel of bacterial proliferation	HSE, (2014b)
A26	Inclusion of *Legionella* detection in microbiological monitoring of air conditioning system	Yes No	0 1	Although *Legionella* detection in the air is not a routine investigation, it can be used in the validation of disinfection methods	HSE, (2014b)

systems, and 13 related to air conditioning systems in the facilities. These data were obtained from official reports filed during microbiological control of the water supply conducted during the study period (2001–2018). An individual risk score, computed from data in the scientific literature regarding risk factors for LD, was assigned to each parameter.

Data on environmental surveys and cases of LD associated with touristic-recreational facilities were obtained from the Regional Epidemiology Center and used in the predictive models of the risk analysis.

2.2. Data analyses

Data analysis was conducted in four steps. In the first step, data from the scientific literature were used to assign an individual risk score to each response for each parameter selected by the panel of experts. In the second step, the dataset obtained from the touristic-recreational facilities was split into two parts (70% and 30%) according to a randomized method reported previously (Xu and Goodacre, 2018). In this step, data on the structural (n. = 9) and water system (n. = 25) features from 70% of the selected facilities were used to determine risk factors through preliminary multivariate analyses associated with three outcomes:

1. Samples positive for *Legionella* in a touristic-recreational facility.
2. Samples positive for *Legionella* in a touristic-recreational facility with an average load exceeding 1000 CFU/L.
3. Clinical cases of LD in a touristic-recreational facility.

The parameters/risk factors related to air conditioning systems were associated (n. = 13) only with the third outcome (clinical cases of LD in a touristic-recreational facility).

We used Poisson regression according to previously applied methodologies (Conza et al., 2014; De Giglio et al., 2019b). Subsequently, only the parameters/risk factors with a *p*-value <0.05 were included in the final model.

The predictions of the final model of Poisson regression for each parameter under study were used to compute an overall risk score per outcome for each touristic-recreational facility, applying the following formula (Cui et al., 2006): $e^{(\alpha+\beta 1X1+\ \beta 2X2+..+\ \beta kXk)}$, where

α = intercept of the model
β = coefficient of the regression model for each risk factor
X = score of each risk factor

In the third step, receiver operating characteristic (ROC) curves were used to compare the risk score identified in the second step for each outcome with their real occurrence. Next, a cut-off value of the risk score for each outcome was determined. Touristic-recreational facilities with a risk score over the cut-off value were considered at risk for the occurrence of the outcome.

In the fourth step, we tested the reliability of the model on the remaining 30% of the dataset from selected facilities (Xu and Goodacre, 2018) as follows:

1. Evaluation of the parameters selected by the final Poisson regression model developed in the second step.

Table 4
Association between specific parameters and water samples positive for *Legionella*.

Risk factor code (listed in Tables 1 and 2)	β	*p*-value
Intercept (α)	10.35378	<0.001
A	0.85984	<0.001
B	0.60602	<0.001
V	0.45157	<0.001
A7	0.37263	<0.001
D	0.36537	<0.001
A5	0.363	<0.001
A6	0.35719	<0.001
I	0.35487	<0.001
A2	0.32301	<0.001
A12	0.28461	<0.001
A1	0.24304	<0.001
E	0.22555	<0.001
A3	−0.26556	<0.001
A9	−0.2958	<0.001
A8	−0.30761	<0.001
Z	−0.37673	<0.001
U	−0.50662	<0.001
A4	−0.57112	<0.001
H	−0.58971	<0.001
G	−0.77249	<0.001
P	−0.82026	<0.001

Table 5
Association between specific parameters and water samples positive for *Legionella* when the average load exceeded 1000 colony-forming units per liter.

Risk factor code (listed in Tables 1 and 2)	β	*p*-value
Intercept (α)	12.7304	<0.001
Q	3.006714	<0.001
A5	1.345379	<0.001
D	1.079889	<0.001
R	0.864863	<0.001
A13	0.831079	<0.001
V	0.774589	<0.001
A11	0.770297	<0.001
O	0.574374	<0.001
A1	0.437369	<0.001
L	0.379814	<0.001
F	0.36049	<0.001
C	0.24795	<0.001
A9	0.137426	<0,001
A12	0.109058	<0.001
A3	0.045603	<0.001
S	0.037794	<0.001
T	−0.07114	<0.001
A6	−0.16148	<0.001
A4	−0.23761	<0.001
A7	−0.26495	<0.001
U	−0.28255	<0.001
A8	−0.32149	<0.001
G	−0.32852	<0.001
A2	−0.3333	<0.001
B	−0.47716	<0.001
Z	−0.53962	<0.001
A	−0.638538	<0.001
E	−0.70117	<0.001
H	−1.41208	<0.001
P	−2.91005	<0.001

2. Classification of each touristic-recreational facility by cut-off values and the three risk scores identified in the third step. This enabled the classification of the touristic-recreational facilities as “at risk” or “not at risk” of being associated with the occurrence of each outcome.
3. Evaluation of the association, for each touristic-recreational facility, between model predictions and observed data for the three outcomes.

Table 6
Association between specific parameters and cases of LD in touristic-recreational facilities.

Risk factor code (listed in Tables 1–3)	β	*p*-value
Intercept (α)	4.12137	<0.001
Q	0.67788	<0.001
L	0.24186	<0.001
A9	0.20287	<0.001
A4	0.14513	<0.001
D	0.12569	0.034705
Z	0.07369	0.006175
A1	−0.0659	<0.001
V	−0.20986	0.006146
A11	−0.31265	<0.001
A22	−0.32601	0.036608
T	−0.47987	<0.001
P	−1.04759	<0.001

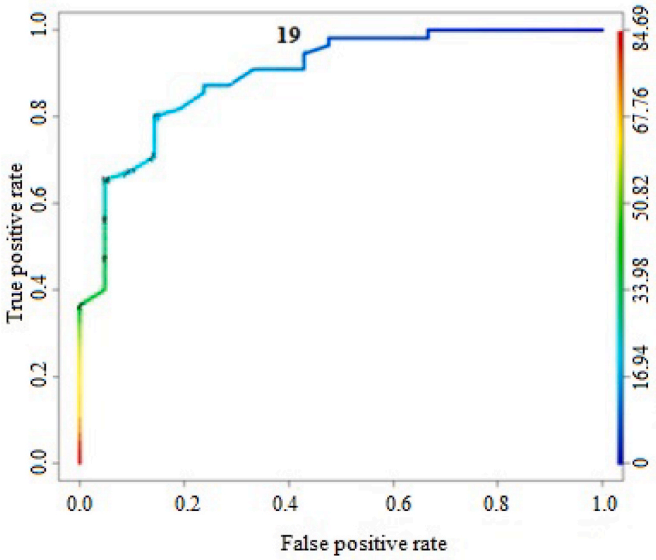

Fig. 1. ROC curve for the occurrence of water samples positive for *Legionella*.

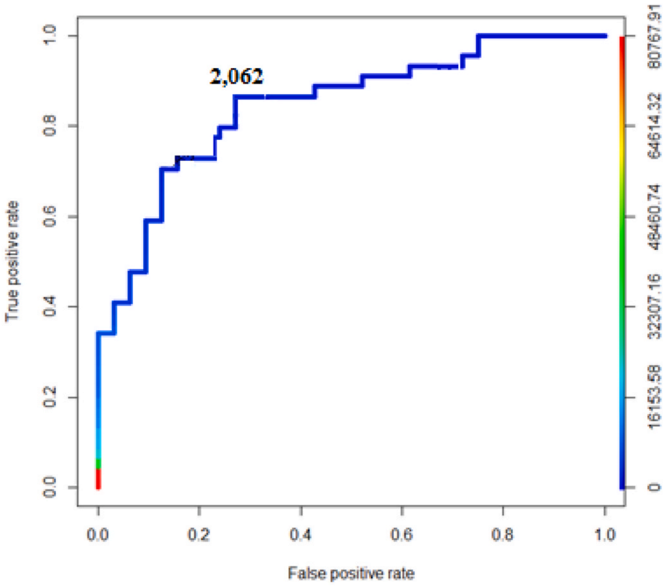

Fig. 2. ROC curve for the occurrence of water samples positive for *Legionella* with an average load exceeding 1000 colony-forming units per liter.

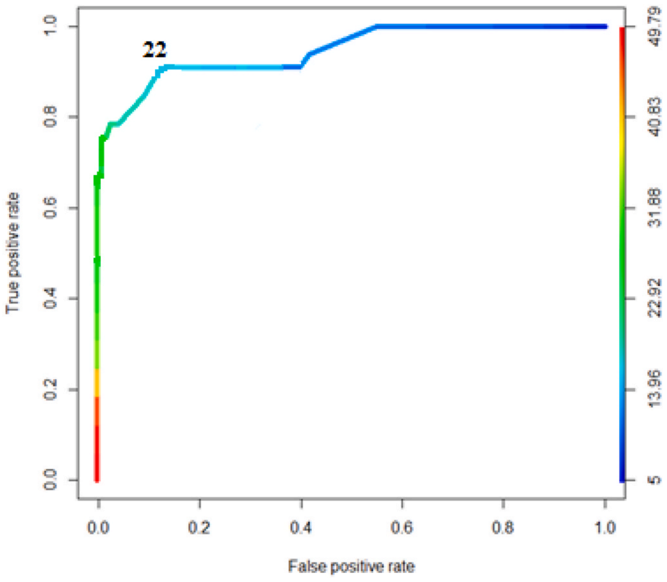

Fig. 3. ROC curve for the occurrence of cases of LD.

R version 3.6.1 was used to perform the statistical tests.

3. Results

3.1. First step

Tables 1–3 list the risk score assigned to each response for each parameter using data from the literature.

3.2. Second step

The microbiological findings in 4153 water samples collected between 2001 and 2018 from 238 touristic recreational facilities were used in the analysis. Tables 4–6 list the results of three final Poisson regression models constructed from data from 70% of the selected tourist-recreational facilities (2898 water samples from 167 facilities). These models were used to compute an overall risk score for each facility with respect to the three outcomes. The overall risk score had a range of 7–54 for the first outcome (samples positive for *Legionella* in the facility), 22–179,871 for the second outcome (samples positive for *Legionella* that had an average load exceeding 1000 CFU/L), and 6–31 for the third outcome (clinical cases of LD in the facility).

Regarding facility type (parameter A), hotels and residences appear to be an influencing factor for findings of *Legionella* in water (first outcome), while guest houses, resorts, and bed and breakfast facilities influenced the second outcome (findings of *Legionella* with an average load exceeding 1000 CFU/L). In terms of the activity period (parameter B), seasonal use appeared to be a risk factor for findings of *Legionella* in water, while facilities used all throughout the year were associated with findings of *Legionella* with an average load exceeding 1000 CFU/L.

When considering the classification of tourist-recreational facilities by the number of stars assigned by the tourism organization (parameter C), the risk that the average load of *Legionella* in water samples would exceed 1000 CFU/L increased in directly proportional manner. An increase of one star in the rating corresponded to a 22% increase in risk.

Facility age (parameter D) increased the risk of *Legionella* and clinical cases of LD. In particular, the risk of *Legionella* with an average load exceeding 1000 CFU/L increased by 194.4% between facility age categories. Long time since the last renovation (parameter E) also increased the risk of *Legionella*, with a 25.3% increase between categories. However, a recent renovation appeared to favor *Legionella* with a load exceeding 1000 CFU/L. Facilities with a superficial water supply (parameter L) were more at risk with respect to both *Legionella* load and clinical cases. Cooling towers (parameter A22) were associated with an increase in cases of LD.

3.3. Third step

The risk scores for each outcome (i.e. positivity of water samples for *Legionella*, average load for each facility, and cases of LD) were calculated for each facility and compared with the observed data from environmental and clinical monitoring. Cut-off values (first outcome: 19; second outcome: 2062; third outcome: 22) were identified through the ROC curves (Figs. 1–3) assuming the sensitivity and specificity levels reported in Table 7 for 70% of the touristic-recreational facilities. Above these values, there was a significant probability of observing the

Table 7
Cut-off values of risk score and methodological sensitivity and specificity of the outcomes under study in 70% of the dataset.

Outcome	Cut-off value	Sensitivity (calculated as true positives divided by true positives plus false negatives)	Specificity (calculated as true negatives divided by true negatives plus false positives)	Accuracy (True Positive + True Negative)/(True Positive + True Negative + False Positive + False Negative)
Water samples positive for *Legionella*	19	92.4% 121/121 + 10	58.3% 21/21 + 15	85.0% (121 + 21)/(121 + 21+15 + 10)
Water samples with a *Legionella* average load exceeding 1000 colony-forming units per liter	2062	86.4% 70/70 + 11	69.8% 60/60 + 26	77.9% (70 + 60)/(70 + 60+26 + 11)
Cases of Legionnaires' disease	22	90.5% 19/19 + 2	89.0% 130/130 + 16	89.2% (19 + 130)/(19 + 130+16 + 2)

Table 8
Cut-off values of risk score and methodological sensitivity, specificity and accuracy of the outcomes under study in 30% of the dataset.

Outcome	Cut-off value	Facility risk scores (range)	Sensitivity (calculated as true positives divided by true positives plus false negatives)	Specificity (calculated as true negatives divided by true negatives plus false positives)	Accuracy (True Positive + True Negative)/(True Positive + True Negative + False Positive + False Negative)
Water samples positive for *Legionella* spp.	19	7–54	87.7% 50/50 + 7	64.3% 9/9 + 5	83.1% (50 + 9)/(50 + 9+5 + 7)
Water samples with a *Legionella* average load exceeding 1000 colony-forming units per liter	2062	25–125,633	86.5% 32/32 + 5	67.86% 23/23 + 11	77.5% (32 + 23)/(32 + 23+11 + 5)
Cases of Legionnaires' disease	22	6–27	90.0% 9/9 + 10	88.5% 54/54 + 7	88.7% (9 + 54)/(9 + 54+7 + 1)

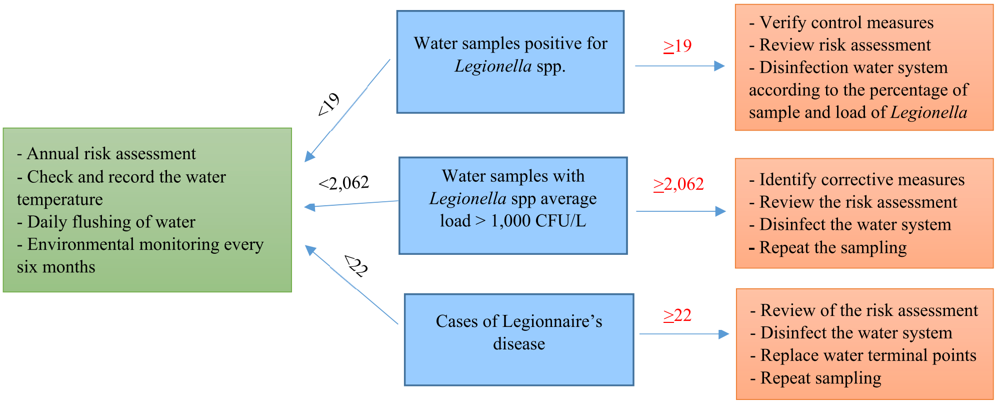

Fig. 4. Conceptual framework for appropriate action based on risk scores.

outcome.

3.4. Fourth step

The reliability test for the model, developed using the remaining 30% of the touristic-recreational facilities studied (1255 water samples from 71 structures) yielded good sensitivity for the purpose of a correct classification of the risk with respect to the three outcomes (Table 8).

The results obtained enabled the design of a conceptual framework (Fig. 4) for implementing appropriate control measures.

4. Discussion

Several studies have assessed legionellosis risk and prevention using various mathematical and statistical models, with incomplete findings. Some authors (Bargellini et al., 2011; Hadjichristodoulou et al., 2006; Kyritsi et al., 2018; Napoli et al., 2010) focused only on environmental risk factors in community settings (including hotels) and sanitary habitats, while others only estimated the clinical risk of legionellosis (dose/response assessment) using a quantitative microbial risk assessment model (Azuma et al., 2013; Bouwknegt et al., 2013). Several guidelines, including Italian ones, establish intervention types using a contamination threshold and/or percentage of water samples positive for *Legionella*, differentiating between touristic-recreational and health facilities. These guidelines also contain a checklist of the structural and managerial features of the systems (cold and hot water, cooling towers, and aeraulic condensers), but such a checklist offers only a preliminary estimate of legionellosis risk.

To our knowledge, the present study represents an innovative model as the cut-off values are specific to each outcome, enabling the prediction of risk of both environmental contamination and cases of LD. We constructed this predictive model using 70% of a large dataset (18 years of clinical and environmental surveillance) and tested the model on the remaining 30% of the dataset to demonstrate its reliability. In addition, our model simultaneously considers a large number of risk factors, quantifying the relative importance of each parameter to the outcomes under study.

Our results confirmed that large facilities (e.g. hotels and residences) appear to be a factor influencing the growth of *Legionella* in the water network. In fact, compared to small structures, their water network is more extensive with possible variable temperatures and biofilm accumulation (Borella et al., 2005; Notiziario ISS, 2019; D'Alò et al., 2019; De Filippis et al., 2017). According to some authors (Völker et al., 2016), seasonal use of facilities can also be a risk factor. In this case, water stagnation can promote the proliferation of *Legionella*, because it allows bacteria to grow, making it difficult to maintain high temperatures and concentrations of disinfectants (Bartram et al., 2007). In reality, our results showed that the structures used all throughout the year had an average *Legionella* load exceeding 1000 CFU/L. This data question variability of the bacterial load as a function of biofilm or amoebae (Napoli et al., 2009; Völker et al., 2016) that host *Legionella* and release high loads in the water network when they are sheared off (Wingender and Fleming, 2011). Furthermore, the culture methods do not detect the real load of contamination as they do not allow to highlight the viable but non-cultivable *Legionella* cells (Borella et al., 2005).

Regarding the risk related to the number of stars, the risk that average load of *Legionella* in water samples exceeded 1000 CFU/L increased in directly proportional manner to number of stars (1–5 stars). According to the Decree of October 21, 2008, the number of stars is directly related to the number of rooms with bathroom and hot and cold water. More precisely, the classification ranges from one star with the number of bathrooms equal to 40% of the rooms to five stars with the number of bathrooms equal to 100% of the rooms. Consequently, the increase in the number of bathrooms determines an increase in the extension of the water network and in the water supply points with a greater risk of *Legionella* contamination.

The value of microbiological monitoring of a water network is still controversial. Since *Legionella* is ubiquitous, its mere presence or absence does not provide a real estimate of the risk of infection (Hadjichristodoulou et al., 2006; Napoli et al., 2009, 2010). Moreover, the increased cost of laboratory investigations and the long time required to isolate *Legionella* are further disadvantages. Thus, an integrated approach for legionellosis risk analysis is necessary to identify which circumstances are favorable for the proliferation of this microorganism. Our model facilitates the planning of environmental controls and prioritization of the facilities most at risk. The model defines a proactive approach to water safety plan, as required by the World Health Organization, allowing early detection of *Legionella* in the water network (Hadjichristodoulou et al., 2006), and supports the recent European Drinking Water Directive (Direttiva [EU] 2020/2184, 2020).

Our study has some limitations. First, the predictive model was constructed from data collected over 18 years, which gives the model robustness but cannot consider changes that occurred in the facilities under study during the sampling period. Second, the number of cases of LD reported over the years is probably underestimated, reflecting a global concern because the etiological diagnosis of LD is often not possible (Napoli et al., 2010). Third, the longitudinal approach of our study showed that the water samples taken at a specific time only provides a snapshot of the microbial situation at that precise moment, therefore it is not representative of the actual situation.

However, the application of our model enabled for the first time the design of a framework for safeguarding both public health and the commercial and legal considerations of touristic facilities (La Russa et al., 2021). This tool allows an objective assessment by local authorities of appropriate control and prevention measures. For this reason, we wish to refine the model, tested on the touristic-recreational facilities of the Apulia Region, and ensure that it can be used in other infrastructural and geographical contexts.

Future developments of this research may concern the use of the multiple fold cross-validation techniques as described by other Authors (Carvajal et al., 2015). In this way it will be possible to avoid any problems, as overfitting and asymmetric sampling, typical of the division of data into two parts (i.e. training/validation) reported in our study. Furthermore, the dataset can be extended to include other factors that could be relevant in the risk analysis (for example, the climatic aspects of the territory).

5. Conclusion

Our study establishes specific cut-off values for each of three outcomes to assess the risk of *Legionella* and LD to a touristic facility. This enabled the creation of a conceptual framework to link the risk analysis to prevention measures.

Credit author statement

Osvalda De Giglio, Christian Napoli, Maria Teresa Montagna: conceptualization, methodology, validation, writing—original draft. Giusy Diella: validation, writing—original draft. Fabrizio Fasano: methodology, software, data formal analysis, writing—original draft. Marco Lopuzzo: investigation, software, data curation. Francesca Apollonio, Marilena D'Ambrosio, Carmen Campanale, Francesco Triggiano: investigation. Giuseppina Caggiano: review and editing.

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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.

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