

Risk Factor Analysis of Children's Exposure to Microbial Pathogens in Playgrounds

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Bacteria are commonly found in soil and may cause health risks to children playing in the outdoor playgrounds with soil, mainly via hand to mouth and pica behaviors. Our study concerned with the risk analysis of infection of a child playing in urban playgrounds in the cities of Patras and Pyrgos in Greece. The presence of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were analyzed in soil samples of these playgrounds. A standardized questionnaire depicted the individual characteristics of each playground and recorded risk factors in playgrounds related to bacterial infections. Furthermore, the distributions of *E. coli*, *S. aureus* and *P. aeruginosa* were analyzed in soil samples. Our results were investigated with beta-Poisson models using the Quantitative Microbial Risk Assessment wiki models to evaluate and construct a probability model of infection for each of these bacteria. The risk of infection was higher during the wet period. The risk was higher for *P. aeruginosa* infection compared to *E. coli* and *S. aureus* ones. Nevertheless, the bacterial concentration was higher for *E. coli* than *P. aeruginosa* and *S. aureus* in both wet and dry periods. Our results provide new data that could contribute in assessing the risks associated with playgrounds where children can unaware play in urban parks.

KEY WORDS: *Escherichia coli*; playground; *Pseudomonas aeruginosa*; risk assessment; soil; *Staphylococcus aureus*

1. INTRODUCTION

Playground soils are common sources and reservoirs of various infectious agents, like bacteria, parasites, and viruses, which may survive, complete their life cycle and remain there, respectively, for long time periods until ingestion occurs (Zenner, Gounel, & Chauve, 2002). Modern urbanization, human and animal (especially feline and canine) population density and composition, environmental and weather condi-

tions, in addition to the significant increase of stray dogs and the extent of the sociofinancial crisis contribute to soil constitution and contamination (Rubel & Wisnivesky, 2005). Playground soils are subject to anthropogenic and natural deposits, posing risks to human health. (Acosta, Cano, Arocena, Debela, & Martínez-Martínez, 2009).

Children are expected to be more susceptible to harmful environmental substances originating from soil than adults, because of their hand-to-mouth behavior (Hubal et al., 2000) and pica behavior (Guney, Zagury, Dogan, & Onay, 2010). Even in the 21st century and in developed countries, bacterial exposure remain an important concern for children. For that reason, in many countries, various institutions and agencies (i.e., Environmental Protection Agency) undertake the responsibility for children's

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environmental health, in order to reduce the impact of environmental exposures for children to a minimum level.

There are several studies on children's exposure to accidents (Chan *et al.*, 2015), heavy metals (Acosta *et al.*, 2009; Glorennec, Lucas, Mandin, & Le Bot, 2012; Guney *et al.*, 2010; Kwon *et al.*, 2004; Luo *et al.*, 2012; Mugoša, Đurović, Pirnat, Bulat, & Barjaktarović-Labović, 2015; Wong & Mak, 1997; Zheng, Liu, Wang, & Liang, 2010, various trace elements (De Miguel, Iribarren, Chacón, Ordoñez, & Charlesworth, 2007), and parasites (Rubel & Wisnivesky, 2005). Therefore, only a few studies have evaluated the children's exposure to bacteria (Kim, Kim, Park, Kim, & Lee, 2014; Staff, Musto, Hogg, Janssen, & Rose, 2012) concerning playgrounds.

The main objectives of our study are:

- (a) To investigate relationships between microbial contamination levels and the special characteristics of playgrounds. Ranking factor (*R*) was prespecified by the researchers as a factor that may depict each playground qualitative characteristics and was assessed based on certain criteria set upon the proposed literature.
- (b) To perform quantitative risk assessment of the probability of infection of three microbes, namely *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* for a 6- to 9-year-old child, playing in a playground for an hour per day.

This is the one of the very few studies to investigate the risk of infection on children relating to the distribution of microbes in the soil of playgrounds as well as the microbiological quality of the playground.

2. MATERIALS AND METHODS

2.1. Scenario Considered

Our study included five general steps, including: (1) exposure environments and concerns, namely outdoor environment and soil contamination, as a result of emerging contaminants, (2) hazard identification, namely relationship between hazard to adverse outcome, (3) dose response model application for each microorganism regarded as hazardous, (4) exposure assessment based on environmental concentrations, and (5) risk characterization.

In our study, a 6–9 years-old child was considered playing in the playground one hour every day, as nor-

mally most playground users are children of this age group. The basic exposure pathways of children playing in municipal playgrounds, involve random direct soil ingestion from:

Hand–mouth contact.

Hand–nose contact.

Hand–eyes contact (McKenzie, Cohen, Sehgal, Williamson, & Golinelli, 2006).

2.2. Location of the Study and Sampling

The study was carried out in playgrounds in urban areas of Patras and Pyrgos, two cities of Peloponnese, in Southern Greece, between June 2018 to March 2019, covering both dry (March–September) and wet (October–April) period. Both cities have a temperate Mediterranean climate.

Sixty out of 80 (75%) randomly selected playgrounds of the Patras and 10 (out of 15) randomly selected playgrounds of Pyrgos were selected. In each period (either wet or dry), playgrounds were sampled in the same week to avoid climatic differences between weeks. The air temperature (°C) was recorded. Twenty gram of soil was collected from five different points from each playground, to be representative, and were combined as composite sample to provide a 100 gr sample per period. Samples were sealed in sterile plastic bags and transported to the laboratory in a portable ice box of 6 °C, in less than 2 h. The soil was analyzed for *E. coli*, *P. aeruginosa*, and *S. aureus* contamination using standard ISO protocols.

The researchers determined the age of the children and the duration of stay at playground upon parents/accompanying persons witness, who were interviewed during data collection process (see Section 2.4).

2.3. Risk Factor Analysis

To perform risk factor analysis, specific data on several factors were required and obtained from literature search. Data search was run from June 2017 to December 2018, to scrutinize the whole available literature included in the following databases: PubMed, Scopus, and Web of Science core correlation. The search terms used to investigate the above-mentioned databases were “microbial risk assessment,” “QMRA,” “playgrounds AND microorganisms,” “*E. coli*,” “*P. aeruginosa*,” and “*S. aureus*,” “QMRA AND playground,” “QMRA AND *E.coli*,”

Table 1. Risk Factors Related to Bacteria Infection in a Playground

Environmental conditions	Human behavior	Climate
Drinking water disposal	Number of children	Soil temperature
Litter bins in the playground	Age of children	Soil moisture
Cleanliness of the playground	Duration of stay	Air temperature
Rusty playground equipment	Random soil ingestion-pica behavior	
Banner sign of prohibit animals on the playground entrance	Hands-eye/hands-mouth/hands-nose/hands-skin contact	
Proper playground fencing	Soil layer on children's hands	
Microorganism survival in the soil	Surface of hand contacting eye/mouth/nose/skin	
Microorganism survival in the host body	Concern for cases in the playground	

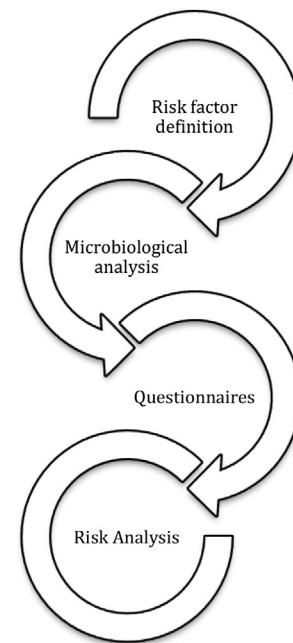
“QMRA AND *P. aeruginosa*,” and “QMRA AND *S. aureus*.” Factors, like surface characteristics were not analyzed, as only sand samples were taken. No behavioral disorders were mentioned in the questionnaires applied in this study.

According to their relative importance, *E. coli*, *S. aureus*, and *P. aeruginosa* infection of 6–9 years old aged children playing in a playground may be related to several risk factors as recorded (Table 1).

The first six parameters (drinking water disposal, litter bins in the playground, cleanliness of the playground, rusty playground equipment, banner sign of prohibit animals on the playground entrance, and proper playground fencing) reflect the quality condition of each playground tested at the time of sampling and are summarized in the so-called ranking factor (*R*) for each playground. *R* was used to categorize the quality of the selected playgrounds. All the rest risk factors related to the presence and spread of microorganisms, hence the probability of infection (P_{inf}), was included in a checklist questionnaire set and completed by the researchers.

2.4. Collection of Data

The data on the above-mentioned potential risk factors were obtained by means of a standardized questionnaire of 24 proper questions, which was conducted upon the proper Official Government Gazette (2029/B/25.7.2014) and completed by the parents/accompanying persons of the children visiting the playgrounds. The study followed the Institutional Review Board rules. Also, an informed consent was obtained from all parents participated in this study. The questionnaires were filled during both periods (See Supporting Information).

**Fig 1.** Research process.

2.5. Risk Assessment Model: Description and Assumptions

To perform risk assessment analysis, data on several specific variables was required. Extensive literature search in databases, such as Web of Science Core Correlation, Index Medicus and PubMed, was completed to obtain all necessary data. The variables modeled in this study are summarized in Fig. 1. In our model, we used (i) as minimum the estimates of the American Industrial Health council, according to which the daily oral intake of soil in children is 0.016 gr/day (Ryan et al., 2014), (ii) as average and

maximum intake values the ones estimated by N & R Consult, namely 0.025 and 0.1 g/day, respectively, (iii) reference values of survival in soil of *E. coli*, *P. aeruginosa*, and *S. aureus* were determined from standards obtained by Farangi et al. (Farhangi, Safari Sinegani, Mosaddeghi, Unc, & Khodakaramian, 2014; USEPA 2011) and QMRAWiki (Holmes Jr, Shirai, Richter, & Kissel, 1999), (iv) random soil intake-pica syndrome was considered as 0.2 g/day (Calabrese et al., 1989), (v) hand-eye, hand-mouth, hand-nose contacts were considered as 1/3.75 min (Farhangi, Sinegani, Mosaddeghi, Unc, & Khodakaramian, 2013), (vi) postaction soil loadings on children's hand was considered as 0.17 mg/cm² (Calabrese et al., 1989; Teunis, Takumi, & Shinagawa, 2004), (vii) the hand surface that comes into contact with mouth, eyes, nose, and skin was supposed to be 240 cm² (Calabrese et al., 1989; USEPA 2011), and (viii) the microorganism survival in the host body as described in Teunis et al. (Teunis et al., 2004).

2.6. Models from the Literature

Dose-response models for this beta-Poisson model were obtained from the published literature by the online QMRA wiki: [http://wiki.camra.msu.edu/index.php?title=1/4Quantitative_Microbial_Risk_Assessment_\(QMRA\)_Wiki](http://wiki.camra.msu.edu/index.php?title=1/4Quantitative_Microbial_Risk_Assessment_(QMRA)_Wiki) (Accessed on 4th October 2020). The models depict the probability of a response, namely *E. coli*, *P. aeruginosa*, and *S. aureus*, given a specific pathogen dose. Based on the most accepted assumption that bacteria are Poisson distributed (Teunis et al., 2004), we used the following models:

(a) Concerning *E. coli*, the dose of exposure and the risk of infection were calculated upon one of the proposed QMRA wiki models, namely beta-Poisson model. Although exponential model was indicated as the preferred one in most circumstances, we chose the beta-Poisson model for Enteroinvasive *E. coli* (EIEC) agent strain 1624, mainly for experimental purposes:

$$P_{infEC} = 1 - \left[1 + \frac{D_{expEC}}{\beta} \right]^{-\alpha}$$

where P_{infEC} is probability of infection by *E. coli* when ingesting a dose, D_{expEC} is dose of exposure, $D_{expEC} = Q_{HM} \times N_{EC} \times t \times R$ (Holmes Jr et al., 1999)

Q_{HM} is the volume of ingestion due to hand-to-mouth-contact with hands per minute, $Q_{HM} = h \times A \times f_{HM}$ (De Man et al., 2014), where h is

the film thickness on hands (0.17 mg/mm², in our case)(Holmes Jr et al., 1999), A is the skin surface area of the hand that touched the mouth (70 cm², in our case) (Boyd et al., 1999), and f_{HM} is the frequency of hand-to-mouth contact (1/2.75 min, as assumed by the researchers in our case)] N_{EC} is *E. coli* density (numbers/cm²), $N = C_{oEC} \times 10^{KT} \times V$ (De Man et al., 2014), C_{oEC} the initial *E. coli* concentration distribution, as found by our laboratory analyses (3373 CFU/ml), K is *E. coli* survival probabilities (2.18E-04), T is the duration of exposure (1 hour per day, in our case), V is the total volume exposure (0.02 g/day, in our case)(Boyd et al., 1999), t is the duration of exposure (1 hour per day, in our case), R is Ranking factor as described and calculated for each sampled playground by the questionnaire (ranged between 0.5 to 1), β is dose response parameter (1.442, in our case)(Teunis et al., 2004), and α is dose response parameter (0.0844, in our case)(Teunis et al., 2004)

b) The dose and risk of infection of *P. aeruginosa* was computed using the beta-Poisson model, as well, as this is proposed by QMRA wiki:

$$P_{infPA} = 1 - \left[1 + \frac{D_{expPA}}{\beta} \right]^{-\alpha} \text{ (Sushil, 2014)}$$

where P_{infPA} is probability of infection by *P. aeruginosa* when ingesting a dose, D_{expPA} is dose of exposure, and $D_{expPA} = Q_{HM} \times N_{PA} \times t \times R$ (Teunis et al., 2004).

where Q_{HM} is the volume of ingestion due to hand-to-mouth-contact with hands per minute, $Q_{HM} = h \times A \times f_{HM}$ (Teunis et al., 2004), where h is the film thickness on hands (0.17 mg/mm², in our case)(Holmes Jr et al., 1999), A is the skin surface area of the hand that touched the mouth (70 cm², in our case)(USEPA 2011), and f_{HM} is the frequency of hand-to-mouth contact (1/2.75 min, as assumed by the researchers in our case)]

N_{PA} is *P. aeruginosa* density (numbers/cm²), $N = C_{oPA} \times 10^{KT} \times V$ (24), C_{oPA} is the initial *P. aeruginosa* concentration distribution, as found by our laboratory analyses (390 CFU/ml), K is *P. aeruginosa* survival probabilities, T is the duration of exposure (1 hour per day, in our case), V is the total volume exposure (0.02 gr/day, in our case)(Boyd et al., 1999), t is the duration of exposure (1 hour per day, in our case), R is Ranking factor as described and calculated for each sampled playground by the questionnaire (ranged between 0.5 to 1), β is dose response parameter (1.442, in our case)(Teunis et al., 2004), and α is dose response parameter (1.9×10^{-01} , in our case)(Sushil, 2014)

Table 2. Summary Statistics of Microbiological Data and Temperature

	Season	Number of Samples tested	°C	SD
Temperature	Dry	64	48.30	8.05
	Wet	76	8.83	7.63
Bacterial type			Mean CFU/g	
<i>E. coli</i>	Dry	64	129.96	474.78
	Wet	76	6,104.05	24,209.62
<i>P. aeruginosa</i>	Dry	64	32.81	181.37
	Wet	76	691.78	2,869.32
<i>S. aureus</i>	Dry	64	0.00	0.00
	Wet	76	5,055.59	5,944.32

c) Concerning *S. aureus* (SA), there was only one available model, the exponential dose response model, which is suggested and preferred in most circumstances, namely:

$$c) P_{infSA} = 1 - \exp(-k \times D_{expSA})$$

where P_{infSA} : probability of infection by *S. aureus* when ingesting a dose, D_{expSA} : dose of exposure, $D_{expSA} = Q_{HM} \times N_{SA} \times t \times R$ (De Man et al., 2014)

where Q_{HM} is the volume of ingestion due to hand-to-mouth-contact with hands per minute, $Q_{HM} = h \times A \times f_{HM}$ (De Man et al., 2014), where h the film thickness on hands (0.17 mg/mm², in our case)(Holmes Jr et al., 1999), A is the skin surface area of the hand that touched the mouth (70 cm², in our case)(Boyd et al., 1999), and f_{HM} is the frequency of hand-to-mouth contact (1/2.75 min, as assumed by the researchers in our case)]

N_{SA} is *S. aureus* density (numbers/cm²), $N = C_{oSA} \times 10^{KT} \times V$ (De Man et al., 2014), C_{oSA} the initial *S. aureus* concentration distribution, as found by our laboratory analyses (2744 CFU/ml), K is *S. aureus* survival probabilities ($K = 7.64E-08$), T is the duration of exposure (1 hour per day, in our case), V is the total volume exposure (0.02 gr/day, in our case)(De Man et al., 2014) t is the duration of exposure (1 hour per day, in our case) R is Ranking factor as described and calculated for each sampled playground by the questionnaire (ranged between 0.5 to 1), and k is constant growth rate (7.64E-08).

2.7. Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) (IBM 2019).

2.8. Spatial GIS analysis

Spatial analysis was prepared using the commercially available software ArcGIS 10.8 (ESRI Inc. Redland, CA). The data included the place of each playground and draw quantities using symbol size to show relative values for each parameter. No normalization used.

3. RESULTS AND DISCUSSION

The summary of the microbiological results and the temperature are reported in Table 2. The main descriptive statistics of the analytical results of the standardized questionnaire completed by parents/accompanying persons are shown in Table 3. Although stricter regulations concerning the children's safety resulted in the modernization of almost all Greek municipal playgrounds, most of the studied playgrounds -with very few exceptions- seem to be of "low quality."

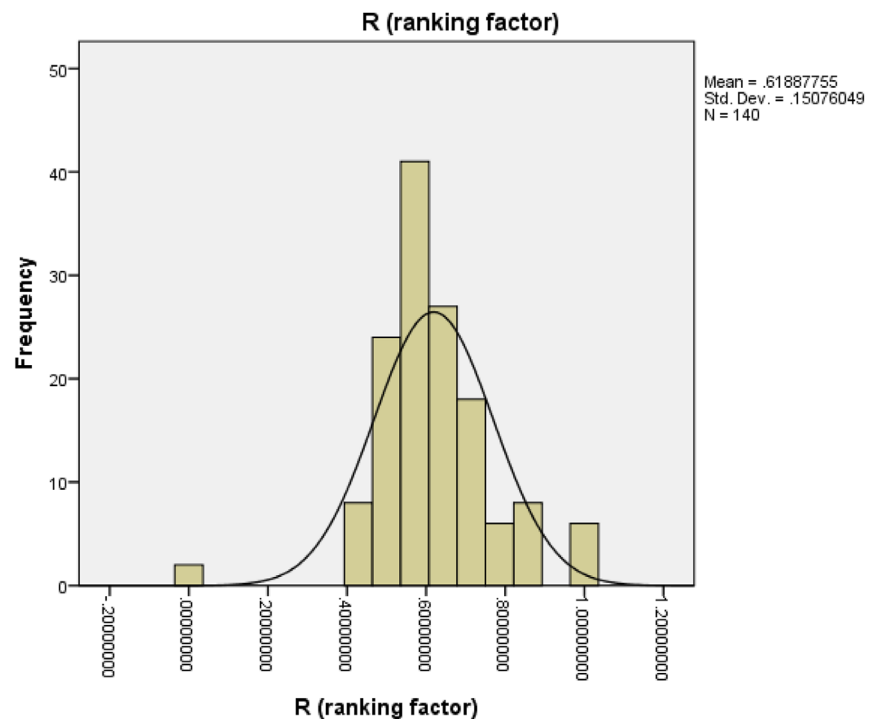
The ranking factor (R) varied from 0.429 (corresponding to the worst playground conditions) to 1 (corresponding to excellent playground conditions) (Fig. 2). The average R to our cases were around 0.61 for either dry or wet period. As opposed to other factors, R constitutes of a summary of agents that are not affected significantly from the period.

Applying the beta-Poisson and the exponential models on our samples, a descriptive summary of all components, namely the R ranking factor, the concentration, the dose of exposure and the risk of infection of *E. coli*, *P. aeruginosa*, and *S. aureus* are shown in Table 4, while the probability of infection of each bacteria is visualized in Fig. 3. It is observed that there is a higher risk of infection in the order of *P. aeruginosa* > *E. coli* > *S. aureus* for a child aged 6–9, when playing in the playground one hour every day. The average bacterial concentrations in

Table 3. Questionnaire Completed by the Parents/Accompanying Persons in the Playgrounds

Question	Categories	Frequency	%
1.How far is the playground from the highway (m)?	300–400	2	1.43
	Other	138	98.57
2.How far is the playground from the railway network (m)?	300–400	2	1.43
	400–500	4	2.86
	500–600	2	1.43
	600–700	4	2.86
	Other	128	91.43
3.Is the playground located less than 300 m away from electromagnetic radiation or high voltage pylons?	Yes	44	31.43
	No	96	68.57
4.Is the playground located in areas that pose risks?	Yes	80	57.14
	No	60	42.86
5.Is the playground visually isolated?	Yes	28	20
	No	112	80
6.Is the playground close to facilities that can harm children's mental health?	Yes	6	4.29
	No	134	95.71
7.Is there easy and secure access to the playground?	Yes	102	72.86
	No	38	27.14
8.Is the playground surrounded by proper fencing?	Natural	6	4.29
	Artificial	48	34.29
	No	86	61.43
9.Does the playground have a main entrance with a minimum opening of 1 m?	Yes	126	90
	No	14	10
10.Does the playground have proper and adequate lighting?	Yes	126	90
	No	14	10
11.Does the playground have sufficient number of seats for accompanying persons?	Yes	118	84.29
	No	22	15.71
12.Is there a faucet with drinking water?	Yes	16	11.43
	No	124	88.57
13.How many faucets of drinking water are there?	1	12	8.57
	Other	128	91.43
14.Are there any litter bins?	Yes	100	71.43
	No	40	28.57
15.Is the playground clean?	Yes	2	1.43
	No	138	98.57
16.Does the playground have emergency telephones?	Yes	6	4.29
	No	134	95.71
17.Are the opening hours listed in the playground?	Strongly ideal	12	8.57
	Very ideal	46	32.86
	Ideal	54	38.57
	Not ideal	28	20
18.Does the playground have suitable equipment for children to play?	Yes	42	30
	No	98	70
19.Does the playground have any rusty equipment?	Very clean	10	7.14
	Clean	70	50
	Unclean	60	42.86
20.Does the playground have any dangerous equipment?	Yes	104	74.29
	No	36	25.71
21.Is there enough space between the equipment?	No	140	100
22.Does the playground have a banner sign for the entry of nonaccompanying children as well as the entry of pets, with the exception of dogs accompanying people with disabilities?	Yes	2	1.43
	No	138	98.57
23.Does the playground have a badge of an accredited playground?	Yes	74	52.86
	No	66	47.14
24.Does the playground have access for people with disabilities?	Yes > 5 m	72	51.43
	Yes < 5 m	54	38.57
	No	14	10

Fig 2. *R* ranking factor as calculated by six risk factors acquired by the questionnaire completed by the parents/accompanying persons.



the soil samples decrease in the same order for either wet or dry periods. The mean *P* infection of *E. coli*, *P. aeruginosa*, and *S. aureus* was 0.003, 0.013, and 0, during dry period respectively and 0.05, 0.08, and 0, in the wet period, respectively. The risk of infection was increased multifold during the wet period (October–March) as opposed to the dry period (April to September), following the increased concentrations of *E. coli*, *P. aeruginosa*, and *S. aureus* in the sampled areas. *S. aureus* was found to be absent during the dry period and log increased in the wet season which was attributed to humidity and temperature. Dwivedi et al. (2016) found that high contamination of playground soil samples may be due to the soil constitution *per se*. Similar results concerning the *E. coli* risk of infection were obtained by Badura et al. (2014) and Matias, Fernandes, Proença, Duarte, & Barroso (2014), who studied soil of playgrounds in Canada and Austria. The *E. coli* survival rate (k) in soil does not differ significantly during either wet or dry period ($k = 0.16$ vs $k = 0.24$, respectively) while concentration increases during the wet period. Therefore, a statistically significant difference in *P* infection between periods is expected. The results of the present study were almost similar to those of Matias et al. (2014), who also concluded that the probability of infection of *P. aerug-*

inosa was higher in the wet period, while analyzing soil samples in playgrounds in Portugal. Additionally, Esmaeil et al. (2015) deduced that *P. aeruginosa* prevails in soil samples which are highly concentrated in heavy metals and this may also be encountered and further studied.

Applying both ANOVA and Mann–Whitney in our data for the probability of infection of *E. coli* and *P. aeruginosa* per period, we concluded that there was a statistically significant difference between them, as the probability of infection of *P. aeruginosa* was much higher. Applying the same statistical tests for the probability of infection of the same bacteria per different city, no statistically significant differences were recorded. The reason for this may be related to the fact that playgrounds in both cities, Patras and Pyrgos, are more or less similar and may be characterized as semiurban environments.

Other comparisons with the t-test were attempted between probability of infection of *E. coli* and *P. aeruginosa*, as well as between the probability of infection of *E. coli* and *S. aureus*, as well as *P. aeruginosa* and *S. aureus* and all comparisons proved to be of no statistical significance.

A correlation of probability of infection of all bacteria was attempted. The correlation results showed that all probabilities of infection are strongly

Table 4. Concentration (Co), Dose of Exposure (Dexp) and Probability of Infection (Pinf) of *Escherichia coli* (EC), *Pseudomonas aeruginosa* (PA), and *Staphylococcus aureus* (SA) as calculated by beta-Poisson and exponential models

	<i>n</i>	Mean	SD	Median
<i>R</i>	140	0.618	0.150	0.571
<i>C_{oEC}</i>	140	3,373.035	1,8035.152	10,000
<i>D_{expEC}</i>	140	14,708.536	8,0677.735	37,826
<i>P_{infEC}</i>	140	0.029	0.062	0.000
<i>C_{oPA}</i>	140	390.535	2,136.749	0.000
<i>D_{expPA}</i>	140	1,318.873	7,197.177	0.000
<i>P_{infPA}</i>	140	0.052	0.114	0.000
<i>C_{oSA}</i>	140	2,744.464	5,045.203	0.000
<i>D_{expSA}</i>	140	6,284.306	11,735.871	0.000
<i>P_{infSA}</i>	140	0.000	0.000	0.000

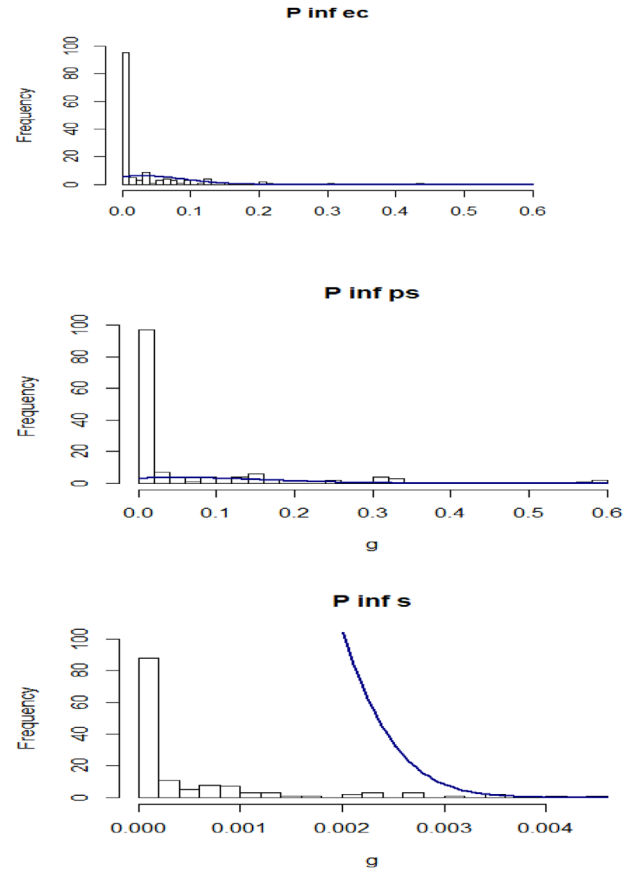


Fig 3. Mean probability of infection of *E. coli* (P inf ec), *P. aeruginosa* (P inf ps), and *S. aureus* (P inf s).

correlated together and this means that eradicating the risk of infection of one bacteria may affect the probability of infection of the other bacteria, as well.

Spatial distribution of the probability of infection of *E. coli*, *P. aeruginosa*, and *S. aureus* in the cities of Patras and Pyrgos cities are separately shown during the wet and dry seasons in Fig. 4. The risk estimation may contribute to targeted, risk-based surveillance of playgrounds from the state, by improving the facilities as well as the health promotion in order to minimize the infections on children and lead to proper decisions.

To our knowledge the present study is the one of the few to assess the risk of infection of *E. coli*, *P. aeruginosa*, and *S. aureus* evaluating the playground quality, the bacterial contamination, and epidemiological risk factors. Although, further studies are needed in order to produce more applicable guidelines for the risk assessments and define realistic

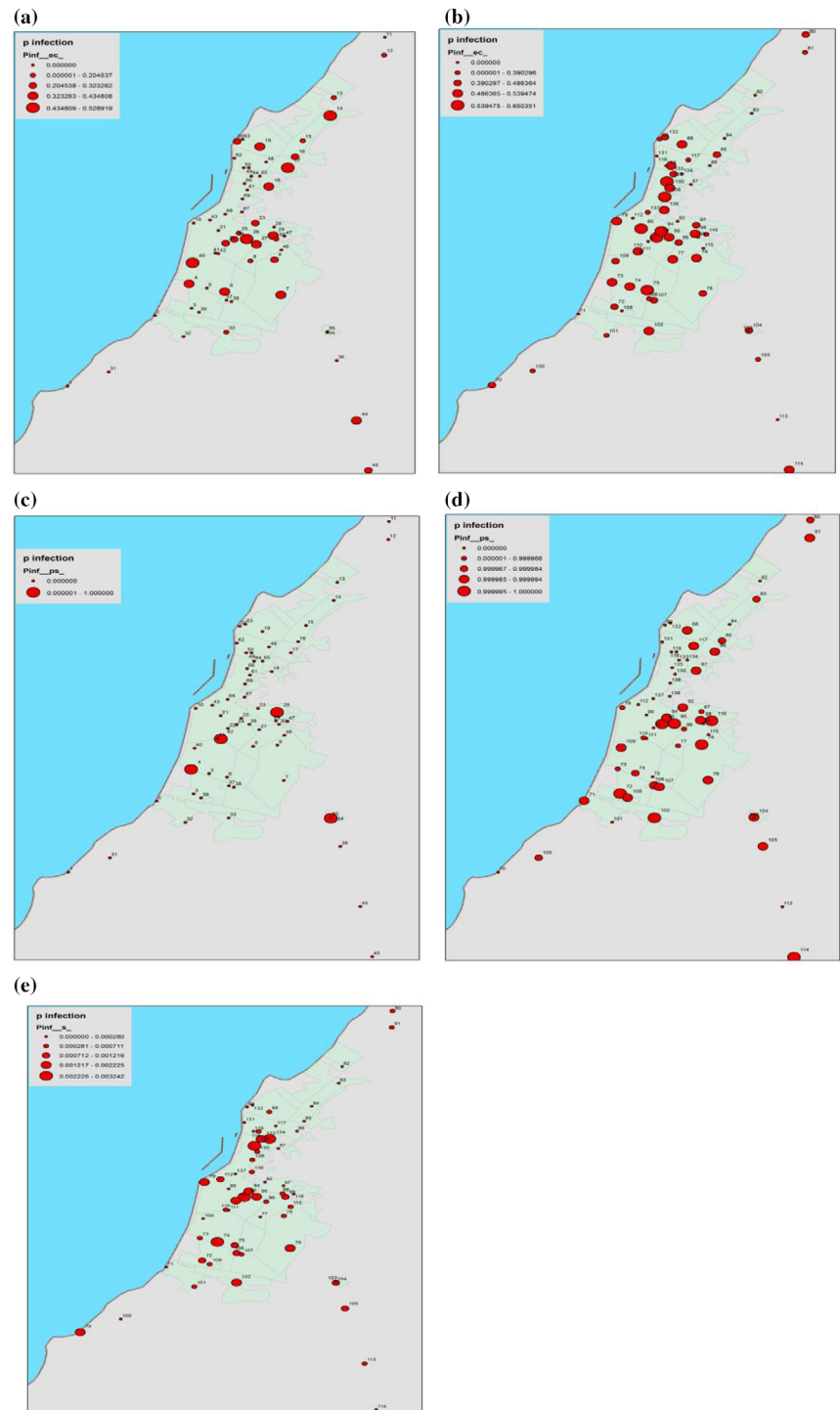


Fig 4. Spatial distribution of P infection of *E. coli*, *P. aeruginosa*, and *S. aureus* in Patras and Pyrgos cities in wet and dry periods. *E. coli*: (P inf ec) 4a (wet period) and 4b (dry), *P. aeruginosa*: (P inf ps) 4c (wet period) and 4d (dry period), and *S. aureus*: (P inf s) 4e (wet period).

cleanup levels of soil contamination, the study is an important contribution to this end.

CONFLICT OF INTERESTS

The authors declare no competing interests.

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